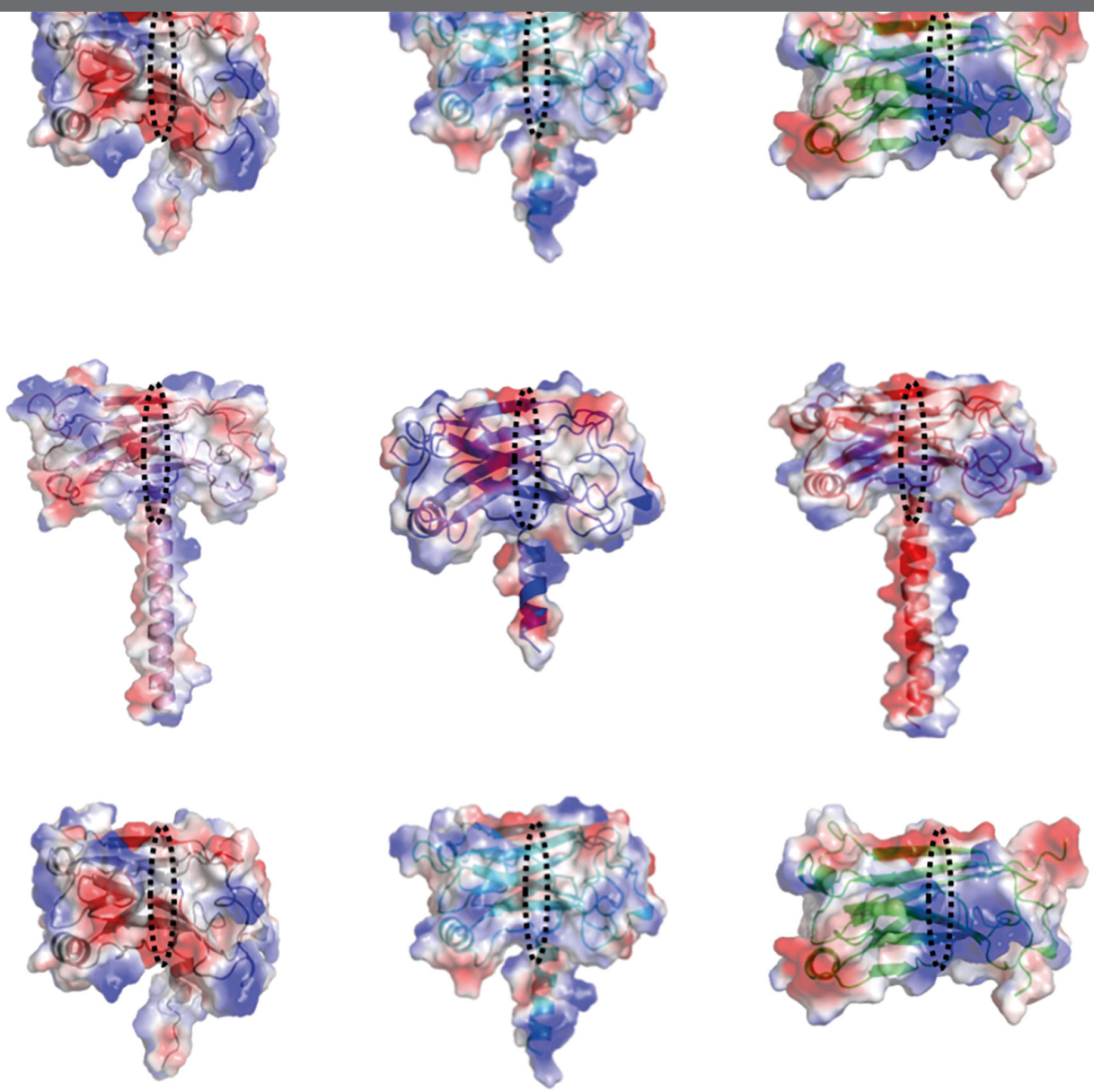
TRAF PROTEINS IN HUMAN DISEASE

EDITED BY: Gail A. Bishop, Ali A. Abdul-Sater and Tania H. Watts
PUBLISHED IN: Frontiers in Immunology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88945-871-4
DOI 10.3389/978-2-88945-871-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

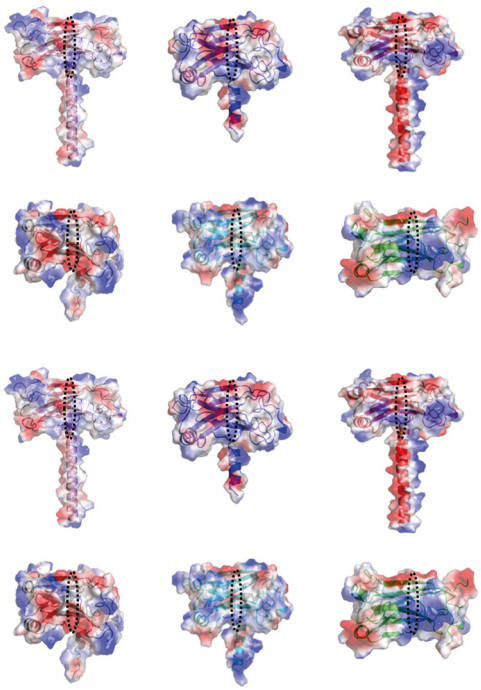
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

TRAF PROTEINS IN HUMAN DISEASE

Topic Editors:
Gail A. Bishop, University of Iowa, United States
Ali A. Abdul-Sater, York University, Canada
Tania H. Watts, University of Toronto, Canada



TRAF domain structure. TRAF1 through 6 NC domain structures are shown as an electrostatic surface presentation rendered using PyMol (https://www.pymol.org/) The black dotted circle indicates the receptor binding region.

Base on Figure 1D of Park HH (2018) Structure of TRAF Family: Current Understanding of Receptor Recognition. Front. Immunol. 9:1999. doi: 10.3389/fimmu.2018.01999

Tumor necrosis factor receptor (TNFR) associated factors (TRAFs) are a family of signaling adaptors first identified as components of TNFR signaling complexes, but now recognized to regulate signal transduction downstream of a diverse array of receptors, including Toll like receptors, antigen receptors and cytokine receptors. TRAF proteins play important roles in many human diseases and processes as both positive and negative regulators. This eBook begins with a review of TRAF structure, followed by reviews on the role of TRAFs in NF-κB and mitogen activated protein kinase (MAPK) signaling as well as in signaling downstream of innate pattern

recognition receptors. Next is a review focused on the role TRAF1 in human disease, while two other reviews focus specifically on the role of TRAF3 in B cells and in bone resorption, respectively. The role of TRAF proteins in cancer is considered in a review article and in an original research contribution. Additional reviews address the role of TRAF proteins in T follicular helper responses, as well as in IL-6 and 4-1BB signaling. Together these articles highlight the diverse and complex role of TRAF proteins as both positive and negative regulators in inflammatory and immune signaling, with impact on many human conditions.

Citation: Bishop, G. A., Abdul-Sater, A. A., Watts, T. H., eds. (2019). TRAF Proteins in Human Disease. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-871-4

Table of Contents

frontiers in Immunology

EDITORIAL
published: 26 February 2019
doi: 10.3389/fimmu.2019.00326

Editorial: TRAF Proteins in Health and Disease

Gail A. Bishop[1,2,3], *Ali A. Abdul-Sater*[4] *and Tania H. Watts*[5]*

[1] Department of Microbiology and Immunology, University of Iowa, Iowa City, IA, United States, [2] Department of Internal Medicine, University of Iowa, Iowa City, IA, United States, [3] Iowa City VA Health Care System, Iowa City, IA, United States, [4] School of Kinesiology and Health Science, Faculty of Health, York University, Toronto, ON, Canada, [5] Department of Immunology, University of Toronto, Toronto, ON, Canada

Keywords: TNFR signaling, TLR signaling, NLR signaling, cytokine signaling, antigen receptor signaling, NF-kB, inflammation, cancer

Editorial on Research topic

TRAF Proteins in Health and Disease

TRAF proteins are a family of signaling adaptors that play diverse roles in signaling by a broad range of receptors involved in immunity and inflammation. First identified as signaling adaptors downstream of TNFR2/CD120b (1), TRAFs have been implicated in regulating signaling by antigen receptors, cytokine receptors, and members of the TNFR superfamily as well as receptors of the innate immune system (2). New insights into the structure of the family as well as a wealth of new studies on regulation of signaling by TRAFs prompted the creation of this special topic. This collection of 11 papers highlights the role of TRAF proteins in diverse signaling pathways as well as their role in a number of biological and disease processes.

There are 7 mammalian TRAFs. TRAFs 1 through 6 share the conserved TRAF domain, responsible for hetero- and homo-oligomerization of TRAF proteins, as well as recruitment to TRAF motifs in the cytoplasmic domains of cell surface receptors, and certain cytoplasmic and nuclear proteins (**Figure 1**). Crystal structures are now available for all 6 TRAF proteins including several recently elucidated complexes of TRAFs with binding partners. As reviewed by Park, comparison of these structures has revealed conserved as well as unique features of binding among the different TRAF proteins. TRAF1 is unusual among TRAF proteins in lacking the RING domain shared by TRAFs 2–6 and the non-conventional TRAF7. Here, Edilova et al. review the role of TRAF1 as a positive regulator of signaling downstream of TNFRs such as CD40, 4-1BB, and LMP1, and as a negative regulator of TLR signaling. They also discuss the potential roles of TRAF1 in human diseases, including arthritis and cancer (Edilova et al.).

NF-κB pathways—including both the canonical and non-canonical/NF-κB2 pathways—play important roles in the functions of TRAF proteins. These pathways, and related MAPK pathways, are the focus of a review by Shi and Sun. Shi and Sun discuss the role of TRAFs 2 and 6 as positive regulators of NF-κB and MAPK signaling, downstream of multiple receptors, the anti-inflammatory role of TRAF2 and 3 in restraining non-canonical NF-κB signaling, as well as TRAF3 as a negative regulator of TLR signaling.

TRAF proteins have important roles in regulation of inflammation through their role in activation of pattern recognition receptors, including Toll-like receptors, RIG-I like receptors, Nod-like receptors, inflammasomes, and STING signaling. In their mini-review, Dhillon et al. discuss the role of TRAFs in both positive and negative regulation of these pathways.

The physiologic importance of TRAFs 2, 3, and 6 was not easy to determine initially, because germline deletion of their genes in mice led to peri-natal lethality with multiple severe developmental abnormalities (3–6). The development of conditional deletion approaches has

OPEN ACCESS

Edited and reviewed by:
Francesca Granucci, Università degli Studi di Milano Bicocca, Italy

***Correspondence:**
Tania H. Watts
tania.watts@utoronto.ca

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *16 January 2019*
Accepted: *07 February 2019*
Published: *26 February 2019*

Citation:
Bishop GA, Abdul-Sater AA and Watts TH (2019) Editorial: TRAF Proteins in Health and Disease. Front. Immunol. 10:326. doi: 10.3389/fimmu.2019.00326

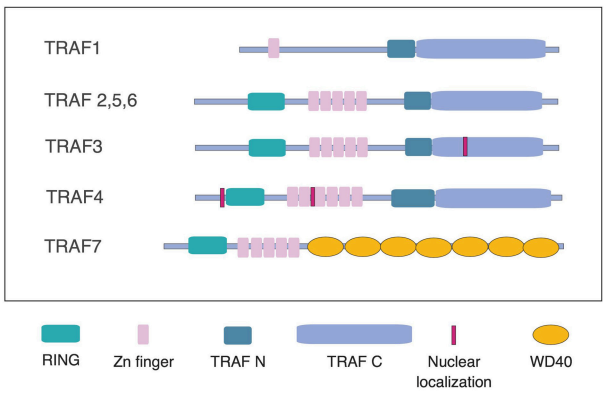


FIGURE 1 | Schematic representation of TRAFs 1 through 7. TRAFs 2,3,5, and 6, share the conserved TRAF domain, consisting of the coiled-coil, TRAFN domain and the TRAF-C domain, also known as a MATH domain, as well as a RING domain and a series of Zn fingers. TRAF1 differs from the other TRAFs in lacking the RING domain. TRAF3 and TRAF4 have nuclear localization sequences. TRAF7 lacks the TRAF domain, which is replaced by a series of WD40 domains (adapted from reference Park).

allowed much more information to be revealed about the *in vivo* functions of these molecules. This has in turn highlighted the cell-type-specific functions that characterize several of the TRAFs. Boyce et al. discuss the roles played by TRAF3 in regulating the balance between osteoblasts and osteoclasts in bone health, as well as disorders detrimentally impacting this balance. Their article summarizes studies showing that TRAF3 can both limit osteoclast formation by limiting signaling through the TNFR superfamily member RANK, as well as inhibiting TNF-induced osteoclast formation. The review by Bishop et al. demonstrates that TRAF3 regulates normal and malignant B cell biology via multiple mechanisms, including the pathways just mentioned as well as by modulating glucose metabolism, and inhibiting targets of the nuclear CREB complex.

Given the critical role of NF-κB in regulating genes associated with inflammation and cellular survival, it is not surprising that TRAF proteins play important roles in cancer. TRAF-dependent signaling pathways are altered in many cancers and there is extensive evidence for both genetic and post-translational alterations in TRAF signaling. In their review, Zhu et al. provide a comprehensive analysis of the Cancer Genome Atlas and the Catalog of Somatic Mutations in Cancer, with respect to genetic alterations in TRAF proteins in cancer. They find that all 7 TRAF family members show alterations in human cancers, with gain of function common for TRAFs 1, 4, 5, and 6, and loss of function commonly seen for TRAFs 3 and 5. In a related original research contribution, Perez-Chacon et al. describe a mouse model in which global overexpression of TRAF3 and Bcl2 results in tumors with features of mature Non-Hodgkin B cell lymphoma. The results suggest that TRAF3 and Bcl2 cooperate to induce neoplasms of mature B cells in mice. Although seemingly at odds with the finding that TRAF3 is frequently lost in human cancer, the review by Dhillon et al. points out that TRAF3 not only inhibits some aspects of TLR function, but can enhance inflammation downstream of TRIF dependent receptors, so TRAF3 can both positively and negatively regulate tumorigenesis. Additionally, it is now clear that TRAFs, particularly TRAF3, can play varied and even divergent cell-type specific functions. As the overexpression of TRAF3 in the mouse model studied in Perez-Chacon et al. is not confined to a single cell type, the phenotype of these mice likely reflects complex interactions between multiple TRAF3-overexpressing cell types.

T cell differentiation, leading to the development of a T follicular helper (Tfh) response, is critical for the development of germinal centers and affinity maturation of the B cell response (7). TRAFs are involved in signaling by a number of receptors that have been implicated in the development of the Tfh response, including the TNFR family members OX40, GITR, and 4-1BB, reviewed in this collection by Pedros et al. ICOS, a critical receptor for Tfh development, shares with TRAF proteins a TBK1 binding motif, and the authors discuss how this TRAF-mimicking signal plays a key role in Tfh development (Pedros et al.).

A more in depth look at the 4-1BB signalosome is provided by Zapata et al., who propose that different TRAF trimer configurations can allow formation of a complex, higher order signalosome with opportunities for recruitment of diverse signaling molecules. The authors also discuss the use of agonists against 4-1BB in cancer therapy and the implications of understanding 4-1BB signaling for design of new cancer treatments (Zapata et al.).

Signaling through the IL-6 receptor (IL-6R) plays an essential role in differentiation of CD4 T cells into Th17 cells (8), cells with important protective functions against extracellular bacteria and fungi (9). However, Th17 cells can also have a pathological role in diseases such as experimental autoimmune encephalomyelitis (EAE) (10), an experimental model for human multiple sclerosis. The IL-6R consists of two chains, the IL-6Rα chain and gp130, shared with other members of the IL-6R family. In this collection, Nagashima et al. discuss how IL-6R in naïve CD4 T cells binds TRAF2 and 5, thereby restricting the binding of JAKs to gp130, and limiting subsequent IL-6-mediated Stat3 activation. Thus, knockdown of TRAF2 or 5 enhances Th17 development and exacerbates EAE.

Overall, the articles in this Frontiers topic address the pivotal roles that TRAF proteins play as positive and negative regulators of inflammation and immunity mediated by a diverse array of receptors and cell types. The fact that each of the TRAF proteins can play both positive and negative roles in particular pathways or contexts, defies simple generalization. The dysregulation of TRAF proteins in cancer and a number of inflammatory diseases, suggests that this is an area that needs further attention. However, the nuanced role of TRAF proteins in each context will need to be carefully evaluated before they can be manipulated therapeutically.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Rothe M, Wong SC, Henzel WJ, Goeddel DV. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* (1994) 78:681–92.
2. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal.* (2013) 8:7. doi: 10.1186/1750-2187-8-7
3. Xu Y, Cheng G, Baltimore D. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity* (1996) 5:407–15.
4. Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, et al. TRAF6 deficiency results in osteopetrosis and defective IL-1, CD40, and LPS signaling. *Genes Dev.* (1999) 13:1015–21.
5. Nguyen LT, Duncan GS, Mirtsos C, Ng M, Speiser DE, Shahinain A, et al. TRAF2 deficiency results in hyperactivity of certain TNFR1 signals and impairment of CD40-mediated responses. *Immunity* (1999) 11:379–89.
6. Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, et al. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev.* (1999) 13:1015–24.
7. Crotty S. T follicular helper cell differentiation, function, and roles in disease. *Immunity* (2014) 41:529–42. doi: 10.1016/j.immuni.2014.10.004
8. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* (2006) 441:235–8. doi: 10.1038/nature04753
9. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* (2008) 28:454–67. doi: 10.1016/j.immuni.2008.03.004
10. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol.* (2005) 6:1133–41. doi: 10.1038/ni1261

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 30 August 2018
doi: 10.3389/fimmu.2018.01999

Structure of TRAF Family: Current Understanding of Receptor Recognition

*Hyun H. Park**

College of Pharmacy, Chung-Ang University, Seoul, South Korea

Tumor necrosis factor receptor–associated factor (TRAF) proteins are key signaling molecules that function in various cellular signaling events including immune response, cell death and survival, development, and thrombosis. Their roles in cellular signaling are mediated mostly by direct interactions with various receptors via the TRAF domain. To determine how specific TRAF domains can interact with various receptors with a limited binding interface and how similar binding interfaces of TRAF family members can recognize their specific binding partners, extensive structural studies on TRAF family proteins have been conducted for several decades. In this review, we discuss the current understanding of the structural and molecular diversity of the TRAF domain and TRAF-binding motifs in many receptors according to available structural information.

Keywords: inflammation, innate immunity, TRAF family, structure, TRAF domain, protein interaction

OPEN ACCESS

Edited by:
Ali Abdul Sater,
York University, Canada

Reviewed by:
Dustin Steven Whitney,
Massachusetts Institute of Technology, United States
Johannes A. Schmid,
Medizinische Universität Wien, Austria

***Correspondence:**
Hyun H. Park
xrayleox@cau.ac.kr

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *04 June 2018*
Accepted: *14 August 2018*
Published: *30 August 2018*

Citation:
Park HH (2018) Structure of TRAF Family: Current Understanding of Receptor Recognition. Front. Immunol. 9:1999. doi: 10.3389/fimmu.2018.01999

INTRODUCTION

Tumor necrosis factor (TNF) receptor–associated factor (TRAF) proteins, which include seven family members (from TRAF1 to TRAF7) in mammals, are key signaling molecules that can transduce signals in various types of receptor-mediated cellular signaling, including tumor necrosis factor receptor (TNF-R), interleukin 1 receptor/Toll-like receptor (TLR), nucleotide-binding oligomerization domain-like receptor (NLR), RIG-I like receptor (RLR), and even cytokine receptor family signaling pathways, and play critical roles in the regulation of the immune system and apoptosis (**Supplementary Table 1**) (1–4). The main feature of TRAF family proteins (except for TRAF1) is the homology RING domain at the N terminus; this domain is found in many E3 ubiquitin ligases and constitutes the core of the ubiquitin ligase catalytic domain and is important for ligase activity (5, 6). Another feature of the members of the TRAF family (except for TRAF7) is the presence of a protein–protein interaction domain of ∼230 amino acid residues, known as the TRAF domain, at the C terminus (**Figure 1A**). The TRAF domain is subdivided into two distinct subdomains: the TRAF-N domain, which is a coiled-coil domain, and the TRAF-C domain, which is composed of seven to eight anti-parallel β-strand folds. TRAF family members form a mushroom-like trimeric structure in solution via the TRAF domain, which is the functional unit of a TRAF (7, 8).

Depending on composition of this domain, TRAF proteins have two main functions: the E3 ubiquitin ligase function and scaffolding function. The scaffolding function of TRAF family members is based mainly on the TRAF domain, which can mediate interactions of various membrane receptors with diverse downstream effector molecules, primarily protein kinases, including IRAKs, RIP1, RIP2, TAK1, MEKK1, and ASK1 (9–12) and several ubiquitin ligases such as members of the cIAP family (13). Although a TRAF is a positive mediator of signaling

events, the antagonistic roles of TRAFs in TNF-R and TLR signaling have been reported (8, 14). The E3 ligase activity of TRAFs has also been intensively studied, and substrates of each family have been identified (**Supplementary Table 2**) (6, 15–18). According to their roles in many critical signaling pathways, TRAFs are related to many human diseases, including cancer, autoimmunity, and inflammatory diseases, and have been suggested as suitable targets for therapeutic intervention (19–23). Because of their important biological roles, structural studies on the TRAF family have increased in number. Specifically, studies have examined how specific TRAF proteins can interact with various receptors through the limited binding interface and how the similar binding interfaces of each TRAF family member can recognize their binding partners. The structures of the TRAF domain of TRAF2, TRAF3, and TRAF6 and their receptor complexes were elucidated around year 2000 (24–26), and those of TRAF1, TRAF4, and TRAF5 and their receptor complexes were examined recently (27–32). In this review, we discuss the current understanding of the structural and molecular diversity of the TRAF domain according to available structural information.

STRUCTURE OF TRAF FAMILY

The presence of the TRAF domain, a ∼180 amino acid protein-interacting domain, is a distinct feature of TRAF family proteins and six TRAF proteins (TRAF1–TRAF6) among the seven in the family, in accordance with this criterion, have been identified as the TRAF family in mammals (3). The TRAF domain can be subdivided into two distinct regions: the TRAF-N domain and TRAF-C domain. Various receptors bind to the TRAF-C domain, while various intracellular signaling molecules bind to the TRAF-N domain. Despite the structural similarity of TRAF domains, each TRAF protein has specific biological functions with specificity to the interacting partners: upstream receptors and downstream effector molecules. The structure of the TRAF domain of TRAF2 was first reported by Dr. Wu's group around 1999 (24), and the structure of TRAF6's TRAF domain was reported 3 years later by the same group (25). Since then, the structures of the TRAF domain of TRAF3 (27), TRAF5 (27), TRAF4 (28–30), and TRAF1 (31) have been reported. The TRAF structures revealed that the TRAF-N domain is a coiled-coil structure, and TRAF-C is composed of seven to eight anti-parallel β-sheet folds (**Figure 1B**). Structural alignment of all six TRAF family members shows that the TRAF-C domain is well-aligned, while the location and length of TRAF-N varies among TRAF family members (**Figure 1C**). Sequence analysis indicates that the length of TRAF-N varies in the family, whereas that of the TRAF-C domain is conserved: the length of TRAF-N of TRAF4 and TRAF6 is relatively shorter, while TRAF3 and TRAF5 are relatively longer (**Supplementary Figure 1**).

Although the overall structures are nearly identical, obvious structural differences have been observed. For example, the length and position of some loops in the TRAF domain of TRAF4 and TRAF6 differ from those of other TRAF family members (**Figure 1C**). Particularly, two loops connecting β5-β6 and β6-β7 of the TRAF4 TRAF domain are relatively longer than those of other TRAFs. The location of the TRAF-N coiled-coil domain also differs in that it is in the outer layer only in the structure of TRAF4 (**Figure 1C**). These slight differences in structure among the TRAF family members may be responsible for their functional differences. Characteristics of the electrostatic surface of the TRAF domain vary within the family, although the TRAF domains of TRAF1, TRAF2, TRAF3, and TRAF5 have similar overall features, with mixed positive and negative charges and several uncharged regions (**Figure 1D**). TRAF4 contains a more negatively charged surface in the middle of the receptor-binding region, whereas TRAF6 contains a more positively charged surface in the receptor-binding region (**Figure 1D**). Because the surface features often determine their mode of interactions with partners, the similar electrostatic surface of the TRAF domain among TRAF1, TRAF2, TRAF3, and TRAF5, namely, diversely charged surfaces, has been shown to be important for accommodating diverse receptors in the same binding pocket with similar modes of interaction. In contrast, different features on the binding surface of functionally different TRAFs, TRAF4, and TRAF6, indicate that TRAF4 and TRAF6 can accommodate different receptors with different modes of interactions.

In solution, the TRAF domain forms a stable functionally important trimer that has a typical mushroom shape; the TRAF-C domain forms the cap and TRAF-N coiled-coil domain forms the stalk (7, 31) (**Figure 1E**). Biochemical and structural analyses show that many interaction hot spots formed by β3, β4, β6, and β7 of the TRAF domains participate in the receptor interaction (**Figure 1E**). On the basis of the available structures of the trimeric TRAF domain, zinc-finger domain, and RING domain, a reconstituted full-length TRAF structure has been modeled (**Figure 1F**). Because there is no evidence of self-association between the zinc-finger domains or RING domains in the TRAF family, the C-terminal TRAF domain, which interacts with trimeric active receptors, forms a functional trimer, while the N-terminal RING domain and zinc-finger domain remain flexible (**Figure 1F**). In this regard, the length of the whole TRAF may be approximately 300Å and the shape is a long rod that is open at one end and closed at the opposite end.

RECEPTOR RECOGNITION BY TRAFs

TRAF family members interact with various receptors and intracellular proteins, including CD40, CD30, Ox40, TRADD, LMP1, TNFR2, RANK, IRAK, RIP2, GPIb, GPVI, and TANK, during specific signaling events. The initial structural and biochemical studies on TRAFs (particularly TRAF2 and TRAF3) and their interacting receptors have revealed that for such interactions, TRAFs use three regions, known as binding hot spots: Hot spot 1, also known as the hydrophobic pocket, is composed of residues from β4, β5, β6, and β7. Hot spot 2, also known as the serine finger, is composed of three serine residues (one serine residue is replaced by alanine in TRAF1) from β6 and the loop connecting β6 and β7. Hot spot 3, also known as the polar pocket, is composed of polar residues from β3 and the loop connecting β3 and β4 (24, 25, 33, 34). The

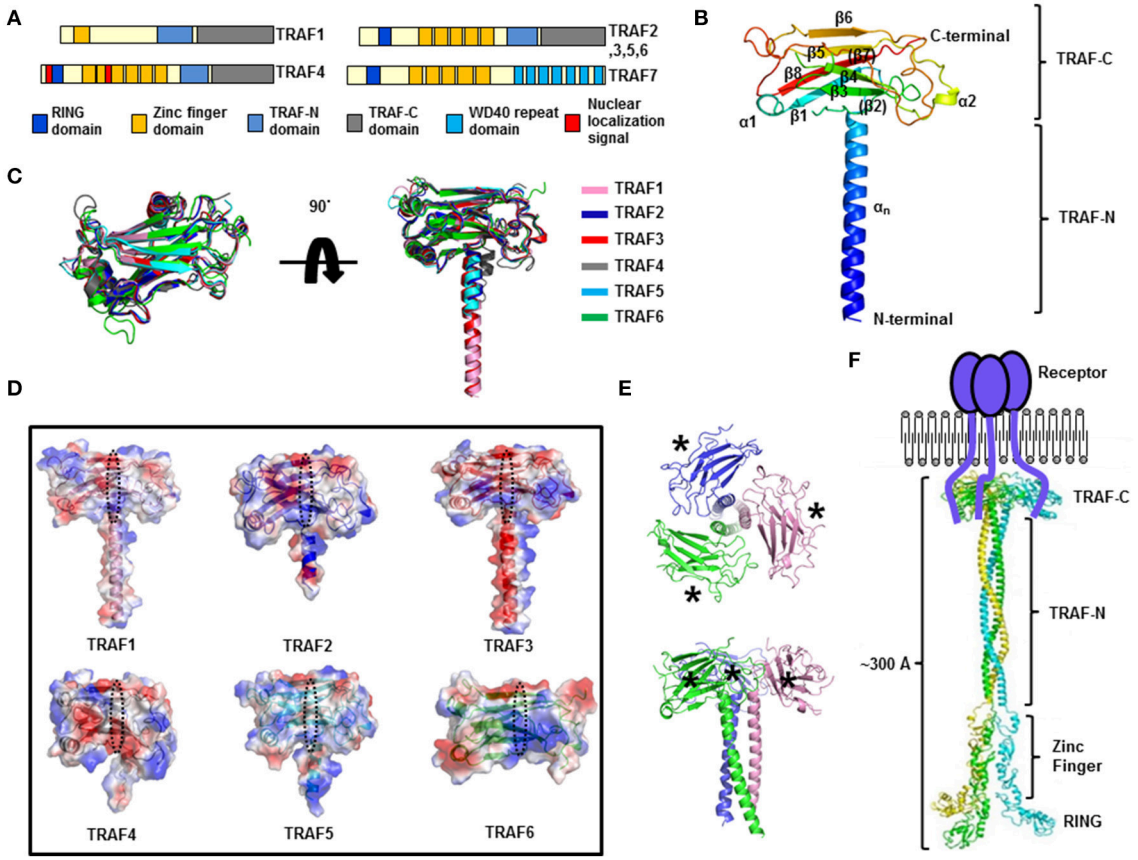
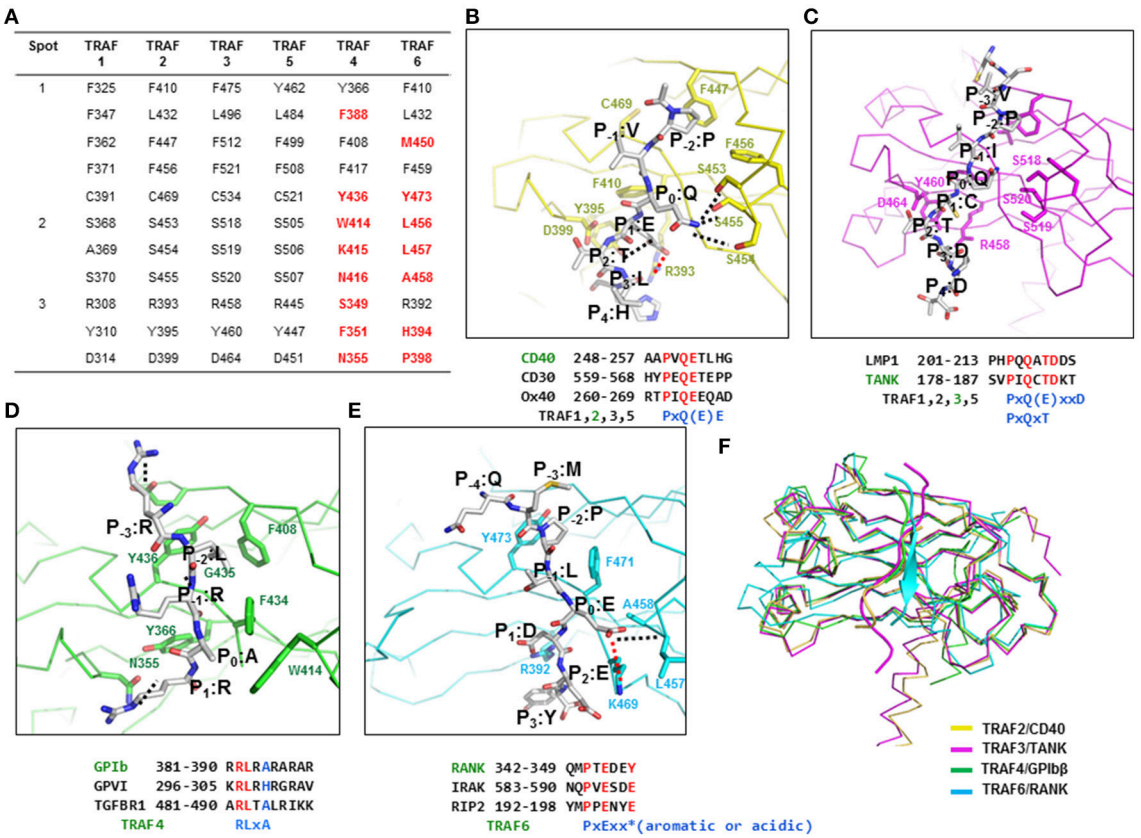


FIGURE 1 | Structure of the TRAF family. **(A)** The domain boundary in TRAF family members. **(B)** A cartoon of the monomeric TRAF domain. The representative TRAF domain of TRAF1 is used to show the overall structure of this domain. The chain from the N to C terminus is colored blue to red. Secondary structures including helices and sheets are labeled. **(C)** Superposition of the structures of the TRAF domain. **(D)** Electrostatic surface representation of the TRAF domain in the TRAF family. PyMol (https://www.pymol.org/) was used to compute qualitative surface electrostatic potential. The receptor-binding region in a TRAF family member is indicated by the black dotted circle. **(E)** A cartoon of the trimeric TRAF domain. Different chains are shown separately in different colors. The top view (upper panel) and side view (lower panel) are presented. The asterisks indicate the receptor-binding region. **(F)** A model of the full-length structure of TRAF family members.

residues in these three hot spots of TRAF1, TRAF2, TRAF3, and TRAF5 are conserved, indicating that these TRAFs share receptor specificity via similar interaction modes (**Figure 2A**). The residues of TRAF4 and TRAF6 in the three binding hot spots of typical TRAFs are not conserved although several residues in hot spot 1 are conserved, indicating that TRAF4 and TRAF6 are unique and do not share binding mode and specificity to their receptors with typical TRAF family members (**Figure 2A**). Current available structures of TARF family with receptor peptides are listed at (**Supplementary Table 3**).

TRAF1, TRAF2, TRAF3, and TRAF5

The typical binding hot spots and modes of interaction of TRAF family members have been well-studied for TRAF2 and TRAF3. The minimal consensus motif in TRAF-binding proteins, including TNF-R family members, CD30, CD40, Ox40, and LMP1, for TRAF2 and TRAF3 interaction is Px(Q or E)E# [x: any amino acid, #: acidic or polar amino acids are favored] (**Figure 2B**). Initial structural research on TRAF2 in complex with various peptides revealed the most conserved amino acid in the TRAF-binding motif to be P_0, or the zero position of the TRAF-binding motif. Based on this labeling strategy, residues of the Px(Q or E)E motif were named as P (P_{-2}), × (P_{-1}), Q or E (P_0), E (P_1), and # (P2). For CD40, residues of the TRAF2-binding motif were named as P (P_{-2}), V (P_{-1}), Q (P_0), E (P_1), T (P_2), and L (P_3) (**Figure 2B**). To accommodate the Px(Q or E)E motif, hot spot 1–forming residues (F410, L432, F447, F456, and C469) in TRAF2 make extensive van der Waal contacts with P at the P_{-2} site. The major structural determinant of Q or E at the P_0 position interacts with residues in hot spot 2 (serine triad, S453, S454, and S455 in TRAF2). Q at position P_0 forms hydrogen bonds with all three serine residues, while E at the P_0 position can form only one hydrogen bond. The carboxylate moiety of the Glu residue at position P_1 engages in an ion-pair interaction with the side chain guanidinium group of R393 and forms a hydrogen bond with Y395 in TRAF2. All residues that are critical for the interaction with the Px(Q or E)E motif are completely conserved in TRAF1, TRAF3, and TRAF5, except that one serine residue in the serine triad is replaced by alanine in TRAF1 (A369), indicating that TRAF1, TRAF2, TRAF3, and



Spot	TRAF 1	TRAF 2	TRAF 3	TRAF 5	TRAF 4	TRAF 6
1	F325	F410	F475	Y462	Y366	F410
	F347	L432	L496	L484	F388	L432
	F362	F447	F512	F499	F408	M450
	F371	F456	F521	F508	F417	F459
	C391	C469	C534	C521	Y436	Y473
2	S368	S453	S518	S505	W414	L456
	A369	S454	S519	S506	K415	L457
	S370	S455	S520	S507	N416	A458
3	R308	R393	R458	R445	S349	R392
	Y310	Y395	Y460	Y447	F351	H394
	D314	D399	D464	D451	N355	P398

FIGURE 2 | The TRAF-binding motif identified by structures of TRAF–receptor complexes. **(A)** Receptor-binding hot spots and conserved amino acid residues (in TRAF1, −2, −3, and −5) that are involved in the interaction with various receptors. The amino acid residues in TRAF4 and −6 that are not conserved are colored in red. **(B–E)** Detailed TRAF–receptor interaction. Close-up view of a CD40 peptide bound to TRAF2 **(B)**, a TANK peptide bound to TRAF3 **(C)**, a GPIbβ peptide bound to TRAF4 **(D)**, and a RANK peptide bound to TRAF6 **(E)**. Red dotted lines and black dotted lines indicate salt bridges and H-bonds, respectively. Amino acid positions of the peptide labeled as P_{-4}-P_3 are shown. TRAF-binding motifs are shown. **(F)** Structural comparison of the TRAF–receptor peptide complex by superposition analysis.

TRAF5 share the same mode of interaction involving the Px(Q or E)E motif.

In addition to this major TRAF-binding motif [Px(Q or E)E motif], the minor motif [Px(Q/E)xxD motif] has been identified in several structural studies including the TRAF2–LMP1 (33) and TRAF3–TANK (35) complexes. Therefore, two minimal consensus motifs for binding to TRAF1, −2, −3, and −5, i.e., Px(Q/E)E as a major motif and Px(Q/E)xxD as a minor motif, have been identified. In the Px(Q/E)xxD motif, the side chains of the residues at positions P_{-2}, P_0, and P_3 are critical for the TRAF interaction, unlike the major binding motif, where the side chains of residues at positions P_{-2}, P_0, and P_1 participate in the interaction (**Figure 2C**). Several modified interactions involving the minor motif have been reported in structural studies on TRAF3 in complex with various receptors, including CD40 (26), LMP1 (36), and TANK (35) and the most recently solved TRAF1–TANK complex (37). In this case, the side chains of the P_{-2}, P_0, and P_2 positions are involved in the TRAF interaction. The conserved amino acid residues at positions P_{-2}, P_0, and P_2 are P at P_{-2}, Q or E at P_0, and T at the P_2 position, forming the PxQxT consensus motif, which is considered an alternative minor TRAF1, −2, −3, and 5-binding motif. In the PxQxT motif, T (P_2) interacts with the conserved aspartic acid residue (D314 in TRAF1 and D464 in TRAF3), whereas the interaction modes of P (P_{-2}) and Q (P_0) are similar to those of the major binding motif. Additionally, D in the minor consensus motif PxQxxD is not important for the interaction with TRAF. The recently solved TRAF1–TANK (SVPIQCTDKT) structure revealed sequence PxQxT as the TRAF1-binding motif (37). In this case, Q at the P_0 position forms a hydrogen bond with S368 of TRAF1. C and T at positions P_1 and P_2, respectively, form hydrogen bonds with D314 of TRAF1.

TRAF4

The TRAF4 structure was determined around the year 2013 by three research groups (28–30). On the basis of these structural studies, TRAF4 was identified as a lipid-binding protein that can modulate tight junctions involved in cell migration; abnormal overexpression of TRAF4 can induce carcinomas by affecting cell migration (29). In 2017, the TRAF4-binding motif was characterized in a study on a TRAF4–receptor complex (32). TRAF4 functions as an adaptor signaling molecule in platelet

receptor–mediated production of reactive oxygen species by directly interacting with two platelet receptors: GPIb-IX-V and GPVI (38); peptides derived from these two platelet receptors have been used to analyze the TRAF4–receptor complex. The structure of the TRAF4–GPIbβ peptide complex has been solved by characterizing TRAF4 behavior and the TRAF4–platelet receptor interaction. If we use the same nomenclature, which denotes the most conserved amino acid position in the TRAF-binding motif as P_0, or the zero position of the TRAF-binding motif, in the bound peptide from GPIbβ receptor, the residues in the RLRAR motif are named as R (P_{-1}), L (P_0), R (P_1), A (P_2), and R (P_3) (**Figure 2D**). Although L (P_0) is the most conserved amino acid position in the TRAF4-binding motif, more appropriate names for residues in the TRAF4-binding motif RLRAR are R (P_{-3}), L (P_{-2}), R (P_{-1}), A (P_0), and R (P_1) according to previously identified receptor-binding hot spots (this notation is used hereafter). The first structure of a receptor complex of TRAF4 indicates that the side chains of R (P_{-3}), L (P_{-2}), A (P_0), and R (P_1) in GPIbβ receptor are involved in the TRAF4 interaction. The two hydrophobic pockets, major and minor, on the surface of TRAF4 are critical for its mode of receptor binding that is different from that of other TRAF family members. The major hydrophobic interaction is formed by L at P_{-2} of the GPIbβ peptide with F408, Y436, and F434 from TRAF4 and the second minor hydrophobic pocket is formed by A at P_0 of GPIbβ with W414 and F434 from TRAF4 (**Figure 2D**). R at the P_{-3} position and another R at the P_1 position form a hydrogen bond with the side chains of E406 and N355, respectively, from TRAF4. After further mutagenesis and interaction analyses using various peptides derived from putative TRAF4-binding receptors, the Arg-Leu motif at positions P_{-3} and P_{-2} was identified as crucial for the TRAF4 interaction, and the Ala residue at position P_0 influences affinity. Replacement of Ala at position P_0 with His (GPVI peptide) or Gly (TGF-β receptor 2 peptides) reduces the binding affinity for TRAF4, and replacement with Arg (NOD2 peptide) abrogates the interaction. This structural study estimated the TRAF4-binding motif for P_{-3} to P_0 as Arg-Leu-X-Ala, where X can be any amino acid and Ala can be replaced by a small uncharged residue.

TRAF6

The mode of interaction of TRAF6 with receptors is unique among TRAF family members and has been revealed by three available structures of complexes including TRAF6–CD40 (25), TRAF6–RANK (25), and TRAF6–MAVS (39). The TRAF6-binding motif is six amino acid residues in length, and the sequence is PxExxZ (x: any amino acid, Z: acidic or aromatic amino acid). Small hydrophobic resides can replace P. In accordance with the typical labeling system, the nomenclature of this motif is P (P_{-2}), × (P_{-1}), E (P_0), × (P_{-1}), × (P_{-2}), and Z (P_{-3}). The residues E235 of CD40, E346 of RANK, and E457 of MAVS have been designated as the P_0 position of TRAF6-binding peptides. The receptor peptide residues corresponding to positions P_{-4} to P_3 of CD40, RANK, and MAVS directly interact with TRAF6. Among the peptide residues, specific side chains of residues at P_{-2}, P_0, and P_3 are the greatest contributors to these interactions. As observed in other TRAF family members, F471 and Y473 of TRAF6 form a hydrophobic pocket to accommodate the P residue at the P_{-2} position (**Figure 2E**). Possible replacement of P residue to A residue at P_{-2} position was studied by mutagenesis on CD40 (25). Because of the absence of the typical serine triad in TRAF6, E at position P_0 in the receptor employs quite a different interaction strategy for incorporation into TRAF6. The carboxylate of E at the P_0 position forms hydrogen bonds with the main chain amide nitrogen atoms of L457 and A458 and engages in an electrostatic interaction with the side chain of K469 (**Figure 2E**). This P_0 interaction is also different from the TRAF4-binding motif in that TRAF4 uses a shallow hydrophobic pocket to bind to A at the P_0 position of the receptor peptide. The residue at position P_3 in CD40 (F238) and RANK (Y349) is adjacent to several aromatic and basic residues of TRAF6, including R392, forming an amino-aromatic interaction (**Figure 2E**).

CONCLUDING REMARKS

Despite the structural similarity of TRAF family members, each TRAF has specific biological functions with specificity to interacting partners: upstream receptors and downstream effector molecules. Because of the critical participation of the TRAF family in various signaling events, functional and structural analyses of these proteins have been conducted for several decades. Intensive studies have revealed that proteins of the TRAF family, except for TRAF7, contain a conserved TRAF domain at the C terminus, which mediates their interaction with upstream receptors and downstream effectors (3). Despite the structural similarity of the TRAF domain within the TRAF family, each domain of each TRAF protein is specific to interacting upstream receptors. In this review, we summarized the current understanding of TRAF-binding motifs of many receptors by examining the structures of all six TRAF family members and complexes of each TRAF with various receptors including recently characterized complexes TRAF4–GPIb (32), TRAF1–TANK (37), TRAF3–Cardif (27), and TRAF6–MAVS (39). Because the sequences of binding hot spots are conserved in TRAF1, −2, −3, and −5, they share the same binding consensus motifs, namely, one major motif, Px(Q/E)E, and two minor motifs: Px(Q/E)xxD, and Px(Q/E)xT. Although possessing nearly identical receptor binding motifs on TRAF2, −3, and −5, recent deep mutational scanning study with TRAF-peptide ligands showed key differences in binding preference. TRAF2, −3, and −5 have a binding preferences on CD40 and TANK with different affinity (40). The recently determined structure of TRAF4 in complex with its receptor GPIbβ revealed a novel mode of binding, which is consistent with the recently discovered receptor specificity of TRAF4, where nonconserved amino acid residues are critical for the interaction with various receptors. The TRAF4-binding motif is R (P_{-3}), L (P_{-2}), x, and A (P_0) [RLxA motif], where x can be any amino acid and Ala can be replaced with a small uncharged residue. The TRAF domain of TRAF6 binds specifically to the consensus

TRAF6-binding motif: PxExxZ (Z: an acidic or aromatic residue).

In conclusion, specificity of TRAFs can be mediated by different organization of binding hot spots. TRAF1, −2, −3, and −5, however, share various common receptors because of almost completely conserved binding hot spots. TRAF4 and TRAF6 are unique member of the TRAF family and have amino acid residues at the receptor-binding site that are completely different from those of other family members. As for the receptor interaction interface of TRAF4, the lower part of the receptor peptide (P_1) binds to TRAF4 at a position similar to that of receptor peptides that bind to TRAF2 and TRAF3, whereas the upper part (P_{-2}, and P_{-1}) is far away from the receptor peptide–binding site in TRAF2 and TRAF3 (**Figure 2F**). Compared to the receptor-binding site of TRAF6, the receptor-binding sites of TRAF1, −2, −3, −4, and −5 do not overlap with the RANK peptide, which binds to TRAF6. Nonetheless, the P_{-2}-binding pocket of TRAF6 is similar to that of other TRAFs, indicating that the region of receptor association in TRAF6 is slightly different from that of other TRAFs (**Figure 2F**). Because of the similarities and differences in the binding hot spots among TRAF family members, they can sometimes share receptors or select unique receptors in various important signaling pathways.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

FUNDING

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) of the Ministry of Education, Science and Technology (NRF-2018R1A2B2003635 and NRF-2017M3A9D8062960) and a grant from the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI17C0155).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2018.01999/full#supplementary-material

REFERENCES

1. Inoue J, Ishida T, Tsukamoto N, Kobayashi N, Naito A, Azuma S, et al. Tumor necrosis factor receptor-associated factor (TRAF) family: adapter proteins that mediate cytokine signaling. *Exp Cell Res.* (2000) 254:14–24. doi: 10.1006/excr.1999.4733
2. Bradley JR, Pober JS. Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene* (2001) 20:6482–91. doi: 10.1038/sj.onc.1204788
3. Chung JY, Park YC, Ye H, Wu H. All TRAFs are not created equal: common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J Cell Sci.* (2002) 115(Pt 4):679–88.
4. So T, Nagashima H, Ishii N. TNF receptor-associated factor (TRAF) signaling network in CD4(+) T-lymphocytes. *Tohoku J Exp Med.* (2015) 236:139–54. doi: 10.1620/tjem.236.139
5. Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. *Annu Rev Biochem.* (2009) 78:399–434. doi: 10.1146/annurev.biochem.78.101807.093809
6. Yang XD, Sun SC. Targeting signaling factors for degradation, an emerging mechanism for TRAF functions. *Immunol Rev.* (2015) 266:56–71. doi: 10.1111/imr.12311
7. Rothe M, Wong SC, Henzel WJ, Goeddel DV. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* (1994) 78:681–92. doi: 10.1016/0092-8674(94)90532-0
8. Zapata JM, Pawlowski K, Haas E, Ware CF, Godzik A, Reed JC. A diverse family of proteins containing tumor necrosis factor receptor- associated factor domains. *J Biol Chem.* (2001) 276:24242–52. doi: 10.1074/jbc.M100354200
9. Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV. TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. *Immunity* (1996) 4:387–96. doi: 10.1016/S1074-7613(00)80252-6
10. Song HY, Regnier CH, Kirschning CJ, Goeddel DV, Rothe M. Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor-kappaB and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. *Proc Natl Acad Sci USA.* (1997) 94:9792–6. doi: 10.1073/pnas.94.18.9792
11. Arch RH, Gedrich RW, Thompson CB. Tumor necrosis factor receptor-associated factors (TRAFs) - a family of adapter proteins that regulates life and death. *Genes Dev.* (1998) 12:2821–30. doi: 10.1101/gad.12.18.2821
12. Hoeflich KP, Yeh WC, Yao Z, Mak TW, Woodgett JR. Mediation of TNF receptor-associated factor effector functions by apoptosis signal-regulating kinase-1 (ASK1). *Oncogene* (1999) 18:5814–20. doi: 10.1038/sj.onc.1202975
13. Song HY, Rothe M, Goeddel DV. The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-kappaB activation. *Proc Natl Acad Sci USA.* (1996) 93:6721–5. doi: 10.1073/pnas.93.13.6721
14. Ye X, Mehlen P, Rabizadeh S, VanArsdale T, Zhang H, Shin H, et al. TRAF family proteins interact with the common neurotrophin receptor and modulate apoptosis induction. *J Biol Chem.* (1999) 274:30202–8. doi: 10.1074/jbc.274.42.30202
15. Vallabhapurapu S, Matsuzawa A, Zhang W, Tseng PH, Keats JJ, Wang H, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling. *Nat Immunol.* (2008) 9:1364–70. doi: 10.1038/ni.1678
16. Alvarez SE, Harikumar KB, Hait NC, Allegood J, Strub GM, Kim EY, et al. Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. *Nature* (2010) 465:1084–8. doi: 10.1038/nature09128
17. Hacker H, Tseng PH, Karin M. Expanding TRAF function: TRAF3 as a tri-faced immune regulator. *Nat Rev Immunol.* (2011) 11:457–68. doi: 10.1038/nri2998
18. Mu Y, Sundar R, Thakur N, Ekman M, Gudey SK, Yakymovych M, et al. TRAF6 ubiquitinates TGFbeta type I receptor to promote its cleavage and nuclear translocation in cancer. *Nat Commun.* (2011) 2:330. doi: 10.1038/ncomms1332
19. Zapata JM, Lefebvre S, Reed JC. Targeting TRAfs for therapeutic intervention. *Adv Exp Med Biol.* (2007) 597:188–201. doi: 10.1007/978-0-387-70630-615
20. Arthur JF, Shen Y, Gardiner EE, Coleman L, Murphy D, Kenny D, et al. TNF receptor-associated factor 4 (TRAF4) is a novel binding partner of glycoprotein Ib and glycoprotein VI in human platelets. *J Thromb Haemost.* (2011) 9:163–72. doi: 10.1111/j.1538-7836.2010.04091.x
21. Starczynowski DT, Lockwood WW, Delehouzee S, Chari R, Wegrzyn J, Fuller M, et al. TRAF6 is an amplified oncogene bridging the RAS and NF-kappaB pathways in human lung cancer. *J Clin Invest.* (2011) 121:4095–105. doi: 10.1172/JCI58818
22. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal.* (2013) 8:7. doi: 10.1186/1750-2187-8-7

23. Zhang L, Zhou F, Garcia de Vinuesa A, de Kruijf EM, Mesker WE, Hui L, et al. TRAF4 promotes TGF-beta receptor signaling and drives breast cancer metastasis. *Mol Cell.* (2013) 51:559–72. doi: 10.1016/j.molcel.2013.07.014
24. Park YC, Burkitt V, Villa AR, Tong L, Wu H. Structural basis for self-association and receptor recognition of human TRAF2. *Nature* (1999) 398:533–8. doi: 10.1038/19110
25. Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, et al. Distinct molecular mechanism for initiating TRAF6 signalling. *Nature* (2002) 418:443–7. doi: 10.1038/nature00888
26. Ni CZ, Welsh K, Leo E, Chiou CK, Wu H, Reed JC, et al. Molecular basis for CD40 signaling mediated by TRAF3. *Proc Natl Acad Sci USA.* (2000) 97:10395–9. doi: 10.1073/pnas.97.19.10395
27. Zhang P, Reichardt A, Liang HH, Aliyari R, Cheng D, Wang YY, et al. Single amino acid substitutions confer the antiviral activity of the TRAF3 adaptor protein onto TRAF5. *Science Signal.* (2012) 5:2003152. doi: 10.1126/scisignal.2003152
28. Niu F, Ru H, Ding W, Ouyang S, Liu ZJ. Structural biology study of human TNF receptor associated factor 4 TRAF domain. *Protein Cell* (2013) 4:687–94. doi: 10.1007/s13238-013-3068-z
29. Rousseau A, McEwen AG, Poussin-Courmontagne P, Rognan D, Nomine Y, Rio MC, et al. TRAF4 is a novel phosphoinositide-binding protein modulating tight junctions and favoring cell migration. *PLoS Biol.* (2013) 11:e1001726. doi: 10.1371/journal.pbio.1001726
30. Yoon JH, Cho YJ, Park HH. Structure of the TRAF4 TRAF domain with a coiled-coil domain and its implications for the TRAF4 signalling pathway. *Acta Crystallogr D Biol Crystallogr* (2014) 70(Pt 1):2–10. doi: 10.1107/S139900471302333X
31. Kim CM, Choi JY, Bhat EA, Jeong JH, Son YJ, Kim S, et al. Crystal structure of TRAF1 TRAF domain and its implications in the TRAF1-mediated intracellular signaling pathway. *Sci Rep.* (2016) 6:25526. doi: 10.1038/srep25526
32. Kim CM, Son YJ, Kim S, Kim SY, Park HH. Molecular basis for unique specificity of human TRAF4 for platelets GPIbbeta and GPVI. *Proc Natl Acad Sci USA.* (2017) 114:11422–7. doi: 10.1073/pnas.1708688114
33. Ye H, Park YC, Kreishman M, Kieff E, Wu H. The structural basis for the recognition of diverse receptor sequences by TRAF2. *Mol Cell* (1999) 4:321–30. doi: 10.1016/S1097-2765(00)80334-2
34. Park YC, Ye H, Hsia C, Segal D, Rich RL, Liou H-L, et al. A novel mechanism of TRAF signaling revealed by structural and functional analyses of the TRADD-TRAF2 interaction. *Cell* (2000) 101:777–87. doi: 10.1016/S0092-8674(00)80889-2
35. Li C, Ni CZ, Havert ML, Cabezas E, He J, Kaiser D, et al. Downstream regulator TANK binds to the CD40 recognition site on TRAF3. *Structure* (2002) 10:403–11. doi: 10.1016/S0969-2126(02)00733-5
36. Wu S, Xie P, Welsh K, Li C, Ni CZ, Zhu X, et al. LMP1 protein from the Epstein-Barr virus is a structural CD40 decoy in B lymphocytes for binding to TRAF3. *J Biol Chem.* (2005) 280:33620–6. doi: 10.1074/jbc.M502511200
37. Kim CM, Jeong JH, Son YJ, Choi JH, Kim S, Park HH. Molecular basis for TANK recognition by TRAF1 revealed by the crystal structure of TRAF1/TANK complex. *FEBS Lett.* (2017) 591:810–21. doi: 10.1002/1873-3468.12584
38. Mackman N. Triggers, targets and treatments for thrombosis. *Nature* (2008) 451:914–8. doi: 10.1038/nature06797
39. Shi Z, Zhang Z, Zhang Z, Wang Y, Li C, Wang X, et al. Structural Insights into mitochondrial antiviral signaling protein (MAVS)-tumor necrosis factor receptor-associated factor 6 (TRAF6) signaling. *J Biol Chem.* (2015) 290:26811–20. doi: 10.1074/jbc.M115.666578
40. Foight GW, Keating AE. Comparison of the peptide binding preferences of three closely related TRAF paralogs: TRAF2, TRAF3, and TRAF5. *Protein Sci.* (2016) 25:1273–89. doi: 10.1002/pro.2881

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 09 August 2018
doi: 10.3389/fimmu.2018.01849

Tumor Necrosis Factor Receptor-Associated Factor Regulation of Nuclear Factor κB and Mitogen-Activated Protein Kinase Pathways

Jian-Hong Shi[1] *and Shao-Cong Sun*[2]*

[1] *Central Laboratory, Affiliated Hospital of Hebei University, Baoding, China,* [2] *Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX, United States*

OPEN ACCESS

Edited by:
Gail Abendroth Bishop,
University of Iowa, United States

Reviewed by:
Hasem Habelhah,
University of Iowa,
United States
Ping Xie,
Rutgers University, The State University of New Jersey,
United States
Weizhou Zhang,
University of Iowa,
United States

***Correspondence:**
Shao-Cong Sun
ssun@mdanderson.org

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *25 June 2018*
Accepted: *26 July 2018*
Published: *09 August 2018*

Citation:
Shi J-H and Sun S-C (2018) Tumor Necrosis Factor Receptor-Associated Factor Regulation of Nuclear Factor κB and Mitogen-Activated Protein Kinase Pathways. Front. Immunol. 9:1849. doi: 10.3389/fimmu.2018.01849

Tumor necrosis factor receptor (TNFR)-associated factors (TRAFs) are a family of structurally related proteins that transduces signals from members of TNFR superfamily and various other immune receptors. Major downstream signaling events mediated by the TRAF molecules include activation of the transcription factor nuclear factor κB (NF-κB) and the mitogen-activated protein kinases (MAPKs). In addition, some TRAF family members, particularly TRAF2 and TRAF3, serve as negative regulators of specific signaling pathways, such as the noncanonical NF-κB and proinflammatory toll-like receptor pathways. Thus, TRAFs possess important and complex signaling functions in the immune system and play an important role in regulating immune and inflammatory responses. This review will focus on the role of TRAF proteins in the regulation of NF-κB and MAPK signaling pathways.

Keywords: tumor necrosis factor receptor-associated factor, nuclear factor κB, mitogen-activated protein kinases, toll-like receptors, tumor necrosis factor receptors, inflammation

INTRODUCTION

Tumor necrosis factor receptor (TNFR)-associated factors (TRAFs) form a family of intracellular signaling molecules, which in mammalian cells includes six typical members (TRAF1–6) and an atypical member (TRAF7) (1, 2). The typical TRAF members share a similar secondary structure, including a homologous C-terminal domain termed TRAF domain and various numbers of zinc fingers. In addition, all TRAF members, except TRAF1, contain a RING domain located in the

Abbreviations: TNFR, tumor necrosis factor receptor; TRAF, tumor necrosis factor receptor-associated factor; NF-κB, nuclear factor κB; IκB, inhibitory κB; IKK, IκB kinase; TAK1, transforming growth factor beta-activated kinase 1; NIK, NF-κB-inducing kinase; BAFFR, BAFF receptor; LTβR, lymphotoxin β receptor; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MKK, MAPK kinase; MAP3K, MAPK kinase kinase; PRR, pattern-recognition receptor; TLR, toll-like receptor; NZF, Npl4 Zinc Finger; IL-1R, IL-1 receptor; IRAK, IL-1R-associated kinase; RANK, receptor activator of nuclear factor kappa-B; MAVS, mitochondrial antiviral signaling protein; MYSM1, myb-like SWIRM and MPN domain 1; NLR, NOD-like receptor; TRADD, TNF receptor-associated death domain; RIP1, receptor-interacting protein kinase 1; S1P, sphingosine-1-phosphate; SphK1, sphingosine kinase 1; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; NDR1, Nuclear Dbf2-related kinase 1; IRF5, interferon regulatory factor 5.

N-terminal region. The TRAF domain mediates oligomerization of TRAF proteins as well as their association with upstream receptors or adaptors and downstream effector proteins (1). The RING domain is best known for its function to mediate protein ubiquitination in a large family of E3 ubiquitinase ligases (3). TRAF6 is a well-characterized E3 ligase that specifically conjugates lysine (K) 63-linked polyubiquitin chains (4). Several other TRAF proteins, TRAF2, TRAF3, and TRAF5, have also been shown to possess K63-specific E3 functions, although the physiological function of their E3 activity is less well defined (1, 2, 5).

Originally identified as signaling adaptors of TNFR2 (6), the TRAF molecules are now known to mediate signal transduction from a large variety of immune receptors, including TNFR superfamily members and other cytokine receptors, pattern-recognition receptors (PRRs), and antigen receptors (1, 2). Among the major downstream pathways regulated by TRAFs are those leading to activation of the nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs), which are in turn important for induction of genes associated with innate immunity, inflammation, and cell survival (7, 8). In addition to these classical functions, novel functions of TRAFs have also been discovered in recent studies. In particular, some TRAF proteins, including TRAF2 and TRAF3, function as negative regulators in some signaling pathways involved in the survival of B cells and inflammatory responses of innate immune cells (9). This review will focus on the role of TRAFs in the regulation of NF-κB and MAPK signaling pathways.

NF-κB AND MAPK PATHWAYS

NF-κB Pathways

Nuclear factor κB is a family of inducible transcription factors that regulate multiple biological processes, including immune and inflammatory responses, cell growth and survival, and oncogenesis (8, 10, 11). Mammalian NF-κB family includes five structurally related members, RelA, RelB, c-Rel, NF-κB1 p50, and NF-κB2 p52, which bind to a conserved DNA element (κB) as various homo- and hetero-dimers in the promoter or enhancer regions of target genes (10). NF-κBs are normally sequestered in the cytoplasm as inactive complexes bound by a family of inhibitory proteins, inhibitory κBs (IκBs), which includes IκBα, IκBβ, and several other structurally related proteins characterized by the presence of an ankyrin-repeat domain mediating binding and inhibition of NF-κBs (10). NF-κB1 and NF-κB2 are produced as precursor proteins, p105 and p100, both of which contain an IκB-like C-terminal portion and function as IκB-like molecules (12, 13). These precursor proteins can be processed by the proteasome, which involves selective degradation of the IκB-like C-terminal portion, thereby producing mature NF-κB p50 and p52, respectively, and disrupting their IκB-like function. During its translation, about half amount of p105 is constitutively processed for p50 production, whereas the remaining p105 functions as an IκB to regulate nuclear translocation of different NF-κB members, including p50, RelA, and c-Rel (13–16). In contrast to the processing of p105, the processing of p100 is tightly controlled and occurs in a signal-inducible manner (17).

Two major signaling pathways, the canonical and noncanonical pathways, mediate activation of the NF-κB members (18). The canonical pathway is based on signal-induced degradation of IκBs, particularly IκBα, which triggers nuclear translocation of p50-containing NF-κB complexes, particularly p50/RelA and p50/c-Rel heterodimers. This pathway relies on activation of a trimeric IκB kinase (IKK) complex, composed of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, NF-κB essential modulator (NEMO) (also called IKKγ). Activation of IKK is typically mediated by transforming growth factor beta-activated kinase 1 (TAK1), a MAPK kinase kinase (MAP3K) that responds to various immune receptor signals and relies on ubiquitination for its catalytic activation and signaling function (19, 20). TAK1 deficiency severely attenuates, although does not completely block, IL-1- and TNFα-induced NF-κB activation (21). Another MAP3K, MEKK3, is also involved in NF-κB activation by different inducers, such as TNFα and IL-1 (22–27). A characteristic of the canonical NF-κB signaling pathway is its rapid and transient kinetics, which is important for preventing deregulated NF-κB functions. The canonical NF-κB pathway plays an important role in regulating diverse immune functions, including innate immunity and inflammation, lymphocyte activation and differentiation, as well as immune tolerance (8, 28).

The noncanonical NF-κB pathway is based on the processing of the NF-κB2 precursor protein p100, which is triggered through site-specific phosphorylation in its C-terminal serine residues (17). This pathway is independent of TAK1 and the trimeric IKK complex but requires NF-κB-inducing kinase (NIK) and its downstream kinase IKKα (17, 18, 29). Unlike the canonical NF-κB pathway, which responds to signals from a large variety of immune receptors, the noncanonical NF-κB pathway selectively responds to a subset of TNFR superfamily members, including CD40, BAFF receptor, lymphotoxin β receptor (LTβR), RANK, TNFR2, TWEAK, CD27, etc. (30, 31). A hallmark of the noncanonical NF-κB signaling is its slow kinetics and dependence on protein synthesis (31). This is largely due to the involvement of an unusual mechanism of NIK regulation. Under normal conditions, NIK steady level is extremely low due to its constitutive degradation *via* the ubiquitin/proteasome pathway, which prevents induction of p100 processing (32). Signal-stimulated noncanonical NF-κB signaling involves stabilization of the *de novo* synthesized NIK, thereby allowing the accumulated NIK to activate IKKα and induce p100 processing. The noncanonical NF-κB pathway is best known for its role in regulating lymphoid organ development, B cell maturation, and osteoclast differentiation. However, recent studies have uncovered additional functions of this pathway and linked this pathway with autoimmune and inflammatory diseases (18).

MAPK Pathways

Mitogen-activated protein kinases form a large family of serine/threonine kinases that respond to diverse extracellular and intracellular stimuli and mediate multiple biological processes, such as cell growth and differentiation, immune and inflammatory responses, and oncogenesis (7, 33). The mammalian MAPK family includes three subfamilies: the extracellular signal-regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the

p38 MAPKs (7, 33). Signal transduction of the MAPKs share a common mechanism, in which an MAPK is phosphorylated and activated by an MAPK kinase (MKK), and the MKK is in turn phosphorylated and activated by an MAP3K. Different MAPKs are regulated by distinct MKKs: MKK1 and MKK2 (also called MEK1 and MEK2) for ERK1 and ERK2, MKK4 and MKK7 for JNKs, and MKK3 and MKK6 for p38. The MAP3Ks for the MAPK signaling cascades vary among different stimulating receptors. In the immune system, MAPK signaling cascades have been extensively studied in innate immune cells stimulated *via* the PRRs, particularly the toll-like receptors (TLRs), where MAPKs mediate induction of various proinflammatory cytokines and chemokines required for host defense and inflammation (7).

In the TLR pathway, TAK1 is a primary MAP3K mediating activation of the JNK and p38 signaling cascades, whereas Tpl2 is the MAP3K mediating activation of the ERK1/2 cascade (34). The TLR-stimulated MAPK signaling involves an interesting crosstalk with the IKK/NF-κB pathway. The NF-κB1 precursor protein p105 forms a stable complex with the MAP3K Tpl2, in which p105 both stabilizes Tpl2 and prevents its phosphorylation of downstream kinases, MKK1/2 (35–39). Upon activation by the TLR signal, IKK phosphorylates p105 to induce its ubiquitin-dependent degradation, which allows the liberated Tpl2 to phosphorylate MKK1/2, leading to activation of ERK1/2. This signaling event is transient, since activated Tpl2 is rapidly degraded due to its instability when dissociated from p105. Therefore, in the TLR pathway, TAK1 functions as a master kinase mediating activation of not only the JNK and p38 MAPK cascades but also the IKK–Tpl2–ERK signaling axis.

TRAF6 AS A MEDIATOR OF NF-κB AND MAPK ACTIVATION

IKK/MAPK Activation

TRAF6 is a prototype of RING domain-containing E3 ubiquitin ligase that functions together with a dimeric E2, composed of Ubc13 and Uev1A, specifically catalyzing the synthesis of K63-linked polyubiquitin chains (4, 19). Accumulating studies have demonstrated a crucial role for TRAF6 in mediating signaling from various TNFRs as well as other immune receptors, such as the IL-1 receptor (IL-1R), IL-17R, TLRs, RIG-I-like receptors, TGFβR, and antigen receptors (20, 40–44). The C-terminal TRAF domain of TRAF6 interacts with a conserved sequence motif, Pro-X-Glu-X-X-(aromatic/acidic residue), present in specific members of the TNFR superfamily, including CD40 and receptor activator of nuclear factor kappa-B (45). Through this molecular interaction, TRAF6 is recruited to the TNFRs in response to ligand stimulation, which is essential for triggering TRAF6 activation and signal transduction. The TRAF6-binding motifs are also present in signaling adaptors of other immune receptors, such as the IL-1R-associated kinases (IRAKs) of the IL-1R and TLR pathways, the mitochondrial antiviral signaling protein of the RIG-I pathway, and Act1 of the IL-17R pathway (41, 44, 46).

The signaling mechanism of TRAF6 has been extensively studied in the TLR and IL-1R pathways (**Figure 1**). TLRs transduce signals *via* common adaptors, MyD88 and TRIF (47). With the exception of TLR3, which signal *via* TRIF, all TLRs, as well as IL-1R, rely on MyD88 for signal transduction, although TLR4 signals through both MyD88 and TRIF. Upon ligand binding, MyD88-dependent TLRs recruit IRAKs (including IRAK1, IRAK2, and IRAK4) *via* the adaptor MyD88 to trigger the formation of a receptor-associated signaling complex that also contains TRAF6 (**Figure 1**). Once activated in the MyD88 signaling complex, TRAF6 functions as an E3 ubiquitin ligase that conjugates K63-linked ubiquitin chains onto itself as well as to other proteins, such as IRAK1 and the IKK regulatory subunit, NEMO (19, 20). Precisely how TRAF6 mediates activation of downstream pathways is incompletely understood. It is generally thought that self-ubiquitinated TRAF6 recruits the downstream kinases TAK1 and IKK to assemble a signaling complex that facilitates TAK1 and IKK activation. In support of this model, both TAK1 and IKK contain regulatory subunits that bind K63-linked polyubiquitin chains (48–50). The TAK1 complex contains two regulatory subunits, TAB 1 and TAB 2 (or TAB 3), and TAB 2 and TAB 3 both contain an Npl4 Zinc Finger type of ubiquitin-binding domain that specifically binds K63-linked polyubiquitin chains (48, 51). The regulatory subunit of IKK, NEMO, also contains a ubiquitin-binding domain, called CC2-LZ (coiled-coil2-lucine zipper) or UBAN (ubiquitin binding in ABIN and NEMO proteins), capable of binding K63 ubiquitin chains (49, 50, 52–54). In further support of this model, mutation of an autoubiquitination site, K124, of TRAF6 attenuates its function in mediating TAK1/IKK activation (55). However, a subsequent study suggests that the E3 ligase activity of TRAF6, although required for TAK1 activation, is dispensable for TRAF6 association with the TAK1 complex (56). Moreover, this study also suggests that TRAF6 autoubiquitination is dispensable for activation of TAK1 and its downstream NF-κB and MAPK pathways by IL-1 and RANKL, whereas TRAF6-mediated NEMO ubiquitination contributes to the activation of IKK and NF-κB (56). Since unconjugated ubiquitin chains are able to activate TAK1 (57), it is also likely that both conjugated and unconjugated K63 ubiquitin chains within the TRAF6/TAK1 complex contribute to TAK1 activation.

Although the E3 ubiquitin ligase activity of TRAF6 is generally believed to be essential for its signaling function, there are controversies. A recent study employing both cell line reconstitution and knockin mouse approaches demonstrates that inactivation of the E3 ligase activity of TRAF6 only partially inhibits its function in mediating activation of NF-κB and MAPK signaling stimulated by IL-1, TLRs, and RANKL (58). It seems that the Pellino family of E3 ligases, particularly Pellino1 and Pellino2, could compensate the E3 function of TRAF6 to mediate K63 ubiquitin chain conjugation for TAK1 activation (58). Since complete deletion of TRAF6 abolishes MyD88-dependent activation of NF-κB and MAPK signaling, these findings suggest that in addition to serving as an E3 ligase, TRAF6 may also function an adaptor.

TRAF6 Regulation

Given the crucial role of TRAF6 in mediating activation of NF-κB and MAPK pathways and inflammation, it is not surprising that the function of TRAF6 is subject to tight control by various regulators (**Figure 1**). An early study suggests that IL-1-stimulated TRAF6 polyubiquitination, an indicator of TRAF6 activation,

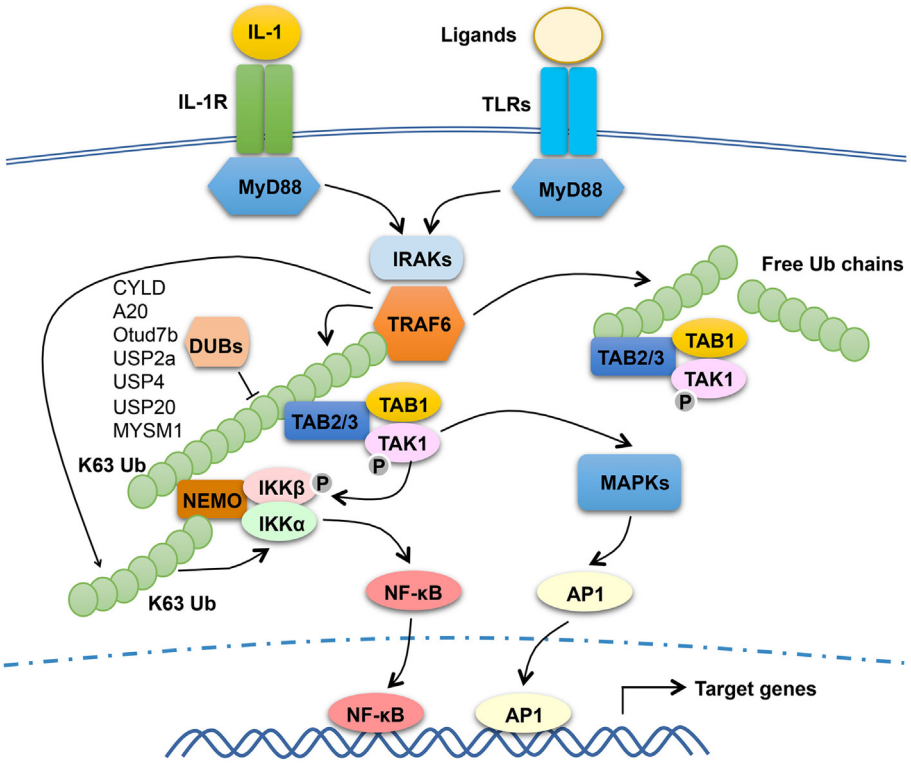


FIGURE 1 | The function and regulation of tumor necrosis factor receptor-associated factor (TRAF)6 in MyD88 signaling pathway. Upon stimulation with IL-1 or toll-like receptor (TLR) ligands, MyD88 recruits IL-1R-associated kinases (IRAKs) (including IRAK1, IRAK2, and IRAK4) and TRAF6 to assemble a MyD88 signaling complex. Once activated in the MyD88 complex, TRAF6 functions as an E3 ubiquitin ligase that catalyzes the synthesis of K63-linked polyubiquitin chains conjugated to itself or NF-κB essential modulator (NEMO) or existing as free ubiquitin chains. The self-ubiquitinated TRAF6 recruits the ubiquitin-dependent kinase transforming growth factor beta-activated kinase 1 (TAK1) and its downstream kinase IκB kinase (IKK) to assemble a signaling complex that facilitates TAK1 and IKK activation. This process requires the TAK1 regulatory subunit TAB 2 (or TAB 3) and the IKK regulatory subunit NEMO, both have ubiquitin-binding functions. Activated TAK1 mediates activation of IKK and mitogen-activated protein kinases (MAPKs), which further activate nuclear factor κB (NF-κB) (RelA- and c-Rel-containing complexes) and AP1. TRAF6 mediated NEMO ubiquitination also contributes to the activation of IKK and NF-κB. Several DUBs have been shown to negatively regulate TRAF6 function through deconjugation of its K63 polyubiquitin chains.

occurs transiently suggesting its regulation by deubiquitination (59). Several DUBs have been implicated in the regulation of TRAF6 activation through deconjugation of its K63 polyubiquitin chains; these include CYLD, A20, Otud7b (also called Cezanne), USP2a, USP4, USP20, and myb-like SWIRM and MPN domain 1 (60–66). Another DUB, YOD1 (also called Otud2), inhibits TRAF6 ubiquitination and signaling function *via* a non-catalytic mechanism that involves binding to the C-terminal TRAF domain of TRAF6 and, thereby, preventing TRAF6 interaction with an activating adaptor protein, p62 (also called Sequestosome-1) (67). It is unclear why there are so many DUBs involved in TRAF6 regulation, but it is likely that they function in different cell types and/or distinct receptor pathways. It is also important to note that the role of some TRAF6-regulating DUBs in regulating TLR signaling and innate immunity is yet to be demonstrated using *in vivo* approaches.

TRAF6 regulation also involves various other factors. A member of the NOD-like receptors (NLR) family, NLRC3, has been shown to inhibit signaling from MyD88-dependent TLRs (68). NLRC3 interacts with and inhibits K63 ubiquitination of TRAF6, thereby negatively regulating TLR-stimulated NF-κB activation. Interestingly, NLRC3-mediated TRAF6 regulation does not influence MAPK signaling pathways (68), indicating a different role for K63 ubiquitination of TRAF6 in regulating NF-κB and MAPK pathways. The molecular mechanism by which NLRC3 inhibits K63 ubiquitination and signaling function of TRAF6 is undefined. A putative E2 molecule, Ube2o binds to TRAF6 and inhibits TRAF6 K63 ubiquitination and activation of NF-κB in the IL-1β and LPS pathways (69). This function of Ube2o is independent of its ubiquitin-conjugating domain and appears to act through disruption of TRAF6 binding to MyD88. TRAF6 is also regulated by a protein kinase, MST4, which phosphorylates TRAF6 at two threonine residues (T463 and T486) in the C-terminal TRAF domain and inhibits the oligomerization and autoubiquitination activity of TRAF6 (70). MST4 knockdown promotes TLR signaling and cytokine induction in cell culture and sensitizes mice to septic shock induction (70). In contrast to the negative role of MST4 in TRAF6 regulation, another kinase, RSK2, positively regulates TRAF6 function and LPS- and IL-1β-stimulated activation of MAPKs and NF-κB (71). RSK2

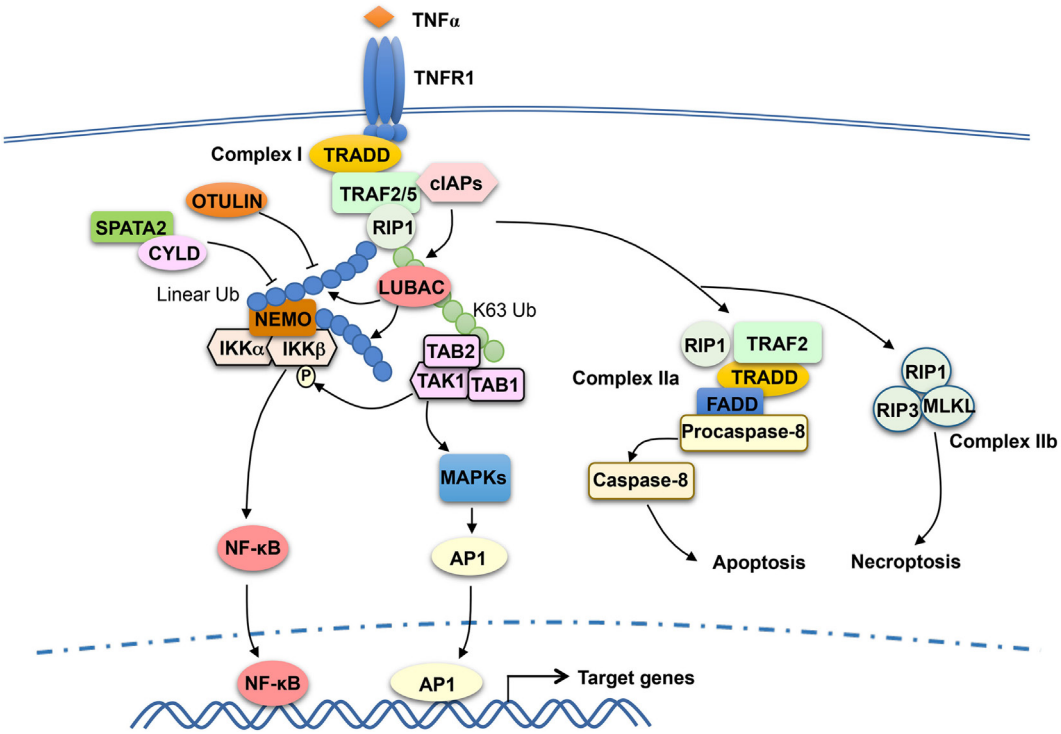


FIGURE 2 | Tumor necrosis factor receptor-associated factor (TRAF)2 and TRAF5 in tumor necrosis factor receptor (TNFR)1 signaling. TNFα binding to TNFR1 triggers the assembly of a TNFR1-associated signaling complex, complex I, which is composed of TNF receptor-associated death domain (TRADD), receptor-interacting protein kinase 1 (RIP1), TRAF2 or TRAF5 (TRAF2/5), and the E3 ubiquitin ligases cIAP1 and cIAP2 (cIAPs). Upon activation, cIAPs conjugate K63-linked ubiquitin chains to RIP1, which facilitates recruitment and activation of the kinase transforming growth factor beta-activated kinase 1 (TAK1) as well as the recruitment of the linear ubiquitin ligase complex LUBAC. LUBAC conjugates linear ubiquitin chains to RIP1 and, thereby, facilitates the requirement of IκB kinase (IKK) *via* the linear ubiquitin-binding function of NF-κB essential modulator (NEMO). Subsequent ubiquitination of NEMO by LUBAC, along with TAK1-mediated IKKβ phosphorylation, results in IKK activation. The activated IKK and TAK1 mediate activation of nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK)/AP1 signaling pathways that promote cell survival. TRAF2 also participates in the subsequent formation of a cytoplasmic complex, complex IIa, which mediates apoptosis induction. When caspase-8 is inhibited, TNFR1 signaling also leads to formation of complex IIb, leading to necroptosis. DUBs, including OTULIN and CYLD, negatively regulate signaling functions of LUBAC by cleaving linear ubiquitin chains. SPATA2, as a high-affinity binding partner of CYLD and LUBAC, facilitates CYLD function by recruiting CYLD to LUBAC.

phosphorylates TRAF6 at three N-terminal serines (S46, S47, and S48), thereby promoting the K63 ubiquitination and signaling function of TRAF6 (71). Future studies should examine whether these negative (T463 and T486) and positive (S46, S47, and S48) phosphorylation sites are phosphorylated *in vivo* along with TLR stimulation.

TRAF2 IN TNFR SIGNALING

TRAF2 has been extensively studied as a mediator of TNFR1 signaling and known to be crucial for TNFα-stimulated NF-κB and MAPK pathways. TRAF2 is essential for TNFα-induced activation JNK, although it is functionally redundant with TRAF5 in the activation of NF-κB (72, 73). TRAF2 does not directly bind TNFR1, but it can be recruited to TNFR1 *via* the adaptor protein TNF receptor-associated death domain (TRADD) (74, 75). The cytoplasmic tail of TNFR1 contains a death domain that mediates interaction with the death domain of TRADD, and TRADD binds to TRAF2 *via* the N-terminal region of TRADD and the C-terminal TRAF domain of TRAF2 (74, 76). TNFR1 signaling involves sequential formation of two different complexes, complex I and complex II, which mediate cell survival and cell death, respectively (77) (**Figure 2**). Complex I is associated with TNFR1 and composed of TRADD, TRAF2 and its close homolog TRAF5, receptor-interacting protein kinase 1 (RIP1), and the E3 ubiquitin ligases cIAP1 and cIAP2 (77). In response to TNFR1 stimulation, cIAP1 and cIAP2 conjugates K63-linked ubiquitin chains to RIP1, which facilitates the recruitment and activation of TAK1 and IKK kinase complexes leading to activation of NF-κB and induction of survival genes (49, 78–80). Following the initial signaling, complex I dissociates from TNFR1 leading to formation of the cytoplasmic complex IIa, composed of TRAF2, RIP1, TRADD, FADD, and procaspase-8. This second complex triggers activation of Caspase-8 and apoptosis, which serves as a checkpoint mechanism mediating cell death when complex I-mediated NF-κB activation fails (77). TNFR1 also induces a complex (complex IIb), composed of RIP1, RIP3, and MLKL, which mediates cell death *via* necroptosis (81).

Like TRAF6, TRAF2 contains an N-terminal RING domain and has been implicated as a K63-specific E3 ubiquitin ligase

(82). However, the RING domain structure of TRAF2 differs significantly from that of TRAF6, and TRAF2 does not interact with Ubc13 and several other E2s (83). A subsequent study suggests that the E3 ligase function of TRAF2 requires a cofactor, sphingosine-1-phosphate (S1P), which binds to the RING domain of TRAF2 and stimulates TRAF2-mediated K63 ubiquitination of RIP1 (84). TRAF2 also interacts with sphingosine kinase 1 (SphK1), one of the isoenzymes catalyzing the generation of S1P, and siRNA-mediated SphK1 silencing attenuates TNFα-stimulated RIP1 ubiquitination and NF-κB activation (84, 85). Whether TRAF2 functions as an adaptor or an E3 ligase in the TNFR1 pathway is still in debate. While TRAF2 has been implicated as an E3 that mediates TNFα-induced RIP1 ubiquitination and NF-κB activation (84, 86), other studies suggest that the RING domain of TRAF2 is dispensable for its function in mediating TNFα-induced canonical NF-κB signaling (87). It is generally believed that the TRAF2-recruited cIAPs function as E3 ligases of RIP1 in the TNFR1 complex I (**Figure 2**). The cIAP-binding motif, but not RING domain, of TRAF2 is essential for TNFR1-stimulated RIP1 ubiquitination and canonical NF-κB activation (87). cIAP1 and cIAP2 directly ubiquitinate RIP1 *in vitro* and are required for TNFα-induced RIP1 ubiquitination and NF-κB activation *in vivo* (78–80).

TRAF2 is functionally redundant with TRAF5 in mediating TNFα-stimulated NF-κB activation. Mouse embryonic fibroblasts (MEFs) deficient in either TRAF2 or TRAF5 are fully functional in TNFα-stimulated NF-κB activation, whereas MEFs lacking both TRAF members are severely attenuated, although not completely defective, in the NF-κB activation (73). These findings confirm the important role of TRAF2 and TRAF5 in TNFα-stimulated NF-κB signaling and also suggest the involvement of additional mechanisms. The role of cIAPs is cell-type specific. shRNA-mediated knockdown of cIAP1 in skeletal myoblasts largely blocked TNFα-stimulated NF-κB signaling, whereas simultaneous knockdown or knockout of cIAP1 and cIAP2 is required for blocking NF-κB activation in MEFs (79, 88).

Recent studies suggest that the signaling function of TNFR1 complex I also involves another E3 ubiquitin ligase, LUBAC, which specifically conjugates linear ubiquitin chains (89, 90). LUBAC is a complex composed of three subunits: SHARPIN, HOIL-1L, and HOIP. In response to TNFR1 stimulation, LUBAC is recruited to the TNFR1 complex I *via* binding to K63 ubiquitin chains conjugated to RIP1 by the cIAPs (89) (**Figure 2**). Within the TNFR1 complex, LUBAC mediates linear ubiquitination of several components of the TNFR1 complex 1, including RIP1 and the IKK regulatory subunit NEMO (91, 92). The LUBAC-catalyzed synthesis of linear ubiquitin chains plays an important role in recruitment and activation of IKK. Unlike TAB 2 and TAB 3, which specifically binds to K63 ubiquitin chains, NEMO preferentially binds to linear ubiquitin chains *via* its ubiquitin-binding domain, UBAN (53, 93–95). The K63 ubiquitin- and linear ubiquitin-conjugated RIP1 molecules recruit TAK1 and IKK complexes, respectively, thereby facilitating IKK activation by TAK1 (**Figure 2**). Linear ubiquitination of NEMO may directly contribute to IKK activation. It has been proposed that linear ubiquitin chain-conjugated NEMO can be bound by another NEMO molecule, which promotes IKK dimerization and catalytic activation (96). It has also been suggested that NEMO binding to linear ubiquitin chains may cause a conformational change in IKK, thereby facilitating IKKβ phosphorylation by TAK1 (97). The signaling function of LUBAC is negatively regulated by DUBs capable of cleaving linear ubiquitin chains, including OTULIN and CYLD (98–100). In addition, the DUB A20 is recruited to the TNFR1 complex *via* binding to linear ubiquitin chains and contributes to the inhibition of NF-κB activation possibly by preventing NEMO recruitment (101, 102).

TRAF2 is also a mediator of TNFR2 signaling. In fact, TRAF2, along with TRAF1, was originally identified as signaling adaptors physically associated with TNFR2 (6). TRAF2 binds to the cytoplasmic domain of TNFR2 and recruits E3 ubiquitin ligases cIAP1 and cIAP2 to the TNFR2 signaling complex, which is important for TNFR2-stimulated NF-κB activation (103, 104). The cytoplasmic tail of TNFR2 contains sequence motifs that are directly bound by TRAF2 (6, 105, 106). TRAF2 also mediates activation of NF-κB and MAPK pathways triggered by other TNFR superfamily members, such as CD40, OX40, 4-1BB, LTβR, and GITR (107–111).

TRAF CONTROL OF NONCANONICAL NF-κB PATHWAY

While TRAF proteins are generally known as signaling adaptors that mediate activation of NF-κB and MAPK pathways, it is now clear that TRAF2 and TRAF3 are pivotal negative regulators of the noncanonical NF-κB pathway (18). An initial study identified TRAF3 as a protein physically interacting with the noncanonical NIK and mediating ubiquitin-dependent NIK degradation (32). TRAF3 knockdown causes NIK stabilization and induction of p100 processing, and signal-induced noncanonical NF-κB activation is associated with TRAF3 degradation and concomitant accumulation of NIK, suggesting that the TRAF3-mediated NIK degradation is a central mechanism of noncanonical NF-κB regulation (32). This finding is corroborated by subsequent studies using TRAF3 knockout mice demonstrating that TRAF3 deficiency causes NIK accumulation and noncanonical NF-κB activation (112–114). Domain mapping analyses revealed an N-terminal motif of NIK, ISIIAQA (amino acids 78–84), which is required for NIK–TRAF3 interaction. A NIK mutant harboring deletion of this motif, named NIKΔ78–84 or NIKΔT3, is stable due to impaired interaction with TRAF3 (32). Transgenic expression of this NIK mutant in B cells causes maximal noncanonical NF-κB activation and B cell survival independently of BAFF, resulting in drastic B cell hyperplasia (115).

Unlike TRAF3, TRAF2 does not bind the N-terminal region of NIK and only weakly interacts with NIK *via* a C-terminal region (32, 116). Interestingly, however, TRAF2 deficiency also causes noncanonical NF-κB activation (114, 117). Moreover, the E3 ubiquitin ligases cIAP1 and cIAP2 were later on found to mediate NIK ubiquitination and degradation (118, 119). Biochemical and genetic evidence suggests that TRAF2, TRAF3, and the cIAPs function together as an E3 ubiquitin ligase complex mediating NIK ubiquitination (120, 121). It has been proposed that within this complex, TRAF2 and TRAF3 bind cIAPs and NIK, respectively, and recruit NIK to the E3 ligases cIAPs *via* TRAF2–TRAF3

dimerization (31, 120, 121). These findings provide mechanistic insight into the functions of small molecule IAP antagonists in cancer treatment. Recent studies suggest that the IAP antagonists not only induce cancer cell death but also promote antitumor immunity and synergize with immune checkpoint inhibitors in mouse models of cancer immunotherapy (122–125). The immunostimulatory action of IAP antagonists is likely due to activation of the NIK-dependent noncanonical NF-κB activation. In addition, the IAP antagonists may also activate innate immune cells in tumor microenvironment. As will be discussed in the following section, disruption of the TRAF–cIAP E3 complex in macrophages promotes production of M1 type of proinflammatory cytokines that facilitate recruitment of antitumor effector T cells to the tumor microenvironment (126).

Precisely how the TRAF–cIAP complex is assembled and regulates NIK stability is incompletely understood. More recent studies suggest that another TRAF member, TRAF1, may also be involved in cIAP E3 complex function and NIK regulation (110, 127). TRAF1 and TRAF2 forms a heterotrimer, TRAF1:(TRAF2)2, which binds cIAP2 more strongly than TRAF2 (127). Since TRAF1 is induced by various cellular stimuli, it may function as a modifier of the TRAF–cIAP complex under certain conditions. In support of this idea, TRAF1 appears to play a role in restraining TCR-stimulated noncanonical NF-κB activation (110). TRAF1 deletion allows murine CD8 T cells to respond to TCR stimulation, in the absence of 4-1BB costimulation, for the activation of noncanonical NF-κB (110). However, the role of TRAF1 in NF-κB signaling is controversial, since another study suggests that TRAF1 directly binds NIK and stabilizes NIK by interfering with NIK association with the TRAF2–cIAP2 complex under overexpression conditions (128). It is important to note, though, the TRAF1–NIK binding is substantially weaker than the TRAF3–NIK interaction (32), and additional studies are required to examine the physiological role of TRAF1 in noncanonical NF-κB regulation.

ANTI-INFLAMMATORY FUNCTION OF TRAF2 AND TRAF3

Anti-Inflammatory Function

Compared to TRAF6, much less is known about the function of TRAF2 and TRAF3 in regulating TLR signaling. Nevertheless, recent studies suggest a role for both TRAF2 and TRAF3 in negatively regulating TLR-stimulated expression of proinflammatory cytokines (9). TRAF3 deficiency promotes proinflammatory cytokine induction, while inhibits type I interferon induction, in macrophages (129, 130). The anti-inflammatory function of TRAF3 has been demonstrated using myeloid cell-conditional TRAF3 knockout (TRAF3-MKO) mice (126, 131). Myeloid cell-specific deletion of TRAF3 does not affect macrophage differentiation, but renders mice hypersensitive to colitis induction in the dextran sodium sulfate (DSS) model (126). The TRAF3-MKO mice also produce elevated levels of IgG3 and IgG2b antibody isotypes in response to T-independent and T-dependent antigens, respectively, likely due to aberrant production of proinflammatory cytokines, such as IL-12 and IL-6 (131). Consistently, the TRAF3 deficiency sensitizes macrophages to *in vitro* induction of proinflammatory cytokines by the TLR4 and TLR3 ligands LPS and polyIC (126, 131). At older ages (15–22 months), the TRAF3-MKO mice spontaneously develop chronic inflammation in multiple organs (131). Interestingly, some of these mice also develop tumors originating from both myeloid cells (histiocytes) and other cell types (including B cells and hepatocytes) that are competent in TRAF3 expression (131). These findings suggest that chronic inflammation in these mutant mice may contribute to the tumorigenesis, although it is also possible that TRAF3 may regulate a tumor-suppressive function of myeloid cells.

An unexpected finding is the anti-inflammatory function of TRAF2 in the TLR signaling pathway (126). Like TRAF3, TRAF2 negatively regulate induction of proinflammatory cytokines by the TLR ligands LPS and polyIC as well as by the cytokine IL-1β, and the myeloid cell-conditional TRAF2 knockout (TRAF2-MKO) mice are hypersensitive to colitis induction in the DSS model (126). The protective role of TRAF2 in colon inflammation has also been revealed using TRAF2 germline knockout mice, which spontaneously develop colitis (132). In addition to its anti-inflammatory role in myeloid cells, TRAF2 appears to inhibit inflammation through protecting intestinal epithelial cells from TNF-induced apoptosis (9, 132). A potential role for TRAF2 and TRAF3 in regulating human inflammatory bowel disease (IBD) is indicated by the finding that the expression level of these TRAF members is upregulated in the colonic tissue of IBD patients (133–135). However, whether the upregulation of TRAF2 and TRAF3 serves as a feedback mechanism to suppress inflammation in the human patients is unclear.

Signaling Mechanism

The finding that both TRAF2 and TRAF3 negatively regulate proinflammatory cytokine induction by TLRs raises the question of whether these TRAF members have a common mechanism of action. TRAF3 has been shown to play a role in regulating receptor-proximal signaling in the MyD88 pathway (136) (**Figure 3**). In response to TLR stimulation, both TRAF6 and TRAF3 are recruited to the MyD88 signaling complex, in which TRAF3 appears to prevent the relocation of the TRAF6 complex from the membrane to the cytoplasm, a signaling step required for activation of the downstream MAPK pathways (136). Interestingly, TLR signaling induces TRAF3 degradation, which is important for optimal activation of MAPKs (136). TRAF3 degradation appears to involve TRAF6-mediated K63 ubiquitination and activation of the E3 ubiquitin ligases cIAP1 and cIAP2, which in turn conjugate K48-linked ubiquitin chains to TRAF3 and cause ubiquitin-dependent TRAF3 degradation in the proteasome (136) (**Figure 3**). TRAF3 degradation also occurs in microglia, resident macrophages of the central nervous system (CNS) (137). In microglia, TRAF3 undergoes degradation and resynthesis along with induction of experimental autoimmune encephalomyelitis (EAE), an animal model of the neuroinflammatory disease multiple sclerosis (137). The TRAF3 degradation also requires the E3 ubiquitin ligase Peli1, which is abundantly expressed in microglia. Peli1 deficiency blocks TRAF3 degradation and causes its accumulation, which contributes to attenuated induction of proinflammatory cytokines and chemokines in

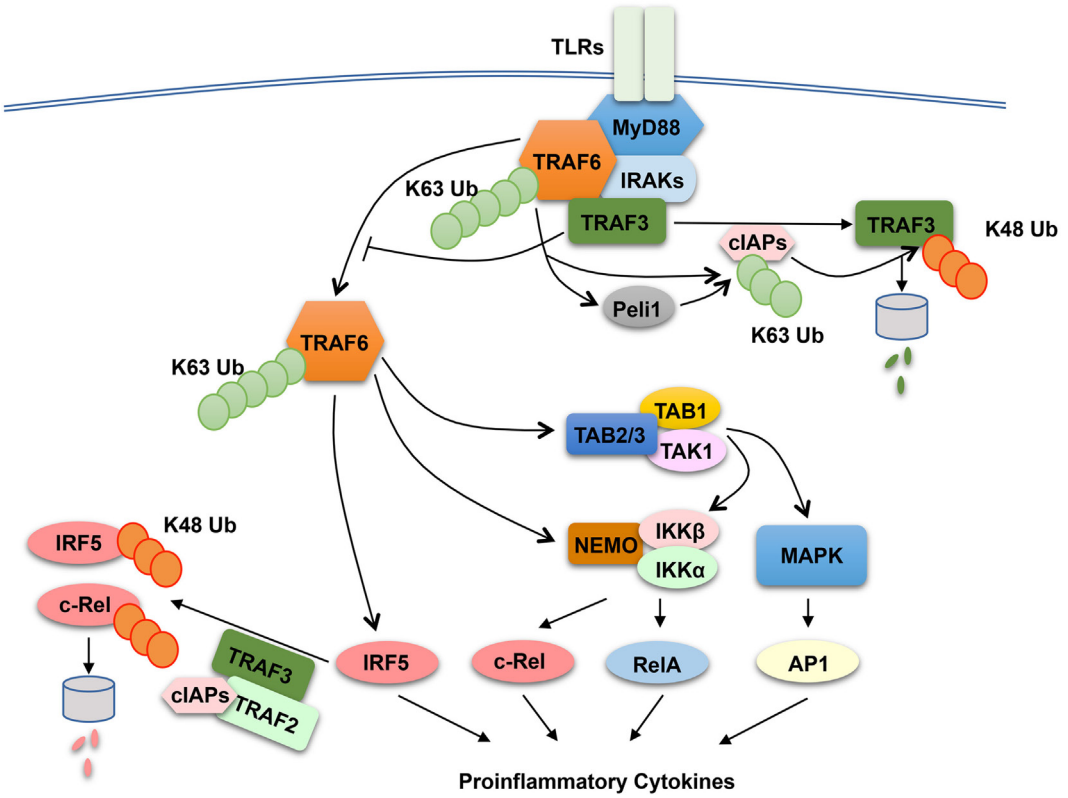


FIGURE 3 | Anti-inflammatory function of tumor necrosis factor receptor-associated factor (TRAF)2 and TRAF3 in toll-like receptor (TLR) signaling pathway. In responds to TLR stimulation, TRAF6 is recruited to the MyD88 signaling complex. Upon activation, the TRAF6 complex relocates from the plasma membrane to the cytoplasm, a signaling step required for activation of the downstream nuclear factor κB and mitogen-activated protein kinase (MAPK) pathways. TRAF3 negatively regulates TLR-stimulated proinflammatory cytokine induction *via* two potential mechanisms. The first is to target the MyD88–TRAF6 complex and prevents the relocation of TRAF6 to the cytoplasm, and the second is to function together TRAF2 and cIAPs to mediate ubiquitin-dependent degradation of two major proinflammatory transcription factors, c-Rel and interferon regulatory factor 5 (IRF5). TLR signaling temporarily overrides the negative signaling function of TRAF3 by inducing TRAF3 proteolysis. In this process, TRAF6 activates cIAPs (cIAP1 and cIAP2) through K63 ubiquitination, which in turn function as E3 ubiquitin ligases to mediate K48-linked ubiquitination and proteolysis of TRAF3. In microglia, Peli1 appears to cooperate with TRAF6 or function downstream of TRAF6 to mediate cIAP activation.

microglia and ameliorated EAE pathogenesis (137). Peli1 appears to cooperate with TRAF6 or function downstream of TRAF6 to mediate cIAP activation, since Peli1 deficiency inhibits TRAF6-mediated induction of cIAP ubiquitination (137) (**Figure 3**). The role of TRAF3 in suppressing CNS inflammation also involves negative regulation of IL-17R signaling (138). TRAF3 binds to IL-17R and interferes with the formation of the IL-17R–Act1–TRAF6 signaling complex and IL-17-stimulated activation of NF-κB and MAPK pathways. Nuclear Dbf2-related kinase 1 inhibits the binding of TRAF3 to IL-17R and, thereby, promotes IL-17R signaling and inflammation (139).

The role of TRAF2 and TRAF3 in MAPK regulation may be cell type specific, since bone marrow-derived macrophages and peritoneal macrophages derived from TRAF2-MKO and TRAF3-MKO mice do not display hyper-activation of MAPKs upon LPS stimulation (126, 131). Another potential mechanism that underlies the anti-inflammatory function of TRAF2 and TRAF3 is suppression of noncanonical NF-κB activation. As discussed in above sections, TRAF2 and TRAF3 are both essential components of the TRAF-cIAP E3 ubiquitin ligase that mediates NIK degradation in noncanonical NF-κB pathway (31). Deficiency in either TRAF2 or TRAF3 in macrophages causes constitutive activation of noncanonical NF-κB, as shown by NIK accumulation and p100 processing (126, 131). However, the noncanonical NF-κB activation does not seem to play an important role, since deletion of NIK in the TRAF2-deficiency macrophages fails to prevent LPS-stimulated hyper-expression of proinflammatory cytokine genes, except a partial inhibition of *Il23a* induction (126). It appears that TRAF2 and TRAF3 negatively regulate two transcription factors, c-Rel and interferon regulatory factor 5 (IRF5), which belong to the NF-κB and IRF families, respectively (126). IRF5 is activated by TRAF6 in the MyD88 signaling pathway, whereas c-Rel is activated by IKK through phosphorylation-dependent degradation of the NF-κB inhibitor IκBα (10, 18). Both c-Rel and IRF5 are crucial mediators of TLR-stimulated proinflammatory cytokine expression (140–145). TRAF2 and TRAF3 control the steady level expression of these transcription factors posttranslationally, which involves a ubiquitin- and proteasome-dependent mechanism (**Figure 3**). In wild-type macrophages, c-Rel and IRF5 undergo constitutive degradation, and

this process is requires TRAF2 and TRAF3, since deletion of either TRAF2 or TRAF3 causes stabilization and accumulation of c-Rel and IRF5 (126). The constitutive degradation of c-Rel and IRF5 also involves cIAPs, since incubation of macrophages with a small molecule IAP antagonist results in accumulation of c-Rel and IRF5. These findings suggest the possibility that the TRAF–cIAP E3 ubiquitin ligase mediates degradation of c-Rel and IRF5 in addition to NIK (**Figure 3**).

Emerging evidence suggests that the anti-inflammatory function of TRAF–cIAP complex in macrophages plays an important role in regulating antitumor immunity. Myeloid cell-specific deletion of TRAF2 promotes the generation of M1 type of macrophages in tumor microenvironment, which promotes recruitment of CD4 and CD8 effector T cells and enhances antitumor immunity (126). Consistently, as discussed in the above section, IAP antagonists promote antitumor immunity and synergize with immune checkpoint inhibitors in mouse models of cancer immunotherapy (122–125). These immunostimulatory effects of IAP antagonists likely involve activation of both the NIK-dependent NF-κB pathway and the activation of M1 type macrophages.

CONCLUDING REMARKS

Tumor necrosis factor receptor-associated factor proteins, particularly TRAF6 and TRAF2, have been established as pivotal mediators of NF-κB and MAPK pathways. In addition, recent studies have identified TRAF2 and TRAF3 as negative regulators of the noncanonical NF-κB pathway and proinflammatory TLR signaling pathways. Accumulating studies have also revealed potential molecular mechanisms that regulate the fate and signaling functions of TRAFs. These research progresses provide novel insights into the mechanism underlying the signaling function of TRAFs and shed light on the molecular basis of human diseases associated with TRAF-dependent signaling pathways. These scientific advances have also suggested new opportunities for designing therapeutic approaches in the treatment of inflammatory diseases and cancer. For example, the IAP antagonists, which induce degradation of cIAPs and, thereby, disruption of the TRAF–cIAP E3 complex, have been tested in animal models of cancer immunotherapy and obtained promising results.

The recent progresses also raise a number of questions to be addressed in future studies. For example, the molecular mechanism by which TRAF6 mediates activation of NF-κB and MAPK pathways is incompletely understood. Although the E3 ligase activity of TRAF6 is important, controversies exist regarding how TRAF6 exerts the ubiquitin-dependent mechanism for activating the NF-κB and MAPK pathways. The signaling mechanism of TRAF2 is also elusive. In particular, whether the E3 ligase activity TRAF2 is required for TNFα-induced NF-κB activation is in debate. Furthermore, our current knowledge is largely based on cell line studies, and it will be important to have a mouse model for studying the role of ubiquitination in TRAF2 function under physiological conditions. Another important question is about the negative roles of TRAFs in regulating signaling. It is important to further define the mechanism by which TRAF2 and TRAF3 function together with cIAPs to mediate ubiquitin-dependent protein degradation and characterize the target proteins of this ubiquitin ligase complex. Finally, how TRAF proteins are regulated in different receptor pathways represents another important area for future research.

AUTHOR CONTRIBUTIONS

Both authors have made significant contributions to this review.

FUNDING

Research is supported by grants from the US National Institutes of Health (AI057555, AI064639, GM84459, and AI104519) for S-CS and by grants from the National Natural Science Foundation of China (No. 31301143) and the Hebei Youth Top-notch Talent Support Program for J-HS.

REFERENCES

1. Ha H, Han D, Choi Y. TRAF-mediated TNFR-family signaling. *Curr Protoc Immunol* (2009) Chapter 11:Unit 11.19D. doi:10.1002/0471142735.im1109ds87
2. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal* (2013) 8(1):7. doi:10.1186/1750-2187-8-7
3. Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. *Annu Rev Biochem* (2009) 78:399–434. doi:10.1146/annurev.biochem.78.101807.093809
4. Deng L, Wang C, Spencer E, Yang L, Braun A, You J, et al. Activation of the IkB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* (2000) 103:351–61. doi:10.1016/S0092-8674(00)00126-4
5. Hacker H, Tseng PH, Karin M. Expanding TRAF function: TRAF3 as a tri-faced immune regulator. *Nat Rev Immunol* (2011) 11(7):457–68. doi:10.1038/nri2998
6. Rothe M, Wong SC, Henzel WJ, Goeddel DV. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* (1994) 78:681–92. doi:10.1016/0092-8674(94)90532-0
7. Arthur JS, Ley SC. Mitogen-activated protein kinases in innate immunity. *Nat Rev Immunol* (2013) 13(9):679–92. doi:10.1038/nri3495
8. Liu T, Zhang L, Joo D, Sun SC. NF-kappaB signaling in inflammation. *Signal Transduct Target Ther* (2017) 2:17023. doi:10.1038/sigtrans.2017.23
9. Yang XD, Sun SC. Targeting signaling factors for degradation, an emerging mechanism for TRAF functions. *Immunol Rev* (2015) 266(1):56–71. doi:10.1111/imr.12311
10. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* (2008) 132:344–62. doi:10.1016/j.cell.2008.01.020
11. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol* (2009) 27:693–733. doi:10.1146/annurev.immunol.021908.132641
12. Beinke S, Ley SC. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. *Biochem J* (2004) 382:393–409. doi:10.1042/BJ20040544
13. Sun SC, Ley SC. New insights into NF-kappaB regulation and function. *Trends Immunol* (2008) 29:469–78. doi:10.1016/j.it.2008.07.003
14. Lin L, DeMartino GN, Greene WC. Cotranslational biogenesis of NF-kappaB p50 by the 26S proteasome. *Cell* (1998) 92:819–28. doi:10.1016/S0092-8674(00)81409-9
15. Sriskantharajah S, Belich MP, Papoutsopoulou S, Janzen J, Tybulewicz V, Seddon B, et al. Proteolysis of NF-kappaB1 p105 is essential for T cell antigen receptor-induced proliferation. *Nat Immunol* (2009) 10(1):38–47. doi:10.1038/ni.1685
16. Yang HT, Papoutsopoulou S, Belich M, Brender C, Janzen J, Gantke T, et al. Coordinate regulation of TPL-2 and NF-kappaB signaling in macrophages by NF-kappaB1 p105. *Mol Cell Biol* (2012) 32(17):3438–51. doi:10.1128/MCB.00564-12

17. Xiao G, Harhaj EW, Sun SC. NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. *Mol Cell* (2001) 7:401–9. doi:10.1016/S1097-2765(01)00187-3
18. Sun SC. The non-canonical NF-kappaB pathway in immunity and inflammation. *Nat Rev Immunol* (2017) 17(9):545–58. doi:10.1038/nri.2017.52
19. Liu S, Chen ZJ. Expanding role of ubiquitination in NF-κB signaling. *Cell Res* (2011) 21:6–21. doi:10.1038/cr.2010.170
20. Hu H, Sun SC. Ubiquitin signaling in immune responses. *Cell Res* (2016) 26(4):457–83. doi:10.1038/cr.2016.40
21. Sato S, Sanjo H, Takeda K, Ninomiya-Tsuji J, Yamamoto M, Kawai T, et al. Essential function for the kinase TAK1 in innate and adaptive immune responses. *Nat Immunol* (2005) 6:1987–1095. doi:10.1038/ni1255
22. Yang J, Lin Y, Guo Z, Cheng J, Huang J, Deng L, et al. The essential role of MEKK3 in TNF-induced NF-kappaB activation. *Nat Immunol* (2001) 2:620–4. doi:10.1038/89769
23. Huang Q, Yang J, Lin Y, Walker C, Cheng J, Liu ZG, et al. Differential regulation of interleukin 1 receptor and toll-like receptor signaling by MEKK3. *Nat Immunol* (2004) 5(1):98–103. doi:10.1038/ni1014
24. Blonska M, Shambharkar PB, Kobayashi M, Zhang D, Sakurai H, Su B, et al. TAK1 is recruited to the tumor necrosis factor-alpha (TNF-alpha) receptor 1 complex in a receptor-interacting protein (RIP)-dependent manner and cooperates with MEKK3 leading to NF-kappaB activation. *J Biol Chem* (2005) 280(52):43056–63. doi:10.1074/jbc.M507807200
25. Qin J, Yao J, Cui G, Xiao H, Kim TW, Fraczek J, et al. TLR8-mediated NF-kappaB and JNK activation are TAK1-independent and MEKK3-dependent. *J Biol Chem* (2006) 281(30):21013–21. doi:10.1074/jbc.M512908200
26. Yao J, Kim TW, Qin J, Jiang Z, Qian Y, Xiao H, et al. Interleukin-1 (IL-1)-induced TAK1-dependent versus MEKK3-dependent NFkappaB activation pathways bifurcate at IL-1 receptor-associated kinase modification. *J Biol Chem* (2007) 282(9):6075–89. doi:10.1074/jbc.M609039200
27. Yamazaki K, Gohda J, Kanayama A, Miyamoto Y, Sakurai H, Yamamoto M, et al. Two mechanistically and temporally distinct NF-kappaB activation pathways in IL-1 signaling. *Sci Signal* (2009) 2(93):ra66. doi:10.1126/scisignal.2000387
28. Sun SC, Chang JH, Jin J. Regulation of nuclear factor-kappaB in autoimmunity. *Trends Immunol* (2013) 34(6):282–9. doi:10.1016/j.it.2013.01.004
29. Senftleben U, Cao Y, Xiao G, Kraehn G, Greten F, Chen Y, et al. Activation of IKKa of a second, evolutionary conserved, NF-kB signaling pathway. *Science* (2001) 293:1495–9. doi:10.1126/science.1062677
30. Hayden MS, Ghosh S. NF-kappaB, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev* (2012) 26(3):203–34. doi:10.1101/gad.183434.111
31. Sun SC. The noncanonical NF-kappaB pathway. *Immunol Rev* (2012) 246(1):125–40. doi:10.1111/j.1600-065X.2011.01088.x
32. Liao G, Zhang M, Harhaj EW, Sun SC. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. *J Biol Chem* (2004) 279(25):26243–50. doi:10.1074/jbc.M403286200
33. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol* (2002) 20:55–72. doi:10.1146/annurev.immunol.20.091301.131133
34. Gantke T, Sriskantharajah S, Ley SC. Regulation and function of TPL-2, an IkappaB kinase-regulated MAP kinase kinase kinase. *Cell Res* (2011) 21(1):131–45. doi:10.1038/cr.2010.173
35. Beinke S, Deka J, Lang V, Belich MP, Walker PA, Howell S, et al. NF-kappaB1 p105 negatively regulates TPL-2 MEK kinase activity. *Mol Cell Biol* (2003) 23(14):4739–52. doi:10.1128/MCB.23.14.4739-4752.2003
36. Waterfield M, Zhang M, Norman LP, Sun SC. NF-kappaB1/p105 regulates lipopolysaccharide-stimulated MAP kinase signaling by governing the stability and function of the Tpl2 kinase. *Mol Cell* (2003) 11:685–94. doi:10.1016/S1097-2765(03)00070-4
37. Beinke S, Robinson MJ, Hugunin M, Ley SC. Lipopolysaccharide activation of the TPL-2/MEK/extracellular signal-regulated kinase mitogen-activated protein kinase cascade is regulated by IkappaB kinase-induced proteolysis of NF-kappaB1 p105. *Mol Cell Biol* (2004) 24:9658–67. doi:10.1128/MCB.24.21.9658-9667.2004
38. Waterfield M, Wei J, Reiley W, Zhang MY, Sun S-C. IKKb is an essential component of the Tpl2 signaling pathway. *Mol Cell Biol* (2004) 24:6040–8. doi:10.1128/MCB.24.13.6040-6048.2004
39. Babu GR, Jin W, Norman L, Waterfield M, Chang M, Wu X, et al. Phosphorylation of NF-kappaB1/p105 by oncoprotein kinase Tpl2: implications for a novel mechanism of Tpl2 regulation. *Biochim Biophys Acta* (2006) 1763:174–81. doi:10.1016/j.bbamcr.2005.12.010
40. Schwandner R, Yamaguchi K, Cao Z. Requirement of tumor necrosis factor receptor-associated factor (TRAF)6 in interleukin 17 signal transduction. *J Exp Med* (2000) 191(7):1233–40. doi:10.1084/jem.191.7.1233
41. Wu H, Arron JR. TRAF6, a molecular bridge spanning adaptive immunity, innate immunity and osteoimmunology. *Bioessays* (2003) 25(11):1096–105. doi:10.1002/bies.10352
42. Lee MS, Kim YJ. Signaling pathways downstream of pattern-recognition receptors and their cross talk. *Annu Rev Biochem* (2007) 76:447–80. doi:10.1146/annurev.biochem.76.060605.122847
43. Sorrentino A, Thakur N, Grimsby S, Marcusson A, von Bulow V, Schuster N, et al. The type I TGF-beta receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. *Nat Cell Biol* (2008) 10:1199–207. doi:10.1038/ncb1780
44. Gu C, Wu L, Li X. IL-17 family: cytokines, receptors and signaling. *Cytokine* (2013) 64(2):477–85. doi:10.1016/j.cyto.2013.07.022
45. Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, et al. Distinct molecular mechanism for initiating TRAF6 signalling. *Nature* (2002) 418(6896):443–7. doi:10.1038/nature00888
46. Liu S, Chen J, Cai X, Wu J, Chen X, Wu YT, et al. MAVS recruits multiple ubiquitin E3 ligases to activate antiviral signaling cascades. *Elife* (2013) 2:e00785. doi:10.7554/eLife.00785
47. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. *Nat Immunol* (2010) 11:373–84. doi:10.1038/ni.1863
48. Kanayama A, Seth RB, Sun L, Ea CK, Hong M, Shaito A, et al. TAB 2 and TAB 3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Mol Cell* (2004) 15:535–48. doi:10.1016/j.molcel.2004.08.008
49. Ea CK, Deng L, Xia ZP, Pineda G, Chen ZJ. Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. *Mol Cell* (2006) 22:245–57. doi:10.1016/j.molcel.2006.03.026
50. Wu CJ, Conze DB, Li T, Srinivasula SM, Ashwell JD. Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation. *Nat Cell Biol* (2006) 8:398–406. doi:10.1038/ncb1384
51. Kulathu Y, Akutsu M, Bremm A, Hofmann K, Komander D. Two-sided ubiquitin binding explains specificity of the TAB 2 NZF domain. *Nat Struct Mol Biol* (2009) 16(12):1328–30. doi:10.1038/nsmb.1731
52. Wagner S, Carpentier I, Rogov V, Kreike M, Ikeda F, Wu CJ, et al. Ubiquitin binding mediates the NF-kB inhibitory potential of ABINs. *Oncogene* (2008) 27:3739–45. doi:10.1038/sj.onc.1211042
53. Lo YC, Lin SC, Rospigliosi CC, Conze DB, Wu CJ, Ashwell JD, et al. Structural basis for recognition of diubiquitins by NEMO. *Mol Cell* (2009) 33(5):602–15. doi:10.1016/j.molcel.2009.01.012
54. Yoshikawa A, Sato Y, Yamashita M, Mimura H, Yamagata A, Fukai S. Crystal structure of the NEMO ubiquitin-binding domain in complex with Lys 63-linked di-ubiquitin. *FEBS Lett* (2009) 583(20):3317–22. doi:10.1016/j.febslet.2009.09.028
55. Lamothe B, Besse A, Campos AD, Webster WK, Wu H, Darnay BG. Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of I kappa B kinase activation. *J Biol Chem* (2007) 282:4102–12. doi:10.1074/jbc.M609503200
56. Walsh MC, Kim GK, Maurizio PL, Molnar EE, Choi Y. TRAF6 autoubiquitination-independent activation of the NFkappaB and MAPK pathways in response to IL-1 and RANKL. *PLoS One* (2008) 3:e4064. doi:10.1371/journal.pone.0004064
57. Xia ZP, Sun L, Chen X, Pineda G, Jiang X, Adhikari A, et al. Direct activation of protein kinases by unanchored polyubiquitin chains. *Nature* (2009) 461(7260):114–9. doi:10.1038/nature08247
58. Strickson S, Emmerich CH, Goh ETH, Zhang J, Kelsall IR, Macartney T, et al. Roles of the TRAF6 and pellino E3 ligases in MyD88 and RANKL signaling. *Proc Natl Acad Sci U S A* (2017) 114(17):E3481–9. doi:10.1073/pnas.1702367114
59. Jensen LE, Whitehead AS. Ubiquitin activated tumor necrosis factor receptor associated factor-6 (TRAF6) is recycled via deubiquitination. *FEBS Lett* (2003) 553(1–2):190–4. doi:10.1016/S0014-5793(03)00998-0
60. Sun SC. Deubiquitylation and regulation of the immune response. *Nat Rev Immunol* (2008) 8:501–11. doi:10.1038/nri2337

61. Yasunaga J, Lin FC, Lu X, Jeang KT. Ubiquitin-specific peptidase 20 targets TRAF6 and human T cell leukemia virus type 1 tax to negatively regulate NF-kappaB signaling. *J Virol* (2011) 85(13):6212–9. doi:10.1128/JVI.00079-11
62. Xiao N, Li H, Luo J, Wang R, Chen H, Chen J, et al. Ubiquitin-specific protease 4 (USP4) targets TRAF2 and TRAF6 for deubiquitination and inhibits TNFalpha-induced cancer cell migration. *Biochem J* (2012) 441(3):979–86. doi:10.1042/BJ20111358
63. He X, Li Y, Li C, Liu LJ, Zhang XD, Liu Y, et al. USP2a negatively regulates IL-1beta- and virus-induced NF-kappaB activation by deubiquitinating TRAF6. *J Mol Cell Biol* (2013) 5(1):39–47. doi:10.1093/jmcb/mjs024
64. Luong le A, Fragiadaki M, Smith J, Boyle J, Lutz J, Dean JL, et al. Cezanne regulates inflammatory responses to hypoxia in endothelial cells by targeting TRAF6 for deubiquitination. *Circ Res* (2013) 112(12):1583–91. doi:10.1161/CIRCRESAHA.111.300119
65. Panda S, Nilsson JA, Gekara NO. Deubiquitinase MYSM1 regulates innate immunity through inactivation of TRAF3 and TRAF6 complexes. *Immunity* (2015) 43(4):647–59. doi:10.1016/j.immuni.2015.09.010
66. Zhou F, Zhang X, van Dam H, Ten Dijke P, Huang H, Zhang L. Ubiquitin-specific protease 4 mitigates toll-like/interleukin-1 receptor signaling and regulates innate immune activation. *J Biol Chem* (2012) 287(14):11002–10. doi:10.1074/jbc.M111.328187
67. Schimmack G, Schorpp K, Kutzner K, Gehring T, Brenke JK, Hadian K, et al. YOD1/TRAF6 association balances p62-dependent IL-1 signaling to NF-kappaB. *Elife* (2017) 6:e22416. doi:10.7554/eLife.22416
68. Schneider M, Zimmermann AG, Roberts RA, Zhang L, Swanson KV, Wen H, et al. The innate immune sensor NLRC3 attenuates toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-kappaB. *Nat Immunol* (2012) 13(9):823–31. doi:10.1038/ni.2378
69. Zhang X, Zhang J, Zhang L, van Dam H, ten Dijke P. UBE2O negatively regulates TRAF6-mediated NF-kappaB activation by inhibiting TRAF6 polyubiquitination. *Cell Res* (2013) 23(3):366–77. doi:10.1038/cr.2013.21
70. Jiao S, Zhang Z, Li C, Huang M, Shi Z, Wang Y, et al. The kinase MST4 limits inflammatory responses through direct phosphorylation of the adaptor TRAF6. *Nat Immunol* (2015) 16(3):246–57. doi:10.1038/ni.3097
71. Yao K, Lee SY, Peng C, Lim DY, Yamamoto H, Ryu J, et al. RSK2 is required for TRAF6 phosphorylation-mediated colon inflammation. *Oncogene* (2018) 37(26):3501–13. doi:10.1038/s41388-018-0167-6
72. Yeh WC, Shahinian A, Speiser D, Kraunus J, Billia F, Wakeham A, et al. Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* (1997) 7:715–25. doi:10.1016/S1074-7613(00)80391-X
73. Tada K, Okazaki T, Sakon S, Kobarai T, Kurosawa K, Yamaoka S, et al. Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF-kappa B activation and protection from cell death. *J Biol Chem* (2001) 276(39):36530–4. doi:10.1074/jbc.M104837200
74. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* (1996) 84:299–308. doi:10.1016/S0092-8674(00)80984-8
75. Shu HB, Takeuchi M, Goeddel DV. The tumor necrosis factor receptor 2 signal transducers TRAF2 and c-IAP1 are components of the tumor necrosis factor receptor 1 signaling complex. *Proc Natl Acad Sci U S A* (1996) 93(24):13973–8. doi:10.1073/pnas.93.24.13973
76. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. *Cell* (1995) 81(4):495–504. doi:10.1016/0092-8674(95)90070-5
77. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* (2003) 114(2):181–90. doi:10.1016/S0092-8674(03)00521-X
78. Bertrand MJ, Milutinovic S, Dickson KM, Ho WC, Boudreault A, Durkin J, et al. cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. *Mol Cell* (2008) 30:689–700. doi:10.1016/j.molcel.2008.05.014
79. Mahoney DJ, Cheung HH, Mrad RL, Plenchette S, Simard C, Enwere E, et al. Both cIAP1 and cIAP2 regulate TNFalpha-mediated NF-kappaB activation. *Proc Natl Acad Sci U S A* (2008) 105:11778–83. doi:10.1073/pnas.0711122105
80. Xu M, Skaug B, Zeng W, Chen ZJ. A ubiquitin replacement strategy in human cells reveals distinct mechanisms of IKK activation by TNFalpha and IL-1beta. *Mol Cell* (2009) 36(2):302–14. doi:10.1016/j.molcel.2009.10.002
81. Shan B, Pan H, Najafov A, Yuan J. Necroptosis in development and diseases. *Genes Dev* (2018) 32(5–6):327–40. doi:10.1101/gad.312561.118
82. Shi CS, Kehrl JH. TNF-induced GCKR and SAPK activation depends upon the E2/E3 complex Ubc13-Uev1A/TRAF2. *J Biol Chem* (2003) 278:15429–34. doi:10.1074/jbc.M211796200
83. Yin Q, Lamothe B, Darnay BG, Wu H. Structural basis for the lack of E2 interaction in the RING domain of TRAF2. *Biochemistry* (2009) 48(44):10558–67. doi:10.1021/bi901462e
84. Alvarez SE, Harikumar KB, Hait NC, Allegood J, Strub GM, Kim EY, et al. Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. *Nature* (2010) 465(7301):1084–8. doi:10.1038/nature09128
85. Xia P, Wang L, Moretti PA, Albanese N, Chai F, Pitson SM, et al. Sphingosine kinase interacts with TRAF2 and dissects tumor necrosis factor-alpha signaling. *J Biol Chem* (2002) 277(10):7996–8003. doi:10.1074/jbc.M111423200
86. Lee TH, Shank J, Cusson N, Kelliher MA. The kinase activity of Rip1 is not required for tumor necrosis factor-alpha-induced IkappaB kinase or p38 MAP kinase activation or for the ubiquitination of Rip1 by Traf2. *J Biol Chem* (2004) 279(32):33185–91. doi:10.1074/jbc.M404206200
87. Vince JE, Pantaki D, Feltham R, Mace PD, Cordier SM, Schmukle AC, et al. TRAF2 must bind to cellular inhibitors of apoptosis for tumor necrosis factor (TNF) to efficiently activate NF-{kappa}B and to prevent TNF-induced apoptosis. *J Biol Chem* (2009) 284(51):35906–15. doi:10.1074/jbc.M109.072256
88. Varfolomeev E, Goncharov T, Fedorova AV, Dynek JN, Zobel K, Deshayes K, et al. c-IAP1 and c-IAP2 are critical mediators of tumor necrosis factor alpha (TNFalpha)-induced NF-kappaB activation. *J Biol Chem* (2008) 283:24295–9. doi:10.1074/jbc.C800128200
89. Sasaki K, Iwai K. Roles of linear ubiquitinylation, a crucial regulator of NF-kappaB and cell death, in the immune system. *Immunol Rev* (2015) 266(1):175–89. doi:10.1111/imr.12308
90. Hrdinka M, Gyrd-Hansen M. The Met1-linked ubiquitin machinery: emerging themes of (De)regulation. *Mol Cell* (2017) 68(2):265–80. doi:10.1016/j.molcel.2017.09.001
91. Haas TL, Emmerich CH, Gerlach B, Schmukle AC, Cordier SM, Rieser E, et al. Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and is required for TNF-mediated gene induction. *Mol Cell* (2009) 36(5):831–44. doi:10.1016/j.molcel.2009.10.013
92. Tokunaga F, Sakata S, Saeki Y, Satomi Y, Kirisako T, Kamei K, et al. Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol* (2009) 11:123–32. doi:10.1038/ncb1821
93. Rahighi S, Ikeda F, Kawasaki M, Akutsu M, Suzuki N, Kato R, et al. Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* (2009) 136(6):1098–109. doi:10.1016/j.cell.2009.03.007
94. Kensche T, Tokunaga F, Ikeda F, Goto E, Iwai K, Dikic I. Analysis of nuclear factor-kappaB (NF-kappaB) essential modulator (NEMO) binding to linear and lysine-linked ubiquitin chains and its role in the activation of NF-kappaB. *J Biol Chem* (2012) 287(28):23626–34. doi:10.1074/jbc.M112.347195
95. Hauenstein AV, Xu G, Kabaleeswaran V, Wu H. Evidence for M1-linked polyubiquitin-mediated conformational change in NEMO. *J Mol Biol* (2017) 429(24):3793–800. doi:10.1016/j.jmb.2017.10.026
96. Fujita H, Rahighi S, Akita M, Kato R, Sasaki Y, Wakatsuki S, et al. Mechanism underlying IkappaB kinase activation mediated by the linear ubiquitin chain assembly complex. *Mol Cell Biol* (2014) 34(7):1322–35. doi:10.1128/MCB.01538-13
97. Zhang J, Clark K, Lawrence T, Peggie MW, Cohen P. An unexpected twist to the activation of IKKbeta: TAK1 primes IKKbeta for activation by autophosphorylation. *Biochem J* (2014) 461(3):531–7. doi:10.1042/BJ20140444
98. Fiil BK, Damgaard RB, Wagner SA, Keusekotten K, Fritsch M, Bekker-Jensen S, et al. OTULIN restricts Met1-linked ubiquitination to control innate immune signaling. *Mol Cell* (2013) 50(6):818–30. doi:10.1016/j.molcel.2013.06.004
99. Keusekotten K, Elliott PR, Glockner L, Fiil BK, Damgaard RB, Kulathu Y, et al. OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell* (2013) 153(6):1312–26. doi:10.1016/j.cell.2013.05.014
100. Takiuchi T, Nakagawa T, Tamiya H, Fujita H, Sasaki Y, Saeki Y, et al. Suppression of LUBAC-mediated linear ubiquitination by a specific interaction between LUBAC and the deubiquitinases CYLD and OTULIN. *Genes Cells* (2014) 19(3):254–72. doi:10.1111/gtc.12128

101. Tokunaga F, Nishimasu H, Ishitani R, Goto E, Noguchi T, Mio K, et al. Specific recognition of linear polyubiquitin by A20 zinc finger 7 is involved in NF-kappaB regulation. *EMBO J* (2012) 31(19):3856–70. doi:10.1038/emboj.2012.241
102. Verhelst K, Carpentier I, Kreike M, Meloni L, Verstrepen L, Kensche T, et al. A20 inhibits LUBAC-mediated NF-kappaB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J* (2012) 31(19):3845–55. doi:10.1038/emboj.2012.240
103. Rothe M, Pan M-G, Henzel WJ, Ayres TM, Goeddel DV. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell* (1995) 83:1243–52. doi:10.1016/0092-8674(95)90149-3
104. Rothe M, Sarma V, Dixit VM, Goeddel DV. TRAF2-mediated activation of NF-kB by TNF receptor 2 and CD40. *Science* (1995) 269:1424–7. doi:10.1126/science.7544915
105. Boucher LM, Marengere LE, Lu Y, Thukral S, Mak TW. Binding sites of cytoplasmic effectors TRAF1, 2, and 3 on CD30 and other members of the TNF receptor superfamily. *Biochem Biophys Res Commun* (1997) 233(3):592–600. doi:10.1006/bbrc.1997.6509
106. Park YC, Burkitt V, Villa AR, Tong L, Wu H. Structural basis for self-association and receptor recognition of human TRAF2. *Nature* (1999) 398(6727):533–8. doi:10.1038/19110
107. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* (2003) 3(9):745–56. doi:10.1038/nri1184
108. Kim YS, Nedospasov SA, Liu ZG. TRAF2 plays a key, nonredundant role in LIGHT-lymphotoxin beta receptor signaling. *Mol Biol Cell* (2005) 25:2130–7. doi:10.1128/MCB.25.6.2130-2137.2005
109. Watts TH. Tnf/Tnfr family members in costimulation of T cell responses. *Annu Rev Immunol* (2005) 23:23–68. doi:10.1146/annurev.immunol.23.021704.115839
110. McPherson AJ, Snell LM, Mak TW, Watts TH. Opposing roles for TRAF1 in the alternative versus classical NF-kappaB pathway in T cells. *J Biol Chem* (2012) 287(27):23010–9. doi:10.1074/jbc.M112.350538
111. Ward-Kavanagh LK, Lin WW, Sedy JR, Ware CF. The TNF receptor superfamily in co-stimulating and co-inhibitory responses. *Immunity* (2016) 44(5):1005–19. doi:10.1016/j.immuni.2016.04.019
112. He JQ, Zarnegar B, Oganesyan G, Saha SK, Yamazaki S, Doyle SE, et al. Rescue of TRAF3-null mice by p100 NF-kappa B deficiency. *J Exp Med* (2006) 203:2413–8. doi:10.1084/jem.20061166
113. Xie P, Stunz LL, Larison KD, Yang B, Bishop GA. Tumor necrosis factor receptor-associated factor 3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. *Immunity* (2007) 27:253–67. doi:10.1016/j.immuni.2007.07.012
114. Gardam S, Sierro F, Basten A, Mackay F, Brink R. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. *Immunity* (2008) 28:391–401. doi:10.1016/j.immuni.2008.01.009
115. Sasaki Y, Calado DP, Derudder E, Zhang B, Shimizu Y, Mackay F, et al. NIK overexpression amplifies, whereas ablation of its TRAF3-binding domain replaces BAFF:BAFF-R-mediated survival signals in B cells. *Proc Natl Acad Sci U S A* (2008) 105:10883–8. doi:10.1073/pnas.0805186105
116. Malinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF-kB induction by TNF, CD95 and IL-1. *Nature* (1997) 385:540–4.
117. Grech AP, Amesbury M, Chan T, Gardam S, Basten A, Brink R. TRAF2 differentially regulates the canonical and noncanonical pathways of NF-kappaB activation in mature B cells. *Immunity* (2004) 21:629–42. doi:10.1016/j.immuni.2004.09.011
118. Varfolomeev E, Blankenship JW, Wayson SM, Fedorova AV, Kayagaki N, Garg P, et al. IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. *Cell* (2007) 131:669–81. doi:10.1016/j.cell.2007.10.030
119. Vince JE, Wong WW, Khan N, Feltham R, Chau D, Ahmed AU, et al. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. *Cell* (2007) 131:682–93. doi:10.1016/j.cell.2007.10.037
120. Vallabhapurapu S, Matsuzawa A, Zhang W, Tseng PH, Keats JJ, Wang H, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling. *Nat Immunol* (2008) 9:1364–70. doi:10.1038/ni.1678
121. Zarnegar BJ, Wang Y, Mahoney DJ, Dempsey PW, Cheung HH, He J, et al. Noncanonical NF-kappaB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nat Immunol* (2008) 9:1371–8. doi:10.1038/ni.1676
122. Dougan M, Dougan S, Slisz J, Firestone B, Vanneman M, Draganov D, et al. IAP inhibitors enhance co-stimulation to promote tumor immunity. *J Exp Med* (2010) 207(10):2195–206. doi:10.1084/jem.20101123
123. Chesi M, Mirza NN, Garbitt VM, Sharik ME, Dueck AC, Asmann YW, et al. IAP antagonists induce anti-tumor immunity in multiple myeloma. *Nat Med* (2016) 22(12):1411–20. doi:10.1038/nm.4229
124. Beug ST, Beauregard CE, Healy C, Sanda T, St-Jean M, Chabot J, et al. Smac mimetics synergize with immune checkpoint inhibitors to promote tumour immunity against glioblastoma. *Nat Commun* (2017) 8:14278. doi:10.1038/ncomms14278
125. Dougan SK, Dougan M. Regulation of innate and adaptive antitumor immunity by IAP antagonists. *Immunotherapy* (2018) 10:787–96. doi:10.2217/imt-2017-0185
126. Jin J, Xiao Y, Hu H, Zou Q, Li Y, Gao Y, et al. Proinflammatory TLR signalling is regulated by a TRAF2-dependent proteolysis mechanism in macrophages. *Nat Commun* (2015) 6:5930. doi:10.1038/ncomms6930
127. Zheng C, Kabaleeswaran V, Wang Y, Cheng G, Wu H. Crystal structures of the TRAF2: cIAP2 and the TRAF1: TRAF2: cIAP2 complexes: affinity, specificity, and regulation. *Mol Cell* (2010) 38:101–13. doi:10.1016/j.molcel.2010.03.009
128. Choudhary S, Kalita M, Fang L, Patel KV, Tian B, Zhao Y, et al. Inducible tumor necrosis factor (TNF) receptor-associated factor-1 expression couples the canonical to the non-canonical NF-kappaB pathway in TNF stimulation. *J Biol Chem* (2013) 288(20):14612–23. doi:10.1074/jbc.M113.464081
129. Häcker H, Redecke V, Blagoev B, Kratchmarova I, Hsu LC, Wang GG, et al. Specificity in toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. *Nature* (2006) 439:204–7. doi:10.1038/nature04369
130. Oganesyan G, Saha SK, Guo B, He JQ, Shahangian A, Zarnegar B, et al. Critical role of TRAF3 in the toll-like receptor-dependent and -independent antiviral response. *Nature* (2006) 439:208–11. doi:10.1038/nature04374
131. Lalani AI, Moore CR, Luo C, Kreider BZ, Liu Y, Morse HC III, et al. Myeloid cell TRAF3 regulates immune responses and inhibits inflammation and tumor development in mice. *J Immunol* (2015) 194(1):334–48. doi:10.4049/jimmunol.1401548
132. Piao JH, Hasegawa M, Heissig B, Hattori K, Takeda K, Iwakura Y, et al. Tumor necrosis factor receptor-associated factor (TRAF) 2 controls homeostasis of the colon to prevent spontaneous development of murine inflammatory bowel disease. *J Biol Chem* (2011) 286(20):17879–11788. doi:10.1074/jbc.M111.221853
133. Qiao YQ, Shen J, Gu Y, Tong JL, Xu XT, Huang ML, et al. Gene expression of tumor necrosis factor receptor associated-factor (TRAF)-1 and TRAF-2 in inflammatory bowel disease. *J Dig Dis* (2013) 14(5):244–50. doi:10.1111/1751-2980.12044
134. Shen J, Qiao Y, Ran Z, Wang T, Xu J, Feng J. Intestinal protein expression profile identifies inflammatory bowel disease and predicts relapse. *Int J Clin Exp Pathol* (2013) 6(5):917–25.
135. Shen J, Qiao YQ, Ran ZH, Wang TR. Up-regulation and pre-activation of TRAF3 and TRAF5 in inflammatory bowel disease. *Int J Med Sci* (2013) 10(2):156–63. doi:10.7150/ijms.5457
136. Tseng PH, Matsuzawa A, Zhang W, Mino T, Vignali DA, Karin M. Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. *Nat Immunol* (2010) 11(1):70–5. doi:10.1038/ni.1819
137. Xiao Y, Jin J, Chang M, Chang JH, Hu H, Zhou X, et al. Peli1 promotes microglia-mediated CNS inflammation by regulating TRAF3 degradation. *Nat Med* (2013) 19(5):595–602. doi:10.1038/nm.3111
138. Zhu S, Pan W, Shi P, Gao H, Zhao F, Song X, et al. Modulation of experimental autoimmune encephalomyelitis through TRAF3-mediated suppression of interleukin 17 receptor signaling. *J Exp Med* (2010) 207(12):2647–62. doi:10.1084/jem.20100703
139. Ma C, Lin W, Liu Z, Tang W, Gautam R, Li H, et al. NDR1 protein kinase promotes IL-17- and TNF-alpha-mediated inflammation by competitively binding TRAF3. *EMBO Rep* (2017) 18(4):586–602. doi:10.15252/embr.201642140

140. Sanjabi S, Hoffmann A, Liou HC, Baltimore D, Smale ST. Selective requirement for c-Rel during IL-12 P40 gene induction in macrophages. *Proc Natl Acad Sci U S A* (2000) 97(23):12705–10. doi:10.1073/pnas.230436397
141. Grumont R, Hochrein H, O'Keeffe M, Gugasyan R, White C, Caminschi I, et al. c-Rel regulates interleukin 12 p70 expression in CD8(+) dendritic cells by specifically inducing p35 gene transcription. *J Exp Med* (2001) 194(8):1021–32. doi:10.1084/jem.194.8.1021
142. Sanjabi S, Williams KJ, Saccani S, Zhou L, Hoffmann A, Ghosh G, et al. A c-Rel subdomain responsible for enhanced DNA-binding affinity and selective gene activation. *Genes Dev* (2005) 19(18):2138–51. doi:10.1101/gad.1329805
143. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, et al. Integral role of IRF-5 in the gene induction programme activated by toll-like receptors. *Nature* (2005) 434(7030):243–9. doi:10.1038/nature03308
144. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, et al. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* (2011) 12(3):231–8. doi:10.1038/ni.1990
145. Eames HL, Corbin AL, Udalova IA. Interferon regulatory factor 5 in human autoimmunity and murine models of autoimmune disease. *Transl Res* (2016) 167(1):167–82. doi:10.1016/j.trsl.2015.06.018

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewers HH and WZ and the handling Editor declared their shared affiliation.

frontiers in Immunology

MINI REVIEW
published: 04 February 2019
doi: 10.3389/fimmu.2019.00104

The Evolving Role of TRAFs in Mediating Inflammatory Responses

Bipandeep Dhillon[1†], *Fatemah Aleithan*[1†], *Zahi Abdul-Sater*[2] *and Ali A. Abdul-Sater*[1*]

[1] School of Kinesiology and Health Science, Muscle Health Research Centre, York University, Toronto, ON, Canada,
[2] Department of Basic Sciences, Phoenicia University, Mazraat El Daoudiyeh, Lebanon

TRAFs [tumor necrosis factor (TNF) receptor associated factors] are a family of signaling molecules that function downstream of multiple receptor signaling pathways and play a pivotal role in the biology of innate, and adaptive immune cells. Following receptor ligation, TRAFs generally function as adapter proteins to mediate the activation of intracellular signaling cascades. With the exception of TRAF1 that lacks a Ring domain, TRAFs have an E3 ubiquitin ligase activity which also contributes to their ability to activate downstream signaling pathways. TRAF-mediated signaling pathways culminate in the activation of several transcription factors, including nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPKs; e.g., ERK-1 and ERK-2, JNK, and p38), and interferon-regulatory factors (IRF; e.g., IRF3 and IRF7). The biological role of TRAFs is largely due to their ability to positively or negatively regulate canonical and non-canonical NF-κB signaling. While TRAF-mediated signaling regulates various immune cell functions, this review is focused on the recent advances in our knowledge regarding the molecular mechanisms through which TRAF proteins regulate, positively and negatively, inflammatory signaling pathways, including Toll–IL-1 receptors, RIG-I like receptors, and Nod-like receptors. The review also offers a perspective on the unanswered questions that need to be addressed to fully understand how TRAFs regulate inflammation.

Keywords: inflammation, innate immunity, TRAF, TLRs, NLR, RLR, STING, TNFR

OPEN ACCESS

Edited by:
Angela Ianaro,
University of Naples Federico II, Italy

Reviewed by:
Shi Yue,
University of Southern California, United States
Michael Paul Gantier,
Hudson Institute of Medical Research, Australia

***Correspondence:**
Ali A. Abdul-Sater
aasater@yorku.ca

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology

Received: *02 October 2018*
Accepted: *14 January 2019*
Published: *04 February 2019*

Citation:
Dhillon B, Aleithan F, Abdul-Sater Z and Abdul-Sater AA (2019) The Evolving Role of TRAFs in Mediating Inflammatory Responses. Front. Immunol. 10:104. doi: 10.3389/fimmu.2019.00104

INTRODUCTION

The Tumor-Necrosis Factor (TNF) Receptor Associated Factor (TRAF) family is comprised of cytoplasmic adaptor proteins involved in transducing downstream effects of a variety of receptors (1). TRAF1 and TRAF2 were first discovered through their association with TNF-R2 (2). Since then four other members have been identified, thus, a total of six known members exist (TRAF1 to TRAF6) (3, 4). The TRAF domain can be divided into a N-terminal coiled-coil region (TRAF-N) and a highly conserved C-terminal Beta-sandwich domain (MATH Domain) (4, 5). It is the MATH domain which allows TRAF molecules to form dimers and recruit downstream effectors to receptors (1). With the exception of TRAF1, all other TRAF members, contain a N-terminal RING finger, followed by a variable number of zinc fingers (1, 4, 6). The RING finger motif allows TRAF molecules to act as E3 ubiquitin ligases (5, 6). As adaptor proteins and E3 ubiquitin ligases involved in several immune pathways, TRAFs ultimately lead to the activation of transcription factors, such as nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPKs; e.g., ERK-1 and ERK-2, JNK, and p. 38), and interferon-regulatory factors (IRF; e.g., IRF3 and IRF7) (5, 6). In addition, TRAF proteins play important roles in embryonic development, tissue homeostasis, stress response, and bone metabolism (3, 6).

Since being discovered in TNF receptor signaling, TRAFs' role has expanded to include involvement in many other inflammatory signaling pathways such as toll-like receptors (TLRs), nucleotide binding-oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and cytokine receptors (4, 6). Aberrant and prolonged activation of inflammation following the activation of these receptors has been associated with debilitating diseases including cancer, atherosclerosis, type II diabetes, and autoimmune diseases (7). Therefore, a number of mechanisms have evolved to negatively regulate these pathways (8). This review is focused on recent studies that identified new roles for TRAF proteins in activating and inhibiting TLR, RLR, and NLR signaling, and emphasizes newly discovered mechanisms of regulating these pathways by targeting TRAF expression and function.

THE ROLE OF TRAFs IN TOLL-LIKE RECEPTOR SIGNALING

Toll-like receptors (TLRs) are a family of transmembrane receptors lining both cellular and endosomal membranes that sense various pathogen-associated-molecular patterns (PAMPs), and danger-associated molecular patterns (DAMPS) (6, 9–11). There are 10 known TLRs in humans that either exist as homo or heterodimers (11). TLRs are characterized by an extracellular ectodomain comprised of leucine-rich repeats (LRRs), which senses the corresponding PAMP or DAMP, a transmembrane domain, and an intracellular Toll/IL-1 receptor (TIR) domain, which induces the downstream response (9, 12). Upon stimulation, TLRs oligomerize, and recruit MyD88, with the exception of TLR3, which recruits TRIF through TIR domain interaction (12). TLR4 can uniquely induce both MyD88-dependent signaling when it's on the plasma membrane and TRIF-mediated signaling when translocated to the endosomal compartment. Subsequently, a signaling cascade is initiated which results in the activation of transcription factors like NF-κB, MAPKs, and IRFs. This ultimately leads to the production of chemokines, cytokines, and other inflammatory mediators, which initiate the innate immune response and prime the adaptive immune response (**Figure 1**) (6, 9, 13).

MyD88-dependent signaling is initiated with the recruitment of the IL-1 receptor-associated kinase (IRAK) 4 which, in turn, recruits and activates, through phosphorylation, IRAK1 and IRAK2 (14). IRAK1/2 recruit TRAF6, which functions as an E3 ubiquitin ligase following its oligomerization via the CC domains (15). This also allows TRAF6 to associate with the E2 ubiquitin complex Uev1A:Ubc13, which together then catalyze the K63-linked polyubiquitination to TRAF6 and other substrates, including TAK1, TAB1, TAB2, and NEMO (IKKγ) (16). This activates TAK1 which co-localizes with the IKK complex and activate IKKβ via phosphorylation (9, 17). Importantly, optimal activation of the IKK complex requires the linear ubiquitination of NEMO (M1-linked) (18–20). This is mediated by a ubiquitin ligase complex termed the Linear UBiquitin chain Assembly Complex (LUBAC) and consists of heme-oxidized IRP2 ubiquitin ligase-1 (HOIL-1), HOIL-1–interacting protein (HOIP), and the Shank-associated RH domain interactor (SHARPIN) (21). Activation of the IKK complex leads to phosphorylation and subsequent degradation of the inhibitor of κB, IκBα, which eventually leads to NF-κB activation (12, 13). TAK1 also induces activation of MAPKs, such as ERK1/2, P38, and JNK, through phosphorylation leading to activation of transcription factors like AP-1 (**Figure 1A**) (12).

In TRIF-mediated signaling, TRIF recruits TRAF3, which catalyzes its own K63-linked polyubiquitination. This leads to the activation of the TBK1 and the non-canonical IKK, IKKε, which in turn phosphorylates IRF3 resulting in its nuclear translocation and the subsequent induction of type 1 IFNs (IFN-Is) (22, 23). With slower kinetics (i.e., late phase), TRIF can also form a complex with TRAF6 and RIP1, to induce the TAK1/IKK axis and the subsequent activation of NF-κB (**Figure 1B**) (11). IFN-Is can also be induced following TLR7 and TLR9 stimulation through the MyD88-dependent pathway. MyD88 forms a complex with TRAF3 which then recruits and activates an IRAK-IKKα complex, which in turn phosphorylates IRF7 resulting in its translocation into the nucleus to induce interferon production (**Figure 1C**) (9).

TRAFs Negatively Regulate TLR Signaling

In addition to activating TLRs, TRAFs can also function as negative regulators of TLR signaling. TRAF3 negatively regulates TLR-mediated MAPK activity, possibly by preventing the release of the TRAF6:TAB1/2:TAK1 complex, but the negative regulation is inhibited by cIAP1/2 which catalyze K48-polyubiquitinated degradation of TRAF3 (**Figure 1A**) (11, 23, 24). Under specific conditions, TRAF2 can dampen TLR mediated cytokine production by causing proteasome-dependent degradation of c-Rel, a member of the NF-κB family, in a mechanism that also requires TRAF3 and cIAP1/2 (25). TRAF5 has also been shown to inhibit TLR-stimulated cytokine production by preventing the interaction between TRAF6 and TAB2 (26). TRAF4 associates with p47phox, a component of cytosolic NADPH oxidase, to negatively regulate TLR signaling by interacting with TRAF6 and TRIF and disrupting their functions (27). Recently, TRAF1 has been shown to attenuate TLR-induced NF-κB signaling by interfering with LUBAC-mediated linear ubiquitination of NEMO (**Figure 1A**) (28). Interestingly, downstream of TLR3 signaling, TRAF1 inhibits TRIF mediated activation of NF-κB, ISRE, and the IFN-β promoter independent of IRF-3 (**Figure 1B**) (29).

Negative Regulation of TLR Signaling by Targeting TRAFs

TLR signaling can also be regulated by targeting the function, expression, or catalytic activity of certain TRAF proteins. Several deubiquitinases (DUB) have been shown to negatively regulate TLR signaling by removing ubiquitin chains from TRAFs or their targets. For instance, A20 is a key regulator of TLR signaling, whereby it targets several aspects of the signaling cascade. It can accomplish this, in part, by directly deubiquitinating TRAF6 (30). Monocyte chemotactic protein-induced protein 1 (MCPIP1) is another DUB that negatively regulates JNK and NF-κB signaling

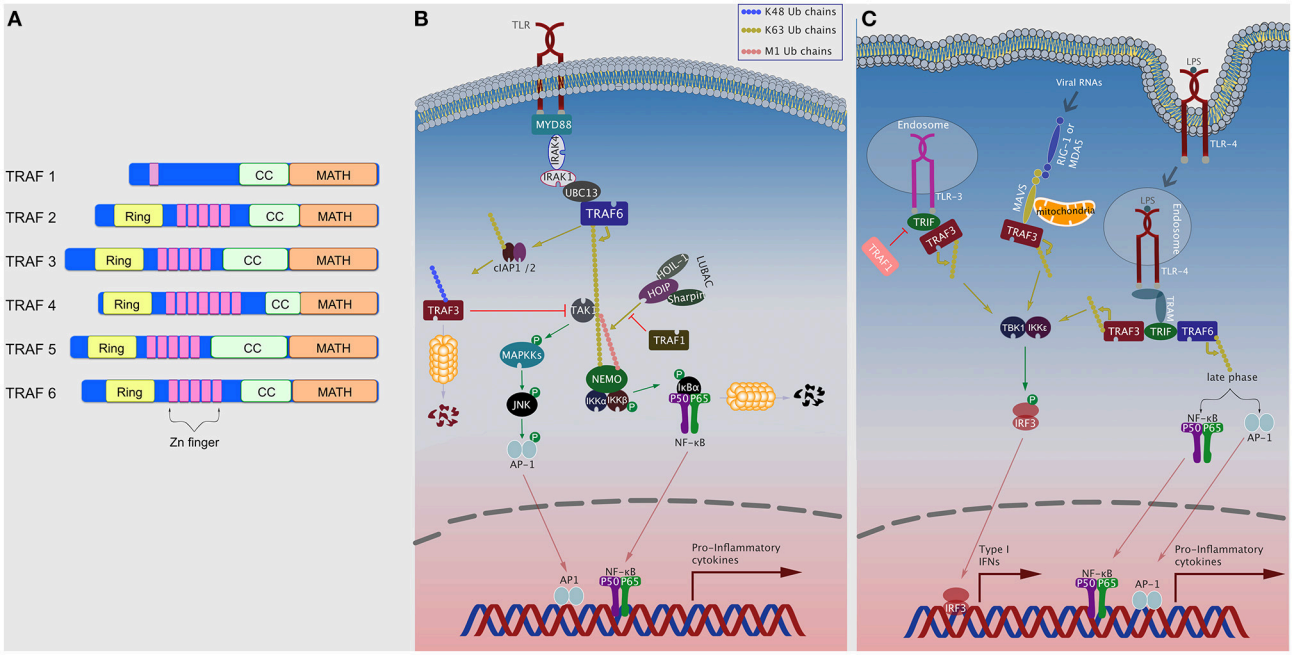


FIGURE 1 | TRAFs in TLR signaling pathways. **(A)** Domain organization of TRAF proteins. Domains shown include Ring, Zinc (Zn) finger, coiled-coil (CC; TRAF-N), and MATH (TRAF-C) **(B)** Plasma membrane TLRs, upon ligand binding, recruit various intracellular signaling elements including TRAF6 to activate NF-κB and MAPK pathways. TRAF1, TRAF3, TRAF4, and TRAF5 can negatively regulate this pathway by different means. **(C)** Following ligand binding, TLR4 translocates to the endosomal compartment and recruits TRIF and TRAF3 to induce the TBK1/IKKε/IRF3 axis, or TRAF6 to induce NF-κB and AP-1 via late phase (slower) kinetics. TLR3 and RLRs can also induce the TBK1/IKKε/IRF3 axis by recruiting TRAF3.

by deubiquitinating TRAF2, TRAF3, and TRAF6 (31). Recently, peroxiredoxin-1 (PRDX1) has been shown to directly interact with TRAF6 ring finger motif and inhibit its ubiquitin-ligase activity, which diminishes NF-κB activation downstream of TLR4 stimulation [(32), p. 1]. Several members of the NLR family, discussed below, have been shown to regulate TLR signaling by targeting TRAF proteins. NLRC3 can attenuate TLR-mediated NF-κB activation by reducing K63-linked polyubiquitination of TRAF6 (33). NLRX1 can interact with TRAF6 to reduce canonical NF-κB activation through the TLR4 mediated pathway [(34), p. 1]. Under normal conditions, NLRX1 associates with TRAF6, but upon TLR4 stimulation, NLRX1 dissociates from TRAF6 and binds to NEMO preventing TRAF6 recruitment of the IKK complex, and subsequent NF-κB activation (35). It's important to note, however, that some of those findings have been controversial in the field as other studies were not able to reach a similar conclusion [(36, 37); (38), p. 1]. NLRP11 inhibits TLR signaling by recruiting RNF19A, an ubiquitin ligase, to catalyze K48-linked polyubiquitination and the subsequent degradation of TRAF6 [(39), p. 11]. NLRP12 reduces non-canonical NF-κB stimulation by interacting with TRAF3 and NIK causing NIK is degraded preventing/reducing cleavage of p100 to p52 (40).

THE ROLE OF TRAFs IN NOD-LIKE RECEPTOR SIGNALING

NOD-like receptors (NLRs) are a family of cytosolic receptors that sample intracellular PAMPs and DAMPs (41–44). These receptors participate in a plethora of cellular processes including: inflammasome assembly, pyroptosis, activation of NF-κB and MAPK pathways, autophagy, IFN signaling, antigen processing and presentation, and ROS production (6, 43, 45). These receptors are characterized by a central nucleotide binding (NOD, also known as NACHT) domain, which allows oligomerization, followed by a C-terminal leucine-rich repeat (LRR) domain, which detects PAMPs and DAMPs and a variable N-terminal domains, which helps induce the downstream response (41–44, 46).

NOD1 and NOD2 are the most studied members of the NLR family [reviewed in Motta et al. (47)]. Upon activation, these receptors oligomerize through their NACHT domains and form a complex with RIPK2, through homotypic CARD-CARD interactions (44). In this complex, RIP2 is associated with multiple E3 ligases, including cIAP1/2, xIAP, TRAF2, and TRAF5, but only cIAP1/2 catalyzes its K63-linked polyubiquitination (44, 48–50). TRAF2 and TRAF5 serve as adaptor molecules to facilitate interaction of cIAP1/2 with RIP2 in this complex (48). RIPK2 then induces K63-linked polyubiquitination of TAK1 and NEMO, which recruits the IKK complex to the platform leading to IKKβ phosphorylation by TAK1 (43, 44). TRAF6 and CARD9 serve as adaptors which allow NOD1 and NOD2 to induce MAPKs and subsequently activate AP-1 transcription factor (51, 52). In addition to NF-κB and MAPK activation, NOD1 and NOD2, induce IFN-I production by forming a complex with RIPK2. This results in the recruitment

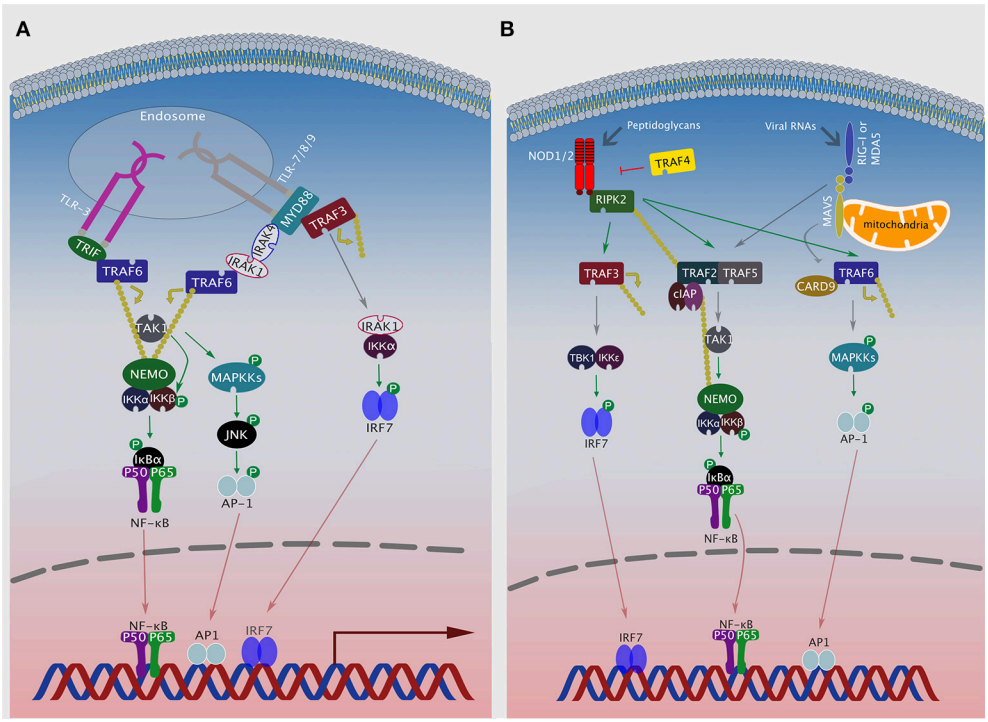


FIGURE 2 | TRAFs in NLR and RLR signaling pathways. **(A)** After ligand recognition, endosomal TLRs recruit TRAF6, either via TRIF (TLR3) or via MyD88/IRAK1/IRAK4 (TLR7, 8 or 9) to activate NF-κB. Additionally, TLR 7, 8, or 9 can recruit MyD88, TRAF3, IRAK1, and IKKα to activate IRF7. **(B)** Ligand activated NOD1 or NOD2 associate with RIPK2, which can then recruit either TRAF3 to activate the TBK1/IKKε/IRF7 axis, TRAF2, and TRAF5 to activate NF-κB or TRAF6 and CARD9 to activate MAPK signaling. Viral RNAs activate RIG-I or MDA5, which then associate with the mitochondrial protein MAVS and activate NF-κB or MAPK signaling by recruiting TRAF2/5 or TRAF6/CARD9, respectively.

of TRAF3 leading to the activation of TBK1/IKKε/IRF7 axis (**Figure 2**) (42, 53).

A few NLR family members (e.g., NLRP1, NLRP3, NLRP6, NLRC4, and NLRC5) are capable of activating inflammasomes (41, 54). Inflammasomes are multimeric protein complexes that play a key role in regulating the secretion of potent cytokines like IL-1β and IL-18. Most inflammasomes are composed of an NLR protein, the zymogen pro-caspase-1, and the adapter protein, apoptosis-associated speck-like protein containing a CARD (ASC) (55, 56). Intriguingly, TRAF3 has been recently shown to play a role in NLRP3 inflammasome activation, as it catalyzes K63-linked polyubiquitination of ASC in order to induce ASC speck formation and inflammasome activation (57). In addition, TRAF2, along with cIAP1/2, mediates K63-linked polyubiquitination of caspase-1 for optimum activity (58). However, TRAF2$^{-/-}$ bone marrow-derived macrophages (BMDMs) show normal inflammasome activation, suggesting that TRAF2 may not even be involved in this pathway (59). Following TLR signaling, TRAF6 promotes the non-transcriptional priming of NLRP3 by inducing its oligomerization and association with ASC (60).

TRAFs Negatively Regulate NLR Signaling

TRAF4 has been shown to act as a negative regulator in NOD2-mediated NF-κB signaling by direct interaction with NOD2. This interaction then allows IKKα to phosphorylate TRAF4, which results in its dissociation from NOD2 and inhibition of NOD2 signaling (61). NLRC3 has been shown to attenuate NLRP3 inflammasome by with ASC for pro-caspase-1 binding (62).

THE ROLE OF TRAFs IN RIG-I-LIKE RECEPTOR SIGNALING

RIG-I-Like receptors (RLRs) are a family of DEAD box helicases that play a crucial role in the innate immune response to viral infections by detecting the presence of viral RNA in the cytosol. RIG-I and MDA5 are the two prototypical members of the RLR family (63). Upon sensing viral RNAs, RLRs dimerize and interact with mitochondrial antiviral signaling adaptor (MAVS, a.k.a. IPS-1, or VISA), with the subsequent formation of a complex that includes among others TRAF2, TRAF3, TRAF5, and TRAF6 [(64); (65), p. 3; (66)]. TRAF proteins then recruit various downstream signaling proteins that culminate in the activation of several transcription factors, including IRF3, NF-κB, and MAPKs.

RIG-I/MDA5 employ TRAF3 to induce IRF3-mediated IFN-I production. Mechanistically, TRAF3 is recruited by MAVS, where it catalyzes its own K63 polyubiquitination followed by recruitment and subsequent activation of TBK1 and IKKε (**Figure 1B**) (67). TRAF2 and TRAF5 play a crucial role in

mediating NF-κB activation after RLRs bind their viral PAMPs, albeit the mechanism remains poorly understood (64, 68). TRAF6 can also be recruited via MAVS, where it activates the TBK1/IKKε/IRF7 as well as the MAPKs/AP-1 signaling axes (**Figure 2**) (66, 69). Intriguingly, the RIG-I-MAVS-TRAF6 signaling axis leads to IKKβ-dependent phosphorylation of NF-κB (70). Furthermore, RIG-I-MAVS-TRAF6 signaling induces K63-ubiquitination of Beclin-1, a critical step for inducing autophagy (71). Finally, TRAF6 interacts with Ubiquitin-specific protease 4 (USP4) to induce NF-κB activation following RLR-simulation (72). This is achieved via targeting of TRAF6 for K48-linked deubiquitination.

Regulation of RLR Signaling by Modulating TRAF Function or Its Interactions

During bacterial infections, the E3 ligase HCTD3 adds K63-linked ubiquitin chains to TRAF3, which enhances the activation of the TBK1/IKKε complex and subsequent production of IFN-Is (73). Conversely, several deubiquitinases, including OTUB1, OTUB2, DUBA, and HSCARG, have been shown to downregulate RLR-mediated IFN-I production by removing K63-linked polyubiquitin chains from TRAF3 or TRAF6 [(74, 75), p. 1; (76)]. MCPIP1, which is known to inhibit JNK and NF-κB signaling by deubiquitinating several TRAFs [see above; (31)], has been recently shown to negatively regulate IFNβ production. Overexpression studies showed that MCPIP1 disrupts the interaction between TRAF3, TBK1, and IKKε, as shown by co-immunoprecipitation, and thereby inhibiting the phosphorylation and translocation of IRF3 into the nucleus (77). There was no evidence to show that this process requires the deubiquitinase activity of MCPIP1. Another deubiquitinase, OTU deubiquitinase 1 (OTUD1), has also been demonstrated to attenuate IFN-I production following RIG-I activation by viral RNAs (78). Mechanistically, OTUD1 deubiquitinates and stabilizes the ubiquitin ligase, Smurf1, which then targets the MAVS/TRAF3/TRAF6 signalosome by mediating K48-linked polyubiquitination and the subsequent degradation of MAVS, TRAF3, and TRAF6 (78). Parkin is another ubiquitin ligase that targets RLR signaling by promoting K48-linked polyubiquitination of TRAF3 and reducing its stability (79). An interesting study demonstrated that linear ubiquitination of NEMO promotes its interaction with TRAF3, which in turn, disrupts the recruitment of TRAF3 to the RIG-I/MAVS complex leading to diminished IFN-I expression (80).

THE ROLE OF TRAFs IN STING SIGNALING

In addition to cytosolic sensors of RNA, DNA sensors in the cytosol are equally crucial in detecting and mounting an inflammatory response against viral and bacterial pathogens. Stimulator of Interferon Genes (STING) is activated directly by second messengers like bacterial cyclic dinucleotides (e.g., c-diAMP and c-diGMP) (81–83) or by cellular cyclic GMP-AMP (cGAMP), which is produced by cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS) upon sensing cytosolic DNA (84). Activation of STING leads to an effective inflammatory response which include the activation of the TBK-1/IRF3 and NF-κB axes. TRAF3 and TRAF6 have been shown to enhance STING-mediated NF-κB and IFN-β promoter activity, albeit in an overexpression system in 293 cells (85). Both TRAF3 and TRAF6 appear to interact with STING (85, 86). Recently, an alternative STING pathway has been revealed in keratinocytes in response to DNA damage. This alternative STING signaling complex includes the tumor suppressor p53 and TRAF6, whereby TRAF6 catalyzes K63-polyubiquitination of STING and activates NF-κB (87). An elegant study by Genhong Cheng's group recently showed that the alternative NF-κB inducing kinase (NIK) can associate with STING and enhance its activation via an alternative NF-κB pathway-independent mechanism (88). Interestingly, they showed that TRAF3, unlike its positive role in RNA-induced IFN-I production, plays an opposite role in the DNA pathway and inhibit IFN-I production by suppressing NIK expression (88).

PERSPECTIVES AND FUTURE DIRECTIONS

There continues to be a great interest in understanding how TRAFs regulate innate immune signaling. Specifically, novel mechanisms have been recently identified to regulate TLR, NLR, and RLR pathways by modulating the ubiquitination status of TRAFs, their stability or their function. However, as most of these regulators seem to be non-redundant, investigating additional novel regulators, and their mechanism of action remains an active area of investigation.

Each individual TRAF protein plays several, sometimes contradictory, roles that are pathway and/or cell specific. For example, a particular TRAF protein might induce lymphocyte survival and maturation while inhibiting a certain inflammatory pathway. Furthermore, most TRAFs function as E3 ligases as well as adapter proteins. Therefore, TRAFs are poor candidates for novel therapies since targeting a TRAF protein could lead to unintended consequences. For these reasons, future studies should focus on assessing the various roles of each TRAF protein in isolation from its other functions. This is especially important when designing therapies for complex inflammatory and autoimmune diseases by targeting TRAFs.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. BD, FA and AA-S wrote the text. A-AS edited the manuscript and conceptualized the figures. ZA-S created the figures.

FUNDING

Our research in this area is funded by the Banting Research Foundation, Grant # 2018-1395 and York University Startup funds.

REFERENCES

1. Arch RH, Gedrich RW, Thompson CB. Tumor necrosis factor receptor-associated factors (TRAFs)—a family of adapter proteins that regulates life and death. *Genes Dev.* (1998) 12:2821–30. doi: 10.1101/gad.12.18.2821
2. Rothe M, Wong SC, Henzel WJ, Goeddel DV. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* (1994) 78:681–92. doi: 10.1016/0092-8674(94)90532-0
3. Chung JY, Park YC, Ye H, Wu H. All TRAFs are not created equal: common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J Cell Sci.* (2002) 115:679–88.
4. Wajant H, Henkler F, Scheurich P. The TNF-receptor-associated factor family: scaffold molecules for cytokine receptors, kinases and their regulators. *Cell Signal.* (2001) 13:389–400. doi: 10.1016/S0898-6568(01)00160-7
5. Lalani AI, Zhu S, Gokhale S, Jin J, Xie P. TRAF molecules in inflammation and inflammatory diseases. *Curr Pharmacol Rep.* (2018) 4:64–90. doi: 10.1007/s40495-017-0117-y
6. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal.* (2013) 8:7. doi: 10.1186/1750-2187-8-7
7. Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease (*). *Annu Rev Immunol.* (2009) 27:621–68. doi: 10.1146/annurev.immunol.25.022106.141627
8. Kondo T, Kawai T, Akira S. Dissecting negative regulation of toll-like receptor signaling. *Trends Immunol.* (2012) 33:449–58. doi: 10.1016/j.it.2012.05.002
9. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol.* (2014) 5:461. doi: 10.3389/fimmu.2014.00461
10. Lin Q, Li M, Fang D, Fang J, Su SB. The essential roles of Toll-like receptor signaling pathways in sterile inflammatory diseases. *Int Immunopharmacol.* (2011) 11:1422–32. doi: 10.1016/j.intimp.2011.04.026
11. Ostuni R, Zanoni I, Granucci F. Deciphering the complexity of Toll-like receptor signaling. *Cell Mol Life Sci.* (2010) 67:4109–34. doi: 10.1007/s00018-010-0464-x
12. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol.* (2010) 11:373–84. doi: 10.1038/ni.1863
13. Keating SE, Bowie AG. Role of non-degradative ubiquitination in interleukin-1 and toll-like receptor signaling. *J Biol Chem.* (2009) 284:8211–5. doi: 10.1074/jbc.R800038200
14. Kollewe C, Mackensen A-C, Neumann D, Knop J, Cao P, Li S, et al. Sequential autophosphorylation steps in the interleukin-1 receptor-associated kinase-1 regulate its availability as an adapter in interleukin-1 signaling. *J Biol Chem.* (2004) 279:5227–36. doi: 10.1074/jbc.M309251200
15. Hu L, Xu J, Xie X, Zhou Y, Tao P, Li H, et al. Oligomerization-primed coiled-coil domain interaction with Ubc13 confers processivity to TRAF6 ubiquitin ligase activity. *Nat Commun.* (2017) 8:814. doi: 10.1038/s41467-017-01290-0
16. Deng L, Wang C, Spencer E, Yang L, Braun A, You J, et al. Activation of the IκB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* (2000) 103:351–61. doi: 10.1016/S0092-8674(00)00126-4
17. Xia Z-P, Sun L, Chen X, Pineda G, Jiang X, Adhikari A, et al. Direct activation of protein kinases by unanchored polyubiquitin chains. *Nature* (2009) 461:114–9. doi: 10.1038/nature08247
18. Gerlach B, Cordier SM, Schmukle AC, Emmerich CH, Rieser E, Haas TL, et al. Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* (2011) 471:591–6. doi: 10.1038/nature09816
19. Ikeda F, Deribe YL, Skanland SS, Stieglitz B, Grabbe C, Franz-Wachtel M, et al. SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* (2011) 471:637–41. doi: 10.1038/nature09814
20. Tokunaga F, Nakagawa T, Nakahara M, Saeki Y, Taniguchi M, Sakata S, et al. SHARPIN is a component of the NF-kappaB-activating linear ubiquitin chain assembly complex. *Nature* (2011) 471:633–6. doi: 10.1038/nature09815
21. Rittinger K, Ikeda F. Linear ubiquitin chains: enzymes, mechanisms and biology. *Open Biol.* (2017) 7:170026. doi: 10.1098/rsob.170026
22. Häcker H, Tseng P-H, Karin M. Expanding TRAF function: TRAF3 as a tri-faced immune regulator. *Nat Rev Immunol.* (2011) 11:457–68. doi: 10.1038/nri2998
23. Tseng PH, Matsuzawa A, Zhang W, Mino T, Vignali DA, Karin M. Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines., Different modes of TRAF3 ubiquitination selectively activate type I interferon and proinflammatory cytokine expression. *Nat Immunol.* (2010) 70:70–5. doi: 10.1038/ni.1819:10.1038/ni.1819
24. Matsuzawa A, Tseng P-H, Vallabhapurapu S, Luo J-L, Zhang W, Wang H, et al. Essential cytoplasmic translocation of a cytokine receptor–assembled signaling complex. *Science* (2008) 321:663–8. doi: 10.1126/science.1157340
25. Jin J, Xiao Y, Hu H, Zou Q, Li Y, Gao Y, et al. Proinflammatory TLR signalling is regulated by a TRAF2-dependent proteolysis mechanism in macrophages. *Nat Commun.* (2015) 6:5930. doi: 10.1038/ncomms6930
26. Buchta CM, Bishop GA. TRAF5 negatively regulates Toll-like receptor signaling in B lymphocytes. *J Immunol.* (2014) 192:145–50. doi: 10.4049/jimmunol.1301901
27. Takeshita F, Ishii KJ, Kobiyama K, Kojima Y, Coban C, Sasaki S, et al. TRAF4 acts as a silencer in TLR-mediated signaling through the association with TRAF6 and TRIF. *Eur J Immunol.* (2005) 35:2477–85. doi: 10.1002/eji.200526151
28. Abdul-Sater AA, Edilova MI, Clouthier DL, Mbanwi A, Kremmer E, Watts TH. The signaling adaptor TRAF1 negatively regulates Toll-like receptor signaling and this underlies its role in rheumatic disease. *Nat Immunol.* (2017) 18:26–35. doi: 10.1038/ni.3618
29. Su X, Li S, Meng M, Qian W, Xie W, Chen D, et al. TNF receptor-associated factor-1 (TRAF1) negatively regulates Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF)-mediated signaling. *Eur J Immunol.* (2006) 36:199–206. doi: 10.1002/eji.200535415
30. Boone DL, Turer EE, Lee EG, Ahmad R-C, Wheeler MT, Tsui C, et al. The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat Immunol.* (2004) 5:1052–60. doi: 10.1038/ni1110
31. Liang J, Saad Y, Lei T, Wang J, Qi D, Yang Q, et al. MCP-induced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF-kappaB signaling. *J Exp Med.* (2010) 207:2959–73. doi: 10.1084/jem.20092641
32. Min Y, Kim M-J, Lee S, Chun E, Lee K-Y. Inhibition of TRAF6 ubiquitin-ligase activity by PRDX1 leads to inhibition of NFKB activation and autophagy activation. *Autophagy* (2018) 14:1347–58. doi: 10.1080/15548627.2018.1474995
33. Schneider M, Zimmermann AG, Roberts RA, Zhang L, Swanson KV, Wen H, et al. The innate immune sensor NLRC3 attenuates Toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-κB. *Nat Immunol.* (2012) 13:823–31. doi: 10.1038/ni.2378
34. Allen IC, Moore CB, Schneider M, Lei Y, Davis BK, Scull MA, et al. NLRX1 protein attenuates inflammatory responses to infection by interfering with the RIG-I-MAVS and TRAF6-NF-κB signaling pathways. *Immunity* (2011) 34:854–65. doi: 10.1016/j.immuni.2011.03.026
35. Xia X, Cui J, Wang HY, Zhu L, Matsueda S, Wang Q, et al. NLRX1 negatively regulates TLR-induced NF-κB signaling by targeting TRAF6 and IKK. *Immunity* (2011) 34:843–53. doi: 10.1016/j.immuni.2011.02.022
36. Ling A, Soares F, Croitoru DO, Tattoli I, Carneiro LAM, Boniotto M, et al. Post-transcriptional inhibition of luciferase reporter assays by the Nod-like receptor proteins NLRX1 and NLRC3. *J Biol Chem.* (2012) 287:28705–16. doi: 10.1074/jbc.M111.333146
37. Soares F, Tattoli I, Wortzman ME, Arnoult D, Philpott DJ, Girardin SE. NLRX1 does not inhibit MAVS-dependent antiviral signalling. *Innate Immun.* (2013) 19:438–48. doi: 10.1177/1753425912467383
38. Tattoli I, Carneiro LA, Jehanno M, Magalhaes JG, Shu Y, Philpott DJ, et al. NLRX1 is a mitochondrial NOD-like receptor that amplifies NF-kappaB and JNK pathways by inducing reactive oxygen species production. *EMBO Rep.* (2008) 9:293–300. doi: 10.1038/sj.embor.7401161
39. Wu C, Su Z, Lin M, Ou J, Zhao W, Cui J, et al. NLRP11 attenuates Toll-like receptor signalling by targeting TRAF6 for degradation via the ubiquitin ligase RNF19A. *Nat Commun.* (2017) 8:1977. doi: 10.1038/s41467-017-02073-3
40. Allen IC, Wilson JE, Schneider M, Lich JD, Roberts RA, Arthur JC, et al. NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-κB signaling. *Immunity* (2012) 36:742–54. doi: 10.1016/j.immuni.2012.03.012

41. Coutermarsh-Ott S, Eden K, Allen IC. Beyond the inflammasome: regulatory NOD-like receptor modulation of the host immune response following virus exposure. *J Gen Virol.* (2016) 97:825–38. doi: 10.1099/jgv.0.000401
42. Elinav E, Strowig T, Henao-Mejia J, Flavell RA. Regulation of the antimicrobial response by NLR proteins. *Immunity* (2011) 34:665–79. doi: 10.1016/j.immuni.2011.05.007
43. Kersse K, Bertrand MJM, Lamkanfi M, Vandenabeele P. NOD-like receptors and the innate immune system: coping with danger, damage and death. *Cytokine Growth Factor Rev.* (2011) 22:257–76. doi: 10.1016/j.cytogfr.2011.09.003
44. Tigno-Aranjuez JT, Abbott DW. Ubiquitination and phosphorylation in the regulation of NOD2 signaling and NOD2-mediated disease. *BBA Mol Cell Res.* (2012) 1823:2022–8. doi: 10.1016/j.bbamcr.2012.03.017
45. Philpott DJ, Sorbara MT, Robertson SJ, Croitoru K, Girardin SE. NOD proteins: regulators of inflammation in health and disease. *Nat Rev Immunol.* (2014) 14:9–23. doi: 10.1038/nri3565
46. Brodsky IE, Monack D. NLR-mediated control of inflammasome assembly in the host response against bacterial pathogens. *Semin Immunol.* (2009) 21:199–207. doi: 10.1016/j.smim.2009.05.007
47. Motta V, Soares F, Sun T, Philpott DJ. NOD-like receptors: versatile cytosolic sentinels. *Physiol Rev.* (2015) 95:149–78. doi: 10.1152/physrev.00009.2014
48. Bertrand MJM, Doiron K, Labbé K, Korneluk RG, Barker PA, Saleh M. Cellular inhibitors of apoptosis cIAP1 and cIAP2 are required for innate immunity signaling by the pattern recognition receptors NOD1 and NOD2. *Immunity* (2009) 30:789–801. doi: 10.1016/j.immuni.2009.04.011
49. Hasegawa M, Fujimoto Y, Lucas PC, Nakano H, Fukase K, Núñez G, et al. A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-κB activation. *EMBO J.* (2008) 27:373–83. doi: 10.1038/sj.emboj.7601962
50. Yang Y, Yin C, Pandey A, Abbott D, Sassetti C, Kelliher MA. NOD2 pathway activation by MDP or *Mycobacterium tuberculosis* infection involves the stable polyubiquitination of Rip2. *J Biol Chem.* (2007) 282:36223–9. doi: 10.1074/jbc.M703079200
51. Abbott DW, Yang Y, Hutti JE, Madhavarapu S, Kelliher MA, Cantley LC. Coordinated regulation of toll-like receptor and NOD2 signaling by K63-linked polyubiquitin chains. *Mol Cell Biol.* (2007) 27:6012–25. doi: 10.1128/MCB.00270-07
52. Zhong X, Chen B, Yang L, Yang Z. Molecular and physiological roles of the adaptor protein CARD9 in immunity. *Cell Death Dis.* (2018) 9:52. doi: 10.1038/s41419-017-0084-6
53. Watanabe T, Asano N, Kitani A, Fuss IJ, Chiba T, Strober W. Activation of type I IFN signaling by NOD1 mediates mucosal host defense against *Helicobacter pylori* infection. *Gut Microbes* (2011) 2:61–5. doi: 10.4161/gmic.2.1.15162
54. Chen G, Shaw MH, Kim Y-G, Nuñez G. NOD-like receptors: role in innate immunity and inflammatory disease. *Annu Rev Pathol.* (2009) 4:365–98. doi: 10.1146/annurev.pathol.4.110807.092239
55. Abdul Sater A, Philpott D. Inflammasomes. In: Ratcliffe MJH, editor. *Encyclopedia of Immunobiology.* Vol. 2. Toronto, ON: University of Toronto and Trinity College (2016). p. 447–53.
56. Abdul-Sater AA, Said-Sadier N, Ojcius DM, Yilmaz O, Kelly KA. Inflammasomes bridge signaling between pathogen identification and the immune response. *Drugs Today* (2009) 45 (Suppl. B) 105–112.
57. Guan K, Wei C, Zheng Z, Song T, Wu F, Zhang Y, et al. MAVS promotes inflammasome activation by targeting ASC for K63-linked ubiquitination via the E3 ligase TRAF3. *J Immunol.* (2015) 194:4880–90. doi: 10.4049/jimmunol.1402851
58. Labbé K, McIntire CR, Doiron K, Leblanc PM, Saleh M. cellular inhibitors of apoptosis proteins cIAP1 and cIAP2 are required for efficient Caspase-1 activation by the inflammasome. *Immunity* (2011) 35:897–907. doi: 10.1016/j.immuni.2011.10.016
59. Vince JE, Wong WW-L, Gentle I, Lawlor KE, Allam R, O'Reilly L, et al. Inhibitor of apoptosis proteins limit RIP3 kinase-dependent interleukin-1 activation. *Immunity* (2012) 36:215–27. doi: 10.1016/j.immuni.2012.01.012
60. Xing Y, Yao X, Li H, Xue G, Guo Q, Yang G, et al. Cutting edge: TRAF6 mediates TLR/IL-1R signaling–induced nontranscriptional priming of the NLRP3 inflammasome. *J Immunol.* (2017) 199:1561–6. doi: 10.4049/jimmunol.1700175
61. Marinis JM, Hutti JE, Homer CR, Cobb BA, Cantley LC, McDonald C, et al. IκB kinase α phosphorylation of TRAF4 downregulates innate immune signaling. *Mol Cell Biol.* (2012) 32:2479–89. doi: 10.1128/MCB.00106-12
62. Eren E, Berber M, Özören N. NLRC3 protein inhibits inflammation by disrupting NALP3 inflammasome assembly via competition with the adaptor protein ASC for pro-caspase-1 binding. *J Biol Chem.* (2017) 292:12691–701. doi: 10.1074/jbc.M116.769695
63. Yoo J-S, Kato H, Fujita T. Sensing viral invasion by RIG-I like receptors. *Curr Opin Microbiol.* (2014) 20:131–8. doi: 10.1016/j.mib.2014.05.011
64. Mikkelsen SS, Jensen SB, Chiliveru S, Melchjorsen J, Julkunen I, Gaestel M, et al. RIG-I-mediated activation of p38 MAPK is essential for viral induction of interferon and activation of dendritic cells: dependence on TRAF2 and TAK1. *J Biol Chem.* (2009) 284:10774–82. doi: 10.1074/jbc.M807272200
65. Saha SK, Pietras EM, He JQ, Kang JR, Liu SY, Oganesyan G, et al. Regulation of antiviral responses by a direct and specific interaction between TRAF3 and Cardif. *EMBO J.* (2006) 25:3257–63. doi: 10.1038/sj.emboj.7601220
66. Yoshida R, Takaesu G, Yoshida H, Okamoto F, Yoshioka T, Choi Y, et al. TRAF6 and MEKK1 play a pivotal role in the RIG-I-like helicase antiviral pathway. *J Biol Chem.* (2008) 283:36211–20. doi: 10.1074/jbc.M806576200
67. Loo YM, Gale M. Immune signaling by RIG-I-like receptors. *Immunity* (2011) 34:680–92. doi: 10.1016/j.immuni.2011.05.003
68. Tang ED, Wang C-Y. TRAF5 is a downstream target of MAVS in antiviral innate immune signaling. *PLoS ONE* (2010) 5:e9172. doi: 10.1371/journal.pone.0009172
69. Konno H, Yamamoto T, Yamazaki K, Gohda J, Akiyama T, Semba K, et al. TRAF6 establishes innate immune responses by activating NF-kappaB and IRF7 upon sensing cytosolic viral RNA and DNA. *PLoS ONE* (2009) 4:e5674. doi: 10.1371/journal.pone.0005674
70. Yoboua F, Martel A, Duval A, Mukawera E, Grandvaux N. Respiratory syncytial virus-mediated NF-kappa B p65 phosphorylation at serine 536 is dependent on RIG-I, TRAF6, and IKK beta. *J Virol.* (2010) 84:7267–77. doi: 10.1128/JVI.00142-10
71. Lee NR, Ban J, Lee NJ, Yi CM, Choi JY, Kim H, et al. Activation of RIG-I-mediated antiviral signaling triggers autophagy through the MAVS-TRAF6-Beclin-1 signaling axis. *Front Immunol.* (2018) 9:2096. doi: 10.3389/fimmu.2018.02096
72. Xu C, Peng Y, Zhang Q, Xu XP, Kong XM, Shi WF. USP4 positively regulates RLR-induced NF-κB activation by targeting TRAF6 for K48-linked deubiquitination and inhibits enterovirus 71 replication. *Sci Rep.* (2018) 8:13418. doi: 10.1038/s41598-018-31734-6
73. Li F, Li Y, Liang H, Xu T, Kong Y, Huang M, et al. HECTD3 mediates TRAF3 polyubiquitination and type I interferon induction during bacterial infection. *J Clin Invest.* (2018) 128:4148–62. doi: 10.1172/JCI120406
74. Kayagaki N, Phung Q, Chan S, Chaudhari R, Quan C, O'Rourke KM, et al. DUBA: a deubiquitinase that regulates type I interferon production. *Science* (2007) 318:1628–32. doi: 10.1126/science.1145918
75. Li S, Zheng H, Mao A-P, Zhong B, Li Y, Liu Y, et al. Regulation of virus-triggered signaling by OTUB1- and OTUB2-mediated deubiquitination of TRAF3 and TRAF6. *J Biol Chem.* (2010) 285:4291–7. doi: 10.1074/jbc.M109.074971
76. Peng Y, Xu R, Zheng X. HSCARG negatively regulates the cellular antiviral RIG-I like receptor signaling pathway by inhibiting TRAF3 ubiquitination via recruiting OTUB1. *PLoS Pathog.* (2014) 10:e1004041. doi: 10.1371/journal.ppat.1004041
77. Chen X, Zhao Q, Xie Q, Xing Y, Chen Z. MCPIP1 negatively regulate cellular antiviral innate immune responses through DUB and disruption of TRAF3-TBK1-IKKε complex. *Biochem Biophys Res Commun.* (2018) 503:830–6. doi: 10.1016/j.bbrc.2018.06.083
78. Zhang L, Liu J, Qian L, Feng Q, Wang X, Yuan Y, et al. Induction of OTUD1 by RNA viruses potently inhibits innate immune responses by promoting degradation of the MAVS/TRAF3/TRAF6 signalosome. *PLoS Pathog.* (2018) 14:e1007067. doi: 10.1371/journal.ppat.1007067
79. Xin D, Gu H, Liu E, Sun Q. Parkin negatively regulates the antiviral signaling pathway by targeting TRAF3 for degradation. *J Biol Chem.* (2018) 293:11996–2010. doi: 10.1074/jbc.RA117.001201

80. Belgnaoui SM, Paz S, Samuel S, Goulet ML, Sun Q, Kikkert M, et al. Linear ubiquitination of NEMO negatively regulates the interferon antiviral response through disruption of the MAVS-TRAF3 complex. *Cell Host Microbe* (2012) 12:211–22. doi: 10.1016/j.chom.2012.06.009
81. Abdul-Sater AA, Grajkowski A, Erdjument-Bromage H, Plumlee C, Levi A, Schreiber MT, et al. The overlapping host responses to bacterial cyclic dinucleotides. *Microbes Infect.* (2012) 14:188–97. doi: 10.1016/j.micinf.2011.09.002
82. Abdul-Sater AA, Tattoli I, Jin L, Grajkowski A, Levi A, Koller BH, et al. Cyclic-di-GMP and cyclic-di-AMP activate the NLRP3 inflammasome. *EMBO Rep.* (2013) 14:900–6. doi: 10.1038/embor.2013.132
83. Burdette DL, Monroe KM, Sotelo-Troha K, Iwig JS, Eckert B, Hyodo M, et al. STING is a direct innate immune sensor of cyclic di-GMP. *Nature* (2011) 478:515–8. doi: 10.1038/nature10429
84. Li T, Chen ZJ. The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. *J Exp Med.* (2018) 215:1287–99. doi: 10.1084/jem.20180139
85. Abe T, Barber GN. Cytosolic-DNA-mediated, STING-dependent proinflammatory gene induction necessitates canonical NF-κB activation through TBK1. *J Virol.* (2014) 88:5328–41. doi: 10.1128/JVI.00037-14
86. Zhong B, Yang Y, Li S, Wang YY, Li Y, Diao F, et al. The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* (2008) 29:538–50. doi: 10.S1074-7613(08)00406-8[pii]10.1016/j.immuni.2008.09.003
87. Dunphy G, Flannery SM, Almine JF, Connolly DJ, Paulus C, Jønsson KL, et al. Non-canonical activation of the DNA sensing adaptor STING by ATM and IFI16 mediates NF-κB signaling after nuclear DNA damage. *Mol Cell* (2018) 71:745–760.e5. doi: 10.1016/j.molcel.2018.07.034
88. Parvatiyar K, Pindado J, Dev A, Aliyari SR, Zaver SA, Gerami H, et al. A TRAF3-NIK module differentially regulates DNA vs RNA pathways in innate immune signaling. *Nature Commun.* (2018) 9:2770. doi: 10.1038/s41467-018-05168-7

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 18 December 2018
doi: 10.3389/fimmu.2018.02969

TRAF1 Signaling in Human Health and Disease

Maria I. Edilova[1], *Ali A. Abdul-Sater*[2] *and Tania H. Watts*[1]*

[1] Department of Immunology, University of Toronto, Toronto, ON, Canada, [2] School of Kinesiology and Health Science, York University, Toronto, ON, Canada

Tumor necrosis factor receptor (TNFR) associated factor 1 (TRAF1) is a signaling adaptor first identified as part of the TNFR2 signaling complex. TRAF1 plays a key role in pro-survival signaling downstream of TNFR superfamily members such as TNFR2, LMP1, 4-1BB, and CD40. Recent studies have uncovered another role for TRAF1, independent of its role in TNFR superfamily signaling, in negatively regulating Toll-like receptor and Nod-like receptor signaling, through sequestering the linear ubiquitin assembly complex, LUBAC. TRAF1 has diverse roles in human disease. TRAF1 is overexpressed in many B cell related cancers and single nucleotide polymorphisms (SNPs) in TRAF1 have been linked to non-Hodgkin's lymphoma. Genome wide association studies have identified an association between SNPs in the 5′ untranslated region of the TRAF1 gene with increased incidence and severity of rheumatoid arthritis and other rheumatic diseases. The loss of TRAF1 from chronically stimulated CD8 T cells results in desensitization of the 4-1BB signaling pathway, thereby contributing to T cell exhaustion during chronic infection. These apparently opposing roles of TRAF1 as both a positive and negative regulator of immune signaling have led to some confusion in the literature. Here we review the role of TRAF1 as a positive and negative regulator in different signaling pathways. Then we discuss the role of TRAF1 in human disease, attempting to reconcile seemingly contradictory roles based on current knowledge of TRAF1 signaling and biology. We also discuss avenues for future research to further clarify the impact of TRAF1 in human disease.

Keywords: TNFR superfamily, signaling, toll-like receptor, linear ubiquitination, cancer, autoimmunity, chronic viral infection

OPEN ACCESS

Edited by:
Stefano Caserta,
University of Hull, United Kingdom

Reviewed by:
Rahul Vijay,
The University of Iowa, United States
James Harris,
Monash University, Australia
Hyun Ho Park,
Chung-Ang University, South Korea

***Correspondence:**
Tania H. Watts
tania.watts@utoronto.ca

Specialty section:
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology

Received: *31 August 2018*
Accepted: *03 December 2018*
Published: *18 December 2018*

Citation:
Edilova MI, Abdul-Sater AA and Watts TH (2018) TRAF1 Signaling in Human Health and Disease. Front. Immunol. 9:2969. doi: 10.3389/fimmu.2018.02969

INTRODUCTION

Tumor necrosis factor receptor (TNFR)-associated factors (TRAF) proteins play important roles in the immune system as key intracellular signaling molecules in TNFR, Toll-like receptor (TLR), cytokine, and antigen receptor signaling pathways (1). While TRAF2 is constitutively expressed and its transcript can be found in almost all tissues, TRAF1 is an NF-κB inducible protein, and under normal conditions has more limited expression in the spleen, lung, and testis (2). Evidence for TRAF1 as both a positive and negative regulator of immune signaling has led to some confusion in the literature. Here we first discuss the role of TRAF1 in TNFR and TLR signaling pathways and then discuss what is known about the impact of TRAF1 in human disease, with references to its specific roles in different pathways, attempting to reconcile these seemingly contradictory roles. Finally, we discuss the outstanding questions in the field and implications for therapy.

ROLE OF TRAF1 IN TNFR SIGNALING

TRAF1 was originally identified along with TRAF2 in immunoprecipitates of TNFR2 (2). TRAF2 is the prototypical TRAF protein and contains a RING finger domain, a series of Zinc fingers followed by the conserved TRAF domain. The TRAF domain, conserved among TRAFs 1 through 6, consists of the TRAF-N, a coiled coil region responsible for homo-, or hetero-oligomerization of TRAF proteins and a C-terminal domain, TRAF-C, also referred to as the meprin and TRAF homology (MATH) domain, which is responsible for TRAF recruitment to the cytoplasmic tails of TNFRs. TRAF1 differs from TRAF2 in lacking the N-terminal RING finger required for NF-κB activation and in having only one Zinc finger (**Figure 1A**) and as such resembles a dominant negative form of TRAF2 (4). The crystal structure of the TRAF1 TRAF domain shows that like other members of the TRAF family, the TRAF C domain forms a mushroom shaped cap and the TRAF N domain forms a stalk with a coiled coil structure, albeit with some specific differences from other TRAFs in the location of several loops in the TRAF domain and the position of the coiled coil α helices (5, 6). The structure of TRAF1 has also been solved in a complex with the protein TANK, TRAF family member-associated NF-kappa B activator (7).

Under normal conditions, TRAF1 expression is largely limited to activated immune cells, including myeloid and lymphoid cells. TRAF1 is present at minimal levels in resting lymphocytes and monocytes and its expression is increased upon activation through the NF-κB pathway (8). TRAF1, along with TRAF2 and the cellular inhibitors of apoptosis (cIAP1 and cIAP2), is required to suppress TNF-induced apoptosis in NF-κB-deficient cell lines (9). *Traf1*$^{-/-}$ mice are viable and fertile and have normal numbers of lymphocytes (10). However, TRAF1-deficient activated and memory T cells have impaired survival (11–13). Conversely, transgenic expression of TRAF1 in mice reduces antigen induced cell death in T cells (14). These data are consistent with a prosurvival role for TRAF1 in lymphocytes.

Role of TRAF1 in the Classical NF-κB Pathway Downstream of TNFRs

Pro-survival members of the TNFR superfamily activate NF-κB and mitogen activated protein kinase (MAPK) pathways. For TNFRI, TRAF proteins are recruited indirectly through TRADD (**Figure 1B**), whereas TNFRs that lack death domains recruit TRAFs directly (1). The TRAF proteins, in turn, recruit cIAP1 or 2. The NF-κB pathway involves three kinds of ubiquitination. cIAPs have E3 ligase activity to add K63-linked polyubiquitin (K63-Ub) to receptor-interacting serine/threonine-protein kinase 1 (RIPK1). The K63-Ub provides a substrate for addition of linear ubiquitin as well as for recruitment of TGFβ-associated kinase (TAK1) and TAK binding protein (TAB), required for Inhibitor of kappa B kinase (IKK) and MAPK activation (15, 16). Linear ubiquitination involves the addition of polyubiquitin polymerized through the M1 position (M1-Ub) (17–19). The addition of M1-Ub is catalyzed by the linear ubiquitin assembly complex (LUBAC), which consists of three subunits: HOIL, HOIP, and SHARPIN. LUBAC is recruited by K63-Ub and can also modify K63-Ub to make hybrid molecules (19). These ubiquitin modifications serve as scaffolds whereby linear ubiquitin recruits the IKK complex, consisting of IKKα, IKKβ, and Nemo/IKKγ, which itself can also be modified by M1- and K63-Ub. K63-Ub recruits TAK/TAB, leading to activation of the IKK complex. M1-Ub is important in NF-κB activation downstream of TNFR as well as TLRs and NLRs (18–22). Activated IKK in turn phosphorylates the Inhibitor of κB (IκB), leading to its K48-Ub modification and degradation, allowing NF-κB translocation into the nucleus (**Figure 1B**) (16).

TRAF1 enhances survival signaling downstream of a subset of TNFR family members, including TNFR1, TNFR2, CD40, 4-1BB (CD137), and the EBV-encoded TNFR family member latent membrane protein (LMP)-1, by enhancing classical NF-κB and MAPK activation (12, 23–29). The coiled coil domain of TRAF1 when mixed with that of TRAF2 spontaneously forms a 1:2 heterotrimer and this complex asymmetrically recruits the BIR domain of cIAP2 (30). The $(TRAF2)_2TRAF1$ coiled coil heterotrimer, is more efficient in recruitment of cellular cIAPs than the TRAF2 homotrimer, and thus TRAF1 provides an NF-κB induced positive feedback loop to enhance TRAF2-dependent signaling (30). *Traf1*$^{-/-}$ dendritic cells (DC) show increased apoptosis and marked deficiency in classical NF-κB activation after CD40 stimulation, implicating TRAF1 in sustaining TRAF2-dependent signaling through CD40 (26). Similarly, in B cells, TRAF1 and TRAF2 were found to cooperate in induction of NF-κB and JNK activation (25). The absence of TRAF1 in T cells leads to impaired NF-κB and ERK activation downstream of 4-1BB and accumulation of the pro-apoptotic molecule BIM (11–13, 28).

TRAF1 Prevention of TRAF2 Degradation

Beyond its demonstrated role in collaboration with TRAF2 in recruiting cIAPs, TRAF1 can prevent proteasome-dependent degradation of TRAF2 downstream of CD40, 4-1BB and TNFR2 signaling, thereby sustaining TRAF2 dependent signaling (12, 23, 25, 26). cIAPs not only add K63-Ub to RIP, but also have E3 ligase activity for adding K48-Ub which can lead to TRAF2 degradation (31). TRAF1 can prevent this effect of cIAPs during TNFR family signaling, although the mechanism of this protection remains to be elucidated. How much of the role of TRAF1 is due to improved recruitment

Abbreviations: C5, complement factor 5; cIAP, cellular inhibitors of apoptosis; EBV, Epstein Barr virus; ERK, extra-cellular signaling related kinase; HOIL, Heme oxidized IRP2 Ub ligase-1, a subunit of LUBAC; HOIP, HOIL interacting protein, a subunit of LUBAC; IκB, inhibitor of kB; IKK, inhibitor of κB kinase; K48-Ub, ubiquitin polymerized through the K48 position; K63-Ub, ubiquitin polymerized through the K63 position; LMP1, latent membrane protein 1 from EBV; LUBAC, linear ubiquitin assembly complex; MAPK, mitogen activated protein kinase; MATH, meprin and TRAF homology; M1-Ub, ubiquitin polymerized through the M1 position; mTOR, mammalian target of rapamycin; NEMO, NF-κB essential modulator (NEMO) also known as IKKγ; NIK, NF-κB inducing kinase; NLR, Nod-like receptor; NZF, Npl4 Zinc finger domains; RA, rheumatoid arthritis; RIPK1, receptor-interacting serine/threonine-protein kinase 1; SHARPIN, Shank-associated RN domain-interacting protein, a component of LUBAC; TAB, TAK binding protein; TAK, TGF β-activated kinase; TIR domain, Toll/IL-1 receptor domain; TLRs, toll-like receptors; TRADD, TNFR associated death domain;TRAFs, TNFR associated factors; TNFR, tumor necrosis factor receptor; TRIF, Tlr domain-containing adaptor inducing interferon-β.

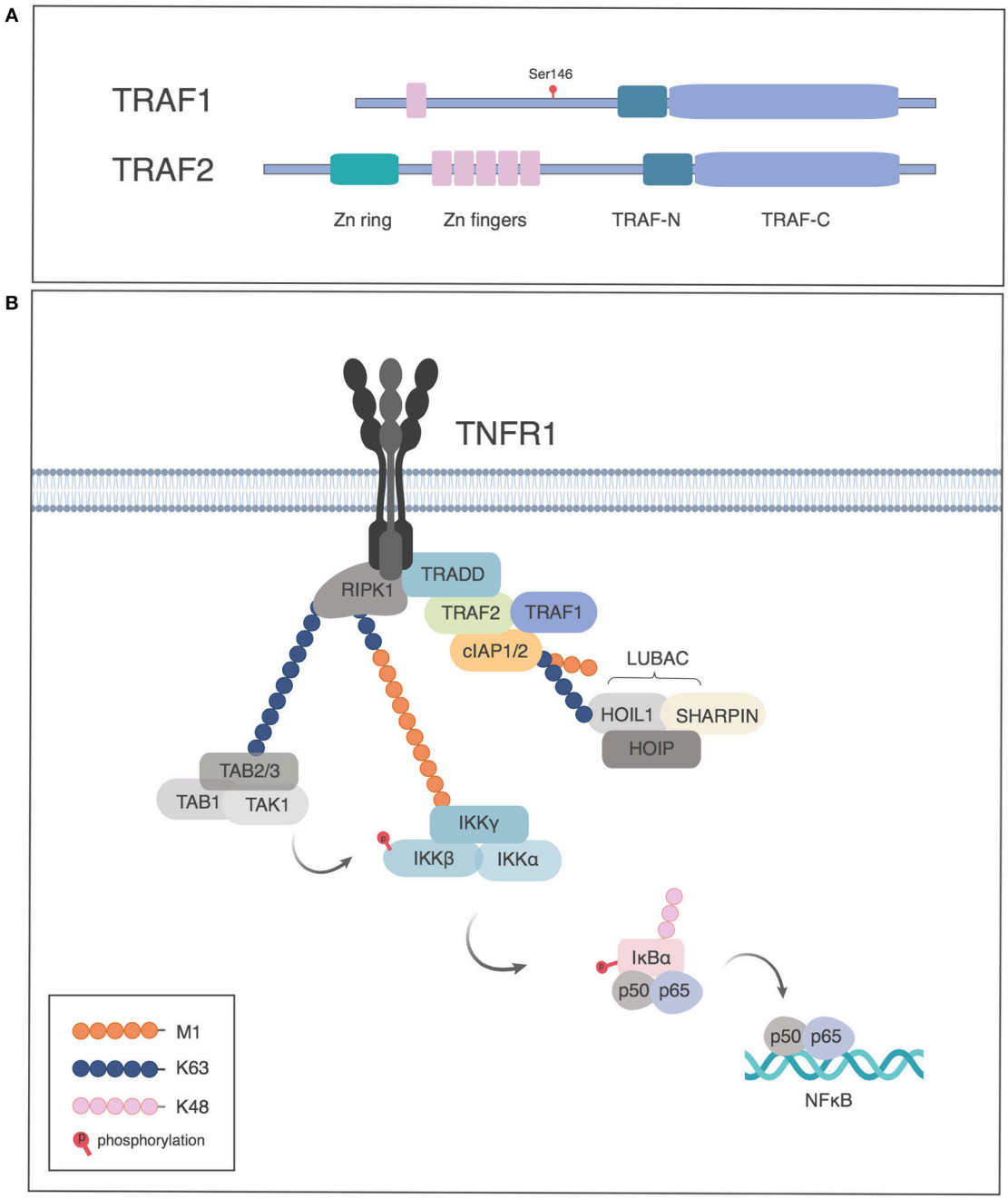


FIGURE 1 | TRAF1 and TRAF 2 proteins in TNFRI signaling. **(A)** Schematic of TRAF1 and 2 structure, indicating the site of phosphorylation of human TRAF1 by PKN1. **(B)** Role of TRAF1 and 2 in activation of NF-κB by TNFRI. TNFRI recruits TNFR associated death domain, TRADD, which in turn recruits TRAF2. TRAF1 associates with the TRADD/TRAF2 complex and the TRAF complex recruits the cellular inhibitors of apoptosis protein (cIAP1 or 2), which have E3 ligase activity to add K63-Ub to RIP1, which leads to recruitment of the TAB-TAK1 complex. The linear ubiquitin assembly complex (LUBAC) is recruited by K63-Ub and adds M1-Ub chains, which in turn recruit the IKK complex through binding NEMO. The TAB/TAK1 complex activates IKK which in turn phosphorylates IκB, leading to proteasome dependent degradation of IκB and release of p50/p65 to the nucleus. Figures generated in Biorender, adapted from Wertz et al. (3).

of cIAP by the TRAF1/2 heterotrimer over the TRAF2 homotrimer (30) and how much is due to TRAF1 preventing TRAF2 degradation, or whether the two are interrelated, is unclear.

Post-translational Modifications of TRAF1

TRAF1 and LUBAC can be co-immunoprecipitated with the TES domain of the EBV-encoded TNFR family member LMP1, when TRAF1, and the TES construct are overexpressed in

HEK293 cells (29). LMP1-dependent signaling results in M1-Ub modification of TRAF1. TRAF2, but not cIAP1 or 2, were found to be important in LUBAC recruitment in this model (29). As TRAF1 was found to increase NF-κB signaling downstream of LMP1, the authors proposed that this M1-Ub modification of TRAF1 was important in IKK recruitment and showed that LMP1 and TRAF1 could co-localize with an M1-Ub sensor that contained the ubiquitin binding domains of ABIN1 and NEMO/IKK-γ (**Figure 2A**). Consistently, knockdown of TRAF1 or HOIP resulted in reduced proliferation of a large cell lymphoma cell line (29).

The protein kinase C related kinase, PKN1 binds to and phosphorylates TRAF1 on serine 146 in human and serine 139 in mouse, but does not phosphorylate TRAF2, 3 or 5 (33), despite binding to TRAF2 (34). Knockdown of PKN1 enhanced basal IKK activation in HeLa cells. In overexpression systems with FLAG-Tagged TRAF1, serine 139 of TRAF1 was found to enhance its recruitment to TNFR2 relative to an alanine mutant, and cells expressing TRAF1 S139A showed enhanced recruitment of TRAF2 to TNFR2 in 293T cells, leading to the suggestion that phosphorylation of TRAF1 allows it to compete with TRAF2 for recruitment to TNFR2 and thereby inhibits NF-κB activation (33). However, the role of PKN1 on TRAF1 biology has yet to be tested in a more physiological setting.

Role of TRAF1 in the Alternate NF-κB Pathway

TRAF1 has also been implicated in regulation of the alternate NF-κB pathway in lymphocytes. The alternate NF-κB pathway involves degradation of p100 protein by NF-κB-inducing kinase (NIK) to the active p52 form. In resting cells, NIK is constitutively degraded due to its ubiquitination by cIAP1 and/or 2. TRAF2 and TRAF3 play non-redundant roles in this process, with TRAF2 bringing in cIAPs to the complex, and TRAF3 bringing in NIK, thereby inducing NIK degradation and preventing constitutive NF-κB activation. Accordingly, mice lacking TRAF2 or 3 have constitutive non-canonical NF-κB signaling and die of lethal inflammation (35, 36). Mice lacking TRAF1 lack this lethal inflammation (10), however T cells lacking TRAF1 are hyper-responsive to anti-CD3 (10). The hyper-responsiveness of *Traf1*$^{-/-}$ T cells to anti-CD3 was later shown to be dependent on NIK and was associated with excessive cytokine production (28). In T cells lacking TRAF1, p100 is processed to p52 in response to anti-CD3 alone, whereas in WT cells p100 processing requires both a TCR signal and a TNFR family signal (28). The role of TRAF1 in limiting non-canonical signaling in anti-CD3 activated T cells may be due its role in preventing TRAF2 degradation or due to its role in enhancing cIAP recruitment. As TRAF1 has only limited expression, it cannot have an essential role in restraining NIK in all cell types, but primarily plays this role in the context of activated lymphocytes. As TCR signaling induces increased expression of p100, it is possible that increased regulation of NIK is required to prevent spontaneous non-canonical NF-κB induction until a costimulatory signal is received (28).

TNFR family members induce activation of the alternate NF-κB pathway by inducing degradation of TRAF2 or TRAF3, usually with delayed kinetics compared to the activation of the classical NF-κB pathway (28, 35, 36). TRAF1 can positively regulate this process (37), likely through recruitment of cIAPs, which are also involved in degrading TRAF3 leading to alternative NF-κB activation (28, 35, 36). Another study, which used overexpression of both TRAF1 and NIK, showed that TRAF1 could bind to NIK and thereby prevent NIK degradation in A549 cells (38); however, the relevance of this interaction in a physiological setting is not clear. Thus, the role of TRAF1 in the alternative NF-κB pathway depends on whether there is active TNFR signaling going on. How TRAF1 promotes TRAF3 degradation to induce the alternative NF-κB activation or prevents cIAP-mediated TRAF2 degradation to allow classical NF-κB activation remains to be elucidated.

Does TRAF1 Also Play a Negative Role in TNFR Signaling?

Overexpression of TRAF1 in cell lines can lead to inhibition of TRAF2-mediated NF-κB activation (39). Additionally, a caspase-induced cleavage product of TRAF1 can interfere with TRAF2-mediated survival signaling (40, 41). This is thought to be due to competition for binding to TRAF binding sites, thereby preventing TRAF2 recruitment. However, as discussed above, transgenic expression of TRAF1 in lymphocytes has a pro-survival effect and loss of TRAF1 impairs T cell survival (11, 14). Thus, in more physiological systems with normal lymphocytes TRAF1 plays a largely positive role in NF-κB signaling. Nonetheless, analysis of TRAF1- deficient mice showed that anti-CD3 stimulated *Traf1*$^{-/-}$ cells hyper-proliferated in response to anti-CD3 alone or in response to TNF and the response of the activated T cells to TNF was specifically blocked by antibodies to TNFR2, leading to the suggestions that TRAF1 is a negative regulator of TNF signaling (10). As discussed above, *Traf1*$^{-/-}$ T cells hyperproliferate due to increased activation of the alternative NF-κB pathway, and these effects might have confounded the interpretation of anti-CD3 activated T cells responding to TNF. In the same study *Traf1*$^{-/-}$ mice were found to have increased TNF-induced skin necrosis. However, as will be discussed below, *Traf1*$^{-/-}$ mice have enhanced responses to TLR signaling, and it is possible that the damage to the skin caused by TNF allowed enhanced TLR-signaling due to skin associated microbes, and thus the negative regulatory role observed might have reflected signals through TLRs, rather than TNFRs. Other studies have clearly shown a role for TRAF1 in enhancing NF-κB signaling downstream of TNFRs (23). Thus, the weight of the evidence suggests that under conditions of physiological expression in viable lymphocytes, TRAF1 plays a largely positive role in NF-κB induction and lymphocyte survival downstream of TNFRs. On the other hand, if caspases are activated, it is possible that the caspase-induced cleavage product of TRAF1 contributes to cell death.

TRAF1 AS A NEGATIVE REGULATOR OF TLR AND NLR SIGNALING

Two studies have demonstrated negative regulation of NF-κB signaling by TRAF1 downstream of TLRs or NLRs, albeit

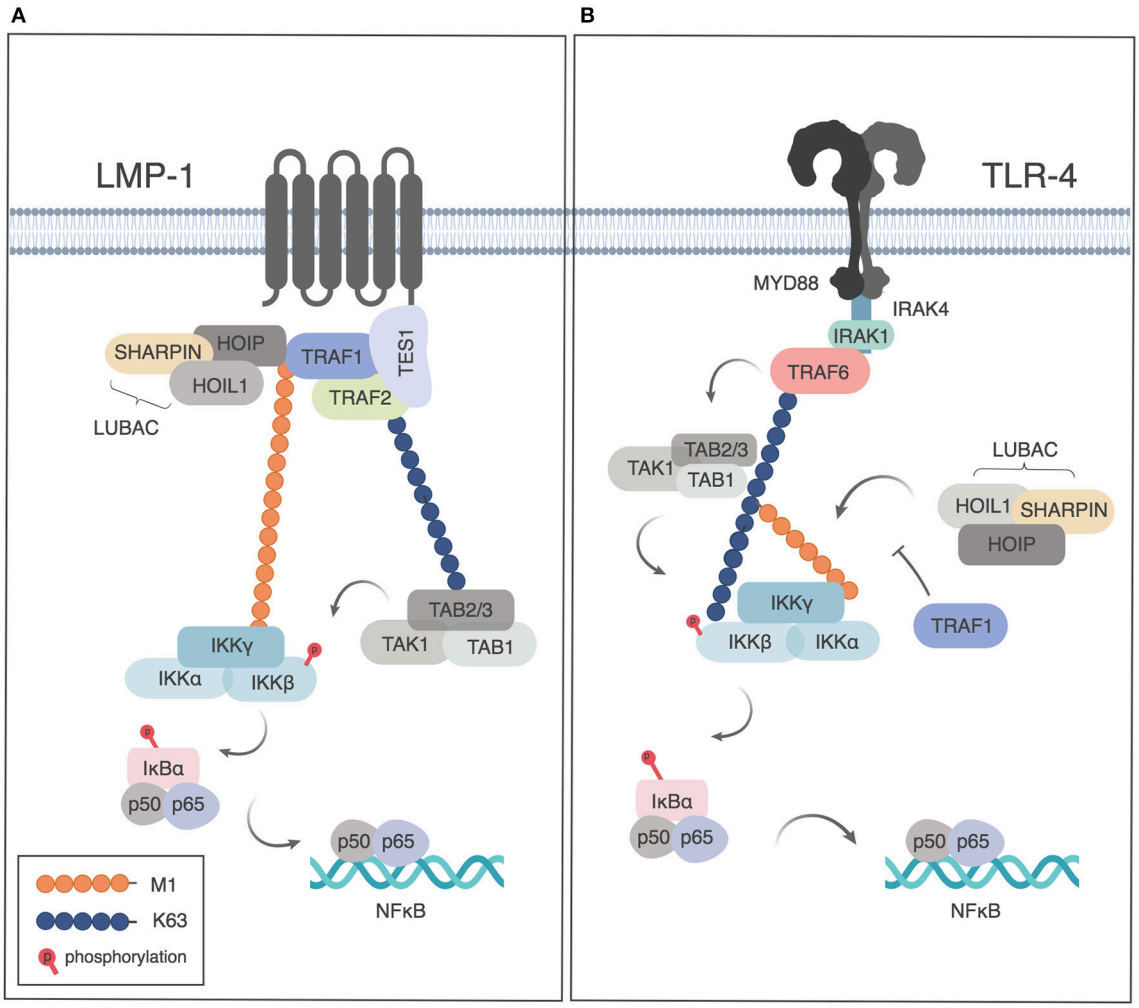


FIGURE 2 | Role of TRAF1 and linear ubiquitination downstream of LMP1 and TLR4. **(A)** TRAF1 is recruited to the TES domain of LMP1 and modified by linear ubiquitination downstream of LMP1 signaling, leading the recruitment of NEMO and NF-κB activation. Adapted from Greenfeld et al. (29) **(B)** Downstream of TLR signaling, TRAF1 sequesters LUBAC, thereby limiting linear ubiquitination of NEMO and restricting NF-κB activation. Figures generated in Biorender, adapted from Abdul-Sater et al. (32).

by different mechanisms. Abdul-Sater et al. showed that TRAF1 binds directly to all three components of LUBAC (SHARPIN, HOIP and HOIL), thereby preventing linear ubiquitination of NEMO, and thus limiting downstream NF-κB activation after TLR or NLR signaling (32) (**Figure 2B**). TRAF1 binding to LUBAC components was dependent on the presence of the MATH domain of TRAF1 and independent of TRAF2 or TNF signaling. TRAF1 binding was largely abrogated by deletion of the HOIP or HOIL NZF domain, a conserved domain required for NEMO recruitment that is found in all 3 LUBAC components (32). As the interaction of TRAF1 with LUBAC components was shown using purified proteins, this demonstrates a role for TRAF1 independently of TRAF2, and thus distinct from its role in TNFR signaling pathways. Of interest, the interaction of TRAF1 with HOIP and HOIL had also been suggested but not further analyzed in a study of protein-protein interactions downstream of microbial stimulation leading to interferon induction (42).

In another study, TRAF1 was identified in a yeast 2-hybrid screen that used the TLR signaling molecule TIR-domain-containing adapter-inducing interferon-β (TRIF) as bait. The TRAF-C domain of TRAF1 was found to bind to the TIR domain of TRIF. TRAF1 overexpression blocked TRIF-dependent NF-κB reporter activation in 293 cells, dependent on the caspase-sensitive cleavage site in TRAF1 (43). However, the physiological role of this cleaved form of TRAF1 in primary cells has not been demonstrated.

SUMMARY TRAF1 SIGNALING

In summary, TRAF1 contributes to signaling in the TNFR signaling pathway as part of a complex with TRAF2, where

it can promote classical NF-κB activation through cIAP recruitment and possibly through stabilization of TRAF2. Later, TRAF1 may also contribute to induction of the alternate NF-κB pathway, again through cIAP recruitment. TRAF1 can also contribute to NF-κB activation independently of cIAPs downstream of LMP1 signaling, through becoming a substrate for linear ubiquitination, possibly contributing to recruitment of IKKγ/NEMO. Conversely, during TLR or NLR signaling, TRAF1 can sequester LUBAC to negatively regulate NF-κB activation. How these opposing roles of TRAF1 in different contexts impact human disease will be discussed in the remainder of the article.

ROLE OF TRAF1 IN CANCER

B Cell Cancers

There is extensive evidence for altered expression of TRAF1 in lymphoid malignancies (44–46). Many human B malignancies including B cell chronic lymphocytic leukemia cells (CLL), non-Hodgkin lymphoma (NHL), and Burkitt's lymphomas exhibit constitutive signaling via TRAF1 binding TNFRs, such as CD30 and the EBV protein LMP1, and this in turn is thought to contribute to high levels of TRAF1 expression via NF-κB signaling (24, 46, 47). Additionally, B-CLL receive signals through CD40L, and this can drive CD40-dependent TRAF1 expression (48, 49). Immunological analysis of NHL revealed TRAF1 overexpression in 48% of cases, and the same study showed the highest levels of TRAF1 protein in refractory CLL (45). Analysis of TRAF1 in Hodgkin–Reed–Sternberg cells of highly proliferating tumors such as Hodgkin lymphoma (HL) and anaplastic large cell lymphoma led to the suggestion that TRAF1 contributes to apoptosis resistance downstream of CD30, and therefore plays an important role in the pathogenesis of classical HL (50). Mediastinal large B-cell lymphoma (MLBCL), a subtype of diffuse large B-cell lymphoma (DLBCL), and HL have a shared survival pathway with high levels of expression of TRAF1 and activation of the NF-κB pathway (51). Anaplastic large-cell lymphomas carrying anaplastic lymphoma kinase (ALK) have a relatively good prognosis, however aggressive forms exist. A translocation that fused the TRAF1 and ALK genes was observed in one patient and was associated with upregulation of ALK and NF-κB pathways. Treatment of TRAF1-ALK cells with proteasome inhibitors, to block the NF-κB pathway, resulted in p50/p52 downregulation and inhibition of lymphoma growth (52–54). Other evidence for the importance of TRAF1 in lymphoma comes from the identification of single nucleotide polymorphisms (SNPs) in the region between TRAF1 and complement factor 5 (TRAF1-C5 locus) that predisposes to lymphoma, although the precise causative SNP has not been identified (55, 56).

The importance of TRAF1 in lymphoma has also been validated in mouse models. Mice that overexpress a truncated form of TRAF2 that is thought to mimic TRAF1 develop lymphadenopathy and splenomegaly due to polyclonal B cell expansion. *In vitro*, these B cells exhibit comparable proliferation rates to wild-type B cells but have markedly increased survival and resistance to apoptosis induced by dexamethasone and chemotherapeutic agents. The histopathologic features of these B cells are consistent with mouse small B cell lymphoma progressing to leukemia and exhibit many similarities to human chronic lymphocytic leukemia (57). A more direct test of the role of TRAF1 in lymphomagenesis was carried out with mice engineered to express a constitutively active NF-κB2 mutant. These mice have expanded peripheral B cell populations and develop small B cell lymphomas. The mutation has no apparent effect on the proliferation of B cells but renders them resistant to apoptosis-induced by cytokine deprivation and mitogenic stimulation. The lymphocytes and lymphoma cells from these transgenic mice express high levels of TRAF1. Importantly, crossing the NF-κB2 mutant mice with *Traf1*$^{-/-}$ mice re-established B cell homeostasis, implicating TRAF1 in the pathogenesis of lymphoma (58).

Other Cancers

According to the Human protein atlas (www.proteinatlas.org), TRAF1 can be found in other cancers besides lymphomas and CLL, including head and neck, melanoma, pancreatic, and thyroid cancers. In addition, as discussed below, recent evidence shows that human squamous cell carcinoma and non-small cell lung carcinomas can show overexpression of TRAF1.

Squamous Cell Carcinoma

In human skin, TRAF1 is expressed at higher levels, as measured by histology, in actinic keratosis as well as in squamous cell carcinoma, compared to normal skin (59, 60). Since UV exposure is thought to contribute to these conditions, Yamamoto et al. tested the role of UV in induction of TRAF1 in mice. They found that TRAF1 was induced and persisted after 3 rounds of UV irradiation. Moreover, TRAF1 was required for carcinogenesis in a UV-induced mouse skin carcinogenesis model (60).

Non-small Cell Lung Carcinoma

Two recent studies reported that TRAF1 is overexpressed in human non-small cell lung cancer and that TRAF1 expression level inversely correlated with patient survival (61, 62). Moreover, in a urethane-induced mouse model, loss of TRAF1 decelerated tumor invasion (61). Knocking down TRAF1 expression in human lung cancer cell lines impaired phosphorylation of the oncogene serine/threonine-protein kinase, BRAF, and affected TRAF2-mediated BRAF Lys48-linked ubiquitination (61). This led to decreased BRAF protein, reduction of downstream MEK and ERK pathway activation and inhibition of growth and differentiation, ultimately leading to death of the lung cancer cells (61). In this study, the TNFR family members involved were not identified, but the studies are consistent with a role for TRAF1 in enhancing TRAF2-mediated signaling in NSCLC. A number of mutations in the TRAF1 gene have been identified in human lung cancer and several other cancers and these are discussed elsewhere in this topic (63).

AUTOIMMUNITY, RHEUMATOID-ARTHRITIS ASSOCIATED SEPSIS, AND CARDIOVASCULAR DISEASE

Genome-wide association studies first identified SNPs in the *TRAF1-C5* locus on chromosome 9 as risk factors for rheumatoid arthritis (RA) in human patients (64–69). In a study of 400 RA patients, Panoulas et al. found that TRAF1/C5 SNP rs3761847 GG homozygote status is also associated with an increased risk of death from sepsis or malignancies but not from cardiovascular disease in patients with established RA (70). In that study, 43.5% of deaths were due to infection, with 30% due to cardiovascular disease and 26% due to malignancy (70). Another study using an inception cohort of 615 recently diagnosed RA patients did not find a link between the TRAF1/C5 SNP rs10818488 and mortality in RA patients or in a non-RA elderly cohort (71). In this RA cohort (71), the leading cause of death was cardiovascular disease with only 9% dying from infections. Thus, differences in causes of death in the different cohorts might have impacted the results. Note that the rs10818488 SNP studied in (71), is in linkage disequilibrium, r^2 value of 0.98, with the rs3761847 SNP studied by Panoulas et al. (70). Interestingly, although the two aforementioned studies found no link between the TRAF1 SNP and cardiovascular disease, a recent study has suggested there could be a link. Hessler et al. identified a TRAF1 SNP, rs2416804 as associated with carotid intima-media thickness, a marker of early stage atherosclerosis and considered a predictor of subsequent cardiovascular events (72). rs2416804 is in linkage disequilibrium with rs3761847, $r^2 = 0.96$. Additional studies with larger cohorts representing more diverse disease outcomes will likely be required to resolve these apparent differences in TRAF1-associated disease outcomes.

SNPs in *TRAF1/C5* have also been implicated in other inflammatory and autoimmune conditions, including autoimmune thyroid diseases, juvenile idiopathic arthritis, and systemic lupus erythematosus (73–78). As several TRAF1 SNPs are in complete or almost complete linkage disequilibrium, the exact causative SNP is not known. Thus, it is not clear if the SNP that affects NHL (55), discussed in the previous section, is the same as the SNPs that affects rheumatic disease.

Increased serum levels of TRAF1 correlate with disease activity and autoantibodies in RA patients. Moreover, SNPs in the *TRAF1-C5* locus may predict the clinical response to anti-TNF therapy (79, 80). The expression of TRAF1 is also significantly higher in inflamed and non-inflamed tissues of patients with inflammatory bowel disease compared to those in control patients (81). However, as TRAF1 is an NF-κB induced gene, the finding of increased TRAF1 in patients with the SNP may relate to the increased inflammatory activity in the patients and not to the direct effect of the SNP. Therefore, to address the role of the TRAF1 SNP in human disease, our group studied healthy donors with the disease associated or disease resistant SNP, rs3761847. It was important to use healthy donors for this study, in order to assess the effect of the SNP on TRAF1 protein levels, independently of chronic inflammation (32).

Surprisingly, our group found that activated T cells as well as monocyte from healthy donors with the disease associated SNP had lower levels of TRAF1 than those with the disease resistant SNP, with an intermediate phenotype in heterozygotes. This finding was somewhat paradoxical given the positive role for TRAF1 in enhancing survival of T lymphocytes (11, 13, 14). However, around the same time our lab had found that *Traf1*$^{-/-}$ mice had increased responses to endotoxin-induced shock (32), and as this is largely a monocyte induced disease, we decided to focus our analysis of the TRAF1 SNP on human monocytes. Consistent with a role for the TRAF1 SNP in inflammation, monocytes from healthy human subjects with the risk associated-SNP produce increased amounts of pro-inflammatory cytokines, including TNF and IL-6 in response to lipopolysaccharide (LPS). As discussed above, further investigation revealed that TRAF1 attenuated TLR-induced cytokine production by sequestering LUBAC, thereby limiting linear ubiquitination of NEMO and limiting NF-κB activation (32). Thus, donors with less TRAF1 protein have enhanced responses to TLR/NLR signaling. These findings suggest that enhanced inflammation due to innate immune signaling likely explains the enhanced disease severity in patients with the risk associated SNP. The findings also suggest that the effects of TRAF1 in limiting innate immune inflammatory signaling outweigh the effects of TRAF1 in sustaining TNFR superfamily signaling in lymphocytes. Indeed, in our study, we showed that when T cell stimulation with anti-CD3 was combined with LPS stimulation of PBMC from donors with or without the TRAF1 risk allele, the effects of TRAF1 on the TLR signaling pathway dominated (32). Over the course of a lifetime, one likely has far more exposure to short-term inflammatory stimuli than to severe infections. Signaling downstream of the TRAF1-dependent TNFR family member 4-1BB is dispensable in mild, as compared to severe influenza infection (82). Thus, the negative regulatory role of TRAF1 in limiting NF-κB during repetitive exposures to innate immune stimuli likely has a more profound effect on overall level of inflammation in humans than the detrimental effects of slightly lowered TRAF1 on TNFR superfamily-induced T cell survival, which might only become apparent during more severe infections.

INFECTIOUS DISEASES

Human Immunodeficiency Virus

During chronic infection, the immune system must be tightly regulated to avoid pathology. These regulatory mechanisms include the persistent upregulation of inhibitory receptors such as PD1 on chronically stimulated T cells, as well as sustained production of anti-inflammatory cytokines, such as IL-10 and TGFβ (83). As many TNFRs are upregulated on activated T cells, this raised the question of how TNFR signaling is regulated during chronic infection. The TNFR family member 4-1BB is a TRAF1 binding TNFR that is normally absent from resting cells, but induced by TCR signaling, and becomes persistently upregulated on antigen-stimulated T cells during chronic infection (84). However, at the chronic phase of chronic

LCMV infection, 4-1BB does not contribute to viral control, as its signaling pathway is desensitized due to TGF-β-dependent TRAF1 degradation in the chronically stimulated CD8 T cells (84). Of note, TRAF1 can be upregulated by common γ chain cytokines, including IL-7, which augments TRAF1 expression in human and mouse T cells. Moreover, treatment of mice with IL-7 prior to provision of an anti-4-1BB agonist restored responses to 4-1BB and lowered viral load (84).

Early in infection, TRAF1 is highly expressed in CD8 T cells responding to HIV, consistent with their activated phenotype. However, with progression of infection, TRAF1 levels are decreased in HIV-specific CD8 T cells in donors followed longitudinally. In a cross-sectional cohort, TRAF1 protein was higher in HIV-specific CD8 T cells from patients who were able to control HIV in the absence of drug treatment, so-called elite controllers, than in chronic progressors (84). Moreover, the frequency of TRAF1^{+} HIV-specific CD8 T cells in infected patients inversely correlated with the frequency of PD-1^{hi} exhausted T cells. The importance of TRAF1 in the CD8 T cells from elite controllers was demonstrated by siRNA-knockdown of TRAF1, which resulted in decreased ability of the CD8 T cells to eliminate HIV-infected CD4 T cells in an *ex vivo* co-culture system. Moreover, knockdown of both TRAF1 and BIM led to enhanced CD8 T cell activity compared to knockdown of TRAF1 alone, consistent with previous findings that 4-1BB can regulate BIM through TRAF1-dependent ERK activation (12, 13, 84).

IL-7 therapy has been used in clinical trials to treat HIV-infected patients whose CD4 T cell counts fail to rebound despite the successful reduction of viral load by anti-retroviral therapy (85–88). One such clinical trial offered the opportunity to monitor TRAF1 levels before or after IL-7 therapy of human subjects. Although sample size was small, there was evidence that some donors increased their level of TRAF1 in HIV-specific T cells as measured 10 weeks after the last IL-7 treatment cycle. Of interest, the level of TRAF1 in the HIV-specific T cells was strongly associated with the level of phospho-ribosomal protein S6, pS6, a downstream target of the metabolic checkpoint kinase mTOR that is associated with cell size. As TRAF1 can enhance MAPK activation downstream of TNFRs, and ERK can enhance mTOR activation through negatively regulating the negative regulator TSC2, this suggests that TRAF1 in T cells may be an important regulator of the mTOR-S6 signaling axis and may contribute to T cell fitness (89).

Hepatitis C Virus

Hepatitis C infection of humans can result in diverse outcomes, from full resolution of infection to long-term chronic infection, which can ultimately lead to liver cirrhosis or hepatocarcinoma. Moreno-Cubero et al. recently examined Hepatitis C virus (HCV)-specific CD8^{+} T cells from patients with progressive infection and those with resolved infection (90). As with chronic HIV infection, progressive exhaustion during persistent infection with HCV was also associated with loss of TRAF1 measured directly *ex vivo* or after *in vitro* TCR stimulation. After *in vitro* T cell receptor stimulation, TRAF1 expression positively correlated with the levels of IL-7R, Mcl-1, and CD107a expression and proliferation intensity and negatively correlating with PD-1 expression. This study also confirmed the results from the HIV study that IL-7 enhanced, whereas TGF-β1 impaired TRAF1 expression in CD8 T cells from infected patients. Consistently, the serum concentration of TGF-β1 was higher in patients with persistent infection than in patients with resolved infection. Moreover, the authors showed that IL-7 plus 4-1BBL treatment *ex vivo* could improve T cell responses of chronically infected patients. In a subset of patients, characterized by slowly progressing liver fibrosis, *in vitro* treatment with anti-PD-L1, in addition to the combination of IL-7 and 4-1BBL, re-established T cell proliferation in individuals with long-lasting persistent infection, once again supporting the idea that TRAF1 is a key regulator involved in supporting specific CD8^{+} T cell responses during chronic viral infection (90).

Epstein-Barr Virus

It has been long established that latent membrane protein-1 (LMP1) is essential for Epstein-Barr virus (EBV)-mediated lymphocyte transformation (44, 91, 92). LMP1 recruits TRAF proteins, including TRAF1 to mimic CD40 receptor signaling in EBV-infected B lymphocytes leading to the activation of NF-κB, MAPK, phosphatidylinositol 3-kinase (PI3-K), IRF7, and STAT pathways (93). TRAF1 is amongst the most highly expressed LMP1-induced target genes and is abundantly expressed in EBV-associated disorders. There is high and consistent TRAF1 overexpression in EBV-induced lymphoproliferations and Hodgkin's disease (44, 94). In addition, many cases of post-transplant lympho-proliferative disease and related disorders are TRAF1 positive (92). Siegler et al. showed that TRAF1 co-localizes with LMP1 in EBV-infected cells in tonsillar cells of infectious mononucleosis patients (95). As discussed earlier, TRAF1 associates with LUBAC and is modified by M1-Ub in the LMP1 signaling complex and this is thought to enhance IKK recruitment and NF-κB activation in EBV-infected cells (29).

CONCLUSIONS AND FUTURE DIRECTIONS

TRAF1 has diverse roles in human health and disease. TRAF1 contributes to control of chronic viral infection and can limit inflammation. This suggests that enhancing TRAF1 expression could be beneficial for both chronic infection and inflammatory diseases. Blocking TGFβ or stimulating IL-7 offer possible interventions for achieving higher TRAF1 in chronically stimulated T cells. Conversely, TRAF1 is dysregulated in cancer, where it likely contributes to a positive feedback loop that perpetuates NF-κB signaling and survival of cancers of mature B cells. TRAF1 also contributes to survival of EBV-dependent cancers through enhancing LMP1- mediated survival signaling. Interfering with TRAF1 in this process could break the cycle of NF-κB activation in these cancers. Human variations in TRAF1 correlate with increased incidence of rheumatic disease, increased mortality from sepsis in RA patients, and increased incidence of NHL. The role of TRAF1 as both a positive and negative regulator of immune responses can be attributed to its

participation in diverse signaling pathways. TRAF1 is important in TNFR superfamily signaling as a complex with TRAF2 and in TLR/NLR signaling independently of TRAF2. What happens in a monocyte responding to both a TLR and a TNF signal? Does TRAF1 limit one and enhance the other simultaneously, and/or are there separate pools of TRAF1 in the cell that engage in these different functions? More work is required to understand how the diverse roles of TRAF1 play out in complex biological systems *in vivo*. TRAF1 interacts with LUBAC as both a substrate in the LMP1 signaling pathway and as an inhibitor in the TLR signaling pathway, with opposite effects on NF-κB activation. How the TRAF1-LUBAC interaction results in distinct outcomes in different signaling complexes will require a precise understanding of the protein-protein interactions involved. Moreover, the fact that TRAF1 can be M1-ubiqutinated by LUBAC and phosphorylated by PKN1 suggest that post-translational modifications will be important in this regulation and need further study.

AUTHOR CONTRIBUTIONS

ME and TW wrote the manuscript with editorial input from AA-S. ME prepared the figures.

FUNDING

Our research in this area is funded by the Canadian Institutes of Health Research grant FDN-143250.

REFERENCES

1. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal* (2013) 8:7. doi: 10.1186/1750-2187-8-7
2. Rothe M, Wong SC, Henzel WJ, Goeddel DV. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* (1994) 78:681–92. doi: 10.1016/0092-8674(94)90532-0
3. Wertz IE, Newton K, Seshasayee D, Kusam S, Lam C, Zhang J, et al. Phosphorylation and linear ubiquitin direct A20 inhibition of inflammation. *Nature* (2015) 528:370–5. doi: 10.1038/nature16165
4. Rothe M, Sarma V, Dixit VM, Goeddel DV. TRAF2-mediated activation of NF-kappa B by TNF receptor 2 and CD40. *Science* (1995) 269:1424–7. doi: 10.1126/science.7544915
5. Kim CM, Choi JY, Bhat EA, Jeong JH, Son YJ, Kim S, et al. Crystal structure of TRAF1 TRAF domain and its implications in the TRAF1-mediated intracellular signaling pathway. *Sci Rep*. (2016) 6:25526. doi: 10.1038/srep25526
6. Park HH. Structure of TRAF family: current understanding of receptor recognition. *Front Immunol*. (2018) 9:1999. doi: 10.3389/fimmu.2018.01999
7. Kim CM, Jeong JH, Son YJ, Choi JH, Kim S, Park HH. Molecular basis for TANK recognition by TRAF1 revealed by the crystal structure of TRAF1/TANK complex. *FEBS Lett*. (2017) 591:810–21. doi: 10.1002/1873-3468.12584
8. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol*. (2003) 3:745–56. doi: 10.1038/nri1184
9. Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin ASJr. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* (1998) 281:1680–3. doi: 10.1126/science.281.5383.1680
10. Tsitsikov EN, Laouini D, Dunn IF, Sannikova TY, Davidson L, Alt FW, et al. TRAF1 is a negative regulator of TNF signaling. enhanced TNF signaling in TRAF1-deficient mice. *Immunity* (2001) 15:647–57. doi: 10.1016/S1074-7613(01)00207-2
11. Sabbagh L, Srokowski CC, Pulle G, Snell LM, Sedgmen BJ, Liu Y, et al. A critical role for TNF receptor-associated factor 1 and Bim down-regulation in CD8 memory T cell survival. *Proc Natl Acad Sci USA*. (2006) 103:18703–8. doi: 10.1073/pnas.0602919103
12. Sabbagh L, Pulle G, Liu Y, Tsitsikov EN, Watts TH. ERK-dependent Bim modulation downstream of the 4-1BB-TRAF1 signaling axis is a critical mediator of CD8 T cell survival *in vivo*. *J Immunol*. (2008) 180:8093–101. doi: 10.4049/jimmunol.180.12.8093
13. Wang C, Wen T, Routy JP, Bernard NF, Sekaly RP, Watts TH. 4-1BBL induces TNF receptor-associated factor 1-dependent Bim modulation in human T cells and is a critical component in the costimulation-dependent rescue of functionally impaired HIV-specific CD8 T cells. *J Immunol*. (2007) 179:8252–63. doi: 10.4049/jimmunol.179.12.8252
14. Speiser DE, Lee SY, Wong B, Arron J, Santana A, Kong YY, et al. A regulatory role for TRAF1 in antigen-induced apoptosis of T cells. *J Exp Med*. (1997) 185:1777–83. doi: 10.1084/jem.185.10.1777
15. Karin M, Gallagher E. TNFR signaling: ubiquitin-conjugated TRAFfic signals control stop-and-go for MAPK signaling complexes. *Immunol Rev*. (2009) 228:225–40. doi: 10.1111/j.1600-065X.2008.00755.x
16. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol*. (2009) 27:693–733. doi: 10.1146/annurev.immunol.021908.132641
17. Kirisako T, Kamei K, Murata S, Kato M, Fukumoto H, Kanie M, et al. A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J*. (2006) 25:4877–87. doi: 10.1038/sj.emboj.7601360
18. Tokunaga F, Sakata S, Saeki Y, Satomi Y, Kirisako T, Kamei K, et al. Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol*. (2009) 11:123–32. doi: 10.1038/ncb1821
19. Hrdinka M, Gyrd-Hansen M. The Met1-linked ubiquitin machinery: emerging themes of (De)regulation. *Mol Cell* (2017) 68:265–80. doi: 10.1016/j.molcel.2017.09.001
20. Damgaard RB, Nachbur U, Yabal M, Wong WW, Fiil BK, Kastirr M, et al. The ubiquitin ligase XIAP recruits LUBAC for NOD2 signaling in inflammation and innate immunity. *Mol Cell* (2012) 46:746–58. doi: 10.1016/j.molcel.2012.04.014
21. Gerlach B, Cordier SM, Schmukle AC, Emmerich CH, Rieser E, Haas TL, et al. Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* (2011) 471:591–6. doi: 10.1038/nature09816
22. Ikeda F, Deribe YL, Skanland SS, Stieglitz B, Grabbe C, Franz-Wachtel M, et al. SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* (2011) 471:637–41. doi: 10.1038/nature09814
23. Wicovsky A, Henkler F, Salzmann S, Scheurich P, Kneitz C, Wajant H. Tumor necrosis factor receptor-associated factor-1 enhances proinflammatory TNF receptor-2 signaling and modifies TNFR1-TNFR2 cooperation. *Oncogene* (2009) 28:1769–81. doi: 10.1038/onc.2009.29
24. Eliopoulos AG, Waites ER, Blake SM, Davies C, Murray P, Young LS. TRAF1 is a critical regulator of JNK signaling by the TRAF-binding domain of the Epstein-Barr virus-encoded latent infection membrane protein 1 but not CD40. *J Virol*. (2003) 77:1316–28. doi: 10.1128/JVI.77.2.1316-1328.2003
25. Xie P, Hostager BS, Munroe ME, Moore CR, Bishop GA. Cooperation between TNF receptor-associated factors 1 and 2 in CD40 signaling. *J Immunol*. (2006) 176:5388–400. doi: 10.4049/jimmunol.176.9.5388
26. Arron JR, Pewzner-Jung Y, Walsh MC, Kobayashi T, Choi Y. Regulation of the subcellular localization of tumor necrosis factor receptor-associated factor (TRAF)2 by TRAF1 reveals mechanisms of TRAF2 signaling. *J Exp Med*. (2002) 196:923–34. doi: 10.1084/jem.20020774
27. Duckett CS, Gedrich RW, Gilfillan MC, Thompson CB. Induction of nuclear factor kappaB by the CD30 receptor is mediated by TRAF1 and TRAF2. *Mol Cell Biol*. (1997) 17:1535–42. doi: 10.1128/MCB.17.3.1535

28. McPherson AJ, Snell LM, Mak TW, Watts TH. Opposing roles for TRAF1 in the alternative versus classical NF-kappaB pathway in T cells. *J Biol Chem*. (2012) 287:23010–9. doi: 10.1074/jbc.M112.350538
29. Greenfeld H, Takasaki K, Walsh MJ, Ersing I, Bernhardt K, Ma Y, et al. TRAF1 coordinates polyubiquitin signaling to enhance epstein-barr virus lmp1-mediated growth and survival pathway activation. *PLoS Pathog.* (2015) 11:e1004890. doi: 10.1371/journal.ppat.1004890
30. Zheng C, Kabaleeswaran V, Wang Y, Cheng G, Wu H. Crystal structures of the TRAF2: cIAP2 and the TRAF1: TRAF2: cIAP2 complexes: affinity, specificity, and regulation. *Mol Cell* (2010) 38:101–13. doi: 10.1016/j.molcel.2010.03.009
31. Li X, Yang Y, Ashwell JD. TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2. *Nature* (2002) 416:345–7. doi: 10.1038/416345a
32. Abdul-Sater AA, Edilova MI, Clouthier DL, Mbanwi A, Kremmer E, Watts TH. The signaling adaptor TRAF1 negatively regulates Toll-like receptor signaling and this underlies its role in rheumatic disease. *Nat Immunol.* (2017) 18:26–35. doi: 10.1038/ni.3618
33. Kato TJr, Gotoh Y, Hoffmann A, Ono Y. Negative regulation of constitutive NF-kappaB and JNK signaling by PKN1-mediated phosphorylation of TRAF1. *Genes Cells* (2008) 13:509–20. doi: 10.1111/j.1365-2443.2008.01182.x
34. Gotoh Y, Oishi K, Shibata H, Yamagiwa A, Isagawa T, Nishimura T, et al. Protein kinase PKN1 associates with TRAF2 and is involved in TRAF2-NF-kappaB signaling pathway. *Biochem Biophys Res Commun.* (2004) 314:688–94. doi: 10.1016/j.bbrc.2003.12.148
35. Vallabhapurapu S, Matsuzawa A, Zhang W, Tseng PH, Keats JJ, Wang H, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling. *Nat Immunol.* (2008) 9:1364–70. doi: 10.1038/ni.1678
36. Zarnegar BJ, Wang Y, Mahoney DJ, Dempsey PW, Cheung HH, He J, et al. Noncanonical NF-kappaB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nat Immunol.* (2008) 9:1371–8. doi: 10.1038/ni.1676
37. Lavorgna A, De Filippi R, Formisano S, Leonardi A. TNF receptor-associated factor 1 is a positive regulator of the NF-kappaB alternative pathway. *Mol Immunol.* (2009) 46:3278–82. doi: 10.1016/j.molimm.2009.07.029
38. Choudhary S, Kalita M, Fang L, Patel KV, Tian B, Zhao Y, et al. Inducible tumor necrosis factor (TNF) receptor-associated factor-1 expression couples the canonical to the non-canonical NF-kappaB pathway in TNF stimulation. *J Biol Chem.* (2013) 288:14612–23. doi: 10.1074/jbc.M113.464081
39. Carpentier I, Beyaert R. TRAF1 is a TNF inducible regulator of NF-kappaB activation. *FEBS Lett.* (1999) 460:246–50. doi: 10.1016/S0014-5793(99)01356-3
40. Henkler F, Baumann B, Fotin-Mleczek M, Weingartner M, Schwenzer R, Peters N, et al. Caspase-mediated cleavage converts the tumor necrosis factor (TNF) receptor-associated factor (TRAF)-1 from a selective modulator of TNF receptor signaling to a general inhibitor of NF-kappaB activation. *J Biol Chem.* (2003) 278:29216–30. doi: 10.1074/jbc.M211090200
41. Leo E, Deveraux QL, Buchholtz C, Welsh K, Matsuzawa S, Stennicke HR, et al. TRAF1 is a substrate of caspases activated during tumor necrosis factor receptor-alpha-induced apoptosis. *J Biol Chem.* (2001) 276:8087–93. doi: 10.1074/jbc.M009450200
42. Li S, Wang L, Berman M, Kong YY, Dorf ME. Mapping a dynamic innate immunity protein interaction network regulating type I interferon production. *Immunity* (2011) 35:426–40. doi: 10.1016/j.immuni.2011.06.014
43. Su X, Li S, Meng M, Qian W, Xie W, Chen D, et al. TNF receptor-associated factor-1 (TRAF1) negatively regulates Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF)-mediated signaling. *Eur J Immunol.* (2006) 36:199–206. doi: 10.1002/eji.200535415
44. Durkop H, Foss HD, Demel G, Klotzbach H, Hahn C, Stein H. Tumor necrosis factor receptor-associated factor 1 is overexpressed in Reed-Sternberg cells of Hodgkin's disease and Epstein-Barr virus-transformed lymphoid cells. *Blood* (1999) 93:617–23.
45. Zapata JM, Krajewska M, Krajewski S, Kitada S, Welsh K, Monks A, et al. TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J Immunol.* (2000) 165:5084–96. doi: 10.4049/jimmunol.165.9.5084
46. Munzert G, Kirchner D, Stobbe H, Bergmann L, Schmid RM, Dohner H, et al. Tumor necrosis factor receptor-associated factor 1 gene overexpression in B-cell chronic lymphocytic leukemia: analysis of NF-kappa B/Rel-regulated inhibitors of apoptosis. *Blood* (2002) 100:3749–56. doi: 10.1182/blood.V100.10.3749
47. Horie R, Watanabe T, Morishita Y, Ito K, Ishida T, Kanegae Y, et al. Ligand-independent signaling by overexpressed CD30 drives NF-kappaB activation in Hodgkin-Reed-Sternberg cells. *Oncogene* (2002) 21:2493–503. doi: 10.1038/sj.onc.1205337
48. Gricks CS, Zahrieh D, Zauls AJ, Gorgun G, Drandi D, Mauerer K, et al. Differential regulation of gene expression following CD40 activation of leukemic compared to healthy B cells. *Blood* (2004) 104:4002–9. doi: 10.1182/blood-2004-02-0494
49. Pascutti MF, Jak M, Tromp JM, Derks IA, Remmerswaal EB, Thijssen R, et al. IL-21 and CD40L signals from autologous T cells can induce antigen-independent proliferation of CLL cells. *Blood* (2013) 122:3010–9. doi: 10.1182/blood-2012-11-467670
50. Durkop H, Hirsch B, Hahn C, Foss HD, Stein H. Differential expression and function of A20 and TRAF1 in Hodgkin lymphoma and anaplastic large cell lymphoma and their induction by CD30 stimulation. *J Pathol.* (2003) 200:229–39. doi: 10.1002/path.1351
51. Savage KJ, Monti S, Kutok JL, Cattoretti G, Neuberg D, De Leval L, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. *Blood* (2003) 102:3871–9. doi: 10.1182/blood-2003-06-1841
52. Feldman AL, Vasmatzis G, Asmann YW, Davila J, Middha S, Eckloff BW, et al. Novel TRAF1-ALK fusion identified by deep RNA sequencing of anaplastic large cell lymphoma. *Genes Chromosomes Cancer* (2013) 52:1097–102. doi: 10.1002/gcc.22104
53. Abate F, Todaro M, van der Krogt JA, Boi M, Landra I, Machiorlatti R, et al. A novel patient-derived tumorgraft model with TRAF1-ALK anaplastic large-cell lymphoma translocation. *Leukemia* (2015) 29:1390–401. doi: 10.1038/leu.2014.347
54. Lawrence K, Berry B, Handshoe J, Hout D, Mazzola R, Morris SW, et al. Detection of a TRAF1-ALK fusion in an anaplastic large cell lymphoma patient with chemotherapy and ALK inhibitor-resistant disease. *BMC Res Notes* (2015) 8:308. doi: 10.1186/s13104-015-1277-7
55. Cerhan JR, Novak AJ, Fredericksen ZS, Wang AH, Liebow M, Call TG, et al. Risk of non-Hodgkin lymphoma in association with germline variation in complement genes. *Br J Haematol.* (2009) 145:614–23. doi: 10.1111/j.1365-2141.2009.07675.x
56. Wang SS, Menashe I, Cerhan JR, Cozen W, Severson RK, Davis S, et al. Variations in chromosomes 9 and 6p21.3 with risk of non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev.* (2011) 20:42–9. doi: 10.1158/1055-9965.EPI-10-0638
57. Zapata JM, Krajewska M, Morse HCIII, Choi Y, Reed JC. TNF receptor-associated factor (TRAF) domain and Bcl-2 cooperate to induce small B cell lymphoma/chronic lymphocytic leukemia in transgenic mice. *Proc Natl Acad Sci USA* (2004) 101:16600–5. doi: 10.1073/pnas.0407541101
58. Zhang B, Wang Z, Li T, Tsitsikov EN, Ding HF. NF-kappaB2 mutation targets TRAF1 to induce lymphomagenesis. *Blood* (2007) 110:743–51. doi: 10.1182/blood-2006-11-058446
59. Wu TF, Li YC, Ma SR, Bing L, Zhang WF, Sun ZJ. Expression and associations of TRAF1, BMI-1, ALDH1, and Lin28B in oral squamous cell carcinoma. *Tumour Biol.* (2017) 39:1010428317695930. doi: 10.1177/1010428317695930
60. Yamamoto H, Ryu J, Min E, Oi N, Bai R, Zykova TA, et al. TRAF1 Is Critical for DMBA/Solar UVR-Induced Skin Carcinogenesis. *J Invest Dermatol.* (2017) 137:1322–32. doi: 10.1016/j.jid.2016.12.026
61. Wang Q, Gao G, Zhang T, Yao K, Chen H, Park MH, et al. TRAF1 is critical for regulating the BRAF/MEK/ERK pathway in non-small cell lung carcinogenesis. *Cancer Res.* (2018) 78:3982–94. doi: 10.1158/0008-5472.CAN-18-0429

62. Wen X, Wang B, Feng T, Yuan W, Zhou J, Fang T. TNF receptor-associated factor 1 as a biomarker for assessment of non-small cell lung cancer metastasis and overall survival. *Clin Respir J.* (2018) 12:2197–203. doi: 10.1111/crj.12789
63. Zhu S, Jin J, Gokhale S, Lu AM, Shan H, Feng J, et al. Genetic alterations of TRAF proteins in human cancers. *Front Immunol.* (2018) 9:2111. doi: 10.3389/fimmu.2018.02111
64. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. *PLoS Med* (2007) 4:e278. doi: 10.1371/journal.pmed.0040278
65. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis–a genomewide study. *N Engl J Med.* (2007) 357:1199–209. doi: 10.1056/NEJMoa073491
66. Chang M, Rowland CM, Garcia VE, Schrodi SJ, Catanese JJ, van der Helm-van Mil AH, et al. A large-scale rheumatoid arthritis genetic study identifies association at chromosome 9q33.2. *PLoS Genet.* (2008) 4:e1000107. doi: 10.1371/journal.pgen.1000107
67. Zervou MI, Sidiropoulos P, Petraki E, Vazgiourakis V, Krasoudaki E, Raptopoulou A, et al. Association of a TRAF1 and a STAT4 gene polymorphism with increased risk for rheumatoid arthritis in a genetically homogeneous population. *Hum Immunol.* (2008) 69:567–71. doi: 10.1016/j.humimm.2008.06.006
68. Han TU, Bang SY, Kang C, Bae SC. TRAF1 polymorphisms associated with rheumatoid arthritis susceptibility in Asians and in Caucasians. *Arthritis Rheum.* (2009) 60:2577–84. doi: 10.1002/art.24759
69. Liang X, Gao Y, Lam TK, Li Q, Falk C, Yang XR, et al. Identifying rheumatoid arthritis susceptibility genes using high-dimensional methods. *BMC Proc.* (2009) 3 (Suppl. 7):S79. doi: 10.1186/1753-6561-3-s7-s79
70. Panoulas VF, Smith JP, Nightingale P, Kitas GD. Association of the TRAF1/C5 locus with increased mortality, particularly from malignancy or sepsis, in patients with rheumatoid arthritis. *Arthritis Rheum.* (2009) 60:39–46. doi: 10.1002/art.24176
71. van Nies JA, Marques RB, Trompet S, de Jong Z, Kurreeman FA, Toes RE, et al. TRAF1/C5 polymorphism is not associated with increased mortality in rheumatoid arthritis: two large longitudinal studies. *Arthritis Res Ther.* (2010) 12:R38. doi: 10.1186/ar2947
72. Hessler N, Geisel MH, Coassin S, Erbel R, Heilmann S, Hennig F, et al. Linkage and association analysis identifies TRAF1 influencing common carotid intima-media thickness. *Stroke* (2016) 47:2904–9. doi: 10.1161/STROKEAHA.116.013943
73. Liang Y, Meng S, Zhang JA, Zhu YF, Li C, Yang XJ, et al. Tumor necrosis factor receptor-associated factor 1 (TRAF1) polymorphisms and susceptibility to autoimmune thyroid disease. *Autoimmunity* (2016) 49:84–9. doi: 10.3109/08916934.2015.1124423
74. Pers YM, Le Blay P, Ludwig C, Rittore C, Tejedor G, Foliwe R, et al. Association of TRAF1-C5 with risk of uveitis in juvenile idiopathic arthritis. *Joint Bone Spine* (2017) 84:305–8. doi: 10.1016/j.jbspin.2016.04.014
75. Kurreeman FA, Goulielmos GN, Alizadeh BZ, Rueda B, Houwing-Duistermaat J, Sanchez E, et al. The TRAF1-C5 region on chromosome 9q33 is associated with multiple autoimmune diseases. *Ann Rheum Dis.* (2010) 69:696–9. doi: 10.1136/ard.2008.106567
76. Albers HM, Kurreeman FA, Houwing-Duistermaat JJ, Brinkman DM, Kamphuis SS, Girschick HJ, et al. The TRAF1/C5 region is a risk factor for polyarthritis in juvenile idiopathic arthritis. *Ann Rheum Dis.* (2008) 67:1578–80. doi: 10.1136/ard.2008.089060
77. Behrens EM, Finkel TH, Bradfield JP, Kim CE, Linton L, Casalunovo T, et al. Association of the TRAF1-C5 locus on chromosome 9 with juvenile idiopathic arthritis. *Arthritis Rheum* (2008) 58:2206–7. doi: 10.1002/art.23603
78. Dimopoulou DG, Zervou MI, Trachana M, Myrthianou E, Pratsidou-Gertsi P, Kardassis D, et al. Investigation of juvenile idiopathic arthritis susceptibility loci: results from a Greek population. *Hum Immunol.* (2013) 74:1194–8. doi: 10.1016/j.humimm.2013.06.018
79. Canhao H, Rodrigues AM, Santos MJ, Carmona-Fernandes D, Bettencourt BF, Cui J, et al. TRAF1/C5 but not PTPRC variants are potential predictors of rheumatoid arthritis response to anti-tumor necrosis factor therapy. *Biomed Res Int.* (2015) 2015:490295. doi: 10.1155/2015/490295
80. Cheng T, Sun X, Wu J, Wang M, Eisenberg RA, Chen Z. Increased serum levels of tumor necrosis factor receptor-associated factor 1 (TRAF1) correlate with disease activity and autoantibodies in rheumatoid arthritis. *Clin Chim Acta* (2016) 462:103–6. doi: 10.1016/j.cca.2016.08.021
81. Qiao YQ, Shen J, Gu Y, Tong JL, Xu XT, Huang ML, et al. Gene expression of tumor necrosis factor receptor associated-factor (TRAF)-1 and TRAF-2 in inflammatory bowel disease. *J Dig Dis.* (2013) 14:244–50. doi: 10.1111/1751-2980.12044
82. Lin GH, Sedgmen BJ, Moraes TJ, Snell LM, Topham DJ, Watts TH. Endogenous 4-1BB ligand plays a critical role in protection from influenza-induced disease. *J Immunol.* (2009) 182:934–47. doi: 10.4049/jimmunol.182.2.934
83. Virgin HW, Wherry EJ, Ahmed R. Redefining chronic viral infection. *Cell* (2009) 138:30–50. doi: 10.1016/j.cell.2009.06.036
84. Wang C, McPherson AJ, Jones RB, Kawamura KS, Lin GH, Lang PA, et al. Loss of the signaling adaptor TRAF1 causes CD8+ T cell dysregulation during human and murine chronic infection. *J Exp Med.* (2012) 209:77–91. doi: 10.1084/jem.20110675
85. Levy Y, Lacabaratz C, Weiss L, Viard JP, Goujard C, Lelievre JD, et al. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. *J Clin Invest.* (2009) 119:997–1007. doi: 10.1172/JCI38052
86. Levy Y, Sereti I, Tambussi G, Routy JP, Lelievre JD, Delfraissy JF, et al. Effects of recombinant human interleukin 7 on T-cell recovery and thymic output in HIV-infected patients receiving antiretroviral therapy: results of a phase I/IIa randomized, placebo-controlled, multicenter study. *Clin Infect Dis.* (2012) 55:291–300. doi: 10.1093/cid/cis383
87. Thiebaut R, Jarne A, Routy JP, Sereti I, Fischl M, Ive P, et al. repeated cycles of recombinant human interleukin 7 in HIV-infected patients with low CD4 T-cell reconstitution on antiretroviral therapy: results of 2 phase II multicenter studies. *Clin Infect Dis.* (2016) 62:1178–85. doi: 10.1093/cid/sciw065
88. Sereti I, Dunham RM, Spritzler J, Aga E, Proschan MA, Medvik K, et al. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. *Blood* (2009) 113:6304–14. doi: 10.1182/blood-2008-10-186601
89. Wang C, Edilova MI, Wagar LE, Mujib S, Singer M, Bernard NF, et al. Effect of IL-7 therapy on phospho-ribosomal protein S6 and TRAF1 expression in HIV-specific CD8 T cells in patients receiving antiretroviral therapy. *J Immunol.* (2018) 200:558–64. doi: 10.4049/jimmunol.1601254
90. Moreno-Cubero E, Subira D, Sanz-de-Villalobos E, Parra-Cid T, Madejon A, Miquel J, et al. According to Hepatitis C Virus (HCV) infection stage, interleukin-7 plus 4-1BB triggering alone or combined with PD-1 blockade increases TRAF1(low) HCV-Specific CD8(+) Cell Reactivity. *J Virol.* (2018) 92:e01443–17. doi: 10.1128/JVI.01443-17
91. E.D. Cahir McFarland, Izumi KM, Mosialos G. Epstein-barr virus transformation: involvement of latent membrane protein 1-mediated activation of NF-kappaB. *Oncogene* (1999) 18:6959–64. doi: 10.1038/sj.onc.1203217
92. Murray PG, Swinnen LJ, Flavell JR, Ragni MV, Baumforth KR, Toomey SM, et al. Frequent expression of the tumor necrosis factor receptor-associated factor 1 in latent membrane protein 1-positive posttransplant lymphoproliferative disease and HIV-associated lymphomas. *Hum Pathol.* (2001) 32:963–9. doi: 10.1053/hupa.2001.27114
93. Devergne OED, Cahir McFarland, Mosialos G, Izumi KM, Ware CF, Kieff E. Role of the TRAF binding site and NF-kappaB activation in Epstein-Barr virus latent membrane protein 1-induced cell gene expression. *J Virol.* (1998) 72:7900–8.

94. Izban KF, Ergin M, Martinez RL, Alkan S. Expression of the tumor necrosis factor receptor-associated factors (TRAFs) 1 and 2 is a characteristic feature of Hodgkin and Reed-Sternberg cells. *Mod Pathol.* (2000) 13:1324–31. doi: 10.1038/modpathol.3880243
95. Siegler G, Kremmer E, Gonnella R, Niedobitek G. Epstein-Barr virus encoded latent membrane protein 1 (LMP1) and TNF receptor associated factors (TRAF): colocalisation of LMP1 and TRAF1 in primary EBV infection and in EBV associated Hodgkin lymphoma. *Mol Pathol.* (2003) 56:156–61. doi: 10.1136/mp.56.3.156

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 24 September 2018
doi: 10.3389/fimmu.2018.02161

TRAF3 as a Multifaceted Regulator of B Lymphocyte Survival and Activation

Gail A. Bishop[1,2,3]*, *Laura L. Stunz*[1] *and Bruce S. Hostager*[1]

[1] Department of Microbiology & Immunology, University of Iowa, Iowa City, IA, United States, [2] Department of Internal Medicine, University of Iowa, Iowa City, IA, United States, [3] Iowa City VA Health Care System, Iowa City, Iowa City, IA, United States

The adaptor protein TNF receptor-associated factor 3 (TRAF3) serves as a powerful negative regulator in multiple aspects of B cell biology. Early *in vitro* studies in transformed cell lines suggested the potential of TRAF3 to inhibit signaling by its first identified binding receptor, CD40. However, because the canonical TRAF3 binding site on many receptors also mediates binding of other TRAFs, and whole-mouse TRAF3 deficiency is neonatally lethal, an accurate understanding of TRAF3's specific functions was delayed until conditional TRAF3-deficient mice were produced. Studies of B cell-specific TRAF3-deficient mice, complemented by investigations in normal and malignant mouse and human B cells, reveal that TRAF3 has powerful regulatory roles that are unique to this TRAF, as well as functions context-specific to the B cell. This review summarizes the current state of knowledge of these roles and functions. These include inhibition of signaling by plasma membrane receptors, negative regulation of intracellular receptors, and restraint of cytoplasmic NF- κB pathways. TRAF3 is also now known to function as a resident nuclear protein, and to impact B cell metabolism. Through these and additional mechanisms TRAF3 exerts powerful restraint upon B cell survival and activation. It is thus perhaps not surprising that TRAF3 has been revealed as an important tumor suppressor in B cells. The many and varied functions of TRAF3 in B cells, and new directions to pursue in future studies, are summarized and discussed here.

Keywords: TRAF, B cell, signal transduction, cytokine, toll-like receptor, TNF receptors, cancer

OPEN ACCESS

Edited by:
Michael R. Gold,
University of British Columbia, Canada

Reviewed by:
Ping Xie,
Rutgers University, The State University of New Jersey, United States
Louis Justement,
University of Alabama at Birmingham, United States

***Correspondence:**
Gail A. Bishop
gail-bishop@uiowa.edu

Specialty section:
This article was submitted to B Cell Biology, a section of the journal Frontiers in Immunology

Received: *06 July 2018*
Accepted: *03 September 2018*
Published: *24 September 2018*

Citation:
Bishop GA, Stunz LL and Hostager BS (2018) TRAF3 as a Multifaceted Regulator of B Lymphocyte Survival and Activation. Front. Immunol. 9:2161. doi: 10.3389/fimmu.2018.02161

INTRODUCTION

We will begin with a brief summary of work leading up to the current understanding of the multiple important roles played by TRAF3 in B lymphocytes; the reader is referred to previous reviews for information on roles of TRAF3 in other cell types (1–6). TRAF3 was discovered as the first protein demonstrated to associate with the cytoplasmic domain of the tumor necrosis factor receptor (TNFR) superfamily member CD40 (7, 8). TRAF3 also binds the C-terminal cytoplasmic domain of the Epstein Barr virus (EBV)-encoded CD40 mimic, Latent Membrane Protein 1 (LMP1) (9). It was inevitable that this newly-identified signaling protein would be experimentally deleted from the mouse germline, the only technology widely available at the time to create "knockout" mice. As with many broadly-expressed signaling proteins, this whole-mouse germline *Traf3* deletion resulted in early lethality (10), and thus could provide only limited hints of TRAF3 protein function, particularly for specific mature cell types. Interestingly, however, this initial report suggested regulation of T-dependent antibody production by TRAF3, a role that was confirmed much later, when T cell-specific TRAF3-deficient mice were made and analyzed (11).

As CD40 was the first identified TRAF3 binding receptor, studies followed examining the role of TRAF3 in CD40 signaling to B cells. Several groups obtained evidence that TRAF3 plays an inhibitory role in both CD40 signaling (12–14), as well as synergistic signaling mediated by cooperation between CD40 and the B cell antigen receptor (BCR) (15, 16). TRAF3 also inhibits signaling to B cells by the BAFF receptor (BAFFR) (17).

Pinning down TRAF3's role precisely was initially prevented by the highly overlapping nature of the major binding site on CD40 (and many other TRAF-binding receptors) for TRAFs 1, 2, 3, and 5 (PXQXT) (18). Thus, the available approaches of mutating the receptor's binding site, and/or mutating the TRAF3 molecule to prevent receptor binding (creating a "dominant negative" TRAF3) could provide important information, but ultimately could not lead to unambiguously interpretable data, because both strategies impact the nature and stoichiometry of binding of other types of TRAFs, in addition to TRAF3. The stoichiometric abnormalities were particularly acute in model systems using exogenous overexpression of TRAF molecules and receptors, such as 293 epithelial cells. For example, a point mutation in the major PXQXT CD40 cytoplasmic domain motif obviates binding of both TRAFs 2 and 3 in artificial systems (18), but when this mutant CD40 molecule is expressed at approximately normal levels in B cells, it binds TRAF3 indistinguishably from WT CD40 (15).

Prior to wide availability of the Cre-Lox system for conditional deletion of specific genes in B cells (19), the challenge of the overlapping TRAF binding site was addressed using modification of gene targeting by homologous recombination, tailored to use in somatic cell lines, which allows complete and specific removal of single types of TRAF molecules (20). When this approach was applied to TRAF3 in B cell lines, a surprising result was obtained. In B cells inducibly expressing transfected LMP1 plus endogenous CD40, removal of TRAF3 enhances CD40 signaling—consistent with earlier reports—but greatly inhibits the typically amplified signaling induced by LMP1 in the same B cells (21). It was subsequently revealed that CD40 and LMP1 bind TRAF3 in distinct ways, contributing to this striking difference (22, 23). Thus, TRAF3 can play distinct roles in regulating signaling to the same cell by different receptors.

Abbreviations: 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; APRIL, a proliferation-inducing ligand; BAD, Bcl-2-associated agonist of cell death; BAFF, B cell activating factor; BCL, B cell lymphoma; BCMA, B cell maturation antigen; B-*Traf3*$^{-/-}$, mice engineered to lack TRAF3 specifically in B cells; CREB, cyclic AMP responsive element binding protein; DLBCL, diffuse large BCL; EBV, Epstein-Barr virus; ERK, extracellular-regulated kinase; Glut1, glucose transporter 1; HXK2, hexokinase 2; cIAP, cellular inhibitor of apoptosis; IκB, inhibitor of kappa B; IKK, IκB kinase; IL, interleukin; IFN, interferon; IRF, IFN response factor; DC, dendritic cells; Jak, Janus kinase; JNK, c-jun kinase; LMP1, latent membrane protein 1; MAPK, mitogen-activated protein kinase; MAP3K, MAP kinase kinase kinase; MM, multiple myeloma; NF-κB, nuclear factor of kappa B; NIK, NF-κB-inducible kinase; NLS, nuclear localization signal; p70S6K, phospho-ribosomal protein S6 kinase beta-1; PI3K, phosphatidyl inositol 3-kinase; Pim, proviral insertion in murine lymphoma; PTP, protein tyrosine phosphatase; siRNA, small interfering RNA; Stat, signal transducer and activator of transcription; TACI, transmembrane and CAML interactor; TCPTP, T cell protein tyrosine phosphatase; TLR, toll-like receptor; TNFR, tumor necrosis factor receptor; TNFRSF, TNFR superfamily; TRAF, TNFR-associated factor.

Following discovery of the mitogen-activated protein kinase kinase kinase (MAP3K) called NF- κB inducing kinase (NIK), and its important role in activation of the non-canonical/NF-κB2 pathway by TNFR superfamily members (24, 25), it was shown that activation of this pathway by CD40 also involves NIK (26). While this finding was initially made in 293 epithelial cell line overexpression models, it was subsequently confirmed in B lymphocytes (27). Importantly, TRAF3 was revealed to be a master regulator of NIK stability in multiple cell types, including B cells (28).

Building upon all these earlier studies, the best information to date on the multiple roles played by TRAF3 in B cells has derived from strains of mice lacking *Traf3* expression specifically in B cells. The first two strains of this type were reported in 2007 (29) and 2008 (30); they revealed a newly appreciated critical role for TRAF3 in restraining B cell homeostatic survival. Consistent with the previously reported role for TRAF3 in reducing NIK stability, TRAF3-deficient primary B cells display constitutive p100 processing and nuclear p52 and Rel B (29). However, subsequent mice made TRAF3-deficient in T lymphocytes, dendritic cells, or macrophages all also display constitutive NF-κB2 activation in the TRAF3-deficient cells, but only TRAF3-deficient B cells display enhanced survival (11, 31, 32). Thus, in addition to receptor-specific roles, TRAF3 has cell-type specific functions, and exerts particularly unique and critical regulation of signaling pathways in B cells. The current state of knowledge of these functions and pathways will be discussed in the following sections, and are summarized in **Figure 1**.

INTERACTIONS BETWEEN TRAF3, THE B CELL ANTIGEN RECEPTOR (BCR) AND TNFR SUPERFAMILY (TNFRSF) RECEPTORS

Numerous TNFRSF members expressed by B lymphocytes interact (or potentially interact) with TRAF3. These receptors include CD27, CD30, BCMA, CD40, BAFFR, TACI, TNFR2, and 4-1BB.

CD40 and CD40^{+}BCR

CD40 is a somewhat unusual member of the TNFRSF in that it activates both the canonical/NF-κB1 and non-canonical/NF-κB2 pathways. TRAF proteins play important roles in regulating signaling pathways activated by CD40 (33). Following engagement of CD40 by CD154 (CD40L) or agonistic antibody, the cytoplasmic domain of CD40 binds TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6 (34). TRAF3 negatively regulates CD40 signaling in B cells (12). Studies of CD40 and TRAF mutants in B cell lines indicate that TRAF3 also has a negative role in regulating synergy between CD40 and the BCR in activation of antibody and cytokine secretion (15). Transformed B cell lines deficient in TRAF3 exhibit markedly enhanced CD40-mediated activation of c-Jun kinase (JNK) and antibody secretion (21), although CD40-mediated signals (including the activation of JNK, p38, ERK, NF- κB1, and NF- κB2), in TRAF3-deficient primary B cells appear only modestly enhanced (29, 30).

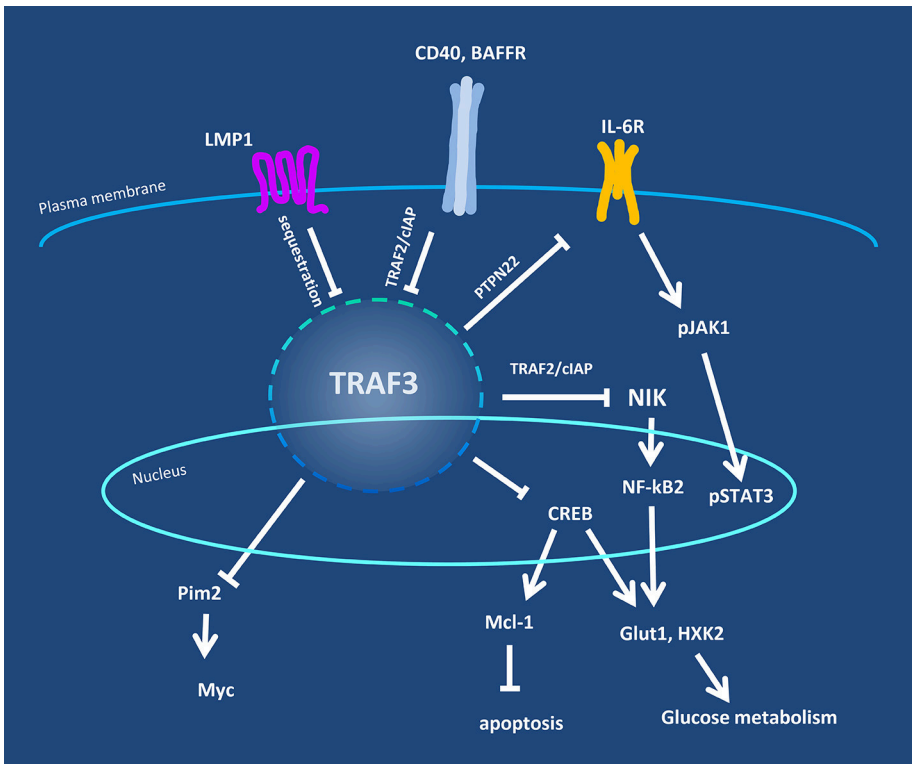


FIGURE 1 | Overview of TRAF3 regulatory pathways in B lymphocytes. Levels of TRAF3 protein and/or its availability in B cells are regulated by cell surface-expressed receptors, exemplified by CD40, BAFFR, and the viral protein LMP1. TRAF3 is in turn responsible for regulation of the activity of additional signaling proteins in the cytoplasm and nucleus, including NIK, Pim2, and CREB. Negative regulatory partners or mechanisms are indicated by the crossbar pointers.

TNFR2 (CD120b)

TNFR2 contributes to the activation of antibody secretion by B cells, mediating autocrine stimulation of B cells by CD40-induced TNF (35). TRAF3 is recruited to this receptor (36), and one role in its regulation of signaling by TNFR2 in B cells may be in the activation of JNK. TNFR2 activates both the canonical and non-canonical NF- κB pathways in B cell lines, although activation of the canonical pathway appears weak (36). TRAF2 contributes to NF- κB signaling activated by TNFR2, but TRAF3 may contribute as well, as B cells deficient in TRAF2 exhibit reduced, but not absent, TNFR2-mediated NF- κB2 activation (36). Further discussion is provided in the section on TRAF3 and B cell cytokine receptors, below.

4-1BB (CD137)

While signaling by 4-1BB (ligand = CD137L, 4-1BBL) has been evaluated in T cells, the effects of 4-1BB signaling in B cells are less well-characterized (reviewed in (37). In mammals, a small population of 4-1BB-expressing B cells expands with age, and in collaboration with cytotoxic T cells may be important in slowing tumor growth (38). 4-1BB, whose expression can be upregulated in B cells by various stimuli, is capable of enhancing B cell proliferation and TNF production [reviewed in (39)]. The cytoplasmic domain of 4-1BB binds TRAF1, TRAF2, and TRAF3 (37). However, the biological roles of TRAF3 in 4-1BB signaling in B cells remain unclear.

CD27

In humans, CD27 is a marker for memory B cells and promotes differentiation of B cells to plasma cells; its ligand is CD70 (40). In epithelial cells, CD27 has been shown to bind TRAF3 (41, 42), where it can potentially inhibit the activation of NF-κB mediated by TRAFs 2 and/or 5 (42). As is the case for 4-1BB, how TRAF3 regulates CD27 function in B cells remains undefined.

CD30

Small numbers of human tonsillar mononuclear cells are $CD30^{+}$ B cells. These cells appear to be a subpopulation of B cells that develop during normal germinal center reactions in lymphoid tissue, and share some transcriptional patterns with Hodgkin lymphoma cells (43). In non-B cell systems, CD30 can bind TRAFs 1, 2, and 3 (44, 45). In mouse T cells, engagement of CD30 by its ligand CD30L/CD153 can activate p38, JNK, and NF-κB. Interestingly, a dominant-negative TRAF2 could inhibit CD30-induced p38 and JNK activity, but not NF-κB activation (46). The role of TRAF3 in CD30 signaling in B cells is currently unclear.

Receptors for B Cell Activating Factor (BAFF) and a Proliferation-Inducing Ligand (APRIL)

TRAF3 plays a major role in signaling by at least one of the receptors for BAFF and APRIL. The cytoplasmic domain of BAFF receptor (BAFFR/CD268) interacts with at least three TRAF proteins, TRAF2, TRAF3, and TRAF6 (47). Engagement of TRAF2/3 by BAFFR leads to proteasomal degradation of TRAF3 and activation of the non-canonical NF- κB2 pathway (see section on NF-κB activation below). Targeted disruption of TRAF3 expression in B cells mimics treatment of normal B cells with BAFF, resulting in enhanced BAFF-independent B cell survival (29). However, experiments with mutant TRAF3 and mutant BAFFR molecules reveal that TRAF3 degradation may be neither necessary nor sufficient for NF-κB2 activation (48). BAFFR also activates the canonical NF-κB1 pathway in B cells, and this signaling event is mediated not by TRAF3, but by TRAF6 (47). Signaling by BAFFR also activates the kinases Syk, phosphatidyl inositol 3-kinase (PI3K), and ERK in B cells (49, 50). The activation of PI3K appears to be TRAF3-independent (50).

The TNFRSF receptors, transmembrane and CAML interactor (TACI) and B cell maturation antigen (BCMA), are also docking sites for BAFF, and a second TNF family member, APRIL. TACI is expressed by activated B cells and plasma cells (51), and interacts with TRAF3 (37). TACI-deficient mice display a marked increase in overall B cell numbers and increased antibody production, indicating an important role in B cell homeostasis (52–54). The role of TACI is complex, however, and not limited to negative regulation of B cells. In humans, *TACI* gene defects are detected in ∼8% of all cases of common-variable immune deficiency (51). TRAF3 inhibits the NF- κB2 pathway activated by TACI in a human kidney epithelial cell line (14). The role of TRAF3 in B cell TACI signaling remains to be described. Function of the BCMA receptor is critical for the long-term survival of antibody-secreting plasma cells (reviewed in (55, 56). While BCMA can bind TRAF3 in non-B cell over-expression systems (57, 58), the role of TRAF3 in BCMA signaling in B cells is not yet defined.

TRAF3 AND TLRS IN B CELLS

Toll-like receptors (TLRs) comprise a group of 13 transmembrane receptors in mammals, expressed on innate immune cells as well as B and T cells [reviewed in (59–61)]. These molecules initiate signaling cascades in response to binding molecules containing certain pathogen or disease associated molecular patterns, thereby regulating the production of type I interferons (IFNs) and other cytokines (60, 62, 63). TRAF6 was initially thought to be the single TRAF involved in TLR signaling (64), but it later became clear that TRAF3 interacts with the TLR pathway adapter proteins MyD88 and TRIF, and regulates TLR signaling using alternative pathways for each (60, 62, 65).

A key finding in the literature showing the importance of TRAF3 to TLR signaling is a case report describing a patient with a history of pediatric *Herpes simplex* viral encephalitis (66). Genetic analysis showed a single allele amino acid substitution in *TRAF3* that exerted a dominant negative effect by mediating a decrease in cellular levels of wild-type TRAF3 protein. The patient's myeloid cells were poor producers of type I IFNs when stimulated *in vitro* with the TLR ligands poly(I:C), LPS or R848, highlighting the importance of TRAF3 in these responses (66).

In studies utilizing mice with *Traf3* deleted specifically in either dendritic cells (DC) or B cells, it was observed that in the absence of TRAF3, DCs respond to signaling through TLRs 3,4,7 and 8 with either no change or decreases in IL6, TNF, and IL-10 (67). TRAF3-deficient DC exhibit a reduced activation-induced type I IFN response (65, 68). In contrast, TRAF3-negative B cells show increased production of TNFα, IL-6, and IL-10 (67). Thus, there is cell-type specific regulation of TLR-driven cytokine production by TRAF3. This difference extends to the IFN pathway, with TRAF3-deficient B cells producing more phosphorylated IFN-response factor 3 (IRF3) and IFNγ-induced protein 10 in response to stimulation through TLRs 3, 4, 7, and 9 (67). Furthermore, B cells lacking TRAF3 also show increased levels of activation induced deaminase and production of isotype-switched immunoglobulins in response to TLR stimulation (67).

In mouse macrophages, *Traf3* mRNA and protein expression is increased as a consequence of TLR2 stimulation (69). Increased TRAF3 levels are critical to enhanced IRF3 activation and *IFNβ* gene induction in response to subsequent signals through TLRs 3 or 4 (69). Conversely, TRAF3 levels can be depleted in B cells after engagement of CD40 and BAFFR, via ubiquitination and degradation of TRAF3 (reviewed in (33). Deubiquitinases can interrupt the process of TRAF3 degradation (70), as well as disrupt the function of signaling complexes containing TRAF3 (6). Thus, regulation of TRAF3 protein levels and modifications can act as a rheostat, affecting the outcome of signaling to B cells and other cell types via TLRs; the direction of the regulation seen appears to be both receptor and cell-type-specific.

TRAF3 AND B CELL CYTOKINE RECEPTORS

As discussed above, TRAF3 serves to regulate TLR signaling to B cells in various ways, impacting a number of downstream TLR-induced events, including cytokine production. However, the involvement of TRAF3 in regulating signals induced by the receptors for such cytokines is much less understood, particularly for the cell of focus in this review, the B lymphocyte.

The first known members of the TNFR superfamily are receptors for the cytokine TNF themselves—TNFR1/CD120a and TNFR2/CD120b. While CD120a is expressed in modest to undetectable levels on B cells, CD120b is robustly expressed (35). The roles played by TRAF2 in signaling to various cells by CD120b is well-documented [reviewed in (71)], including signals to B cells, in which TNFR2 plays important roles in Ig production (35, 36). Because TRAF2 binds CD120b, and TRAF2 often forms heterodimers with TRAF3, it was predicted that TRAF3 is also a CD120b-associated protein (71). This prediction was subsequently confirmed for HEK293 epithelial

cells transfected with plasmids encoding CD120b and TRAF3; in this system, TRAF3 inhibits NF-κB and JNK activation induced by exogenous over-expression of both CD120b and TRAF2 (72). In B lymphocytes, endogenously-expressed CD120b also binds TRAF3, and recruits this adapter to membrane lipid rafts (36). Similar to CD40 signaling to B cells, CD120b engagement induces both TRAF2 and TRAF3 degradation (36). However, how B cell TRAF3 regulates CD120b signaling in lymphocytes remains to be discovered, an important knowledge gap.

The receptor for the cytokine IL-17 binds TRAF3 when both the receptor and TRAF3 are exogenously overexpressed in the fibroblast cell line HeLa or the epithelial cell line HEK293 (73). Using the same model systems, it was subsequently shown that TRAF3 competes for IL-17 receptor binding with the pro-inflammatory kinase nuclear Dbf2-related kinase (74). Whether these associations can be confirmed for endogenous levels of these proteins in immune cell types will be of great interest for future investigation.

Conditional *Traf3*-deficient mice, produced by Cre-Lox technology (29), revealed two additional cytokine receptors that are regulated by TRAF3 in lymphocytes. Mice lacking TRAF3 in T cells have a 2-3-fold increase in natural T regulatory cells (Treg), attributable to enhanced IL-2 receptor (IL-2R) signaling to pre-Treg (75). In WT T cells, TRAF3 mediates recruitment of the phosphatase T cell protein tyrosine phosphatase (TCPTP, also known as PTPN2) to the IL-2R. TCPTP de-phosphorylates the IL-2R-associated Janus kinase (Jak) 2 and the transcription factor signal transducer and activator of transcription (Stat) 5 (75). Thus, in TRAF3-deficient T cells, there is enhanced Jak2 and Stat5 phosphorylation, and amplified signaling through the IL-2R (75). It is not yet known whether IL-2R signaling is altered by TRAF3 in B cells; given the striking cell-type specificity of TRAF3-mediated regulation, this is an interesting knowledge gap to be addressed.

The IL-6R is the cytokine receptor for which we have the most detailed understanding of the regulatory role for TRAF3 in B cells to date. Investigation of this relationship was prompted by the observation that B-*Traf3*$^{-/-}$ mice, in addition to their phenotype of increased homeostatic survival of all B cells (29, 30), display a 2-3-fold increase in CD138^{+} plasma cells (76). This increase disappears in B-*Traf3*$^{-/-}$ mice bred to IL-6$^{-/-}$ mice, although B-*Traf3*$^{-/-}$ mice have no increase in IL-6R levels, nor of serum IL-6 (76). These results implicated IL-6R signaling as responsible for the enlarged plasma cell compartment. Upon investigation it was revealed that B cells lacking TRAF3, similar to the situation with the IL-2R in TRAF3-deficient T cells described above, show elevated IL-6-induced phosphorylation of Jak1 and Stat3, the signaling molecule pair equivalent to that of Jak2 and Stat5 for the IL-2R. In both cases, normal human peripheral blood T or B cells transduced with siRNA targeting human TRAF3 also display increased lymphokine-mediated Stat phosphorylation (75, 76). As in T cells, the mechanistic explanation for this phenotype is that TRAF3 is recruited to the IL-6R upon cytokine binding, to which it recruits a phosphatase—in the case of B cell IL-6R, this is PTPN22 (76). Consistent with these results, *Ptpn22*$^{-/-}$ mice also display an increased plasma cell compartment and elevated pStat3 following IL-6 signaling (76). It will be exciting to determine how widespread this novel role for TRAF3 in recruitment of phosphatases is, and its specificity in regards to both cell and receptor type.

REGULATION OF NF- κB CYTOPLASMIC PATHWAYS

Most, if not all members of the TNFRSF are capable of activating NF- κB. In general, family members that interact with TRAF2 or TRAF6 induce the canonical NF-κB1 pathway through activation of inhibitor of kappa B (I κB) kinase β (IKKβ) (77). IKKβ is responsible for phosphorylating NF-κB inhibitory IκB proteins, flagging them for poly-ubiquitination and proteasomal degradation. Destruction of these inhibitors releases components of the canonical pathway, such as p50, to transit to the nucleus and initiate transcription of various genes [reviewed in (78)]. TNFRSF members such as CD40 and BAFFR that bind TRAF3, also often activate the non-canonical NF-κB2 pathway [reviewed in (79)]. Interestingly, in this pathway, TRAF3 serves a negative regulatory function crucial for normal B cell homeostasis [reviewed in (33)]. In the context of CD40 and BAFFR signaling, and potentially in signaling by other receptors that interact with TRAF3, the recruitment of TRAF3 to the receptor disrupts TRAF3's inhibitory activity in the cytoplasm, allowing activation of the NF-κB2 pathway. In unstimulated cells, TRAF3 forms a complex with TRAF2 and cellular inhibitors of apoptosis (cIAPs) 1 and/or 2 [reviewed in (80)]. This complex regulates the MAP3K NF-κB inducing kinase (NIK), an enzyme that phosphorylates and activates IKKα, which in turn is responsible for mediating the phosphorylation of p100, a precursor component of the NF-κB2 pathway. In unstimulated cells, the TRAF3/cIAP1/2 complex induces the post-translational modification of NIK with K48-linked poly-ubiquitin, targeting it for proteasomal degradation. This prevents NIK from contributing to the phosphorylation of p100 by activating IKKα, which would otherwise lead to p100 processing into active p52, an important component of the NF-κB2 pathway. Engagement of CD40, or other TNFRSF members that interact with TRAF3, can direct the ubiquitination activity of cIAP1/2 on to TRAF3 itself, resulting in its ubiquitination and degradation. The decrease in cytoplasmic TRAF3 leads to accumulation of NIK in the cytosol, which is then able to process p100, leading to translocation of p52 (often as a heterodimer with RelB) into the nucleus [reviewed in (80)]. The degradation of TRAF3 may not be strictly required for this to occur (48); its redirection away from NIK may suffice. TRAF3 may also help regulate the canonical NF-κB1 pathway, by interfering with the binding of other TRAFs to the cytoplasmic domains of stimulatory receptors such as CD40 (30). In addition to its regulation of NF-κB2, NIK appears to regulate NF-κB1 through its activation of the IKK complex (81). The regulation of NIK levels by TRAF3 may therefore also modulate NIK-mediated NF-κB1 activity.

B CELL TRAF3 AND NUCLEAR FUNCTIONS

TRAFs, with the exception of TRAF4, are generally considered to be cytoplasmic proteins, and their function has mostly been studied with respect to interactions that take place in the cytoplasm of cells. However, TRAF3, but not other TRAFs, associates with p62 nucleoporin in a HEK293T epithelial cell overexpression system (82). Recently B cell TRAF3 was revealed as a resident nuclear protein; in this role, it functions to restrain transcriptional activation mediated by cyclic AMP response element binding protein (CREB), with which it displays preferentially nuclear association (83). In TRAF3-deficient B cells, there are increased levels of nuclear CREB. This appears to be because in WT B cells, TRAF3 recruits TRAF2 to the nucleus to mediate K48-linked polyubiquitination of CREB, followed by CREB degradation (83). As a result, the pro-survival CREB target Mcl-1 is increased at both mRNA and protein levels in TRAF3-deficient B cells (83). This Mcl-1 increase is consistent with their enhanced homeostatic survival (29). TRAF3 was also shown to contain a functional nuclear localization sequence (NLS) in the TRAFC domain; transfection of TRAF3-deficient B cells with TRAF3 containing a mutated NLS allowed the generation of cells with TRAF3 located predominantly in the cytoplasm. These B cells also show increased CREB-regulated transcription, while cells transfected with WT TRAF3, present both in the cytoplasm and nucleus, do not (83). Although our focus in this review is B cells, it is worth noting that nuclear localization of TRAF3 has also been identified in endothelial cells and neurons (84–86) and TRAF3 forms a transcriptional complex with TRAF2, phospho-RNA Polymerase II and p65/RelA in Neuro2a cells activated through CD40 (84). CD40 is expressed on antigen-presenting cells, including B cells, so this result is of particular interest. It will be interesting and informative to identify additional nuclear binding partners and functions for TRAF3 in both B cells and other cell types.

TRAF3 AND B CELL METABOLISM

B lymphocytes are seldom studied as a key cell type regulating mammalian metabolism, so it is unsurprising that the impact of TRAF3 upon B cell metabolism is to date an understudied topic—but one with intriguing initial findings, as described below. In recent years, manipulation of amounts of TRAF3 in different cell types revealed TRAF3-mediated regulation of a number of metabolic events, leading to striking *in vivo* effects in animal models. In several mouse models of obesity, genetic deletion of *Traf3* in macrophages and neutrophils alleviates a number of hallmarks of obesity-related inflammation. These include insulin resistance, hyperglycemia, glucose intolerance and hepatic steatosis, as well as liver and adipose tissue production of inflammatory cytokines. Conversely, the amounts of these cytokines are increased in the liver and adipose tissue of lean mice (87). A similar pattern was seen for hepatocyte TRAF3, which is low in fasted mice, but increased when glucose levels are elevated by various metabolic manipulations. As with mice lacking myeloid TRAF3, deletion of hepatocyte TRAF3 reduces metabolic abnormalities seen in obese mouse models, while overexpression of TRAF3 in the liver induces metabolic abnormalities and suppresses insulin signaling (88).

Another non-immune cell type in which TRAF3 is reported to regulate metabolic pathways is neural stem cells. These cells are of interest because maternal diabetes is associated with increased neural tube defects, in which caspase-induced apoptosis is thought to play an important role. *Traf3* is a target of microRNA-322; in a mouse model of diabetes, maternal disease and high glucose decreases microRNA while *Traf3* expression and caspase-mediated apoptosis of neural stem cells is increased. Use of a microRNA-322 mimic or inhibition of *Traf3* expression blocks both these effects (89).

In B lymphocytes, TRAF3 functions to restrain rather than promote glucose metabolism, emphasizing the context-dependent nature of TRAF3 functions. TRAF3-deficient B cells express elevated levels of the glucose transporter Glut1 and the glycolytic enzyme Hexokinase 2 (HXK2) (90). This is relevant to the frequent loss of TRAF3 function in B cell malignancies (see below), and also with the well-discussed roles of HXK2, Glut1, and glucose metabolism in many types of cancers [reviewed in (91, 92)]. Consistent with their overexpression of Glut1 and HXK2, $TRAF3^{-/-}$ B cells show enhanced glucose uptake both *in vitro* and *in vivo* (90), as well as increased anaerobic glycolysis and oxidative phosphorylation, without changes in reactive oxygen species or mitochondrial mass (90). Interestingly, although the enhanced viability of $TRAF3^{-/-}$ B cells is not abrogated by deficiency in the TRAF3-regulated kinase NIK (48), TRAF3-controlled Glut1 levels and glucose uptake return to normal if TRAF3-deficient B cells are also rendered NIK-deficient (90).

Increased glucose utilization by $TRAF3^{-/-}$ B cells does contribute to their increased homeostatic survival, however, and renders them more sensitive than $TRAF3^{+/+}$ B cells to death induced by glucose deprivation (90). Human B cell lymphoma (BCL) cell lines also display an inverse correlation between Glut1 and TRAF3 expression, and cell lines with relatively lower TRAF3 expressed show increased sensitivity to glucose deprivation (90). As described above, B cell TRAF3 is a resident nuclear protein that induces degradation of CREB, hence inhibiting transcription of CREB-promoted survival proteins, such as Mcl-1 (83). In the absence of glucose, this upregulation of Mcl-1 induced by TRAF3 deficiency is abrogated (90). Thus, B cell TRAF3 as a metabolic reprogramming protein has particular relevance for B cell malignancies, discussed in more detail below.

TRAF3-MEDIATED REGULATION OF PIM2 AND C-MYC

As described above, TRAF3 limits B cell survival by altering the stability of key kinases and transcription factors through post-translational modification. Recently, it was found that TRAF3 also inhibits expression of the transcriptionally-regulated pro-survival kinase proviral insertion in murine lymphoma 2 (Pim2) (93, 94), a kinase required for the cytokine BAFF to promote B

cell survival (95). Interestingly, Pim2 is overexpressed in multiple human cancer types (96, 97), including the B cell malignancies most frequently associated with *TRAF3* deficiency—multiple myeloma (MM) and B cell lymphoma (BCL) (98, 99). TRAF3-deficient primary B cells, as well as MM and BCL cell lines, display an inverse relationship between TRAF3 and Pim2 protein levels (93, 94). TRAF3-deficient B cells have enhanced phosphorylation of the Pim2 targets Bcl-2-associated agonist of cell death (BAD), phospho-ribosomal protein S6 kinase beta-1 (p70S6K), and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) (93, 94), which abrogates BAD-induced apoptosis (100), and relieves p70S6K and 4E-BP1 -mediated translational repression (101). Transcription-independent elevation of the proto-oncogenic protein c-Myc is also observed in TRAF3-deficient B cells. This increase is associated with a striking decrease in K48-linked poly-ubiquitination of c-Myc in these cells. Interestingly, siRNA to Pim2 also reduces these increased levels of c-Myc (93, 94). Consistent with this relationship, combined pharmacological targeting of c-Myc and Pim2 proved significantly more effective in promoting B cell apoptosis than either alone, and TRAF3-deficient B cells are especially sensitive to these drugs (93, 94). The reported cardiac toxicity of single-agent Pim inhibition has limited its clinical utility (102); combining Pim inhbitors at lower doses with c-Myc inhibition could potentially address this problem, particularly in TRAF3-deficient B cell malignancies.

TRAF3 AND B CELL MALIGNANCIES

Human *TRAF3* mutations associated with B cell malignancies were first described in the plasma cell cancer, MM; the nature of such mutations is such that they are expected to be loss-of-function alternations (103, 104). It is now reported that *TRAF3* is one of the top ten mutated genes found in ∼ 65% of cases of human MM (105); overall, 15–20% of human MM display *TRAF3* mutations. *TRAF3* gene mutations or loss have also been reported in Hodgkin's disease (106), Waldenstrom's macroglobulinemia (107) and various types of BCL (108). The percentage of BCL with *TRAF3* mutations or deletions varies among studies, but up to 15% of human Diffuse Large BCL (DLBCL) examined show *TRAF3* genetic changes (109–111). Monoallelic deletions of *TRAF3* are the most common finding (107, 109, 110) and these deletions tend to be large, with mapped deletions from 13 human DLBCL showing a minimum common region of about 600 kb (110). A recent paper analyzing DLBCL by genetic subtype based on clusters of genetic changes showed that *TRAF3* gene loss is frequently, although not exclusively, associated with mutations in *BCL6* and *Notch2* (BN2 subtype) and *Notch1* (N1 subtype) of DLBCL (111). Non-Hodgkin lymphomas are the most common cancers in pet dogs (110). An examination of 84 such canine BCL showed an unexpectedly high 44% bearing *Traf3* mutations, with 30% of these being somatic changes and 14% single-allele germline mutations (110).

As discussed above and elsewhere in this issue, TRAF3 protein plays an important role in the regulation of B cell NF-κB2 activity. Thus, it is not surprising that *TRAF3* gene deletions and mutations in human B cell cancers correlate with an increased NF-κB transcriptional signature (104, 107, 112). However, enforced expression of the NF-κB2-activating kinase NIK in mouse germinal center B cells does not lead to rapid development of BCL, unless *Bcl6* over-expression is also enforced (109). Consistent with this finding, mice engineered to lack TRAF3 in their B cells (B-*Traf3*$^{-/-}$), described in earlier sections, do not develop spontaneous BCL until ∼ 8 months of age (113).

In B-*Traf3*$^{-/-}$ mice, B cells exhibit an abnormally long lifespan, resulting in accumulation of B cells in various tissues (29). In these mice, NF-κB2 activity is constitutively elevated in B cells (29). However, as mentioned earlier, the enhanced B cell lifespan is due not only to NF-κB2 activity, but also to other factors, including enhanced CREB activity, with the latter resulting in increased expression of the pro-survival protein Mcl-1 (83). These mice, with *Traf3* deleted in the transitional stage of B cell development using CD19^{Cre} (29), are particularly prone to develop high grade marginal zone BCL with high penetrance (113), consistent with their especially high accumulation of marginal zone B cells (29). These BCL are monoclonal or oligoclonal (113), indicating that absence of *Traf3* is not in itself sufficient to cause BCL, but the enhanced viability such loss confers upon B cells is likely to potentiate their extended survival in the presence of additional mutations.

While *TRAF3* genetic loss is associated with B cell malignancies, this is not the only mechanism by which a B cell can become TRAF3 protein-deficient, with the tumor-predisposing consequences discussed above. Our laboratory recently reported the results of TRAF3 protein staining of several 100 human DLBCL samples, which revealed that more than 30% of these BCL had low to undetectable TRAF3 protein expression (114). It was previously demonstrated that the EBV transforming protein LMP1 binds TRAF3 with considerably enhanced affinity, compared to the normal cellular receptor that it mimics, CD40 (21, 23). Thus, we examined whether B cell expression of LMP1 is associated with sequestration of TRAF3 in the plasma membrane, resulting in decreased availability of TRAF3 to downregulate various pro-survival signaling pathways discussed above; this was found to be the case (114). It is also well-documented that signaling to B cells via CD40 or BAFFR leads to poly-ubiquitination and degradation of TRAF3 [reviewed in (33)]. A decrease in TRAF3 protein expression in B cell tumors without detectable *TRAF3* gene changes could thus also be the result of chronic signaling through CD40 or BAFFR, or other receptors that activate TRAF3 degradation. This is an intriguing possibility for future investigation. Thus, many more B cell cancers may be impacted by the biologic pro-survival impact of TRAF3 deficiency than even the significant number impacted by *TRAF3* gene loss.

CONCLUSIONS

Although prior reviews have discussed TRAF3 functions in general [e.g., see (115, 116)], the underlying assumption has been that functions defined in one cell type or model system apply to all cell types and TRAF3-binding receptors. However,

as discussed in the present review, while some roles for TRAF3 overlap between cell types, there are many and varied biological roles for this pleiotropic signaling protein that are quite context-specific. TRAF3 is particularly important in regulating B lymphocytes, due to its B-cell-specific role in restraining homeostatic survival. As discussed above, TRAF3 also has many additional roles in B cell biology (**Figure 1**), many of which contribute to its increasingly-appreciated function as a B cell tumor suppressor. Our discussions above also highlight many interesting knowledge gaps that remain to be filled in understanding B cell TRAF3.

AUTHOR CONTRIBUTIONS

GB, BH, and LS all contributed to planning, writing, and editing this review. GB was responsible for final organization.

REFERENCES

1. Saha SK, Cheng G. TRAF3: a new regulator of type I IFNs. *Cell Cycle* (2006) 5:804–07. doi: 10.4161/cc.5.8.2637
2. Engel D, Seijkens T, Poggi M, Sanati M, Thevissen L, Beckers L, et al. The immunobiology of CD154-CD40-TRAF interactions in atherosclerosis. *Sem Immunol* (2009) 21:308–12. doi: 10.1016/j.smim.2009.06.004
3. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal.* (2013) 8:7. doi: 10.1186/1750-2187-8-7
4. Yi Z, Wallis AM, Bishop GA. Roles of TRAF3 in T cells: many surprises. *Cell Cycle* (2015) 14:1156–63. doi: 10.1080/15384101.2015.1021524
5. So T, Nagashima H, Ishii N. TRAF signaling network in $CD4^+$ T lymphocytes. *Tohoku J Exp Med.* (2015) 236:139–54. doi: 10.1620/tjem.236.139
6. Yang XD, Sun SC. Targeting signaling factors for degradation, an emerging mechanism for TRAF functions. *Immunol Rev.* (2015) 266:56–71. doi: 10.1111/imr.12311
7. Hu HM, O'Rourke K, Boguski MS, Dixit VM. A novel RING finger protein interacts with the cytoplasmic domain of CD40. *J Biol Chem.* (1994) 269:30069–72.
8. Cheng G, Cleary AM, Ye Z, Hong DI, Lederman S, Baltimore D. Involvement of CRAF1, a relative of TRAF, in CD40 signaling. *Science* (1995) 267:1494–8. doi: 10.1126/science.7533327
9. Mosialos G, Birkenback M, Yalamanchili R, VanArsdale T, Ware C, Kieff E. The EBV transforming protein LMP1 engages signaling proteins for the TNF-R family. *Cell* (1995) 80:389–99. doi: 10.1016/0092-8674(95)90489-1
10. Xu Y, Cheng G, Baltimore D. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity* (1996) 5:407–15. doi: 10.1016/S1074-7613(00)80497-5
11. Xie P, Kraus ZJ, Stunz LL, Liu Y, Bishop GA. TRAF3 is required for T cell-mediated immunity and TCR/CD28 signaling. *J Immunol.* (2011) 186:143–55. doi: 10.4049/jimmunol.1000290
12. Hostager BS, Bishop GA. Cutting Edge: Contrasting roles of TRAF2 and TRAF3 in CD40-mediated B lymphocyte activation. *J Immunol.* (1999) 162:6307–11.
13. He L, Grammer AC, Wu X, Lipsky PE. TRAF3 forms heterotrimers with TRAF2 and modulates its ability to mediate NF-κB activation. *J Biol Chem.* (2004) 279:55855–65. doi: 10.1074/jbc.M407284200
14. Hauer J, Püschner S, Ramakrishnan P, Simon U, Bongers M, Federle C, et al. TRAF3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF-κB pathway by TRAF-binding TNFRs. *Proc Natl Acad Sci USA.* (2005) 102:2874–9. doi: 10.1073/pnas.0500187102
15. Haxhinasto SA, Hostager BS, Bishop GA. Cutting Edge: molecular mechanisms of synergy between CD40 and the BCR: Role for TRAF2 in receptor interaction. *J Immunol.* (2002) 169:1145–9. doi: 10.4049/jimmunol.169.3.1145
16. Haxhinasto SA, Bishop GA. A novel interaction between PKD and TRAFs regulates BCR-CD40 synergy. *J Immunol.* (2003) 171:4655–62. doi: 10.4049/jimmunol.171.9.4655
17. L-Xu G, H.-Shu B, TRAF3 is associated with BAFF-R and negatively regulates BAFF-R-mediated NF-κB activation and IL-10 production. *J Immunol.* (2002) 169:6883–9. doi: 10.4049/jimmunol.169.12.6883
18. Pullen SS, T.Dang TA, Crute JJ, Kehry MR. CD40 signaling through TRAFs. Binding site specificity and activation of downstream pathways by distinct TRAFs. *J Biol Chem.* (1999) 274:14246–54. doi: 10.1074/jbc.274.20.14246
19. Rickert RC, Roes J, Rajewsky K. B lymphocyte-specific, Cre-mediated mutagenesis in mice. *Nucl Acids Res.* (1997) 25:1317–8. doi: 10.1093/nar/25.6.1317
20. Hostager BS, Haxhinasto SA, Rowland SR, Bishop GA. TRAF2-deficient B lymphocytes reveal novel roles for TRAF2 in CD40 signaling. *J Biol Chem.* (2003) 278:45382–90. doi: 10.1074/jbc.M306708200
21. Xie P, Hostager BS, Bishop GA. Requirement for TRAF3 in signaling by LMP1, but not CD40, in B lymphocytes. *J Exp Med.* (2004) 199:661–71. doi: 10.1084/jem.20031255
22. Ni C, Welsh K, Leo E, Wu H, Reed JC, Ely KR. Molecular basis for CD40 signaling mediated by TRAF3. *Proc Natl Acad Sci (USA).* (2000) 97:10395–99. doi: 10.1073/pnas.97.19.10395
23. Wu S, Xie P, Welsh K, Li C, Ni C, Zhu X, et al. LMP1 protein from EBV is a structural decoy in B lymphocytes for binding to TRAF3. *J Biol Chem.* (2005) 280:33620–6. doi: 10.1074/jbc.M502511200
24. Malinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF-κB induction by TNF, CD95, and IL-1. *Nature* (1997) 385:540–44. doi: 10.1038/385540a0
25. Yin L, Wu L, Wesche H, Arthur CD, White JM, Goeddel DV, et al. Defective LT-β receptor-induced NF-κB transcriptional activity in NIK-deficient mice. *Science* (2001) 291:2162–5. doi: 10.1126/science.1058453
26. Coope HJ, Atkinson GP, Huhse B, Belich M, Janzen J, Holman MJ, et al. CD40 regulates the processing of NF-κB2 p100 to p52. *EMBO J.* (2002) 21:5375–85. doi: 10.1093/emboj/cdf542
27. Razani B, Zarnegar B, Ytterberg AJ, Shiba T, Dempsey PW, Ware CF, et al. Negative feedback in noncanonical NF-κB signaling modulates NIK stability through IKKα-mediated phosphorylation. *Sci Signal* (2010) 3:ra41. doi: 10.1126/scisignal.2000778
28. Liao G, Zhang M, Harhaj EW, S.-Sun C. Regulation of NIK by TRAF3-induced degradation. *J Biol Chem.* (2004) 279:26243–50. doi: 10.1074/jbc.M403286200
29. Xie P, Stunz LL, Larison KD, Yang B, Bishop GA. TRAF3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. *Immunity* (2007) 27:253–67. doi: 10.1016/j.immuni.2007.07.012
30. Gardam S, Sierro S, Basten A, Mackay F, Brink R. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. *Immunity* (2008) 28:391–401. doi: 10.1016/j.immuni.2008.01.009
31. Xie P, Poovassery JS, Stunz LL, Smith SM, Schultz ML, Carlin LE, et al. Enhanced TLR responses of TRAF3-deficient B lymphocytes. *J Leukoc Biol.* (2011) 90:1149–57. doi: 10.1189/jlb.0111044
32. Lalani AI, Luo C, Han Y, Xie P. TRAF3: a novel tumor suppressor gene in macrophages. *Macrophage* (2015) 2:e1009. doi: 10.14800/macrophage.1009
33. Lin WW, Hostager BS, Bishop GA. TRAF3, ubiquitination, and B lymphocyte regulation. Immunol Rev (2015) 266:46-55. doi: 10.1111/imr.12299
34. Bishop GA, Hostager BS. The CD40-CD154 interaction in B cell-T cell liaisons. *Cytokine Growth Fact Rev.* (2003) 14:297–309. doi: 10.1016/S1359-6101(03)00024-8
35. Hostager BS, Bishop GA. Role of TRAF2 in the activation of IgM secretion by CD40 and CD120b. *J Immunol.* (2002) 168:3318–22. doi: 10.4049/jimmunol.168.7.3318
36. Munroe ME, Bishop GA. Role of TRAF2 in distinct and overlapping CD40 and TNFR2/CD120b-mediated B lymphocyte activation. *J Biol Chem.* (2004) 279:53222–31. doi: 10.1074/jbc.M410539200

37. Yi Z, Lin WW, Stunz LL, Bishop GA. Roles for TRAF3 in lymphocyte functions. *Cytokine Growth Fact Rev.* (2014) 25:146–56. doi: 10.1016/j.cytogfr.2013.12.002
38. Lee-Chang C, Bodogai M, Moritoh K, Olkhanud PB, Chan AC, Croft M, et al. Accumulation of 4-1BBL$^+$ B cells in the elderly induces the generation of granzyme-B$^+$ CD8$^+$ T cells with potential antitumor activity. *Blood* (2014) 124:1450–9. doi: 10.1182/blood-2014-03-563940
39. Vinay DS, Kwon BS. 4-1BB signaling beyond T cells. *Cell Mol Immunol.* (2011) 8:281–84. doi: 10.1038/cmi.2010.82
40. Nolte MA, van Olffen RW, van Gisbergen KP, van Lier RA. Timing and tuning of CD27-CD70 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology. *Immunol Rev.* (2009) 229:216–31. doi: 10.1111/j.1600-065X.2009.00774.x
41. Akiba H, Nakano H, Nishinaka S, Sindo M, Kobata T, Tasuta C, et al. CD27, a member of the TNF-R superfamily, activates NF-κB and SAPK/JNK via TRAF2, TRAF5, and NIK. *J Biol Chem.* (1998) 273:13353–8. doi: 10.1074/jbc.273.21.13353
42. Yamamoto H, Kishimoto T, Minamoto S. NF-kB activation in CD27 signaling: Involvement of TRAFs in its signaling and identification of functional region of CD27. *J Immunol* (1998) 161:4753–9.
43. Weniger MA, Tiacci E, Schneider S, Arnolds J, Ruschenbaum S, Duppach J, et al. Human CD30$^+$ B cells represent a unique subset related to Hodgkin lymphoma cells. *J Clin Invest.* (2018) 128:2996–3007. doi: 10.1172/JCI95993
44. Gedrich RW, Gilfillan MC, Duckett CS, Van Dongen JL, Thompson CB. CD30 contains two binding sites with different specificities for members of the TRAF family of signal transducing proteins. *J Biol Chem.* (1996) 271:12852–8. doi: 10.1074/jbc.271.22.12852
45. Boucher LM, Marengere LE, Lu Y, Thukral S, Mak TW. Binding sites of cytoplasmic effectors TRAF1, 2, and 3 on CD30 and other members of the TNFR superfamily. *Biochem Biophys Res Commun.* (1997) 233:592–600. doi: 10.1006/bbrc.1997.6509
46. Harlin H, Podack E, Boothby M, Alegre ML. TCR-independent CD30 signaling selectively induces IL-13 production via a TRAF/p38 MAPK-dependent mechanism. *J Immunol.* (2002) 169:2451–60. doi: 10.4049/jimmunol.169.5.2451
47. Hildebrand JM, Luo Z, Manske MK, Price-Troska T, Ziesmer SC, Lin W, et al. A BAFF-R mutation associated with Non-Hodgkin's lymphoma exhibits altered TRAF association and reveals new insights into proximal BAFF-R signaling. *J Exp Med.* (2010) 207:2569–79. doi: 10.1084/jem.20100857
48. Lin WW, Hildebrand JM, Bishop GA. A complex relationship between TRAF3 and non-canonical NF-κB2 activation in B lymphocytes. *Front Immunol.* (2013) 4:477. doi: 10.3389/fimmu.2013.00477
49. Schweighoffer E, Vanes L, Nys J, Cantrell DA, McCleary S, Smithers N, et al. The BAFFR transduces survival signals by co-opting the BCR signaling pathway. *Immunity* (2013) 38:475–88. doi: 10.1016/j.immuni.2012.11.015
50. Jellusova J, Miletic AV, Cato MH, Lin WW, Hu Y, Bishop GA, et al. Context-specific BAFF-R signaling by the NF-κB and PI3K pathways. *Cell Rep.* (2013) 5:1022–34. doi: 10.1016/j.celrep.2013.10.022
51. Mackay F, Schneider P. TACI, an enigmatic BAFF/APRIL receptor, with new unappreciated biochemical and biological properties. *Cyt. Growth Factor Rev.* (2008) 19:263–76. doi: 10.1016/j.cytogfr.2008.04.006
52. Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T. et al. Activation and accumulation of B cells in TACI-deficient mice. *Nat Immunol.* (2001) 2:638–43. doi: 10.1038/89790
53. von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral immune response by TACI. *Immunity* (2001) 14:573–82. doi: 10.1016/S1074-7613(01)00130-3
54. Seshasayee D, Valdez P, Yan M, Dixit VM, Tumas D, Grewal IS. Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor. *Immunity* (2003) 18:279–88. doi: 10.1016/S1074-7613(03)00025-6
55. Coquery CM, Erickson LD. Regulatory roles of the TNFR BCMA. *Crit Rev Immunol.* (2012) 32:287–305. doi: 10.1615/CritRevImmunol.v32.i4.10
56. Sakai J, Akkoyunlu M. The role of BAFF system molecules in host response to pathogens. *Clin Microbiol Rev.* (2017) 30:991–1014. doi: 10.1128/CMR.00046-17
57. Hatzoglou A, Roussel J, Bourgeade MF, Rogier E, Madry C, Inoue J, et al. TNFR family member BCMA associates with TRAF1, TRAF2, and TRAF3 and activates NF-B, elk-1, JNK, and p38 MAPK. *J Immunol.* (2000) 165:1322–30. doi: 10.4049/jimmunol.165.3.1322
58. Shu HB, Johnson H. BCMA is a receptor for the TNF family member TALL-1. *Proc Natl Acad Sci USA.* (2000) 97:9156–61. doi: 10.1073/pnas.160213497
59. Takeda K, Akira S. TLRs in innate immunity. *Int Immunol.* (2005) 17:1–14. doi: 10.1093/intimm/dxh186
60. Buchta CM, Bishop GA. TLRs and B cells: functions and mechanisms. *Immunol Res.* (2014) 59:12–22. doi: 10.1007/s12026-014-8523-2
61. Rahman AH, Taylor DK, Turka LA. The contribution of direct TLR signaling to T cell responses. *Immunol Res.* (2009) 45:25–36. doi: 10.1007/s12026-009-8113-x
62. Häcker H, Redecke V, Blagoev B, Kratchmarova I, Hsu LC, Wang GG, et al. Specificity in TLR signalling through distinct effector functions of TRAF3 and TRAF6. *Nature* (2006) 439:204–7. doi: 10.1038/nature04369
63. Perkins DJ, Vogel SN. Space and time: New considerations about the relationship between TLRs and type I IFNs. *Cytokine* (2015) 74:171–4. doi: 10.1016/j.cyto.2015.03.001
64. Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* (1998) 2:253–8. doi: 10.1016/S1097-2765(00)80136-7
65. Oganesyan G, Saha SK, Guo B, He JQ, Shahangian A, Zarnegar B, et al. Critical role of TRAF3 in the TLR-dependent and independent antiviral response. *Nature* (2006) 439:208–11. doi: 10.1038/nature04374
66. Pérez de Diego R, Sancho-Shimizu V, Lorenzo L, Puel A, Plancoulaine S, Picard C, et al. Human TRAF3 adaptor molecule deficiency leads to impaired TLR3 response and susceptibility to Herpes simplex encephalitis. *Immunity* (2010) 33:400–11. doi: 10.1016/j.immuni.2010.08.014
67. Xie P, Brown L, Stunz L, Bishop G. TRAF3 inhibits signaling by TLRs in B lymphocytes. *FASEB J.* (2008) 22.
68. Hildebrand JM, Yi Z, Buchta CM, Poovassery JS, Stunz LL, Bishop GA. Roles of TRAF3 and TRAF5 in immune cell function. *Immunol Rev.* (2011) 244:55–74. doi: 10.1111/j.1600-065X.2011.01055.x
69. Perkins DJ, Polumuri SK, Pennini ME, Lai W, Xie P, Vogel SN. Reprogramming of murine macrophages through TLR2 confers viral resistance via TRAF3-mediated, enhanced interferon production. *PLoS Pathog.* (2013) 9:e1003479. doi: 10.1371/journal.ppat.1003479
70. Hu H, Brittain GC, Chang JH, Puebla-Osorio N, Jin J, Zal A, et al. OTUD7B controls non-canonical NF-κB activation through deubiquitination of TRAF3. *Nature* (2013) 494:371–4. doi: 10.1038/nature11831
71. Cabal-Hierro L, Lazo PS. Signal transduction by TNF receptors. *Cell Signal.* (2012) 24:1297–305. doi: 10.1016/j.cellsig.2012.02.006
72. Cabal-Hierro L, Rodriguez M, Artime N, Iglesias J, Ugarte L, Prado MA. et al. TRAF-mediated modulation of NF-κB and JNK activation by TNFR2. *Cell Signal.* (2014) 26:2658–66. doi: 10.1016/j.cellsig.2014.08.011
73. Zhu S, Pan W, Shi P, Gao H, Zhao F, Song X, et al. Modulation of EAE through TRAF3-mediated suppression of IL-17 receptor signaling. *J Exp Med.* (2010) 207:2647–62. doi: 10.1084/jem.20100703
74. Ma C, Lin W, Liu Z, Tang W, Gautam R, Li H, et al. NDR1 protein kinase promotes IL-17- and TNF-α-mediated inflammation by competitively binding TRAF3. *EMBO Rep.* (2017) 18:586–602. doi: 10.15252/embr.201642140
75. Yi Z, Lin WW, Stunz LL, Bishop GA. The adaptor TRAF3 restrains the lineage determination of thymic regulatory T cells by modulating signaling via the receptor for IL-2. *Nat Immunol.* (2014) 15:866–74. doi: 10.1038/ni.2944
76. Lin WW, Yi Z, Stunz LL, Maine CJ, Sherman LA, Bishop GA. The adaptor protein TRAF3 inhibits IL-6 receptor signaling in B cells to limit plasma cell development. *Sci Signal.* (2015) 8:ra88. doi: 10.1126/scisignal.aaa5157
77. Bishop GA. TRAF3 as a powerful and multi-talented regulator of lymphocyte functions. *J Leuk Biol.* (2016) 100:919–26. doi: 10.1189/jlb.2MR0216-063R
78. Bonizzi G, Karin M. The two NF-κB activation pathways and their role in innate and adaptive immunity. *TRENDS Immunol.* (2004) 25:280–8. doi: 10.1016/j.it.2004.03.008
79. Bishop GA, Stunz LL, Hostager BS. TNFR superfamily signaling pathways in immune cells. *Encyclopedia Immunobiol.* (2016) 3:115–23. doi: 10.1016/B978-0-12-374279-7.11016-1
80. Sun SC. The noncanonical NF-κB pathway. *Immunol Rev.* (2012) 246:125–40. doi: 10.1111/j.1600-065X.2011.01088.x

81. Zarnegar B, Yamazaki S, He JQ, Cheng G. Control of canonical NF-kB activation through the NIK-IKK pathway. *Proc Natl Acad Sci USA*. (2008) 105:3503–8. doi: 10.1073/pnas.0707959105
82. Gamper C, van Eyndhoven WG, Schweiger E, Mossbacher M, Koo B, Lederman S. TRAF-3 interacts with p62 nucleoporin, a component of the nuclear pore central plug thta binds classical NLS-containing import complexes. *Mol Immunol*. (2000) 37:73–84. doi: 10.1016/S0161-5890(00)00015-8
83. Mambetsariev N, Lin WW, Stunz LL, Hanson BM, Hildebrand JM, Bishop GA. Nuclear TRAF3 is a negative regulator of CREB in B cells. *Proc Natl Acad Sci USA*. (2016) 113:1032–7. doi: 10.1073/pnas.1514586113
84. El Hokayem J, Brittain GC IV, Nawaz Z, Bethea JR. TRAFs 2 and 3 form a transcriptional complex with phosho-RNA polymerase II and p65 in CD40L activated Neuro2a cells. *Mol Neurobiol*. (2017) 54:1301–13. doi: 10.1007/s12035-016-9742-4
85. Urbich C, Mallat Z, Tedgui A, Clauss M, Zeiher AM, Dimmeler S. Upregulation of TRAF-3 by shear stress blocks CD40-mediated endothelial activation. *J Clin Invest*. (2001) 108:1451–8. doi: 10.1172/JCI200113620
86. Wu Y, Zheng M, Wang S, Song C, Wang C, Xiao Y, et al. Spatiotemporal pattern of TRAF3 expression after rat spinal cord injury. *J Mol Histol*. (2014) 45:541–53. doi: 10.1007/s10735-014-9575-2
87. Chen Z, Shen H, Sun C, Yin L, Tang F, Zheng P, et al. Myeloid cell TRAF3 promotes metabolic inflammation, insulin resistance, and hepatic steatosis in obesity. *Am J Physiol Endocrinol Metab*. (2015) 308:E460–9. doi: 10.1152/ajpendo.00470.2014
88. Chen Z, Canet MJ, Sheng L, Xiong Y, Yin L, Rui L. Hepatocyte TRAF3 promotes insulin resistance and type 2 diabetes in mice with obesity. *Mol Metab*. (2015) 4:951–60. doi: 10.1016/j.molmet.2015.09.013
89. Gu H, Yu J, Dong D, Zhou Q, Wang JY, Yang P. The miR-322-TRAF3 circuit mediates the pro-apoptotic effect of high glucose on neural stem cells. *Toxicol Sci*. (2015) 144:186–96. doi: 10.1093/toxsci/kfu271
90. Mambetsariev N, Lin WW, Wallis AM, Stunz LL, Bishop GA. TRAF3 deficiency promotes metabolic reprogramming in B cells. *Nat Sci Rep*. (2016) 18:35349. doi: 10.1038/srep35349
91. Mathupala SP, Ko YH, Pedersen PL. Hexokinase II: Cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. *Oncogene* (2006) 25:4777–86. doi: 10.1038/sj.onc.1209603
92. Hamanaka RB, Chandel NS. Targeting glucose metabolism for cancer therapy. *J Exp Med*. (2012) 209:211–5. doi: 10.1084/jem.20120162
93. Mambetsariev N. *Mechanisms of TRAF3-Mediated Regulation of B Cell Survival*. Doctoral dissertation, The University of Iowa (2016).
94. Whillock AL, Mambetsariev N, Lin WW, Stunz LL, Bishop GA. TRAF3 regulates Pim2 and c-Myc-mediated B cell survival. *J Immunol*. (2018) 200 (Suppl 1) 116.7.
95. Woodland RT, Fox CJ, Schmidt MR, Hammerman PS, Opferman JT, Korsmeyer SJ, et al. Multiple signaling pathways promote BLyS-dependent B-cell growth and survival. *Blood* (2008) 111:750–60. doi: 10.1182/blood-2007-03-077222
96. Amaravadi R, Thompson CB. The survival kinases Akt and Pim as potential pharmacological targets. *J Clin Invest*. (2005) 115:2618–24. doi: 10.1172/JCI26273
97. Nawijn M, Alendar A, Berns A. For better or worse: the role of pim oncogenes in tumorigenesis. *Nat Rev Cancer* (2011) 11:23–34. doi: 10.1038/nrc2986
98. Keane NA, Reidy M, Natoni A, Raab MS, O'Dwyer M. Targeting the Pim kinases in MM. *Blood Cancer J*. (2015) 5:e325. doi: 10.1038/bcj.2015.46
99. Jinesh GG, Mokkapati S, Zhu K, Morales EE. Pim kinase isoforms: devils defending cancer cells from therapeutic and immune attacks. *Apoptosis* (2016) 21:1203–13. doi: 10.1007/s10495-016-1289-3
100. Yan B, Zemskova M, Holder S, Chin V, Kraft A, Koskinen PJ, et al. THe PIM-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. *J Biol Chem*. (2003) 278:45358–67. doi: 10.1074/jbc.M307933200
101. Fox CJ, Hammerman PS, Thompson CB. The pim kinases control rapamycin-resistant T cell survival and activation. *J Exp Med*. (2005) 201:259–66. doi: 10.1084/jem.20042020
102. Mondello P, Cuzzocrea S, Mian M. Pim kinases in hematological malignancies: where are we now and where are we going? *J Hematol Oncol*. (2014) 7:95. doi: 10.1186/s13045-014-0095-z
103. Annunziata CM, Davis RE, Demchenko Y, Bellamy W, Gabrea A, Zhan F, et al. Frequent engagement of the classical and alternative NF-κB pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* (2007) 12:115–30. doi: 10.1016/j.ccr.2007.07.004
104. Keats JJ, Fonesca R, Chesi M, Schop R, Baker A, W.-Chng J, et al. Promiscuous mutations activate the noncanonical NF-kB pathway in multiple myeloma. *Cancer Cell* (2007) 12:131–44. doi: 10.1016/j.ccr.2007.07.003
105. San Miguel JF. Introduction to a series of reviews on multiple myeloma. *Blood* (2015) 125:3039–40. doi: 10.1182/blood-2015-01-613596
106. Otto C, Giefing M, Massow A, Vater I, Gesk S, Schlesner M, et al. Genetic lesions of the TRAF3 and MAP3K14 genes in classical HL. *Br J Hematol*. (2012) 157:702–8. doi: 10.1111/j.1365-2141.2012.09113.x
107. Braggio E, Keats JJ, Leleu X, Van Wier S, Jimenez-Zepeda VH, Valdez R, et al. Identification of copy number abnormalities and inactivating mutations in two negative regulators of NF-kB signaling pathways in Waldenstrom's Macroglobulinemia. *Cancer Res*. (2009) 69:3579–88. doi: 10.1158/0008-5472.CAN-08-3701
108. Vaque JP, Martinez N, Batlle-Lopez A, Perez C, Montes-Moreno S, Sanchez-Beato M, et al. B-cell lymphoma mutations: improving diagnostics and enabling targeted therapies. *Haematologica* (2014) 99:222–31. doi: 10.3324/haematol.2013.096248
109. Zhang B, Calado DP, Wang Z, Fröhler S, Köchert K, Qian Y, et al. An oncogenic role for alternative NF-kB siganling in DLBCL revealed upon deregulated BCL6 expression. *Cell Rep*. (2015) 11:715–26. doi: 10.1016/j.celrep.2015.03.059
110. Bushell KR, Kim Y, Chan FC, Ben-Neriah S, Jenks A, Alcaide M, et al. Genetic inactivation of TRAF3 in canine and human B-cell lymphoma. *Blood* (2015) 125:999–1005. doi: 10.1182/blood-2014-10-602714
111. Schmitz R, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ, et al. Genetics and pathogenesis of DLBCL. *N Engl J Med*. (2018) 378:1396–407. doi: 10.1056/NEJMoa1801445
112. Nagel D, Vincendeau M, Eitelhuber AC, Krappmann D. Mechanisms and consequences of constitutive NF-κB activation in B-cell lymphoid malignancies. *Oncogene* (2014) 33:5655–65. doi: 10.1038/onc.2013.565
113. Moore CR, Liu Y, Shao C, Covey LR, Morse HC, III, Xie P. Specific deletion of TRAF3 in B lymphocytes leads to B-lymphoma development in mice. *Leukemia* (2012) 26:1122–7. doi: 10.1038/leu.2011.309
114. Bangalore-Prakash P, Stunz LL, Mambetsariev N, Whillock AL, Hostager BS, Bishop GA. The oncogenic membrane protein LMP1 sequesters TRAF3 in B-cell lymphoma cells to produce functional TRAF3 deficiency. *Blood Adv*. (2017) 1:2712–3. doi: 10.1182/bloodadvances.2017009670
115. He JQ, Oganesyan G, Saha SK, Zarnegar B, Cheng G. TRAF3 and its biological function. *Adv Exp Biol Med*. (2007) 597:48–59. doi: 10.1007/978-0-387-70630-6_4
116. Häcker H, Tseng PH, Karin M. Expanding TRAF function: TRAF3 as a tri-faced immune regulator. *Nat Rev Immunol*. (2011) 11:457–68. doi: 10.1038/nri2998

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 28 September 2018
doi: 10.3389/fimmu.2018.02263

Bone Remodeling and the Role of TRAF3 in Osteoclastic Bone Resorption

Brendan F. Boyce, Jinbo Li, Lianping Xing and Zhenqiang Yao*

Department of Pathology and Laboratory Medicine, Center for Musculoskeletal Research, University of Rochester Medical Center, Rochester, NY, United States

Skeletal health is maintained by bone remodeling, a process in which microscopic sites of effete or damaged bone are degraded on bone surfaces by osteoclasts and subsequently replaced by new bone, which is laid down by osteoblasts. This normal process can be disturbed in a variety of pathologic processes, including localized or generalized inflammation, metabolic and endocrine disorders, primary and metastatic cancers, and during aging as a result of low-grade chronic inflammation. Osteoclast formation and activity are promoted by factors, including cytokines, hormones, growth factors, and free radicals, and require expression of macrophage-colony stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL) by accessory cells in the bone marrow, including osteoblastic and immune cells. Expression of TNF receptor-associated factor 6 (TRAF6) is required in osteoclast precursors to mediate RANKL-induced activation of NF-κB, which is also necessary for osteoclast formation and activity. TRAF3, in contrast is not required for osteoclast formation, but it limits RANKL-induced osteoclast formation by promoting proteasomal degradation of NF-κB-inducing kinase in a complex with TRAF2 and cellular inhibitor of apoptosis proteins (cIAP). TRAF3 also limits osteoclast formation induced by TNF, which mediates inflammation and joint destruction in inflammatory diseases, including rheumatoid arthritis. Chloroquine and hydroxychloroquine, anti-inflammatory drugs used to treat rheumatoid arthritis, prevent TRAF3 degradation in osteoclast precursors and inhibit osteoclast formation *in vitro*. Chloroquine also inhibits bone destruction induced by ovariectomy and parathyroid hormone in mice *in vivo*. Mice genetically engineered to have TRAF3 deleted in osteoclast precursors and macrophages develop early onset osteoporosis, inflammation in multiple tissues, infections, and tumors, indicating that TRAF3 suppresses inflammation and tumors in myeloid cells. Mice with TRAF3 conditionally deleted in mesenchymal cells also develop early onset osteoporosis due to a combination of increased osteoclast formation and reduced osteoblast formation. TRAF3 protein levels decrease in bone and bone marrow during aging in mice and humans. Development of drugs to prevent TRAF3 degradation in immune and bone cells could be a novel therapeutic approach to prevent or reduce bone loss and the incidence of several common diseases associated with aging.

Keywords: osteoclast, osteoblast, NF-kappaB, RANK, TNF, TRAF3, TRAF6

OPEN ACCESS

Edited by:
Gail Abendroth Bishop,
University of Iowa, United States

Reviewed by:
Bruce Hostager,
University of Iowa, United States
Parameswaran Ramakrishnan,
Case Western Reserve University, United States

****Correspondence:***
Brendan F. Boyce
brendan_boyce@urmc.rochester.edu

Specialty section:
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

Received: *10 July 2018*
Accepted: *11 September 2018*
Published: *28 September 2018*

Citation:
Boyce BF, Li J, Xing L and Yao Z (2018) Bone Remodeling and the Role of TRAF3 in Osteoclastic Bone Resorption. Front. Immunol. 9:2263. doi: 10.3389/fimmu.2018.02263

INTRODUCTION

The skeleton provides support for propulsion by skeletal muscles as well as vital protection for internal organs, including the brain and heart. It is also a repository for calcium and other elements that get deposited in bone as it mineralizes during bone formation and are released from bone when it is being remodeled. In this way, bone participates in the control of calcium levels in the blood and tissues (1) to mediate numerous cellular functions, including contraction of skeletal and cardiac muscles (2). Bone remodeling is a normal physiological process that maintains skeletal integrity after skeletal development by removing small foci of damaged or effete bone from bone surfaces and replacing them with new bone (3, 4). By this mechanism, the skeleton is continuously renewed throughout life.

During embryonic development, bone is formed by osteoblasts, specialized mesenchyme-derived cells that lay down layers (lamellae) of matrix composed of mainly type 1 collagen (3, 5), which is mineralized a few days later. Numerous other non-collagenous proteins are also deposited in the bone matrix, including osteocalcin, sialoproteins, glycoproteins, proteoglycans, TGFβ, bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs) (6, 7). These proteins and minerals are released from bone during bone resorption and in increased amounts in numerous pathologic processes in which bone destruction is elevated. They can influence the behavior of cells in the bone microenvironment and outside the skeleton, particularly in pathologic processes in which remodeling is increased (5, 8, 9). During development, long bones are formed initially in cartilage molds roughly in the shape that the bones will have before birth (3). The cartilage is resorbed by TRAP-positive osteoclasts, but it is also removed by chondroclasts, poorly characterized tartrate–resistant acid phosphatase (TRAP)-negative cells that perform this function in mice in the absence of RANKL or RANK expression (10, 11). This is based on remodeling and removal of the much of the cartilage in bone metaphyses beneath growth plates and its replacement by bone in the absence of TRAP-positive cells in RANKL$^{-/-}$ and RANK$^{-/-}$ (**Figure 1**) mice (10, 11). Growth plates form at the proximal and distal ends of embryonic long bones. These plates consist of columns of small resting proliferating chondrocytes and larger hypertrophic chondrocytes, which lay down matrix that calcifies at the interface between them and the bone marrow (3). This calcified matrix is resorbed and replaced by bone, which also is calcified. Hypertrophic chondrocytes express RANKL to attract osteoclast precursors from adjacent sinusoids in the marrow (10), and like osteoblastic cells, they also express osteoprotegerin (OPG) (12, 13), a decoy receptor for RANKL, that prevents RANKL binding to RANK to limit osteoclast formation (4, 10). 1, 25 dihydroxy Vitamin D3, BMP2 and Wnt/β-catenin signaling proteins also are expressed by these chondrocytes in which they regulate expression of RANKL (10).

In the adult skeleton, bone remodeling begins with removal of microscopic foci of calcified bone matrix by osteoclasts, which form trenches on bone surfaces, called resorption lacunae. Osteoclast formation requires expression of M-CSF and RANKL by accessory cells in the bone microenvironment and of their receptors by osteoclast precursors (4, 14). Signaling downstream from these receptors regulates the differentiation of osteoclast precursors into osteoclasts as well as the resorptive activity and survival of osteoclasts. The M-CSF receptor is a tyrosine kinase that phosphorylates and activates downstream signaling molecules (15). In contrast, RANK does not possess kinase activity and recruits TRAFs, which are adaptor proteins that form complexes that activate mitogen-activated protein kinases (MAPKs), NF-κB and activator protein-1 (AP-1) signaling (16). TRAFs play important positive and negative regulatory roles in RANKL-induced osteoclast formation and activation (16, 17) in normal bone remodeling and in many pathologic processes affecting the skeleton in which bones can weaken to the point where they can fracture readily. This review will briefly describe the mechanisms that regulate bone remodeling, with emphasis on osteoclast formation in normal and pathologic processes, and the roles that TRAFs play in osteoclast and osteoblast formation and function, focusing on the evolving roles of TRAF3.

BONE REMODELING

In response to normal wear and tear and mechanical forces and the aging process, bone is continuously remodeled in the adult skeleton by a process in which damaged or effete microscopic portions of bone are removed by osteoclasts and subsequently are replaced by new bone, which is laid down by osteoblasts (3, 18, 19). On trabecular surfaces of spongy (cancellous) bone, bone remodeling units (BRUs) are trench-shaped structures that osteoclasts form by degrading the matrix. They erode to a mean depth of ∼60 μm and then tend to work their way along lamellae of collagen, which were laid down previously by osteoblasts, and typically create relatively smooth-bottomed trenches during normal remodeling. The bases of these trenches are marked by the reversal line, a dark line seen in sections stained with H&E and other stains. Osteoclastic resorption is less orderly in pathologic processes in which resorption rates are increased, resulting in reversal lines that are typically irregular and can give the bone a mosaic pattern, seen most classically in Paget's disease of bone (20). Osteoclasts also remodel the more dense cortical bone that encases and protects spongy bone by forming roughly circular tunnels through it. These tunnels are almost completely filled in with new bone to form structures called osteons, which have a small central nutrient artery and vein. This remodeling process involves complex interactions between osteoclastic and osteoblastic cells that couple bone formation to these sites of resorption where coupling factors released from the bone matrix and by osteoclasts attract osteoblast precursors to the site (5).

To initiate bone resorption, osteoclasts first produce hydrochloric acid, which dissolves the mineral in bone, and then they secrete metalloproteases, which breakdown the collagenous matrix (10). Osteoclasts secrete H^+ ions through protons pumps and Cl^- ions pass through chloride channels on the cell membrane on their undersurface adjacent to calcified bone (10, 21). Mutations in the genes involved in matrix demineralization and dissolution account for the majority of human cases of osteopetrosis (3, 10, 21, 22). The osteoclast cell

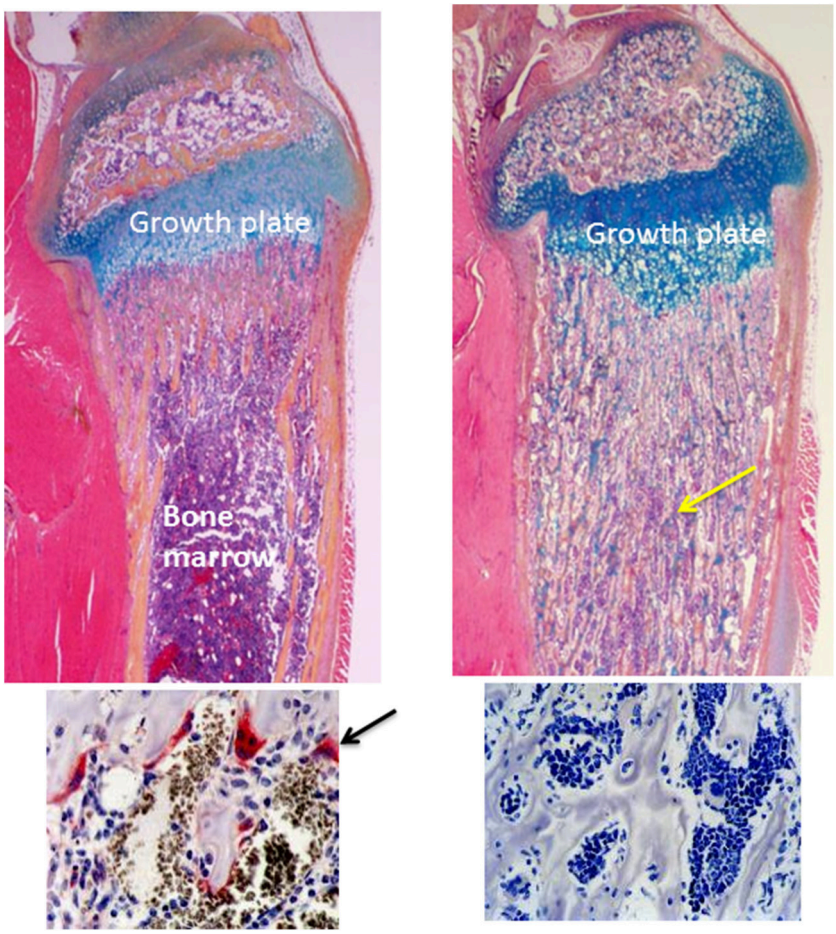


FIGURE 1 | Normal and osteopetrotic tibial bones from wild-type and RANK$^{-/-}$ mice. The upper panels are H&E-stained longitudinal sections of tibiae from 4-weeks-old mice showing a normal growth plate and underlying metaphyseal trabecular bone and bone marrow from a wild-type mouse (left panel) and a thickened growth plate and unremodeled osteopetrotic bone (yellow arrow) filling the medullary cavity from a RANK$^{-/-}$ mouse (right panel). The lower panels are TRAP-stained sections of the bone beneath the growth plate showing TRAP-positive (red, arrow) osteoclasts in the wild-type tibia and absence of osteoclasts and TRAP staining in the RANK$^{-/-}$ tibia.

membrane folds to form finger-like processes called the ruffled border that greatly increases the cell surface area for secretion of bone-degrading acid and enzymes (10, 21). Osteoclast cell membranes form a roughly circular tight junction with the bone surface around the ruffled border, called the sealing zone, which effectively creates an enclosed extracellular lysosomal compartment that protects cells in resorption lacunae from the low pH (∼5.5) under the cells. The main osteoclast proteolytic enzyme, cathepsin K, functions most effectively at this pH to degrade the matrix after the mineral has been dissolved (10, 21). Degraded matrix particles are passed through the osteoclast cytoplasm to the outer surface of the cell from which they are released into the resorption lacunae (23), where there are nutrient-carrying afferent sinusoids as well as efferent sinusoids that remove these particles to the bloodstream (10). The lacunae appear to be covered by a thin collagenous membrane called a canopy that isolates the lacunae to protect the adjacent bone marrow from the resorptive process (24). Osteoclasts die by apoptosis in the deepest parts of BMUs behind the advancing edges for the resorption lacunae (25, 26), and cytokines released from bone resorption, such as TGFβ1 promote osteoclast apoptosis (27) and attract osteoblast progenitors to the site (28), while macrophages (29) appear to be involved in preparation of the resorbed surface for new bone formation by osteoblasts.

There are estimated to be >1 million BRUs (also called basic multicellular units; BMUs) in the normal adult skeleton, and their numbers increase in many pathologic conditions in response to increased production of cytokines, hormones and growth factors. In many of these conditions, including infections (30, 31), inflammatory/auto-immune diseases (e.g., rheumatoid arthritis) (32), endocrine disorders (20, 33), and metastatic cancers (34, 35) that spread to bone, these factors typically increase bone resorption and inhibit bone formation, leading to generalized bone loss (osteoporosis) or to localized, radiologically lytic lesions.

REGULATION OF OSTEOCLAST FORMATION AND FUNCTION

Osteoclasts are multinucleated cells that form by fusion of hematopoietic myeloid precursors typically in the bone marrow adjacent to bone surfaces. They can be recognized in H&E-stained sections, and more readily in sections stained histochemically for tartrate-resistant acid phosphatase (TRAP), which osteoclasts secrete (**Figure 1**). Expression of TRAP is not required for normal bone resorption, but serum TRAP levels correlate positively with the level of skeletal resorption (36). Osteoclast precursors are formed in the bone marrow and are attracted from there into the bloodstream by sphingosine-1 phosphate (S1P) (37), which is produced in large amounts by red blood cells. They are attracted back into the bone marrow to resorption lacunae by RANKL (10, 38) expressed by osteoblastic and immune cells. They are also recruited by CXCL12/SDF1 (39) and by S1P (40), expressed by osteoblastic/stromal cells and osteoclasts, respectively.

Osteoclasts can also form outside the skeleton in a variety of pathologic lesions in humans, including the relatively common giant cell tumor of tendon sheath and the closely related pigmented villonodular tenosynovitis [(41); **Figure 2**]. Mesenchymal cells in these soft tissue lesions express RANKL and M-CSF (42), which presumably attract osteoclast precursors from the bloodstream and induce their differentiation into osteoclasts. Osteoclasts can also be observed, sometimes in large numbers, in a small percentage of primary carcinomas (43, 44), including breast, lung, pancreas, and bladder, and in some soft tissue sarcomas, but the molecular mechanisms that induce their formation in these lesions are unknown. Macrophages, like osteoclasts, are derived from myeloid precursors and can comprise up to 40% of the cells in malignant tumors. Tumor cells attract and activate these macrophages (45), which are called tumor-associated macrophages (TAMs). TAMs have multiple functions, some supportive of tumor cell growth and invasion (46), others inhibitory (47). Macrophage/monocytes are also present in benign lesions, including giant cell tumor of tendon sheath and pigmented villonodular tenosynovitis. Some of these cells fuse to form the multinucleated osteoclasts in these lesions, but others can fuse to form TRAP-negative polykaryons [**Figure 2**; (48)] and these multinucleated cells do not resorb bone. TRAP-negative giant cells can form in numerous other pathologic settings in response to a variety of factors, including cholesterol from dead normal or tumor cells (**Figure 2**), foreign agents, such as some bacteria and viruses, and surgically implanted graft materials, and their function in these conditions is to degrade them. It is possible that osteoclasts and their mononuclear precursors, like TAMs, have positive or negative influences on the behavior of malignant cells in tumors outside the skeleton, but this has not been studied to date.

M-CSF is expressed by osteoblast lineage cells in the bone marrow and induces expression of RANK by osteoclast precursors, which further differentiate and fuse with one another to form osteoclasts in response to RANKL (5, 10). RANKL is expressed in BMUs in the bone marrow by accessory cells, including osteoblastic/stromal cells (5, 18), B lymphocytes (49) and T lymphocytes (4, 10, 50, 51). RANKL is also expressed and secreted by osteocytes (52, 53), the most abundant cells in bone. Osteocytes start their existence as matrix-forming osteoblasts on bone forming surfaces. Most osteoblasts die by apoptosis when their matrix forming mission has been completed (26), but some of them become embedded within the uncalcified matrix, called osteoid, as it is being formed, and the others remain on the bone surface as flat lining cells. When osteoid becomes mineralized, osteoblasts remain "trapped" and form osteocytes in the calcified bone until they are released during a subsequent remodeling cycle. Osteocytes have numerous dendritic processes that allow them to communicate with each other within the bone and with lining cells on the surfaces of fully calcified bone (54). It is believed that, as a result of this syncytial arrangement, osteocytes can respond to mechanical forces and detect areas of bone that have become damaged and need to be removed by osteoclasts (54).

Osteocytes express both cell membrane-bound and secreted form of RANKL (52). Interestingly, osteocyte-derived RANKL is not required for the formation and activation of osteoclasts that resorb bone during embryonic development in mice, but it is required for normal bone remodeling in the adolescent and adult mouse skeleton (53, 55). RANKL also activates osteoclasts and maintains their survival along with M-CSF in resorption lacunae for up to ∼30 days, the average lifespan of osteoclasts. Mice and humans deficient in RANKL, RANK or M-CSF or its receptor *c-fms* develop osteopetrosis (10, 22), which is characterized by failure of removal of mineralized bone matrix from the medullary cavities of long bones and vertebrae during embryonic development (**Figure 1**). Consequently, osteopetrotic bones are radio-opaque on X-ray and have a typical diagnostic club-shape to their ends because the resorption of cortical bone on the periosteum at metaphyses that gives the ends a concave configuration does not occur. Despite their sclerotic appearance, osteopetrotic bones are weaker than normal bones (22), because the bone formed during development is typically composed of woven, rather than lamellar bone, which is stronger than woven bone.

Osteoblast precursors, like osteoclast precursors, appear to circulate in the blood and are attracted to BMUs by molecules released during bone resorption, including various cytokines, chemokines and growth factors (56), and other osteoclast products, including S1P and collagen fragments (57). Osteoblasts, derived from mesenchymal precursors in the bone marrow, positively and negatively regulate osteoclast formation and activation: osteoblast precursors (presumably at the advancing edges of BMUs) and osteocytes express M-CSF and RANKL to drive and maintain resorption (5, 10). Osteoblast precursors destined to become osteoblasts appear to be attracted to the deeper parts of BMUs after osteoclasts undergo apoptosis. At this site, they form a layer of cells on the lacunar surface and lay down lamellae of bone matrix. Osteoblasts and osteocytes also express osteoprotegerin (OPG), a decoy receptor for RANKL that binds to RANKL and prevents it from binding to RANK to limit osteoclast formation and activation (10, 58). They also express other factors, including Leucine-rich repeat-containing G-protein-coupled receptor 4 (LGR4), a recently identified additional receptor for RANKL (59) that also competes with RANK. LGR4 activates $G\alpha_q$ and GSK3-β

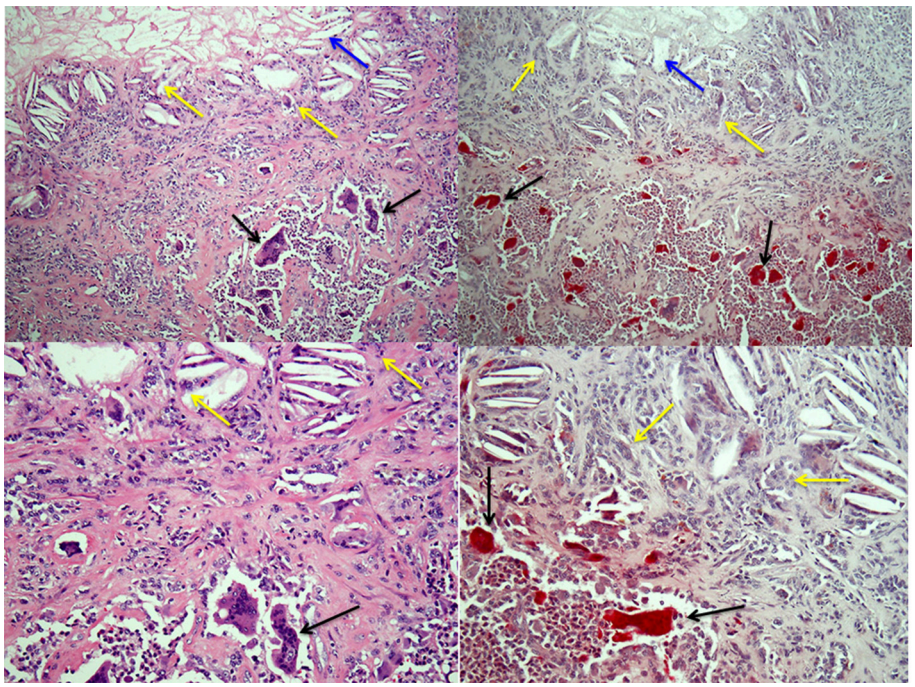


FIGURE 2 | Osteoclasts and multinucleated foreign-body type giant cells in giant cell tumor of tendon sheath. Left-hand panels show H&E-stained sections of a giant cell tumor of tendon sheath with a mix of collagenous stroma, mononuclear cells and osteoclasts (black arrows) in the lower halves of the images and cholesterol clefts (blue arrows) with multinucleated giant cells (yellow arrows) below an area of necrosis in the tumor in the upper halves. The right-hand panels show low and high power images of the lesion with TRAP-positive osteoclasts and TRAP-negative multinucleated giant cells associated with the cholesterol clefts.

signaling, which suppresses expression and activity of NFATc1 (59), a transcription factor required for osteoclast formation (10, 58). The precise details of which subsets of osteoblastic cells promote and inhibit osteoclast formation and where they are located precisely in resorption lacunae remain to be determined. Osteoclasts and their precursors can also positively and negatively regulate osteoblast formation (3, 5), but exactly where these subsets of cells are located in BMUs also remains to be determined.

THE RANKL/RANK/OPG SIGNALING SYSTEM IN OSTEOCLAST FORMATION AND ACTIVATION

RANKL expression by osteocytes and accessory cells in bone marrow attracts osteoclast precursors from the bloodstream to resorption lacunae where expression of RANK by precursors is increased in response to M-CSF (60). RANK expression is also induced in osteoclast precursors by the transcription factors, PU.1 and microphthalmia-induced transcription factor (MITF) (61, 62) during the early stages of commitment of these cells to osteoclast differentiation, as well as by IL-34 (63), Wnt5a (64), and TNF (10, 65), which is the major inflammation-inducing cytokine in RA. Many of the accessory cells that express RANKL also express OPG to limit osteoclast formation, and the relative concentrations of these cytokines appear to be a major determinant of the level of bone resorption in normal and disease states (3, 58). A human monoclonal antibody to RANKL has been approved by the FDA for the treatment of a variety of osteolytic bone diseases, including osteoporosis, metastatic bone disease, and multiple myeloma (66, 67).

RANK is expressed by a growing number of cell types in addition to immune cells in bone marrow. These include dendritic cells, which are activated by RANKL expressed by T cells, mammary gland milk-producing cells (58), which fail to develop in RANKL$^{-/-}$ and RANK$^{-/-}$ mice during pregnancy, and consequently mutant mothers are unable to feed their pups. Breast and prostate cancers in humans also express RANK (58), and RANKL/RANK signaling has been implicated in breast cancer metastasis to bone. A few cases of RANK deficiency have been reported in humans (68), but activating mutations in *TNFRSF11A* (the gene encoding RANK) are more common (68). These are associated with early-onset (juvenile) Paget's disease of bone, familial expansile osteolysis and expansile skeletal hyperphosphatasia (69, 70).

OPG is secreted by osteoblasts in response to most of the factors that promote RANKL expression by these cells and in this way it limits osteoclast formation, activity and survival, and the subsequent bone destruction (58). OPG is also expressed by cells in numerous other organs, including the heart, liver, kidney, and spleen, and has been implicated in cardiovascular disease, diabetes, and hypertension (71). Homozygous partial deletions of *TNFRSF11B* (the gene encoding

OPG) have been reported in some patients with juvenile Paget's disease, resulting in osteoporosis and increased risk of fractures (72). An inactivating deletion in exon 3 of *TNFRSF11B* is associated with increased bone turnover and deformities of long bones, acetabular protrusion, and kyphosis in some children with idiopathic hyperphosphatasia, an autosomal recessive disease (3, 73).

Wnt/β-catenin signaling regulates osteoblast formation and differentiation from MSCs (74), but it also regulates osteoclast formation. For example, Wnt5a induces RANK expression in osteoclast precursors (64) and canonical Wnt/β-catenin signaling promotes OPG expression by osteoblastic cells (75). In addition, Wnt3a (76) and Wnt16 (75, 77) limit osteoclast formation not only through canonical Wnt signaling, but also through non-canonical signaling by inhibiting RANKL-mediated NF-κB-induced NFATc1 expression. Wnt4a also inhibits osteoclast formation. Wnt4a prevents the formation of a RANKL-induced TRAF6-Tak1-Tab2 complex and instead promotes formation of a Tak1-Tab2-NlK complex, thereby limiting NF-κB p65 nuclear translocation. Through these actions Wnt4a inhibits ovariectomy-induced osteoporosis (76, 78). In addition, activation of β-catenin signaling in early OCPs promotes their differentiation into osteoclasts, but inhibits OC formation in more differentiated precursors (79). Thus, Wnt signaling can have positive and negative regulatory roles in osteoclast formation and activation.

ROLES FOR TRAFS AND NF-κB SIGNALING IN OSTEOCLAST FORMATION AND ACTIVATION

RANK is a member of the TNF superfamily of receptors, which lack intrinsic protein kinase activity to activate downstream signaling. These receptors recruit a number of proteins to their cytoplasmic domains, including TRAFs, to mediate downstream signaling. In response to RANKL, RANK recruits TRAFs 1, 2, 3, 5, and 6 in OCPs (10, 58); of these, only TRAF6 appears to be necessary for osteoclast formation, since only TRAF6$^{-/-}$ mice are osteopetrotic. Two lines of TRAF6$^{-/-}$ mice were generated independently, and surprisingly one has no OCs, while the other has many osteoclasts that do not resorb bone (10, 58), suggesting that TRAF6 has essential roles in both OC formation and activation. Why these knockout mice have different OC phenotypes has not been explained, but this may reflect different knockout strategies. RANKL/RANK signaling through TRAF6 activates several pathways in OCPs to promote their differentiation and activation. These include NF-κB, c-Jun N-terminal kinase (JNK), c-myc, and phospholipase Cγ/calcineurin/NFATc1 (10, 58).

NF-κB signaling was discovered unexpectedly to be essential for osteoclast formation before the discovery of RANKL or RANK when p50/p52 double knockout were generated. These mice formed no osteoclasts or TRAP-positive mononuclear cells in their bone marrow cavities, which were filled with unremodeled trabecular bone, typical of severe osteopetrosis (80, 81). Subsequent studies showed that the defect in RANKL-induced osteoclast formation from precursor cells in the double knockout mice could be prevented *in vitro* by overexpression of c-fos or NFATc1, indicating that c-fos or NFATc1 acts downstream of NF-κB signaling (82). Other pathways activated by RANKL/RANK/TRAF6 signaling mediate activation of osteoclasts, including Src and mitogen-activated protein kinase kinase 6 (MKK6)/p38/MITF, and to prevent their apoptosis, for example Src and ERK (10, 58).

TRAF2$^{-/-}$ mice die during embryonic development or within 2–3 weeks after birth (83, 84), similar to TRAF3$^{-/-}$ mice (85), making examination of the roles of these TRAFs in skeletal development and in post-natal osteoclast and osteoblast formation challenging. TRAF2$^{-/-}$ and TRAF3$^{-/-}$ mice were reported to have normally formed, but shorter limbs than their WT littermates, suggesting that they were not osteopetrotic. Using fetal liver transplantation as a source of osteoclast precursors from TRAF2$^{-/-}$ mice, another group reported that TRAF2 is required for full TNF-, but not RANKL-induced osteoclastogenesis (86), consistent with TRAF2 having a non-essential function in osteoclastogenesis. Mice deficient in TRAFs 1, 4, and 5 appear to have normal skeletal development (87, 88).

ROLES FOR TRAF3 IN OSTEOCLASTIC CELLS

RANKL efficiently processes non-canonical NF-κB protein p100 into p52 and thus induces full osteoclast differentiation. In contrast, TNF does not efficiently process p100 to 52 and this limits osteoclast differentiation (89). Interestingly, TRAF3 protein levels parallel those of p100 during osteoclast differentiation. For example, TNF increases p100 and TRAF3 protein level, associated with limited osteoclast formation, while RANKL induces degradation of TRAF3 protein leading to processing of p100 to p52 and lower p100 levels, associated with increased osteoclast formation (89), consistent with TRAF3 negatively regulating osteoclast formation by preventing p100 processing into p52. Indeed, knockdown of TRAF3 expression promoted TNF induction of osteoclast formation, associated with increased levels of NF-κB inducing kinase (NIK) and enhanced p100 processing to p52 (89). Consistent with this, over-expression of TRAF3 inhibited RANKL-induced osteoclast formation, associated with decreased p100 processing to p52, decreased NIK, RelB and RelA levels as well as a decrease in the osteoclast formation markers, NFATc1 and c-Fos (17). This is consistent with previous studies showing that TRAF3 suppresses both canonical and non-canonical NF-κB signaling (90, 91) and that transgenic mice over-expressing a form of NIK that lacks the TRAF3 binding domain develop osteoporosis due to increased osteoclast formation and activity (92).

Ubiquitination is a common pathway for protein degradation, which can be carried out by proteasomes or lysosome/autophagosomes. Original studies indicated that the proteasome inhibitor, MG-132, did not prevent RANKL-induced TRAF3 degradation (89), but different lysosomal inhibitors, including chloroquine (CQ) and NH4Cl, blocked RANKL-induced degradation of TRAF3 (17). Similarly, the autophagy inhibitors, bafilomycin and 3-Methyladenine, also prevented RANKL-induced TRAF3 degradation. Consistent with this,

RANKL promoted TRAF3 co-localization with LAMP2, which CQ blocked (17).

To further explore the role of TRAF3 in osteoclasts, Xiu et al. (17) generated mice with TRAF deleted in osteoclast lineage cells by crossing $TRAF3^{fl/fl}$ mice with lysozyme M^{cre} and cathepsin K^{cre} mice. Lysozyme M targets all myeloid precursor cells, including osteoclast precursors, while cathepsin K targets committed osteoclast precursors and osteoclasts since it is expressed by these cells and is the main metalloproteinase secreted by osteoclasts to dissolve bone matrix. They found that both lines of mice with conditional deletion of TRAF3 had normal skeletal development and phenotype, but they developed early onset osteoporosis due to increased osteoclast formation and activity (17). Treatment of bone marrow macrophages from both lines of transgenic mice with M-CSF and low doses of RANKL resulted in more and larger osteoclasts, which formed earlier than that from wild type littermate mice. These transgenic mice developed more severe bone loss after ovariectomy, but unlike in wild type mice, chloroquine did not prevent ovariectomy-induced bone loss and the associated increased osteoclastogenesis (17). Other investigators have generated these mice using lysozyme M^{cre} mice and reported that 68% of mice aged 15–22-months-old developed various chronic inflammatory lesions, infections or tumors, including B cell lymphomas (93), indicating that TRAF3 in myeloid cells has anti-inflammatory and anti-neoplastic functions. TRAF3 levels decrease in monocytes from humans during aging due to proteasomal degradation (94), and Li et al. have reported that TRAF3 levels decrease in bone in mice during aging (95).

Chloroquine has been used for decades to treat and prevent malaria and is still used in some parts of the world as a first-line anti-inflammatory drug for autoimmune diseases, including RA and systemic lupus erythematosus. Hydroxychloroquine replaced chloroquine in the 1970s and 80s in the US and Europe as an anti-inflammatory drug. Hydroxychloroquine also inhibits bone resorption *in vitro* and *in vivo* (96). Thus, chloroquine or its analogs, including hydroxychloroquine could potentially be used to treat osteoporosis and other osteolytic diseases, particularly if they could be targeted to bone and away from other tissues to reduce side effects, which limit the amount of these drugs that can be administered to patients. To this end, Yao et al. generated bone-targeted conjugates of chloroquine and hydroxychloroquine by linking them to a bisphosphonate, which has high binding affinity for hydroxyapatite, but minimal or no anti-osteoclastic activity (97). Bone-targeted chloroquine more effectively inhibited osteoclast formation and bone resorption *in vitro* and *in vivo* than chloroquine (97). They are currently are testing these *in vitro* and *in vivo* in models of RA and age-related bone loss.

TRAF3 IN TNF-INDUCED OSTEOCLAST FORMATION

TNF, like RANKL, induces osteoclast formation by sequentially activating NF-κB/c-fos/NFATc1 signaling (82) and enhancing IκB-α phosphorylation in osteoclast precursors (98). Unlike RANKL, TNF recruits TRAF2, but not TRAF6 to its receptors (86, 99). In fact, TRAF6 appears to negatively regulate TNF-induced canonical NF-κB signaling, based on enhanced TNF-induced expression of IL-6, CXCL1 and GM-CSF in TRAF6-deficient mouse embryonic fibroblasts, associated with enhanced IκB kinase activation and IκB-α degradation (100). We and others have reported that TNF can induce osteoclast formation from WT, $RANKL^{-/-}$, and $RANK^{-/-}$ osteoclast precursors *in vitro* as well as *in vivo* in $RANKL^{-/-}$ and $RANK^{-/-}$ mice when the mice are also deficient in the inhibitory NF-κB protein, p100, which limits osteoclast formation (89, 101). These findings indicate that TNF can induce osteoclast formation independent of RANKL. In contrast, other investigators reported earlier that priming of precursors by RANKL was necessary for TNF induction of osteoclastogenesis (102). This discrepancy may reflect differences in the *in vitro* approaches used by these labs. Despite this controversy, TNF stimulates the expression of RANKL by accessory cells as its major mechanism to indirectly enhance bone resorption, as evidenced by the report that synoviocytes appear to be the major source of RANKL in inflamed joints in RA (14). In contrast to RANKL signaling, which causes TRAF3 degradation, TNF signaling increases protein levels of TRAF3 in osteoclast precursors to limit osteoclast formation (89). As a result, RANKL promotes non-canonical NIK-mediated p100 proteasomal processing to p52, while TNF does not (89). In addition, TNF dose-dependently reduces RANKL-induced osteoclast formation *in vitro* by increasing p100 protein levels in osteoclast precursors (89). Interestingly, RANKL signaling also reduces TNF-induced TRAF3 levels to enhance osteoclast differentiation in the absence of TRAF6 (98). Consistent with this, deletion of TRAF3 in osteoclast progenitor cells enhanced TNF-induced osteoclast formation (98). Thus, TRAF3 and p100 can combine to limit osteoclastogenesis induced by TNF, which also induces expression of other inhibitors of osteoclastogenesis, including IRF-8 and the Notch-induced RPB-Jκ (103).

ROLES FOR TRAF3 IN OSTEOBLASTIC CELLS

More recently, the role of TRAF3 has also been investigated in osteoblast progenitors by crossing $TRAF3^{fl/fl}$ mice with $Prx1^{cre}$ mice (95). Prx1 targets mesenchymal progenitor cells, including osteoblastic and chondroblastic cells. These conditional knockout (cKO) mice have normal skeletal development and bone mass until at least 3-months of age, but they develop early onset osteoporosis by 9-months-old through a combination of increased bone resorption and decreased bone formation (95). A role for TRAF3 in mesenchymal cells has not been reported previously, and the mechanisms whereby TRAF3 protects against age-related bone loss are under investigation. However, these recent findings suggest that therapeutic prevention of TRAF3 degradation *in vivo* could increase bone mass in a variety of diseases by preventing bone destruction and promoting bone formation.

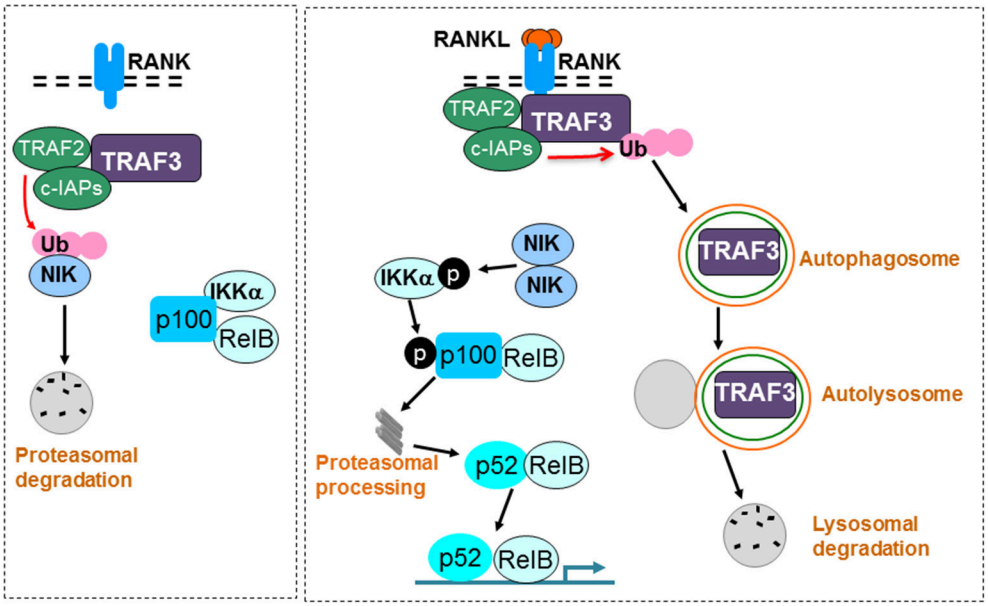


FIGURE 3 | RANKL signaling induces TRAF3 degradation to promote osteoclast formation. A TRAF3/TRAF2/cIAP complex constitutively induces ubiquitination (Ub) and proteasomal degradation of NIK in unstimulated osteoclast precursors. As a consequence, p100 and RelB remain in the cytoplasm in an inactive complex with the inhibitory NF-κB protein, IKKα (left panel). RANKL binding to RANK induces ubiquitination and autophagolysosomal degradation of TRAF3, allowing accumulation of NIK, which phosphorylates and activates IKKα. IKKα then phosphorylates p100, leading to its proteasomal processing to p52. As a result, p52/RelB dimers translocate to the nucleus of osteoclast precursors to promote osteoclast formation (right panel).

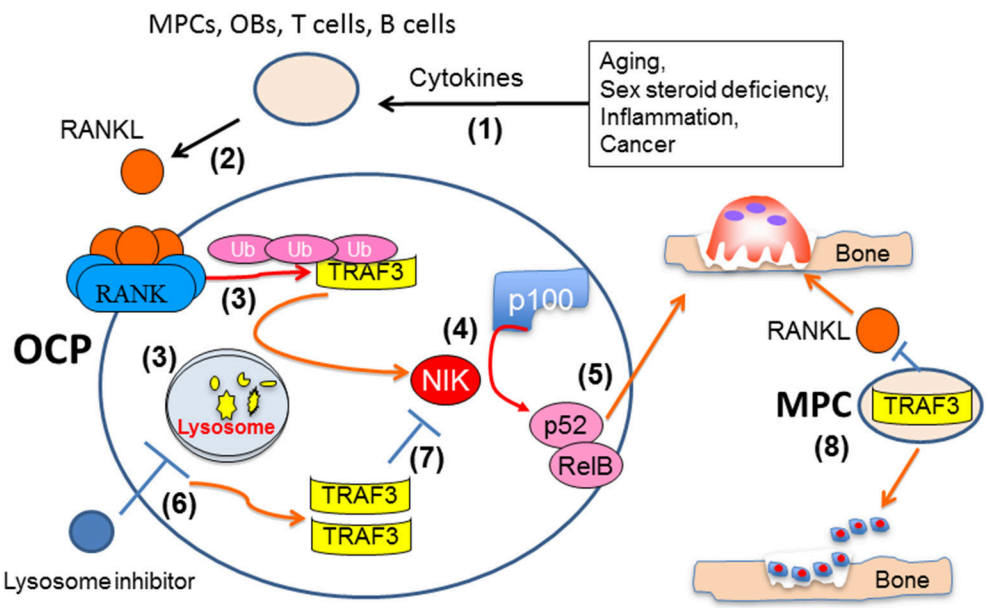


FIGURE 4 | Mechanisms influencing TRAF3 functions and degradation in osteoclast and osteoblast precursors. Multiple pathologic processes and aging can increase production of cytokines, such as TNF, IL-1 and IL-6 (1), to increase production of RANKL by accessory cells, including mesenchymal progenitor (MPCs), osteoblastic (OB), and T and B lymphocytes (2). RANKL binding to RANK in osteoclast precursors results in TRAF3 ubiquitination and lysosomal degradation (3), thus allowing NF-κB-inducing kinase (NIK) to mediate proteasomal processing of p100 to 52 (4) and formation of p52/RelB heterodimers to promote osteoclast formation and bone resorption (5). Inhibitors of lysosomal degradation, such as chloroquine, can prevent degradation of TRAF3, which will promote NIK degradation and inhibit osteoclast formation (7). TRAF3 expression in MPCs also promotes their differentiation into OBs and limits their production of RANKL to maintain bone formation and restrict bone resorption (8).

SUMMARY

Normal skeletal development and bone remodeling require the formation and activation of osteoclasts, which are derived from myeloid precursors in the bone marrow. Osteoclasts are formed and activated in response to RANKL, which is expressed by osteoblastic and immune cells in bone. RANKL activates NF-κB signaling in osteoclast precursors by recruiting TRAF6 to its receptor, RANK, and this leads to activation of a number of signaling pathways in these cells that induce osteoclast formation and activation. RANKL signaling also induces autophagosomal degradation of TRAF3 by TRAF2 and cIAPs (**Figure 3**). This facilitates osteoclast formation by inhibiting TRAF3-induced proteasomal degradation of NIK and promoting p100 processing to p52. Mice with TRAF3 conditionally deleted in osteoclast precursor cells develop early-onset osteoporosis due to increased osteoclast formation. Inhibition of TRAF3 degradation by the autophagosomal inhibitor drug, chloroquine, inhibits osteoclast formation and prevents ovariectomy-induced osteoporosis in mice. The finding that mice with TRAF3 deleted in mesenchymal precursors have increased bone resorption and decreased bone formation, points to TRAF3 having a positive regulatory role in osteoblastic precursors that could be targeted therapeutically to not only inhibit bone resorption, but also stimulate bone formation in common diseases associated with decreased bone mass. These findings suggest that drugs, like chloroquine or cIAP antagonists (104), that inhibit TRAF3 degradation could prevent bone destruction by inhibiting osteoclast formation and stimulating bone formation by enhancing mesenchymal progenitor cell differentiation into osteoblasts in a variety of bone diseases (**Figure 4**).

FUTURE DIRECTIONS AND GAPS IN KNOWLEDGE

Chloroquine or hydroxychloroquine are FDA-approved drugs for the treatment of autoimmune diseases, including rheumatoid arthritis, and may not be ideal drugs to treat age- or menopause-related bone loss because of their known side effects, including blindness that can affect up to 1% of patients, that limit the doses that can be administered (105, 106). Nevertheless, chloroquine and hydroxychloroquine are being studied in clinical trials of patients with multiple myeloma (107, 108) in which they appear to augment the effects of proteasome inhibitors by inducing myeloma cell apoptosis (109). In this setting chloroquine could also potentially inhibit the associated bone resorption and perhaps stimulate new bone formation. One future direction should be attempts to develop small molecule inhibitors that could prevent TRAF2/cIAP-induced TRAF3 degradation. cIAP antagonists have already been developed by a number of pharmaceutical companies as chemotherapeutic agents to promote cancer cell apoptosis with promising results (104) However, one of these cIAP inhibitors appears to also stimulate bone resorption in male, but not female mice, and thus this class of molecules could have detrimental effects on the skeleton of men treated with them as part of a chemotherapeutic regimen for cancer (110).

Findings to date suggest that post-translational modification, rather than increased gene expression, is the major mechanism regulating TRAF3 levels and functions in osteoclast precursors to mediate bone loss in conditions associated with increased bone resorption (17, 89). However, the molecular mechanisms regulating *TRAF3* gene expression in bone cells in normal or pathologic remodeling remain to be determined and could also be a potential target for upregulation in future studies. TRAF3 could also have roles in mature osteoblasts that have yet to be examined.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication .

FUNDING

BB and ZY are supported by funding from the National Institute of Arthritis and Musculoskeletal and Skin Diseases and the National Institute of Aging of the NIH, USA (grant numbers AR43510, AR070984, and AG049994). LX is supported by funding from the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the NIH, USA (grant numbers AR063650 and AR069789).

REFERENCES

1. Veldurthy V, Wei R, Oz L, Dhawan P, Jeon YH, Christakos S. Vitamin D, calcium homeostasis and aging. *Bone Res.* (2016) 4:16041. doi: 10.1038/boneres.2016.41
2. Gehlert S, Bloch W, Suhr F. $Ca2^{+}$-dependent regulations and signaling in skeletal muscle: from electro-mechanical coupling to adaptation. *Int J Mol Sci.* (2015) 16:1066–95. doi: 10.3390/ijms16011066
3. Boyce BF, Zuscik MJ, Xing L. Biology of bone and cartilage. *Genet Bone Biol Skeletal Dis.* (2018) 23:3–24. doi: 10.1016/B978-0-12-804182-6.00011-3
4. Martin TJ, Sims NA. RANKL/OPG; critical role in bone physiology. *Rev Endocr Metab Disord.* (2015) 16:131–9. doi: 10.1007/s11154-014-9308-6
5. Sims NA, Martin TJ. Coupling signals between the osteoclast and osteoblast: how are messages transmitted between these temporary visitors to the bone surface? *Front Endocrinol (Lausanne)* (2015) 6:41. doi: 10.3389/fendo.2015.00041
6. Bonewald LF. The amazing osteocyte. *J Bone Miner Res.* (2011) 26:229–38. doi: 10.1002/jbmr.320
7. Crane JL, Cao X. Bone marrow mesenchymal stem cells and TGF-beta signaling in bone remodeling. *J Clin Invest.* (2014) 124:466–72. doi: 10.1172/JCI70050
8. Croucher PI, McDonald MM, Martin TJ. Bone metastasis: the importance of the neighbourhood. *Nat Rev Cancer* (2016) 16:373–86. doi: 10.1038/nrc.2016.44
9. Waning DL, Mohammad KS, Reiken S, Xie W, Andersson DC, John S, et al. Excess TGF-beta mediates muscle weakness associated with bone metastases in mice. *Nat Med.* (2015) 21:1262–71. doi: 10.1038/nm.3961

10. Boyce BF. Advances in osteoclast biology reveal potential new drug targets and new roles for osteoclasts. *J Bone Miner Res.* (2013) 28:711–22. doi: 10.1002/jbmr.1885
11. Xing L, Chen D, Boyce BF. Mice deficient in NF-kappaB p50 and p52 or RANK have defective growth plate formation and post-natal Dwarfism. *Bone Res.* (2013) 1:336–45. doi: 10.4248/BR201304004
12. Wang B, Jin H, Shu B, Mira RR, Chen D. Chondrocytes-specific expression of osteoprotegerin modulates osteoclast formation in metaphyseal bone. *Sci Rep.* (2015) 5:13667. doi: 10.1038/srep13667
13. Weitzmann MN. The role of inflammatory cytokines, the RANKL/OPG axis, and the immunoskeletal interface in physiological bone turnover and osteoporosis. *Scientifica* (2013) 2013:125705. doi: 10.1155/2013/125705
14. Danks L, Komatsu N, Guerrini MM, Sawa S, Armaka M, Kollias G, et al. RANKL expressed on synovial fibroblasts is primarily responsible for bone erosions during joint inflammation. *Ann Rheum Dis.* (2016) 75:1187–95. doi: 10.1136/annrheumdis-2014-207137
15. Stanley ER, Chitu V. CSF-1 receptor signaling in myeloid cells. *Cold Spring Harb Perspect Biol.* (2014) 6:a021857. doi: 10.1101/cshperspect.a021857
16. Park JH, Lee NK, Lee SY. Current understanding of RANK signaling in osteoclast differentiation and maturation. *Mol Cells* (2017) 40:706–13. doi: 10.14348/molcells.2017.0225
17. Xiu Y, Xu H, Zhao C, Li J, Morita Y, Yao Z, et al. Chloroquine reduces osteoclastogenesis in murine osteoporosis by preventing TRAF3 degradation. *J Clin Invest.* (2014) 124:297–310. doi: 10.1172/JCI66947
18. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys.* (2008) 473:139–46. doi: 10.1016/j.abb.2008.03.018
19. Hadjidakis DJ, Androulakis, II. Bone remodeling. *Ann N Y Acad Sci.* (2006) 1092:385–96. doi: 10.1196/annals.1365.035
20. Feng X, McDonald JM. Disorders of bone remodeling. *Annu Rev Pathol.* (2011) 6:121–45. doi: 10.1146/annurev-pathol-011110-130203
21. Novack DV, Mbalaviele G. Osteoclasts-key players in skeletal health and disease. *Microbiol Spectr.* (2016) 4:1–19. doi: 10.1128/microbiolspec.MCHD-0011-2015
22. Teti A, Econs MJ. Osteopetroses, emphasizing potential approaches to treatment. *Bone* (2017) 102:50–9. doi: 10.1016/j.bone.2017.02.002
23. Vaananen HK, Laitala-Leinonen T. Osteoclast lineage and function. *Arch Biochem Biophys.* (2008) 473:132–8. doi: 10.1016/j.abb.2008.03.037
24. Hauge EM., Qvesel D, Eriksen EF, Mosekilde L, Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *J Bone Miner Res.* (2001) 16:1575–82. doi: 10.1359/jbmr.2001.16.9.1575
25. Boyce BF, Hughes DE, Wright KR, Xing L, Dai A. Apoptosis in bone cells. In: *Novel Approaches to Treatment of Osteoporosis*. Berlin, NY: Springer-Verlag (2001). pp 22.
26. Hughes DE, Boyce BF. Apoptosis in bone physiology and disease. *Mol Pathol.* (1997) 50:132–7. doi: 10.1136/mp.50.3.132
27. Hughes DE, Dai A, Tiffee JC, Li HH, Mundy GR, Boyce BF. Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-beta. *Nat Med.* (1996) 2:1132–6. doi: 10.1038/nm1096-1132
28. Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, et al. TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. *Nat Med.* (2009) 15:757–65. doi: 10.1038/nm.1979
29. Sinder BP, Pettit AR, McCauley LK. Macrophages: their emerging roles in bone. *J Bone Miner Res.* (2015) 30:2140–9. doi: 10.1002/jbmr.2735
30. Raynaud-Messina B, Bracq L, Dupont M, Souriant S, Usmani SM, Proag A, et al. Bone degradation machinery of osteoclasts: An HIV-1 target that contributes to bone loss. *Proc Natl Acad Sci USA.* (2018) 115:E2556–65. doi: 10.1073/pnas.1713370115
31. Walker Harris V, Brown TT. Bone loss in the HIV-infected patient: evidence, clinical implications, and treatment strategies. *J Infect Dis.* (2012) 205:S391–8. doi: 10.1093/infdis/jis199
32. Amarasekara DS, Yu J, Rho J. Bone loss triggered by the cytokine network in inflammatory autoimmune diseases. *J Immunol Res.* (2015) 2015:832127. doi: 10.1155/2015/832127
33. Takahashi N, Udagawa N, Suda T. Vitamin D endocrine system and osteoclasts. *Bonekey Rep.* (2014) 3:495. doi: 10.1038/bonekey.2014.17
34. Le Pape F, Vargas G, Clezardin P. The role of osteoclasts in breast cancer bone metastasis. *J Bone Oncol.* (2016) 5:93–5. doi: 10.1016/j.jbo.2016.02.008
35. Ottewell PD. The role of osteoblasts in bone metastasis. *J Bone Oncol.* (2016) 5:124–7. doi: 10.1016/j.jbo.2016.03.007
36. Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, Vaananen HK. Tartrate-resistant acid phosphatase 5b: a novel serum marker of bone resorption. *J Bone Miner Res.* (2000) 15:1337–45. doi: 10.1359/jbmr.2000.15.7.1337
37. Ishii M, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J, et al. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature* (2009) 458:524–8. doi: 10.1038/nature07713
38. Xing L, Schwarz EM, Boyce BF. Osteoclast precursors, RANKL/RANK, and immunology. *Immunol Rev.* (2005) 208:19–29. doi: 10.1111/j.0105-2896.2005.00336.x
39. Shahnazari M, Chu V, Wronski TJ, Nissenson RA, Halloran BP. CXCL12/CXCR4 signaling in the osteoblast regulates the mesenchymal stem cell and osteoclast lineage populations. *FASEB J.* (2013) 27:3505–13. doi: 10.1096/fj.12-225763
40. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proc Natl Acad Sci USA.* (2008) 105:20764–9. doi: 10.1073/pnas.0805133106
41. van der Heijden L, Gibbons CL, Dijkstra PD, Kroep JR, van Rijswijk CS, Nout RA, et al. The management of diffuse-type giant cell tumour (pigmented villonodular synovitis) and giant cell tumour of tendon sheath (nodular tenosynovitis). *J Bone Joint Surg Br.* (2012) 94:882–8. doi: 10.1302/0301-620X.94B7.28927
42. Fiocco U, Sfriso P, Lunardi F, Pagnin E, Oliviero F, Scagliori E, et al. Molecular pathways involved in synovial cell inflammation and tumoral proliferation in diffuse pigmented villonodular synovitis. *Autoimmun Rev.* (2010) 9:780–4. doi: 10.1016/j.autrev.2010.07.001
43. Priore SF, Schwartz LE, Epstein JI. An expanded immunohistochemical profile of osteoclast-rich undifferentiated carcinoma of the urinary tract. *Mod Pathol.* (2018) 31:984–8. doi: 10.1038/s41379-018-0012-z
44. Zagelbaum NK, Ward MF II, Okby N, Karpoff H. Invasive ductal carcinoma of the breast with osteoclast-like giant cells and clear cell features: a case report of a novel finding and review of the literature. *World J Surg Oncol.* (2016) 14:227. doi: 10.1186/s12957-016-0982-6
45. Sousa S, Brion R, Lintunen M, Kronqvist P, Sandholm J, Monkkonen J, et al. Human breast cancer cells educate macrophages toward the M2 activation status. *Breast Cancer Res.* (2015) 17:101. doi: 10.1186/s13058-015-0621-0
46. Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers (Basel)* (2014) 6:1670–90. doi: 10.3390/cancers6031670
47. Goswami KK, Ghosh T, Ghosh S, Sarkar M, Bose A, Baral R. Tumor promoting role of anti-tumor macrophages in tumor microenvironment. *Cell Immunol.* (2017) 316:1–10. doi: 10.1016/j.cellimm.2017.04.005
48. McNally AK, Anderson JM. Macrophage fusion and multinucleated giant cells of inflammation. *Adv Exp Med Biol.* (2011) 713:97–111. doi: 10.1007/978-94-007-0763-4_7
49. Meednu N, Zhang H, Owen T, Sun W, Wang V, Cistrone C, et al. Production of RANKL by memory B cells: a link between B cells and bone erosion in rheumatoid arthritis. *Arthritis Rheumatol.* (2016) 68:805–16. doi: 10.1002/art.39489
50. Li J, Wang Q, Yang R, Zhang J, Li X, Zhou X, et al. BMI-1 mediates estrogen-deficiency-induced bone loss by inhibiting reactive oxygen species accumulation and T cell activation. *J Bone Miner Res.* (2017) 32:962–73. doi: 10.1002/jbmr.3059
51. Takayanagi H, Ogasawara K, Hida S, Chiba T, Murata S, Sato K, et al. T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma. *Nature* (2000) 408:600–5. doi: 10.1038/35046102
52. Xiong J, O'Brien CA. Osteocyte RANKL: new insights into the control of bone remodeling. *J Bone Miner Res.* (2012) 27:499–505. doi: 10.1002/jbmr.1547
53. Xiong J, Piemontese M, Onal M, Campbell J, Goellner JJ, Dusevich V, et al. Osteocytes, not osteoblasts or lining cells, are the main source of the RANKL required for osteoclast formation in remodeling bone. *PLoS ONE* (2015) 10:e0138189. doi: 10.1371/journal.pone.0138189

54. Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell. and more. *Endocr Rev.* (2013) 34:658–90. doi: 10.1210/er.2012-1026
55. Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med.* (2011) 17:1231–4. doi: 10.1038/nm.2452
56. Teti A. Mechanisms of osteoclast-dependent bone formation. *Bonekey Rep.* (2013) 2:449. doi: 10.1038/bonekey.2013.183
57. Barrow AD, Raynal N, Andersen TL, Slatter DA, Bihan D, Pugh N, et al. OSCAR is a collagen receptor that costimulates osteoclastogenesis in DAP12-deficient humans and mice. *J Clin Invest.* (2011) 121:3505–16. doi: 10.1172/JCI45913
58. Nagy V, Penninger JM. The RANKL-RANK Story. *Gerontology* (2015) 61:534–42. doi: 10.1159/000371845
59. Luo J, Yang Z, Ma Y, Yue Z, Lin H, Qu G, et al. LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. *Nat Med.* (2016) 22:539–46. doi: 10.1038/nm.4076
60. Arai A, Mizoguchi T, Harada S, Kobayashi Y, Nakamichi Y, Yasuda H, et al. Fos plays an essential role in the upregulation of RANK expression in osteoclast precursors within the bone microenvironment. *J Cell Sci.* (2012) 125:2910–7. doi: 10.1242/jcs.099986
61. Ishii J, Kitazawa R, Mori K, McHugh KP, Morii E, Kondo T, et al. Lipopolysaccharide suppresses RANK gene expression in macrophages by down-regulating PU.1 and MITF. *J Cell Biochem.* (2008) 105:896–904. doi: 10.1002/jcb.21886
62. Lu SY, Li M, Lin YL. Mitf induction by RANKL is critical for osteoclastogenesis. *Mol Biol Cell.* (2010) 21:1763–71. doi: 10.1091/mbc.e09-07-0584
63. Chen Z, Buki K, Vaaraniemi J, Gu G, Vaananen HK. The critical role of IL-34 in osteoclastogenesis. *PLoS ONE* (2011) 6:e18689. doi: 10.1371/journal.pone.0018689
64. Maeda K, Kobayashi Y, Udagawa N, Uehara S, Ishihara A, Mizoguchi T, et al. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat Med.* (2012) 18:405–12. doi: 10.1038/nm.2653
65. Zhang YH, Heulsmann A, Tondravi MM, Mukherjee A, Abu-Amer Y. Tumor necrosis factor-alpha (TNF) stimulates RANKL-induced osteoclastogenesis via coupling of TNF type 1 receptor and RANK signaling pathways. *J Biol Chem.* (2001) 276:563–8. doi: 10.1074/jbc.M008198200
66. Cosman F. Long-term treatment strategies for postmenopausal osteoporosis. *Curr Opin Rheumatol.* (2018) 30:420–6. doi: 10.1097/BOR.0000000000000509
67. Wilcock A, Charlesworth S, Toller CS, Girish R, Mihalyo M, Howard P. Denosumab: AHFS 92:24. *J Pain Symptom Manage.* (2018) 56:295–301. doi: 10.1016/j.jpainsymman.2018.05.021
68. Sobacchi C, Schulz A, Coxon FP, Villa A, Helfrich MH. Osteopetrosis: genetics, treatment and new insights into osteoclast function. *Nat Rev Endocrinol.* (2013) 9:522–36. doi: 10.1038/nrendo.2013.137
69. Coudert AE, de Vernejoul MC, Muraca M, Del Fattore A. Osteopetrosis and its relevance for the discovery of new functions associated with the skeleton. *Int J Endocrinol.* (2015) 2015:372156. doi: 10.1155/2015/372156
70. Crockett JC, Mellis DJ, Scott DI, Helfrich MH. New knowledge on critical osteoclast formation and activation pathways from study of rare genetic diseases of osteoclasts: focus on the RANK/RANKL axis. *Osteoporos Int.* (2011) 22:1–20. doi: 10.1007/s00198-010-1272-8
71. Lieb W, Gona P, Larson MG, Massaro JM, Lipinska I, Keaney JF Jr., et al. Biomarkers of the osteoprotegerin pathway: clinical correlates, subclinical disease, incident cardiovascular disease, and mortality. *Arterioscler Thromb Vasc Biol.* (2010) 30:184 9–54. doi: 10.1161/ATVBAHA.109.199661
72. Whyte MP, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN, McAlister WH, et al. Osteoprotegerin deficiency and juvenile Paget's disease. *N Engl J Med.* (2002) 347:175–84. doi: 10.1056/NEJMoa013096
73. Cundy T, Hegde M, Naot D, Chong B, King A, Wallace R, et al. A mutation in the gene TNFRSF11B encoding osteoprotegerin causes an idiopathic hyperphosphatasia phenotype. *Hum Mol Genet.* (2002) 11:2119–27. doi: 10.1093/hmg/11.18.2119
74. Glass DA II, Karsenty G. *In vivo* analysis of Wnt signaling in bone. *Endocrinology* (2007) 148:2630–4. doi: 10.1210/en.2006-1372
75. Glass DA II, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* (2005) 8:751–64. doi: 10.1016/j.devcel.2005.02.017
76. Weivoda MM, Ruan M, Hachfeld CM, Pederson L, Howe A, Davey RA, et al. Wnt signaling inhibits osteoclast differentiation by activating canonical and noncanonical cAMP/PKA pathways. *J Bone Miner Res.* (2016) 31:65–75. doi: 10.1002/jbmr.2599
77. Moverare-Skrtic S, Henning P, Liu X, Nagano K, Saito H, Borjesson AE, et al. Osteoblast-derived WNT16 represses osteoclastogenesis and prevents cortical bone fragility fractures. *Nat Med.* (2014) 20:1279–88. doi: 10.1038/nm.3654
78. Yu B, Chang J, Liu Y, Li J, Kevork K, Al-Hezaimi K, et al. Wnt4 signaling prevents skeletal aging and inflammation by inhibiting nuclear factor-kappaB. *Nat Med.* (2014) 20:1009–17. doi: 10.1038/nm.3586
79. Wei W, Zeve D, Suh JM, Wang X, Du Y, Zerwekh JE, et al. Biphasic and dosage-dependent regulation of osteoclastogenesis by beta-catenin. *Mol Cell Biol.* (2011) 31:4706–19. doi: 10.1128/MCB.05980-11
80. Franzoso G, Carlson L, Xing L, Poljak L, Shores EW, Brown KD, et al. Requirement for NF-kappaB in osteoclast and B-cell development. *Genes Dev.* (1997) 11:3482–96. doi: 10.1101/gad.11.24.3482
81. Iotsova V, Caamano J, Loy J, Yang Y, Lewin A, Bravo R. Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. *Nat Med.* (1997) 3:1285–9. doi: 10.1038/nm1197-1285
82. Yamashita T, Yao Z, Li F, Zhang Q, Badell IR, Schwarz EM, et al. NF-kappaB p50 and p52 regulate receptor activator of NF-kappaB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor differentiation by activating c-Fos and NFATc1. *J Biol Chem.* (2007) 282:18245–53. doi: 10.1074/jbc.M610701200
83. Lee SY, Reichlin A, Santana A, Sokol KA, Nussenzweig MC, Choi Y. TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. *Immunity* (1997) 7:703–13. doi: 10.1016/S1074-7613(00)80390-8
84. Yeh WC, Shahinian A, Speiser D, Kraunus J, Billia F, Wakeham A, et al. Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* (1997) 7:715–25. doi: 10.1016/S1074-7613(00)80391-X
85. Xu Y, Cheng G, Baltimore D. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity* (1996) 5:407–15. doi: 10.1016/S1074-7613(00)80497-5
86. Kanazawa K, Kudo A. TRAF2 is essential for TNF-alpha-induced osteoclastogenesis. *J Bone Miner Res.* (2005) 20:840–7. doi: 10.1359/JBMR.041225
87. Nakano H, Sakon S, Koseki H, Takemori T, Tada K, Matsumoto M, et al. Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. *Proc Natl Acad Sci USA.* (1999) 96:9803–8. doi: 10.1073/pnas.96.17.9803
88. Shiels H, Li X, Schumacker PT, Maltepe E, Padrid PA, Sperling A, et al. TRAF4 deficiency leads to tracheal malformation with resulting alterations in air flow to the lungs. *Am J Pathol.* (2000) 157:679–88. doi: 10.1016/S0002-9440(10)64578-6
89. Yao Z, Xing L, Boyce BF. NF-kappaB p100 limits TNF-induced bone resorption in mice by a TRAF3-dependent mechanism. *J Clin Invest.* (2009) 119:3024–34. doi: 10.1172/JCI38716
90. Bista P, Zeng W, Ryan S, Bailly V, Browning JL, Lukashev ME. TRAF3 controls activation of the canonical and alternative NFkappaB by the lymphotoxin beta receptor. *J Biol Chem.* (2010) 285:12971–8. doi: 10.1074/jbc.M109.076091
91. Zarnegar B, Yamazaki S, He JQ, Cheng G. Control of canonical NF-kappaB activation through the NIK-IKK complex pathway. *Proc Natl Acad Sci USA.* (2008) 105:3503–8. doi: 10.1073/pnas.0707959105
92. Yang C, McCoy K, Davis JL, Schmidt-Supprian M, Sasaki Y, Faccio R, et al. NIK stabilization in osteoclasts results in osteoporosis and enhanced inflammatory osteolysis. *PLoS ONE* (2010) 5:e15383. doi: 10.1371/journal.pone.0015383
93. Lalani AI, Moore CR, Luo C, Kreider BZ, Liu Y, Morse HC III, et al. Myeloid cell TRAF3 regulates immune responses and inhibits inflammation and tumor development in mice. *J Immunol.* (2015) 194:334–48. doi: 10.4049/jimmunol.1401548

94. Molony RD, Nguyen JT, Kong Y, Montgomery RR, Shaw AC, Iwasaki A. Aging impairs both primary and secondary RIG-I signaling for interferon induction in human monocytes. *Sci Signal.* (2017) 10:eaan2392. doi: 10.1126/scisignal.aan2392
95. Li J, Yao Z, Xiu Y, Yin X, Xing L, Boyce BF. Deletion of TRAF3 specifically in mesenchymal progenitor cells results in age-related osteoporosis through effects on both osteoblasts and osteoclasts. In: *ASBMR 2014 Annual Meeting (No. FR0362)*. Houston, TX (2014).
96. Both T, Zillikens MC, Schreuders-Koedam M, Vis M, Lam WK, Weel A, et al. Hydroxychloroquine affects bone resorption both *in vitro* and *in vivo*. *J Cell Physiol.* (2018) 233:1424–33. doi: 10.1002/jcp.26028
97. Yao Z, Hou X, Lei W, Xiao L, Ebetino FH, K BR, et al. Bone-targeted chloroquine inhibits osteoclastogenesis and bone resorption more effectively than chloroquine. In: *ASBMR 2016 Annual Meeting (No. SU0161)*. Atlanta, GA (2016).
98. Yao Z, Lei W, Duan R, Li Y, Luo L, Boyce BF. RANKL cytokine enhances TNF-induced osteoclastogenesis independently of TNF receptor associated factor (TRAF) 6 by degrading TRAF3 in osteoclast precursors. *J Biol Chem.* (2017) 292:10169–79. doi: 10.1074/jbc.M116.771816
99. Mizukami J, Takaesu G, Akatsuka H, Sakurai H, Ninomiya-Tsuji J, Matsumoto K, et al. Receptor activator of NF-kappaB ligand (RANKL) activates TAK1 mitogen-activated protein kinase kinase kinase through a signaling complex containing RANK, TAB2, and TRAF6. *Mol Cell Biol.* (2002) 22:992–1000. doi: 10.1128/MCB.22.4.992-1000.2002
100. Funakoshi-Tago M, Kamada N, Shimizu T, Hashiguchi Y, Tago K, Sonoda Y, et al. TRAF6 negatively regulates TNFalpha-induced NF-kappaB activation. *Cytokine* (2009) 45:72–9. doi: 10.1016/j.cyto.2008.10.010
101. O'Brien W, Fissel BM, Maeda Y, Yan J, Ge X, Gravallese EM, et al. RANK-independent osteoclast formation and bone erosion in inflammatory arthritis. *Arthritis Rheumatol.* (2016) 68:2889–900. doi: 10.1002/art.39837
102. Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL. TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J Clin Invest.* (2000) 106:1481–8. doi: 10.1172/JCI11176
103. Zhao B, Ivashkiv LB. Negative regulation of osteoclastogenesis and bone resorption by cytokines and transcriptional repressors. *Arthritis Res Ther.* (2011) 13:234. doi: 10.1186/ar3379
104. Asano M, Hashimoto K, Saito B, Shiokawa Z, Sumi H, Yabuki M, et al. Design, stereoselective synthesis, and biological evaluation of novel tri-cyclic compounds as inhibitor of apoptosis proteins (IAP) antagonists. *Bioorg Med Chem.* (2013) 21:5725–37. doi: 10.1016/j.bmc.2013.07.020
105. Lloyd LA, Hiltz JW. Ocular complications of chloroquine therapy. *Can Med Assoc J.* (1965) 92:508–13.
106. Tehrani R, Ostrowski RA, Hariman R, Jay WM. Ocular toxicity of hydroxychloroquine. *Semin Ophthalmol.* (2008) 23:201–9. doi: 10.1080/08820530802049962
107. Montanari F, Lu M, Marcus S, Saran A, Malankar A, Mazumder A. A Phase II trial of chloroquine in combination with bortezomib and cyclophosphamide in patients with relapsed and refractory multiple myeloma. In: *ASH Annual Meeting*. San Francisco, CA (2014) 124:5775.
108. Vogl DT, Stadtmauer EA, Tan KS, Heitjan DF, Davis LE, Pontiggia L, et al. Combined autophagy and proteasome inhibition: a phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. *Autophagy* (2014) 10:1380–90. doi: 10.4161/auto.29264
109. Jarauta V, Jaime P, Gonzalo O, de Miguel D, Ramirez-Labrada A, Martinez-Lostao L, et al. Inhibition of autophagy with chloroquine potentiates carfilzomib-induced apoptosis in myeloma cells *in vitro* and *in vivo*. *Cancer Lett.* (2016) 382:1–10. doi: 10.1016/j.canlet.2016.08.019
110. Zarei A, Yang C, Gibbs J, Davis JL, Ballard A, Zeng R, et al. Manipulation of the alternative NF-κB pathway in mice has sexually dimorphic effects on bone. *JBMR Plus* (2018). doi: 10.1002/jbm4.10066. [Epub ahead of print].

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer BH and handling Editor declared their shared affiliation at the time of the review.

frontiers in Immunology

REVIEW
published: 20 September 2018
doi: 10.3389/fimmu.2018.02111

Genetic Alterations of TRAF Proteins in Human Cancers

*Sining Zhu[1,2], Juan Jin[1,3], Samantha Gokhale[1,2], Angeli M. Lu[1], Haiyan Shan[1,4], Jianjun Feng[1,5] and Ping Xie[1,6]**

[1] Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ, United States, [2] Graduate Program in Cellular and Molecular Pharmacology, Rutgers University, Piscataway, NJ, United States, [3] Department of Pharmacology, Anhui Medical University, Hefei, China, [4] Department of Obstetrics and Gynecology, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, China, [5] Engineering Research Center of the Modern Technology for Eel Industry, Ministry of Education of the People's Republic of China, Fisheries College of Jimei University, Xiamen, China, [6] Member, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, United States

The tumor necrosis factor receptor (TNF-R)-associated factor (TRAF) family of cytoplasmic adaptor proteins regulate the signal transduction pathways of a variety of receptors, including the TNF-R superfamily, Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and cytokine receptors. TRAF-dependent signaling pathways participate in a diverse array of important cellular processes, including the survival, proliferation, differentiation, and activation of different cell types. Many of these TRAF-dependent signaling pathways have been implicated in cancer pathogenesis. Here we analyze the current evidence of genetic alterations of *TRAF* molecules available from The Cancer Genome Atlas (TCGA) and the Catalog of Somatic Mutations in Cancer (COSMIC) as well as the published literature, including copy number variations and mutation landscape of TRAFs in various human cancers. Such analyses reveal that both gain- and loss-of-function genetic alterations of different TRAF proteins are commonly present in a number of human cancers. These include pancreatic cancer, meningioma, breast cancer, prostate cancer, lung cancer, liver cancer, head and neck cancer, stomach cancer, colon cancer, bladder cancer, uterine cancer, melanoma, sarcoma, and B cell malignancies, among others. Furthermore, we summarize the key *in vivo* and *in vitro* evidence that demonstrates the causal roles of genetic alterations of TRAF proteins in tumorigenesis within different cell types and organs. Taken together, the information presented in this review provides a rationale for the development of therapeutic strategies to manipulate TRAF proteins or TRAF-dependent signaling pathways in different human cancers by precision medicine.

Keywords: TRAFs, cancer, oncogenes, tumor suppressor genes, NF-κB, MAPK

OPEN ACCESS

Edited by:
Tania H. Watts,
University of Toronto, Canada

Reviewed by:
Juan M. Zapata,
Instituto de Investigaciones Biomédicas Alberto Sols (IIBM), Spain
Shao-Cong Sun,
University of Texas MD Anderson Cancer Center, United States

***Correspondence:**
Ping Xie
xie@dls.rutgers.edu

Specialty section:
This article was submitted to Molecular Innate Immunity, a section of the journal Frontiers in Immunology

Received: *29 July 2018*
Accepted: *28 August 2018*
Published: *20 September 2018*

Citation:
Zhu S, Jin J, Gokhale S, Lu AM, Shan H, Feng J and Xie P (2018) Genetic Alterations of TRAF Proteins in Human Cancers. Front. Immunol. 9:2111. doi: 10.3389/fimmu.2018.02111

INTRODUCTION

The tumor necrosis factor receptor (TNF-R)-associated factor (TRAF 1–7) family of cytoplasmic adaptor proteins regulates the signal transduction pathways of a variety of receptors, including the TNF-R superfamily, Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and cytokine receptors (1–4). TRAF proteins function as both adaptor proteins and E3 ubiquitin ligases to regulate receptor signaling, leading to the activation of canonical and noncanonical nuclear factor-κBs (NF-κB1 and NF-κB2), mitogen-activated protein kinases

(MAPKs: ERK1/2, JNK1/2, and p38), or interferon-regulatory factors (IRFs: IRF3, IRF5, and IRF7) (1–4). The TRAF-dependent signaling pathways participate in a diverse array of important cellular processes, including the survival, proliferation, differentiation, activation, and stress responses of different cell types (1–4). Many of these TRAF-dependent signaling pathways have been implicated in cancer pathogenesis.

With the rapid progress made in next-generation deep sequencing technology and the tremendous efforts put forth on whole genome/exome/transcriptome sequencing and copy number variation (CNV) analyses of cancers at the post-genome era, it has become increasingly clear that genetic alterations of TRAF proteins are commonly present in various human cancers. Here we analyze the current evidence of genetic alterations of *TRAF* molecules available from the Cancer Genome Atlas (TCGA) (5) and the Catalog of Somatic Mutations in Cancer (COSMIC) (6) as well as the published literature, including the landscape of genetic alterations and the map of recurrent mutations in *TRAF* molecules in different types of human cancers. Moreover, we summarize the key *in vivo* and *in vitro* evidence that demonstrates the causal roles of genetic alterations of *TRAF* proteins in tumorigenesis within different cell types and organs. Collectively, the information presented in this review identifies *TRAF* proteins and TRAF-dependent signaling pathways as important therapeutic targets in specific human cancers.

TRAF1

Landscape of Genetic Alterations

According to the TCGA and COSMIC datasets of sample size n $>$ 100, the frequency of genetic alterations of *TRAF1* is generally $<$4% in human cancers (**Figure 1A**). The eight human cancers with relatively higher genetic alterations of *TRAF1* are pancreatic cancer (3.7%) (7), skin cutaneous melanoma (2.9%) (TCGA, PanCancer Atlas), esophageal cancer (2.8%) (TCGA, PanCancer Atlas), stomach cancer (2.7%) (8), sarcoma (2.4%) (9), ovarian cancer (2.3%) (TCGA, Provisional), lung cancer (2.3%) (10), and prostate cancer (2%) (TCGA, Provisional). The most common genetic alterations of *TRAF1* are gene amplification (copy gain) and mutation. Deep deletion (copy loss) is less common but also detected in several types of human cancers (**Figure 1**). Truncation is rare for *TRAF1* in human cancers.

Overview and Map of Recurrent Mutations

To date, there are 139 different mutations of the *TRAF1* gene detected in human cancers, comprising 80% (111/139) mutations that alter the protein sequence of *TRAF1* and 20% (28/139) coding silent mutations (**Table 1**). In the *TRAF* family, *TRAF1* has the lowest count of recurrent mutations. Only 29% (32/111) of the coding-altering mutations of *TRAF1* are recurrent and have been detected in at least two patients with various cancers. Almost all the recurrent mutations of *TRAF1* are missense mutations (94%, 30/32) except one nonsense mutation (truncation) and one fusion (**Table 1** and **Figure 2**). These recurrent mutations occurred across 24 different amino acids that are distributed in all the major domains of the TRAF1 protein (**Figure 3**). Interestingly, missense mutations of two specific amino acids are detected in more than three patients: R70C or H in the linker between the Zinc finger and the coiled-coil domain, and M182I of the coiled-coil (also known as TRAF-N) domain of the *TRAF1* protein (**Figure 3**). The R70 mutations are detected in 4 patients with stomach, colon, and colorectal cancers (TCGA) (11–13). M182I is documented in 4 patients with melanoma and chronic lymphocytic leukemia (CLL) (14, 15). The functional significance of R70C/H and M182I mutations of *TRAF1* remains to be determined.

Fusion

There is only one fusion of the *TRAF1* gene detected in human cancers, the TRAF1-ALK fusion that has been detected in five patients with anaplastic large cell lymphoma (ALCL) (16–19). All five cases contain the identical in frame fusion of *TRAF1* and *ALK* that generates a chimeric protein linking the N-terminal 1–294 aa of *TRAF1* to the entire intracellular domain of *ALK* (1,058–1,620 aa), including its kinase domain (16–19). Interestingly, expression of the TRAF1-ALK fusion protein leads to constitutive activation of the *ALK* and NF-κB pathways as demonstrated by the elevated levels of phosphorylated *ALK* (pALK) and STAT3 (pSTAT3) as well as nuclear p50 NF-κB1 and p52 NF-κB2 in ALCL cells (18). Similar to wild type (WT) TRAF1, the TRAF1-ALK fusion protein also binds to TRAF2 in co-immunoprecipitation experiments (18), suggesting the involvement of *TRAF2* in the activation of NF-κB pathways. Furthermore, treatment of patient ALCL cells expressing the TRAF1-ALK fusion protein with proteasome inhibitors that decrease NF-κB1/2 or a selective *ALK* inhibitor (CEP28122) results in significant inhibition on lymphoma growth but could not eradicate lymphoma cells (18). Thus, constitutive activation of NF-κB1/2 pathways contributes to the neoplastic phenotype of TRAF1-ALK-expressing ALCL.

In vivo Causal Oncogenic Roles

Gene amplification is the most common *TRAF1* genetic alteration in human cancers. *TRAF1* expression is ubiquitously elevated in skin squamous cell carcinoma (SSCC), non-small cell lung cancer (NSCLC), Hodgkin lymphomas (HLs) and non-Hodgkin lymphomas (NHLs) (20–25). Notably, *TRAF1* protein is consistently elevated in B cell leukemias and lymphomas without evidence of gene amplification (1, 23). In this case, *TRAF1* upregulation might be the result of epigenetic alterations and/or aberrant activation of NF-κB1/2, as *TRAF1* is a direct target gene of NF-κB (23, 26, 27). Interestingly, *TRAF1* expression levels are increased in chronic lymphocytic leukemia (CLL) cells from patients with refractory disease, suggesting a role for *TRAF1* in the progression of this disease and in the development of chemoresistance (23). Furthermore, genetic association studies identify *TRAF1* as a susceptibility gene for risk of CLL (28). Thus, human evidence implicates *TRAF1* as a candidate oncogene. Indeed, *in vivo* evidence obtained from mouse models demonstrates the causal oncogenic roles of *TRAF1* in the skin, lung, T cells, and B cells (**Table 2**). TRAF1$^{-/-}$ mice exhibit increased skin sensitivity to TNFα-induced necrosis and reduced skin tumor formation induced by DMBA/chronic solar UV

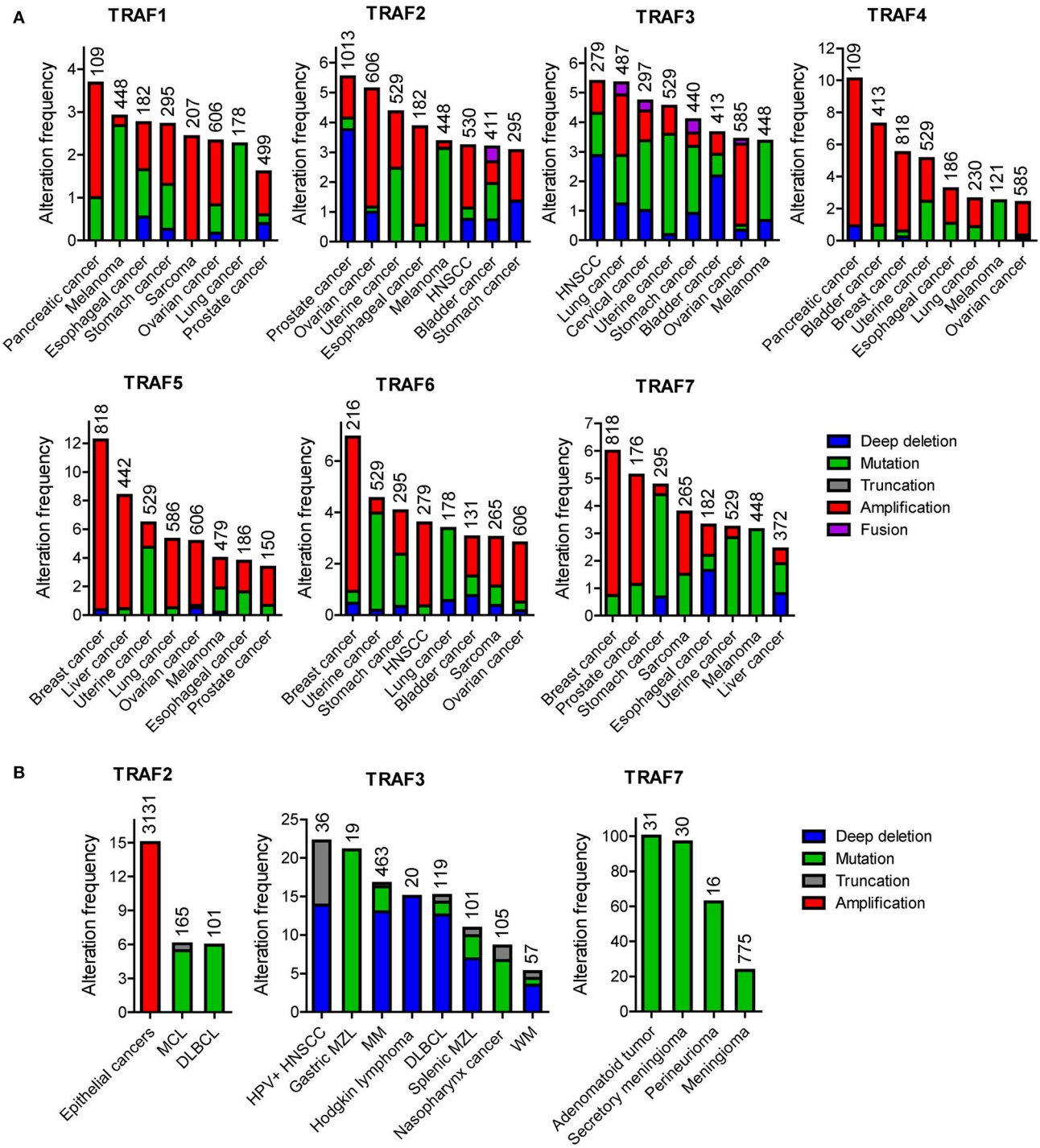


FIGURE 1 | Landscape of genetic alterations of the *TRAF* family in human cancers. **(A)** Representative results retrieved from TCGA. For each *TRAF* gene, eight cancer types that exhibit relatively higher frequency of genetic alterations were selected and datasets with relatively larger sample size ($n > 100$) are shown. **(B)** Frequent genetic alterations recognized in the published literature. Genetic alterations shown include deep deletion (copy number loss), mutation (missense mutation, frameshift insertion or deletion, and in frame insertion or deletion), truncation (nonsense mutation), amplification (copy number gain), and fusion. The sample size of each dataset is indicated on top of each bar in the graphs.

TABLE 1 | Summary of the number of different types of mutations of TRAF proteins detected in human cancers.

Type of mutation	TRAF1		TRAF2		TRAF3		TRAF4		TRAF5		TRAF6		TRAF7	
	All	Recurrent	All	Recurrent	All	Recurrent	All	Recurrent	All	Recurrent	All	Recurrent	All	Recurrent
CODING ALTERING														
Missense	96	30	168	75	166	75	86	39	137	49	132	38	281	161
Frameshift	7	0	13	10	41	21	6	1	8	2	6	1	15	5
Truncation	5	1	9	4	23	9	8	3	9	5	9	2	8	3
In frame deletion	0	0	5	2	2	1	2	1	2	1	1	0	8	2
In frame insertion	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Splice mutation	2	0	5	0	7	1	2	0	4	0	4	0	6	2
Fusion	1	1	5	1	14	1	1	0	0	0	0	0	6	1
Subtotal	111	32	205	92	253	108	105	44	160	57	152	41	326	174
CODING SILENT														
Synonymous	24	5	25	3	24	6	18	1	24	5	23	5	39	7
Intronic mutation	4	1	7	0	3	0	0	0	4	2	3	0	11	2
Total	139	38	237	95	280	114	123	45	188	64	178	46	376	183

radiation (UVR) (20, 29). Mechanistically, TRAF1 enhances the ubiquitination of ERK5 and is required for UVR-induced ERK5 phosphorylation and the expression of AP-1 family members (c-Fos/c-Jun) in keratinocytes and epithelial cells (20). TRAF1$^{-/-}$ mice also show reduced lung tumorigenesis induced by i.p. administration of urethane (30). In this lung cancer model, TRAF1 affects TRAF2-mediated K48-linked ubiquitination and degradation of BRAF, and thereby promotes the survival and proliferation of lung cancer cells (30). Consistent with studies of the TRAF1-ALK fusion protein in ALCL, transgenic mice overexpressing *TRAF1* in T cells exhibit decreased antigen-induced apoptosis of CD8 T cells (35), while TRAF1$^{-/-}$ mice display impaired survival and altered proliferation of T cells in response to the 4-1BB-NF-κB2 and T cell receptor (TCR)-NF-κB1 signaling pathways, respectively (29, 31–34). In line with the evidence of *TRAF1* overexpression in HLs and NHLs, *TRAF1* deficiency inhibits the spontaneous development of small B cell lymphoma in a transgenic mouse model that expresses the human lymphoma-associated NF-κB2 mutant p80HT specifically in lymphocytes (p80HT tg mice) (**Table 2**) (27). Taken together, these findings identify *TRAF1* as a therapeutic target in skin cancer, lung cancer, and T cell and B cell lymphomas.

Key Oncogenic Pathways

In addition to the above *TRAF1*-dependent oncogenic pathways (ERK5-AP1, BRAF-ERK, NF-κB1, and NF-κB2) that have been verified in both human cancers and *in vivo* mouse models, several oncogenic pathways involving TRAF1 have been suggested by studies using patient samples, cultured human cancer cells or xenograft models. These include: (1) CD30-TRAF1 in HL and ALCL tumors (22, 91); (2) TNF-R1/2-TRAF1/TRAF2-JNK/NF-κB in cervical and colon cancer cells (92); (3) Wnt/β-catenin-NF-κB-TRAF1/iNOS in colon, breast and liver cancer cells (93, 94); and (4) TWEAK-Fn14-TRAF1 in solid tumors (95–97). Further investigation of these signaling pathways using TRAF1$^{-/-}$ or *TRAF1*-transgenic animal models would provide new insights on the roles and mechanisms of *TRAF1* in cancer pathogenesis.

TRAF2

Landscape of Genetic Alterations

The frequency of genetic alterations of *TRAF2* is generally <6% in human cancers (**Figure 1A**) based on the TCGA and COSMIC datasets of sample size n > 180. The eight human cancers with relatively higher genetic alterations of *TRAF2* are prostate cancer (5.5%) (98), ovarian cancer (5.1%) (TCGA, Provisional), uterine cancer (4.4%) (TCGA, PanCancer Atlas), esophageal cancer (3.9%) (TCGA, PanCancer Atlas), skin cutaneous melanoma (3.4%) (TCGA, PanCancer Atlas), head and neck squamous cell carcinoma (HNSCC, 3.2%) (TCGA, Provisional), bladder cancer (3.2%) (TCGA, PanCancer Atlas), and stomach cancer (3.1%) (8). Notably, although not cataloged in TCGA, mutations of *TRAF2* are recognized as one of the most frequent somatic mutations in mantle cell lymphoma (MCL, 6.1%, 10/165) (99–101) and diffuse large B-cell lymphoma (DLBCL, 6%, 6/101) (**Figure 1B**) (102). In addition, *TRAF2* has been identified as an oncogene that is recurrently amplified and rearranged in 15% of human epithelial cancers (**Figure 1B**) (103). Thus, the most common genetic alterations of *TRAF2* are deep deletion, gene amplification and mutation (**Figure 1**). Truncation and fusion of *TRAF2* are relatively rare but also detected in human cancers (**Figure 1**).

Overview and Map of Recurrent Mutations

There are 237 different mutations of *TRAF2* detected in human cancers, comprising 86% (205/237) mutations that change the protein sequence of *TRAF2* and 14% (32/237) coding silent mutations (**Table 1**). Notably, 45% (92/205) of the coding-altering mutations of *TRAF2* are recurrently detected in at least

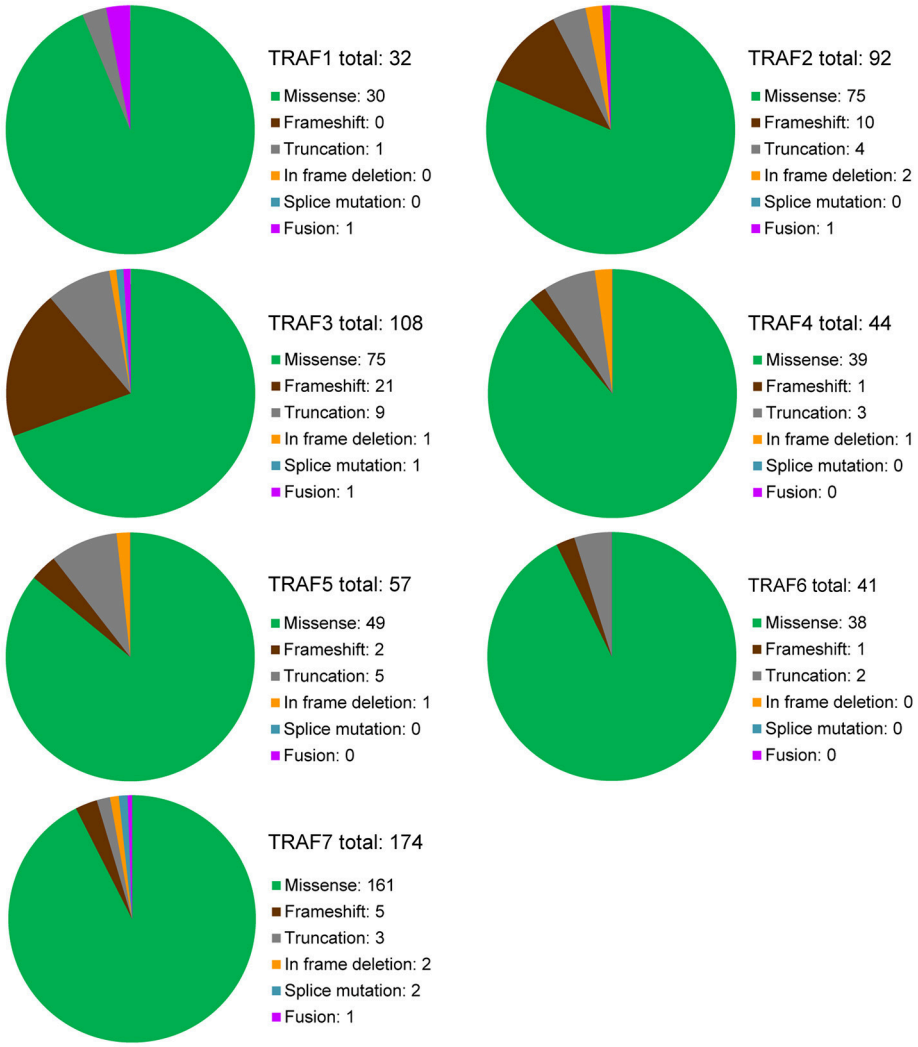


FIGURE 2 | Overview of recurrent mutations of the *TRAF* family in human cancers. Recurrent mutations of the *TRAF* family that are identified in at least 2 cancer patients are summarized in this figure. The composition of recurrent mutation types are shown in a pie graph for each *TRAF* gene. The total count of recurrent mutations and the actual count of each category of recurrent mutation for each *TRAF* gene are indicated in each pie graph.

two cancer patients. Recurrent mutations of *TRAF2* are more complex than those of *TRAF1*, including not only missense mutations (82%, 75/92), but also frameshifts (11%, 10/92), truncations (4%, 4/92), in frame deletions (2%, 2/92) and fusion (1%, 1/92) (**Table 1** and **Figure 2**). *TRAF2* recurrent mutations are identified at 52 different amino acids that are almost evenly distributed in all the structural motifs and domains of the TRAF2 protein (**Figure 3**). Interestingly, four mutation hotspots of *TRAF2* are detected in more than 5 cancer patients, specifically P9, G10, R372, and Q457 (**Figure 3**). In particular, the frameshift deletion occurred at P9 (P9fs*77) is found in 16 patients with colon cancer, colorectal cancer (CRC), uterine cancer, stomach cancer, and sarcoma, and an additional missense mutation at P9 (P9S) is also detected in a CRC patient (TCGA) (12, 104–108). The amino acid right next to P9, G10, also exhibits similar frameshift deletion (G10fs*76) or insertion (G10fs*70) or missense mutation (G10D) in five patients with colon cancer, CRC, gallbladder cancer, and glioblastoma (TCGA) (105, 106, 109). Missense mutations at R372 (R372C, H or S) of the TRAF-C domain of *TRAF2* are detected in eight patients with HNSCC, melanoma, and prostate, uterine, cervical, stomach, and liver cancers (TCGA; COSMIC) (110–113). Another amino acid of the TRAF-C domain, Q457, shows complex mutations, including a truncation (Q457*), a frameshift insertion (Q457fs*277), and missense mutations (Q457K or L) in six patients of HNSCC, oral squamous cell carcinoma (OSCC), stomach cancer, melanoma, and breast cancer (TCGA; COSMIC) (8, 114). Frameshift mutations occurring at P9 and G10 are functionally equivalent to deletion of *TRAF2*. Missense mutations at R372 and the complex mutations at Q457 of the TRAF-C domain of *TRAF2* are predicted to result in inactivation of the TRAF2 protein (99–102).

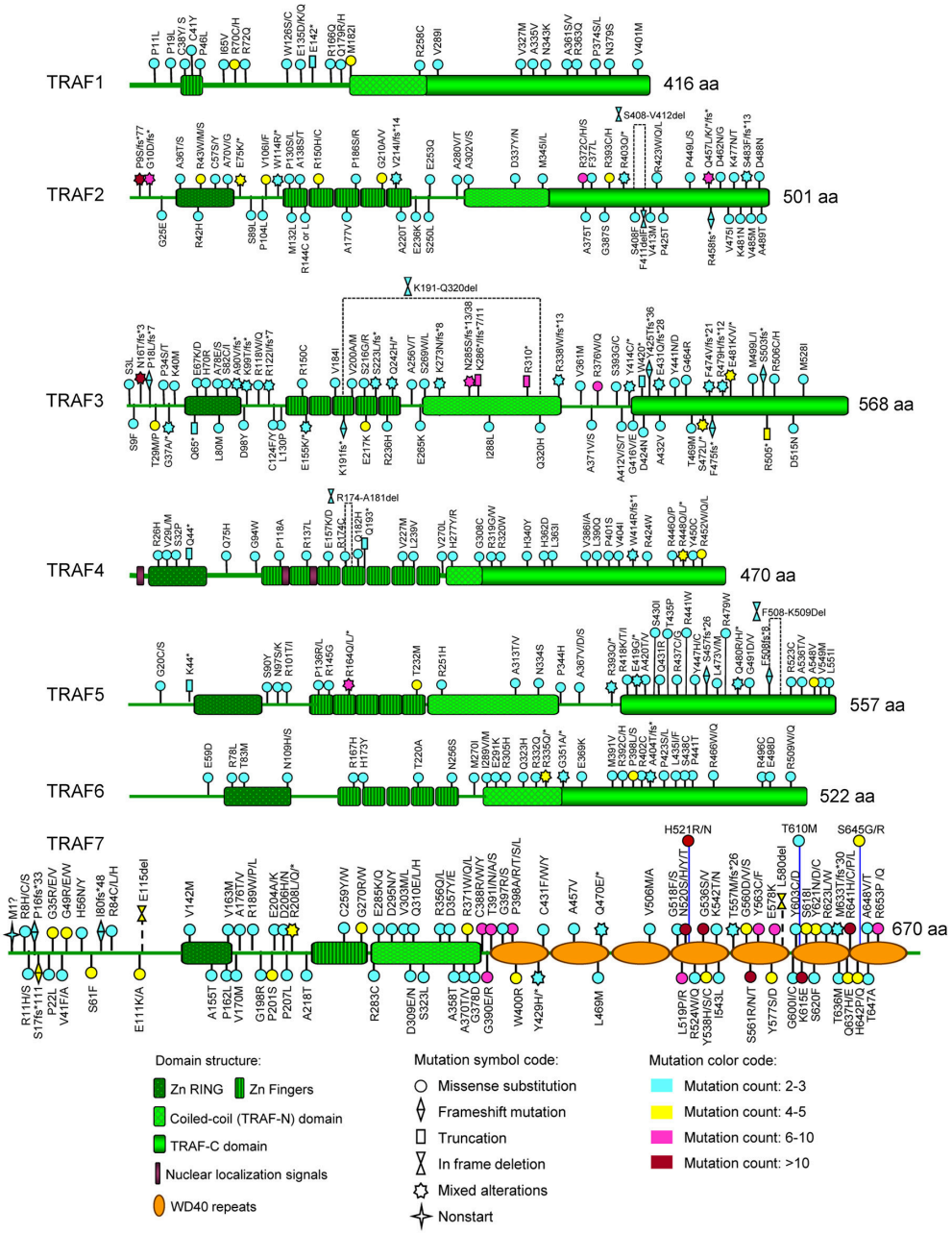


FIGURE 3 | Map of recurrent *TRAF* mutations of human cancers on the TRAF proteins. The distribution of recurrent mutations on the domain structure of each TRAF protein is depicted in this figure. The domain structures of TRAF proteins shown include the zinc RING (Zn RING), zinc fingers (Zn Fingers), coiled-coil (TRAF-N) domain, TRAF-C domain, nuclear localization signals, and WD40 repeats. For each recurrent mutation, the nature of the mutation is indicated by a mutation symbol code and the patient count is indicated by a color code as shown at the bottom legend of the figure. The actual amino acid changes are also given for each recurrent mutation: letter change, missense mutation; *, Nonsense mutation (truncation); fs*, frameshift insertion or deletion; del, in frame deletion.

Fusion

There are five different fusions of the *TRAF2* gene detected in human cancers, including *TRAF2-CCDC183* in breast and bladder cancers, *TRAF2-CACNA1B* in bladder cancer, *TMEM141-TRAF2* in breast cancer (TCGA), *TRAF2-NOTCH1* in ovarian cancer (106) and *NTRK2-TRAF2* in melanoma (115). Among these, only the *TRAF2-CCDC183* fusion is recurrently detected in two patients with breast cancer and bladder cancer (TCGA). Functional contribution of these *TRAF2* fusions to cancer pathogenesis is currently unclear.

In vivo Tumor Suppressive Roles

Inactivating mutations of *TRAF2* are frequently detected in human MCL and DLBCL, resulting in elevated activation of NF-κB1 and NF-κB2 in malignant B cells (99–102). *TRAF2* is also involved in human MALT lymphomagenesis induced by the oncogenic cIAP2-MALT1 fusion protein through the interaction between TRAF2 and the BIR1 domain of cIAP2 portion of the fusion protein, leading to activation of the TRAF2-RIP1-NF-κB pathway (116). Consistent with the human evidence, B cell-specific TRAF2$^{-/-}$ (B-TRAF2$^{-/-}$) mice exhibit expanded B cell compartment in lymphoid organs due to constitutive NF-κB2 activation and survival advantage independent of the B cell survival factor BAFF (**Table 2**) (117). Similarly in TRAF2DN-tg mice that express a dominant negative form of *TRAF2* specifically in lymphocytes (Igh-TRAF2DN), inhibition of TRAF2 also leads to splenomegaly and lymphadenopathy due to constitutive NF-κB2 activation and increased numbers of B cells (40, 42). Remarkably, TRAF2DN/Bcl-2 double-transgenic mice spontaneously develop small B cell lymphoma progressing to leukemia with many similarities to human CLL (**Table 2**) (41, 42). Thus, TRAF2 acts as a tumor suppressor in B lymphocytes primarily by inhibiting the NF-κB2 pathway through the well-established cIAP1/2-TRAF2-TRAF3-NIK axis (48, 54, 118).

Genetic alterations of *TRAF2* are detected in 1–2% of human liver cancers, including deletion, mutation and amplification (TCGA, PanCancer Atlas) (119). In human hepatocellular carcinoma (HCC), low expression of *TRAF2* and its interacting partner RIP1 is associated with an unfavorable prognosis (43). In line with human evidence, deletion of both *TRAF2* and RIP1 in liver parenchymal cells (LPC) leads to spontaneous development of hepatocellular carcinoma, which results from extensive hepatocyte apoptosis due to hyperactivation of caspase-8 but impaired NF-κB activation induced by TNFα (**Table 2**) (43). Interestingly, TRAF2 also suppresses TNFα-induced necroptosis in hepatocytes by constitutively interacting with MLKL, thereby disrupting the TNFα-induced RIPK3-MLKL association and necroptosome formation. Induced *TRAF2* deletion in adult mice results in rapid lethality, in conjunction with increased hepatic necroptosome assembly (**Table 2**) (44). Therefore, TRAF2 protects hepatocytes from death and tumorigenesis by inhibiting both the TNFα-TNFR1-TRADD-FADD-caspase 8 apoptosis and TNFα-TNFR1-RIPK1-RIPK3-MLKL necroptosis pathways.

Genetic alterations of *TRAF2* are detected in 3–4% of human HNSCC and melanoma (**Figure 1A**). In cultured HNSCC cell lines, TRAF2 is required for cellular proliferation by acting in the TNFα-TNFR1-TRADD-TRAF2-RIPK1-TAK1-IKK-NF-κB pathway (120). In primary human keratinocytes, exposure to UV light triggers association of TRAF2 with TNF-R1 to induce NF-κB activation and inflammation (121). Keratinocyte-specific TRAF2$^{-/-}$ mice exhibit psoriatic skin inflammation associated with apoptotic death and epidermal hyperplasia, which is dependent on TNFα, constitutive NF-κB2 activation and inflammatory cytokine production (45). Further in support of a role for TRAF2 in skin tumorigenesis, mutations of the TRAF2-deubiquitinating enzyme CYLD are identified in patients with familial cylindromatosis, a condition that results in benign tumors of skin appendages, and CYLD$^{-/-}$ mice are highly susceptible to chemically induced skin tumors (122). Similarly, genetic alterations of *TRAF2* are also identified in 2.7% (12/439) of human colon cancers (TCGA, PanCancer Atlas). In cultured primary human colon cancer cells, TRAF2 mediates the apoptosis by acting in the AMPK-ASK1-TRAF2-JNK-p53 axis in response to chemotherapies (123). Consistent with a potential role of TRAF2 in colon tumorigenesis, germline TRAF2$^{-/-}$ mice spontaneously develop severe colitis, which results from TNFα-TNFR1-mediated apoptosis of TRAF2$^{-/-}$ colonic epithelial cells and altered colonic microbiota (37). Interestingly, myeloid cell-specific ablation of *TRAF2* markedly exacerbates DSS-induced colitis in mice due to enhanced TLR-induced proinflammatory cytokine expression in macrophages (46). This is caused by constitutively elevated levels of the transcription factors c-Rel and IRF5 that are targeted for proteasome-dependent degradation by the cIAP1/2-TRAF2-TRAF3 E3 ubiquitin ligase complex (46). Together, the above evidence consistently supports a suppressive role for TRAF2 in skin and colon tumorigenesis.

It is also noteworthy that genetic alterations of *TRAF2* are detected in 2.6% (7/265) of human sarcomas (TCGA) and TRAF2$^{-/-}$ mice display decreased viability of skeletal muscle tissue because of defective TNFα-induced NF-κB activation in myotubes (**Table 2**) (38). Additionally, specific deletion of *TRAF2* in T cells results in decreased numbers of CD8 naïve and memory T cells as well as NKT cells, due to impaired IL-15-induced signaling in these cells (**Table 2**). However, evidence of *TRAF2* genetic alteration in T cell neoplasms is still lacking. Potential causal roles of *TRAF2* dysregulation in muscle or T cell tumorigenesis remain to be elucidated.

Key Signaling Pathways in Cancer Pathogenesis

In addition to the above TRAF2-dependent tumor suppressive pathways verified in both human cancers and *in vivo* mouse models, several important tumor suppressive pathways involving TRAF2 have been suggested by evidence obtained from cultured human cancer cells or xenograft models. These are: (1) IRE1α-TRAF2-ASK1-JNK in the apoptosis of melanoma, lung cancer and OSCC cells induced by chemotherapies or ER stress (124–126); (2) the TRAF2-caspase-2 complex in mediating DNA damage- or chemotherapy-induced apoptosis of breast, cervical and lung cancer cells, in which TRAF2-mediated ubiquitination of caspase-2 stabilizes the caspase-2 dimer complex and enhances its activity to fully commit the cell to apoptosis (127, 128); (3) TRAF2-mediated inhibition of constitutive NF-κB2 activation, cell proliferation, and anchorage-independent growth in pancreatic cancer, and a similar TRAF2-mediatied inhibition of the Eva1-induced NF-κB2-Sox2/CD15/CD49f pathway in the stemness of glioblastoma (129, 130); and (4) TRAF2-mediated K63-linked ubiquitination of MLST8 that disrupts the MLST8-SIN1-mTORC2-Akt pathway in the Kras-driven lung tumorigenesis (131). Together, these data suggest that *TRAF2* is a tumor suppressor in many human cancers.

Interestingly however, increasing evidence indicates that TRAF2 also plays oncogenic roles in epithelial cancers and some

TABLE 2 | *In vivo* evidence of the causal roles of genetic alterations of the TRAF family in cancer pathogenesis.

Mouse models	Cancer-related phenotype	References
TRAF1		
TRAF1$^{-/-}$	Increased skin sensitivity to TNFα-induced necrosis	(29)
	Reduced skin tumors induced by DMBA/solar UVR due to defective UVR-induced ERK5 phosphorylation	(20)
	Reduced lung tumors induced by urethane i.p. administration due to increased TRAF2-mediated ubiquitination and degradation of BRAF	(30)
	Enhanced T cell proliferation in response to TCR-NF-κB1 signaling	(29, 31)
	Impaired CD8 and memory T cell survival in response to 4-1BB-NF-κB2 signaling	(31–34)
TRAF1-tg	Decreased antigen-induced apoptosis of CD8 T lymphocytes	(35)
p80HT tg/TRAF1$^{-/-}$	Reduced development of small lymphocytic lymphoma	(27)
TRAF2		
TRAF2$^{-/-}$	Early lethality, reduced TNFα-mediated JNK activation	(36)
	Spontaneous severe colitis and TNFα-dependent apoptosis of colonic epithelial cells	(37)
	Decreased viability of skeletal muscle tissue due to impaired TNFα-induced NF-κB activation in myotubes	(38)
B cell KO: TRAF3flox/flox, CD19-Cre	Prolonged B cell survival, splenomegaly and lymphadenopathy due to constitutive NF-κB2 activation, but defective CD40-induced NF-κB1 activation and proliferation	(39)
B cell tg: Igh-TRAF2DN (ΔN240aa) tg	Lymphadenopathy and splenomegaly due to increased number of B cells	(40, 41)
Igh-TRAF2DN (ΔN240aa)/Bcl-2 tg	Spontaneously development of small lymphocytic lymphoma	(41, 42)
Liver parenchymal cell KO: TRAF2flox/flox, Ripk1flox/flox, Alfp-Cre	Spontaneous development of hepatocellular carcinoma due to extensive hepatocyte apoptosis, caspase 8 hyperactivation and impaired TNFα-induced NF-κB activation	(43)
Induced KO: TRAF2flox/flox, Rosa-creERT2	Rapid lethality that is dependent on Ripk3, TNFR1, DR5 and Fas signaling and increased hepatic necroptosome assembly and necroptosis	(44)
Keratinocyte KO: TRAF2flox/flox, K14-Cre	Psoriatic skin inflammation and epidermal hyperplasia that is partially dependent on TNFα, constitutive NF-κB2 activation and inflammatory cytokine expression	(45)
Myeloid cell KO: TRAF2flox/flox, LysM-Cre	Exacerbated DSS-induced colitis due to increased TLR-induced inflammatory cytokine production caused by elevated c-Rel and IRF5 protein levels in macrophages	(46)
T cell KO: TRAF2flox/flox, Lck-Cre	Decreased NKT cells and CD8 naïve and memory T cells due to impaired IL-15 signaling in NKT cells and defective IL-15-induced proliferation of CD8 T cells	(47)
TRAF3		
TRAF3$^{-/-}$	Early lethality, which could be resued by compound loss of p100 NF-κB2 or NIK	(48–50)
	Defective antigen-induced T cell proliferation	(49)
B cell KO: TRAF3flox/flox, CD19-Cre	Expanded B cell compartment, splenomegaly and lymphadenopathy due to prolonged B cell survival caused by constitutive NF-κB2 activation	(39, 51)
	Spontaneous development of splenic marginal zone lymphoma and B1 lymphoma	(52)
	Enhanced signaling by TLR3, TLR4, TLR7, and TLR9 in B cells	(53)
	Accelerated CD40-induced phosphorylation of JNK, p38, and ERK	(54)
B cell Tg: Igh-TRAF3 Tg	Spontaneous plasmacytosis, autoimmunity, inflammation and cancer, particularly squamous cell carcinomas of the tongue and salivary gland tumors	(55)
Myeloid cell KO: TRAF3flox/flox, LysM-Cre	Spontaneous development of histiocytic sarcoma, B lymphoma, liver cancer, or chronic inflammation that often affect multiple organs in aging mice	(56)
	Exacerbated DSS-induced colitis due to increased TLR-induced inflammatory cytokine production caused by elevated c-Rel and IRF5 protein levels in macrophages	(46)
T cell KO: TRAF3flox/flox, CD4-Cre	Impaired T cell proliferation in response to co-engagement of TCR and CD28	(57)
	Increased number of Treg cells due to enhanced IL-2 signaling	(57, 58)
	Impaired IL-15-induced iNKT cell proliferation and survival	(59)
	Reduced number of CD8 central memory T cells due to impaired IL-15 signaling	(60)
TRAF4		
TRAF4$^{-/-}$	Defects in embryonic development and neurulation	(61–63)

(Continued)

TABLE 2 | Continued

Mouse models	Cancer-related phenotype	References
	Reduced migration of DCs	(64)
	Reduced skin tumors induced by DMBA/TPA due to diminished IL-17A–induced ERK5 activation and epidermal hyperplasia	(65)
	Blunted airway inflammation and Th2 cytokine production in response to IL-25 administration due to defective IL-25R-Act1 signaling	(66)
TRAF5		
TRAF5$^{-/-}$	Defective CD40-induced proliferation and surface molecule upregulation in B cells	(67)
	Decreased CD40 plus IL-4-induced Ig production in B cells	(67)
	Impaired CD27-induced survival and proliferation in CD4 and CD8 T cells	(67, 68)
	Defective GITR-induced proliferation, IL-2 production and NF-κB/p38/ERK1/2 activation in CD4 T cells	(69)
	Enhanced OX40-induced Th2 differentiation of CD4 T cells and exacerbated Th2-driven lung inflammation	(70)
	Enhanced IL-6-induced CD4 Th17 differentiation due to increased IL-6-gp130-STAT3 signaling and exaggerated Th17-driven experimental autoimmune encephalomyelitis	(71)
	Exacerbated DSS-induced colitis and increased NF-κB activation in the colon	(72)
CD40LMP1-tg/TRAF5$^{-/-}$	Reduced spleen and LN size compared to CD40LMP1-tg mice, decreased serum IL-6 and autoantibodies, and decreased LMP1-mediated JNK activation in B cells.	(73)
TRAF6		
TRAF6$^{-/-}$	Reduced number of immature B cells in the bone marrow	(74)
	Defective differentiation of osteoclasts, DCs, and Treg cells	(74–77)
	Defective IL-1, CD40, LPS and RANK signaling	(74, 75)
	Loss of NF-κB activity in the epithelia and vasculature during development	(78)
	Impaired NGF-p75NTR-induced NF-κB activation and survival in Schwann cells	(79)
	Defective BDNF-p75NTR-induced JNK activation and apoptosis in neurons	(79, 80)
Hematopoietic KO:TRAF6flox/flox, Vav-Cre	Decreased basal IKKβ-NF-κB activation, impaired hematopoietic stem cell self-renewal and loss of hematopoietic stem/progenitor cells (HSPCs)	(81)
B cell KO: TRAF6flox/flox, CD19-Cre	Reduced number of mature B cells in the bone marrow and spleen, defective development of B1 B cells, and defective CD40 and TLR signaling in B cells	(82)
T cell KO: TRAF6flox/flox, CD4-Cre	Multiorgan inflammation and hyperactivation of TCR-PI3K-Akt signaling in CD4 T cells	(83)
	Defects in generating CD8 memory T cells due to impaired AMPK-activation and mitochondrial fatty acid oxidation in response to growth factor withdrawal	(84)
	Increased Th17 differentiation due to increased sensitivity of CD4 T cells to TGFβ-induced Smad2/3 activation and proliferation arrest	(85)
	Impaired OX40-induced Th9 differentiation due to defective OX40-NIK-NF-κB2 signaling	(86)
Intestinal epithelial cell KO: TRAF6flox/flox, Villin-Cre	Exacerbated DSS-induced colitis due to altered gut microbiota, which is independent of TLR signaling in intestinal epithelial cells	(87)
Skeletal muscle KO:TRAF6flox/flox, MCK-Cre	Minimal muscle loss in response to transplanted tumor growth due to defective activation of NF-κB, ubiquitin-proteasome and autophagy-lysosomal systems	(88)
	Improved regeneration of myofibers upon injury due to upregulated Notch signaling but downregulated NF-κB activation and inflammatory cytokine production	(89)
	Reduced starvation-induced skeletal muscle atrophy due to increased phosphorylation of Akt and FoxO3a and decreased AMPK activation	(90)

Direct evidence in tumorigenesis is highlighted in blue font.

other neoplasms. Consistent with the frequent amplification of *TRAF2* detected in human epithelial cancers (**Figure 1B**) (103), *TRAF2* expression is higher in prostate cancer (133), pancreatic cancer (132), lung cancer (134), stomach cancer (135), colon cancer (136), glioblastoma (137) than in normal tissues. Increased *TRAF2* expression is recognized as a prognostic factor in pancreatic cancer (132), stomach cancer (135), and and glioblastoma (137). Importantly, suppression of *TRAF2* in cancer cells harboring a *TRAF2* copy number gain inhibits proliferation, NF-κB activation, anchorage-independent growth, and tumorigenesis (103). Knockdown of *TRAF2* also enhances TRAIL-induced apoptosis in prostate cancer (133) and inhibits the growth but induces radiosensitization of lung cancer and glioblastoma cells (134). Thus, TRAF2 is required for the maintenance of the malignant state in certain cancer cells containing *TRAF2* amplification or overexpression, and TRAF2

protein levels also regulate the sensitivity of cancer cells to chemotherapy and radiotherapy.

A variety of TRAF2-dependent oncogenic pathways have been reported based on studies of patient samples, cultured human cancer cells or xenograft models. Examples include: (1) TRAF2-NEMO-p65-NF-κB1-Bcl2/XIAP/Survivin/TNFα/IL-1/IL-8/HIF-1α in the migration, invasion, metastasis, or drug resistance of breast, stomach and pancreatic cancer cells as well as DLBCL (135, 138–140); (2) EGF-EGFR-TRAF2-RSK2-AP1 in the growth of colon cancer cells (136) and EGFR-TRAF2-RIP1-IKK-NF-κB1 in the resistance to chemotherapy (EGFR inhibitors) in lung cancer cells (141); (3) cIAP1-cIAP2-TRAF2-IKKε-TBK1-IRF3/7/NF-κB1/STAT3 in the tumorigenesis of breast cancer, in which IKKε is amplified in 30% of patients (142, 143); (4) Although TRAF2 is generally considered as a K63-specific E3 ubiquitin ligase (144), a few studies reported TRAF2-mediated K48-linked ubiquitination and degradation of Caspase 8 in the switch of the DR5-Caspase 8 apoptotic pathway to the DR5-Cbl-TRAF2-JNK-AP1-MMP1 invasion/metastasis pathway or the cytoprotective TRAF2-RIPK1-JNK autophagic survival pathway following TRAIL treatment in HNSCC, prostate, lung, stomach, colorectal, and bladder cancer cells (145–148); (5) S100A9-CD147-TRAF2-cdc42 in the metastasis of melanoma (149); (6) TNFα-TRAF2-NF-κB1/AP1-COX2/IL-6/IL-8-PGE2-NOS2 and NOS2-NO-IRE1α-TRAF2-NF-κB1/AP1-COX2/IL-6/IL-8-PGE2 in the growth of breast cancer (150); (7) CD95-TRAF2-NF-κB1/AP1-IL-8/uPA in the invasion of pancreatic cancer (132); and (8) TWEAK-Fn14-TRAF2-SGEF-RhoG-Rac1 in the migration and invasion of glioma (151). Taken together, it is perplexing that both tumor suppressive and oncogenic roles of TRAF2 have been reported in the same type of human cancers. The exact roles of TRAF2 may be dependent on the genetic alteration context and malignant stage of the cancer cells as well as the nature of the environmental cue and treatment regimen.

TRAF3

Landscape of Genetic Alterations

The frequency of genetic alterations of *TRAF3* is generally <6% in human cancers (**Figure 1A**) according to the TCGA and COSMIC datasets of sample size n > 250. The eight human cancers with relatively higher genetic alterations of *TRAF3* are HNSCC (5.4%) (113), lung cancer (5.3%) (TCGA, PanCancer Atlas), cervical cancer (4.7%) (TCGA, PanCancer Atlas), uterine cancer (4.5%) (TCGA, PanCancer Atlas), stomach cancer (4.1%) (TCGA, PanCancer Atlas), bladder cancer (3.6%) (152), ovarian cancer (3.4%) (TCGA, PanCancer Atlas), and skin cutaneous melanoma (3.4%) (TCGA, PanCancer Atlas). Interestingly however, a subgroup among the 279 cases of HNSCC cataloged in TCGA, the human papilloma virus-positive (HPV+) HNSCC tumors, has much higher frequency (22%, 8/36) of deep deletions and truncations of *TRAF3* than the HPV- HNSCC tumors (**Figure 1B**) (113). Notably, although not cataloged in TCGA, deletions and mutations of *TRAF3* are recognized as one of the most frequent genetic alterations in a variety of B cell malignancies (153), including gastric marginal zone lymphoma (MZL, 21%) (154), multiple myeloma (MM, 17%) (155, 156), HL (15%) (157), DLBCL (14.3%) (158), splenic MZL (10%) (159), and Waldenstrom's macroglobulinemia (WM, 5.3%) (160) (**Figure 1B**). Furthermore, somatic mutations of *TRAF3* are also frequently detected in human nasopharyngeal cancer (NPC, 8.6%) (161) (**Figure 1B**). Together, the most common genetic alteration of *TRAF3* is deep deletion, followed by mutation and then amplification. Truncation and fusion of *TRAF3* are less common but also detected in several different types of human cancers (**Figure 1**).

Overview and Map of Recurrent Mutations

There are 280 different mutations of *TRAF3* detected in human cancers, comprising 90% (253/280) mutations that change the protein sequence of TRAF3 and 10% (27/280) coding silent mutations (**Table 1**). Approximately 43% (108/253) of the coding-altering mutations of *TRAF3* are recurrently detected in at least two cancer patients. Among all the *TRAF* genes, *TRAF3* recurrent mutations exhibit the most complex pattern and include the highest frequencies of frameshift mutations (19%, 21/108) and truncations (8%, 9/108). *TRAF3* recurrent mutations also include 69% (75/108) missense mutations, 1% (1/108) in frame deletion, 1% splice mutation, and 1% fusion (**Table 1** and **Figure 2**). These recurrent mutations occurred at 67 amino acid positions that are distributed in almost the entire length of the TRAF3 protein (**Figure 3**).

Five mutation hotspots of *TRAF3* are identified in more than 5 cancer patients, specifically N16, N285, K286, R310, and R376 (**Figure 3**). *TRAF3* mutations at N16 have the highest patient count, including the missense mutation (N16T) identified in 10 patients with HNSCC (COSMIC) and the frameshift deletion (N16fs*3) detected in a patient with splenic MZL (162, 163). Mutations at the two consecutive amino acids N285 and K286 of the coiled-coil domain of TRAF3 exhibit the most complex pattern. N285 contains frameshift deletion (N285fs*38), frameshift insertion (N285fs*13) and missense mutation (N285S) identified in 8 patients with HNSCC, MZL, NPC, CRC, stomach cancer and uterine cancer (TCGA; COSMIC) (12, 107, 161, 164, 165). Similarly, K286 exhibits frameshift deletion (K286fs*7 or fs*11) and truncation (K286*) detected in six patients with B cell malignancies, including MM, CLL and WM (155, 160, 166, 167). A third amino acid of the coiled-coil domain, R310, is consistently targeted by truncation (R310*) as detected in 8 patients with DLBCL, MM, HNSCC, cervical cancer and uterine cancer (TCGA) (113, 155, 158, 166, 168). Missense mutations at R376 (R376W or Q) located in the linker between the coiled-coil and TRAF-C domains of TRAF3 are detected in six patients with lung cancer, CRC, SSCC, and melanoma (TCGA; COSMIC) (14, 108, 169). Many of these truncations, frameshifts and missense mutations have been shown to result in inactivation of TRAF3 by disrupting its interaction with NIK, thereby inducing constitutive NF-κB2 activation (155, 156, 158, 159, 170). Thus, most of the recurrent genetic alterations of *TRAF3* identified in human cancers cause complete loss or inactivation of the TRAF3 protein.

Fusion

There are 14 different fusions of *TRAF3* detected in human cancers, including *TRAF3-WDR20* in stomach and uterine

cancers, four fusions of *TRAF3-MYO16*, *TRAF3-RCOR1*, *TRAF3-KLC1*, and *EVL-TRAF3* in breast cancer, *TRAF3-SFXN1* in cervical cancer, *UBR5-TRAF3* in HNSCC, two fusions of *TRAF3-ZNF839* and *TRAF3-MARK3* in kidney cancer, two fusions of TRAF3-BMP3 and *SLC22A23-TRAF3* in lung cancer, *TRAF3-IFNL1* in ovarian cancer, *TRAF3-ITPK1* in pheochromocytoma and *TRAF3-SIVA1* in stomach cancer (TCGA). Among the 14 fusions, only the *TRAF3-WDR20* fusion is recurrently detected in two patients with stomach cancer and uterine cancer (TCGA, PanCancer Atlas). However, the functional significance of these TRAF3 fusions is currently unknown.

In vivo Causal Roles in Cancer Pathogenesis

Similar to *TRAF2* and also consistent with the frequent deletions and inactivating mutations of TRAF3 identified in human B cell malignancies (**Figure 1B**), a tumor suppressive role for TRAF3 in B lymphocytes has been demonstrated by *in vivo* evidence obtained from mouse models. As shown for B-TRAF2$^{-/-}$ mice, B cell-specific TRAF3$^{-/-}$ (B-TRAF3$^{-/-}$) mice also exhibit severe peripheral B cell hyperplasia due to prolonged survival of mature B cells independent of BAFF, which results from constitutive NF-κB2 activation (39, 51). These B-TRAF3$^{-/-}$ mice spontaneously develop splenic MZL or B1 lymphoma at high incidence (52). Interestingly, B-TRAF3$^{-/-}$ mice also have doubled number of plasma cells due to enhanced responsiveness to IL-6 (171). Mechanistically, TRAF3 inhibits the IL-6-IL-6R-JAK1-STAT3 survival and differentiation pathway in plasma cells by facilitating the association of PTPN22 with JAK1 (171). Furthermore, the EBV-encoded oncoprotein LMP1 sequesters TRAF3 to produce functional TRAF3 deficiency in human and mouse B lymphoma cells (172, 173). Intriguingly, lymphocyte-specific TRAF3 transgenic mice also develop plasmacytosis, autoimmunity, inflammation, and cancers, which are likely caused by hyper-responsiveness of B cells to antigens and TLR agonists (55). Thus, TRAF3 acts as a tumor suppressor in naïve B cells, but an appropriate and balanced level, neither too high nor too low, of TRAF3 is required to maintain the homeostasis of plasma cells and protect them from tumorigenesis.

Interestingly, specific deletion of TRAF3 from myeloid cells (granulocytes, monocytes, and macrophages) leads to spontaneous development of histiocytic sarcomas derived from TRAF3$^{-/-}$ tissue-resident macrophages in aging mice (56, 174). The pathogenic mechanisms are likely related to the enhanced TLR-induced inflammatory responses observed in TRAF3$^{-/-}$ macrophages through constitutive activation of NF-κB2, c-Rel, and IRF5, as described for TRAF2$^{-/-}$ macrophages (56, 174). Two other mouse models with functional relevance to TRAF3, Dok1$^{-/-}$Dok2$^{-/-}$Dok3$^{-/-}$ mice and humanized TLR7/TLR8 transgenic mice, also spontaneously develop histiocytic sarcomas (175, 176). DOK3, a negative regulator of protein tyrosine kinase (PTK)-mediated signaling, has recently been identified as a TRAF3-interacting protein (177). Similar to TRAF3$^{-/-}$ macrophages, DOK3$^{-/-}$ macrophages are defective in the TLR3-IRF3-IFNβ antiviral pathway (177). TRAF3 is also a transducer of TLR7 and TLR8 signaling through direct interaction with MyD88 (1). Transgenic expression of human TLR7/TLR8 in mice deficient for endogenous TLR7/TLR8 drives inflammation and proliferative histiocytosis, which can be reversed by compound deletion of MyD88 (176). Collectively, the above *in vivo* evidence indicates that TRAF3 is a tumor suppressor in macrophages and that dysregulation of the TLR-MyD88-TRAF3-Dok3 axis in macrophages plays causal roles in the pathogenesis of histiocytic sarcoma. However, because histiocytic sarcoma in humans is a rare malignancy with sparse pathologic and cytogenetic data (178, 179), potential TRAF3 genetic alterations in human histiocytic sarcomas require future investigation.

In addition to the phenotype of histiocytic sarcoma, aging myeloid cell-specific TRAF3$^{-/-}$ (M-TRAF3$^{-/-}$) mice spontaneously develop chronic inflammation and other cancers that often affect multiple organs including the gastrointestinal tract (56). Similar to M-TRAF2$^{-/-}$ mice, young adult M-TRAF3$^{-/-}$ mice exhibit exacerbated DSS-induced colitis with increased levels of inflammatory cytokines produced by TRAF3$^{-/-}$ macrophages in response to TLR signaling (46). Notably, another mouse model with functional relevance to TRAF3, NLRP12$^{-/-}$ mice, is highly susceptible to colitis and colitis-associated colon cancer (180). NLRP12 interacts with both TRAF3 and NIK, and NLRP12$^{-/-}$ cells have constitutively activated NF-κB2 associated with a decreased protein level of TRAF3 (180). Interestingly, both NLRP12$^{-/-}$ hematopoietic and non-hematopoietic cells contribute to inflammation, but the latter dominantly contributes to colon tumorigenesis (180). In line with the *in vivo* data, mutations and deletions of TRAF3 are detected in 2.3% (10/439) of human colon cancers (TCGA, PanCancer Atlas). Furthermore, miR-32-TRAF3-mediated inhibition of the NIK-NF-κB2 pathway protects human colorectal epithelium against colorectal cancer in response to a diet of non-digestible carbohydrates (181). Thus, TRAF3 appears to act in both epithelial cells and myeloid cells to suppress colon tumorigenesis by inhibiting the NF-κB2 and TLR-induced inflammatory pathways.

Although most evidence identifies TRAF3 as a tumor suppressor, studies of the T cell-specific TRAF3$^{-/-}$ (T-TRAF3$^{-/-}$) mouse model suggest an oncogenic role for TRAF3 in T cells. Despite their constitutive NF-κB2 activation, TRAF3$^{-/-}$ T cells exhibit impaired proliferation and activation in response to TCR and CD28 co-stimulation (39, 57). T-TRAF3$^{-/-}$ mice show defects in T cell-mediated immunity and IL-15-induced proliferation and survival of iNKT cells, and also have reduced number of CD8 central memory T cells (**Table 2**) (57, 59, 60). Consistent with these *in vivo* data, TRAF3 is required for the proliferation of human neoplastic ALCL cells in culture (182). Silencing of TRAF3 in ALCL cells not only results in aberrant activation of the NIK-NF-κB2 pathway, but also affects the continued PI3K-AKT and JAK-STAT signaling (182). Therefore, distinct tumor suppressive and oncogenic roles of TRAF3 in different cellular contexts have been revealed from studies of both human and mouse models.

Key Tumor Suppressive Pathways

In addition to the above TRAF3-dependent tumor suppressive pathways verified in both human cancers and *in vivo* mouse models, several additional tumor suppressive pathways involving TRAF3 have been suggested by studies of cultured human cancer cells or xenograft models. These include: (1) TRAF3-mediated inhibition of the oncogenic RelB-SMAD4 association that represses TGFβ-SMAD target gene expression to promote the tumorigenesis of lung cancer, in which TRAF3 is targeted by RAS-NDP52-mediated autophagic degradation via the NDP52-TRAF3 interaction (183); (2) LIGHT-LTβR-TRAF3/TRAF5-ROS-ASK1-Caspase3 in the apoptosis of human colon cancer and hepatoma cells (184); (3) membrane-bound CD40L-CD40-TRAF3-p40phox-ROS-ASK1-MKK4-JNK-AP1-caspase 9/3/Bax/Bak in the apoptosis of human bladder and CRC cells but not normal epithelial cells (185–187); and (4) TRAF3-mediated inhibition of the oncogenic RIP2-NF-κB1/NF-κB2/p38-Bcl-xL pathway in the survival and proliferation of glioblastoma cells (188). Taken together, available evidence supports that TRAF3 acts as a tumor suppressor in a variety of cell types, but we cannot rule out that TRAF3 upregulation might also alter normal cell homeostasis in the same or other cell types and therefore contribute to transformation, as it has been observed in B cells and T cells.

TRAF4

Landscape of Genetic Alterations

The frequency of genetic alterations of *TRAF4* is generally <11% in human cancers (**Figure 1A**) based on the TCGA and COSMIC datasets of sample size n > 100. The eight human cancers with relatively higher genetic alterations of *TRAF4* are pancreatic cancer (10.1%) (7), bladder cancer (7.3%) (152), breast cancer (5.5%) (189), uterine cancer (5.1%) (TCGA, PanCancer Atlas), esophageal cancer (3.2%) (TCGA, Provisional), lung cancer (2.6%) (190), melanoma (2.5%) (191), and ovarian cancer (2.4%) (TCGA, PanCancer Atlas). The most common genetic alteration of *TRAF4* is amplification, followed by mutation (**Figure 1**). Deep deletion, truncation and fusion of *TRAF4* are relatively rare in human cancers.

Overview and Map of Recurrent Mutations

There are 123 different mutations of *TRAF4* detected in human cancers, comprising 85% (105/123) mutations that cause changes in the amino acid sequence of *TRAF4* and 15% (18/123) coding silent mutations (**Table 1**). About 42% (44/105) of the coding-altering mutations of the *TRAF4* gene are recurrent and detected in at least two cancer patients, including mostly missense mutations (89%, 39/44), 3 truncations, 1 frameshift deletion, and 1 in frame deletion (**Table 1** and **Figure 2**). *TRAF4* recurrent mutations occurred at 32 different amino acids that are distributed in the entire length of the *TRAF4* protein but are relatively enriched in the TRAF-C domain (**Figure 3**). Only two specific amino acids, R448 and R452 located at the C-terminal TRAF-C domain, are mutated in more than 3 patients (**Figure 3**). For R448, mixed missense mutations (R448Q or L) and a truncation (R448*) are identified in 4 patients with prostate cancer, uterine cancer, HNSCC, and OSCC (192–195). For R452, missense mutations (R452W or Q or L) are detected in four patients with uterine, colorectal and lung cancers (10, 108, 193). Further studies are needed to determine whether such missense mutations in the TRAF-C domain result in loss- or gain-of-function of TRAF4.

Fusion

There is only one fusion of *TRAF4* detected in human cancers, the *TRAF4-FASN* fusion identified in a glioma patient (TCGA), with currently unknown functional significance.

In vivo Causal Oncogenic Roles

Available human evidence indicates that gene amplification is the most common *TRAF4* genetic alteration in cancers and that *TRAF4* expression is ubiquitously elevated in many human cancers (196–204). This suggests that *TRAF4* overexpression may play causal roles in cancer initiation, progression and metastasis. Similar to classical oncogenes (such as c-Myc and K-ras), *TRAF4* is also required for ontogenic processes and TRAF4$^{-/-}$ mice show defects in embryonic development and neurulation (61, 62, 205). Interestingly, TRAF4$^{-/-}$ dendritic cells (DCs) derived from the null mice exhibit reduced *in vivo* and *in vitro* migration (64). Furthermore, recent *in vivo* evidence obtained from mouse models demonstrates the causal oncogenic roles of *TRAF4* in skin tumorigenesis (**Table 2**) (65). *TRAF4* deficiency substantially diminishes IL-17A-induced ERK5 activation and epidermal hyperplasia in mice. In the DMBA/TPA-induced skin cancer model, TRAF4$^{-/-}$ mice exhibit remarkably reduced tumor incidence and tumor numbers. Mechanistically, *TRAF4* bridges the interaction between Act1 and MEKK3 in response to IL-17A signaling, and therefore is required for the activation of the downstream MEK5-ERK5-Steap4/p63 pathway. The transcription factor p63 directly induces *TRAF4* expression in keratinocytes, promoting positive feedback on TRAF4 in the epidermis and thus sustaining the activation of the TRAF4-ERK5 axis to induce keratinocyte proliferation and skin tumorigenesis (65). These *in vivo* findings are reinforced by the examination of human SSCC samples, which also demonstrates the presence of the IL-17A-Act1-TRAF4-MEKK3-MEK5-ERK5-Steap4/p63 pathway (65). Together, both human and *in vivo* mouse evidence supports an oncogenic role for TRAF4.

Key Oncogenic Pathways

In addition to the established IL-17A-TRAF4-ERK5 axis, a variety of potential *TRAF4*-dependent oncogenic pathways have been suggested by studies of patient samples, cultured human cancer cells or their xenografts in immunodeficient mice. These include: (1) TRAF4-Akt/NF-κB-Glut1/HK2/RSK4/Slug in the proliferation and metastasis of lung and breast cancer cells as well as the migration and epithelial-mesenchymal transition (EMT) of hepatocellular carcinoma cells (HCC) (199, 203, 206); (2) TGFβ-TβRI-TRAF4-Smurf1/Smurf2/USP15-SMAD2/TAK1-N-cadherin/Fibronectin/Vimentin/SMA in the migration, EMT, and metastasis of breast cancer cells (200, 207); (3) SRC3-TRAF4-mediated inhibition of the USP7-p53 interaction, leading to the loss of p53 deubiquitination/stabilization and thus the resistance

to cytotoxic drugs and stress in breast cancer (208); (4) NGF-TrkA-TRAF4-Akt/p38-IL-6/Integrins/COX2 in the metastasis of prostate cancer cells (204); (5) TNFα-TRAF4/TRAF2-NF-κB1 in the survival and proliferation of breast cancer cells (209); (6) TRAF4-mediated up-regulation and nuclear translocation of β-catenin in the Wnt/β-catenin-cyclin D1/c-myc/Bcl-2/MMPs pathway that promote the growth and migration of OSCC and breast cancer cells (210, 211); (7) TRAF4-mTOR-p70S6K-S6 in the proliferation of breast cancer cells (212); and (8) the TRAF4-phosphoinositide (PIP) interaction at tight junctions that favors breast cancer cell migration (213). It would be interesting to verify these TRAF4-dependent oncogenic pathways using *in vivo* models.

TRAF5

Landscape of Genetic Alterations

The landscape of *TRAF5* genetic alterations is similar to that of *TRAF4*. The frequency of genetic alterations of *TRAF5* is generally <13% in human cancers (**Figure 1A**) according to the TCGA and COSMIC datasets of sample size n > 140. The eight human cancers with relatively higher genetic alterations of *TRAF5* are breast cancer (12.2%) (189), liver cancer (8.4%) (TCGA, Provisional), uterine cancer (6.4%) (TCGA, PanCancer Atlas), lung cancer (5.3%) (TCGA, Provisional), ovarian cancer (5.1%) (TCGA, Provisional), melanoma (4.0%) (TCGA, Provisional), esophageal cancer (3.8%) (TCGA, Provisional), and prostate cancer (3.3%) (214). As described for *TRAF4*, the most common genetic alteration of *TRAF5* is also amplification, followed by mutation (**Figure 1A**). Deep deletion, truncation and fusion of *TRAF5* are rare events in human cancers.

Overview and Map of Recurrent Mutations

There are 188 different mutations of *TRAF5* detected in human cancers, comprising 85% (160/188) mutations that alter the amino acid sequence of *TRAF5* and 15% (28/188) coding silent mutations (**Table 1**). Approximately 36% (57/160) of the coding-altering mutations of *TRAF5* are recurrent in human cancers. Similar to *TRAF4*, *TRAF5* recurrent mutations also include mostly missense mutations (85%, 49/57), but also some truncations (9%, 5/57), frameshift deletions (4%, 2/57), and an in frame deletion (2%, 1/57) (**Table 1** and **Figure 2**). These recurrent mutations occurred at 36 different amino acids that are mainly enriched in the TRAF-C domain but also scattered in other regions of the TRAF5 protein (**Figure 3**). Mutations of three specific amino acids, R164, T232, and A548, are detected in more than three patients (**Figure 3**). Complex alterations of R164 of the zinc finger motif, including truncation (R164*) and missense mutations (R164Q or L), are detected in six patients with uterine, colon and bile duct cancers and DLBCL (TCGA) (12, 193, 215). Another missense mutation of the zinc finger motif, T232M, is detected in four patients with colon, breast, and prostate cancers (TCGA; COSMIC) (98). Missense mutation A548V of the TRAF-C domain is identified in four patients with uterine, cervical, stomach, and breast cancers (TCGA) (107). The functional consequences of these recurrent *TRAF5* mutations await further investigation.

In vivo Causal Oncogenic Roles

Although not cataloged in TCGA, *TRAF5* mutations are detected in 5% (5/101) of human DLBCL (102). *TRAF5* expression is upregulated in human splenic MZL (216). In addition, apoptosis-resistant B cell-acute lymphoblastic leukemia (B-ALL) cells have aberrantly higher protein level of TRAF5 and TRAF6 in response to irradiation than apoptosis-proficient B-ALL cells (217). The above evidence suggests that TRAF5 may be oncogenic in B cells. Consistent with human evidence, B cells of TRAF5$^{-/-}$ mice show defects in CD40-induced proliferation and up-regulation of surface molecules and activation markers as well as CD40 plus IL-4-induced Ig production (**Table 2**) (67). Using a chimeric CD40-LMP1 transgenic (CD40LMP1-tg) mouse model that mimics the B cell hyperactivation induced by the EBV-encoded oncoprotein LMP1 (218), Kraus et al. demonstrated that TRAF5 is a critical mediator of the *in vivo* functions of LMP1 (73). *TRAF5* deficiency reverses the CD40-LMP1-induced enlargement of the spleen and lymph nodes, decreases the serum levels of IL-6 and autoantibodies that are elevated by CD40-LMP1-tg expression, and also inhibits LMP1-mediated JNK activation in B lymphocytes (**Table 2**) (73). Together, both human and mouse evidence supports an oncogenic role for TRAF5 in B cells that appears to be required for LMP1-mediated B lymphomagenesis and is likely also involved in spontaneous B lymphomagenesis initiated by genetic alterations.

Additionally, available *in vivo* evidence indicates the importance of TRAF5 in the survival, proliferation and differentiation of different T cell subsets as detailed in **Table 2**, suggesting that TRAF5 malfunction may contribute to T cell malignancies. However, the evidence of *TRAF5* alterations in human T cell lymphomas/leukemias is still lacking.

TRAF5-Dependent Signaling Pathways in Human Cancer Cells

In addition to the signaling pathways of B cells and T cells revealed by the *in vivo* studies of TRAF5$^{-/-}$ mice, a number of TRAF5-dependent signaling pathways have been proposed based on the studies of patient samples, cultured human cancer cells or their xenografts in immunodeficient mice. These include: (1) CD30-TRAF2/TRAF5-NIK-IKKα-NF-κB-IL-13 in the survival of Hodgkin-Reed-Sternberg (H-RS) cells of HL (219, 220) and a similar CD30v-TRAF2/TRAF5-NIK-NF-κB pathway in acute myeloid leukemia (AML) and ALL (221); (2) LIGHT-LTβR-TRAF3/TRAF5-ROS-ASK1-Caspase 3 in the apoptosis of human colon cancer and hepatoma cells (184); (3) upregulated TRAF5-NF-κB in the migration and invasion of glioma cells, in which TRAF5 is directly targeted for degradation by the tumor suppressor Numbl (222); (4) TRAF5/TRAF6-NF-κB-Vimentin/TWIST1/SNAIL2/MMP13/IL-11 in the EMT and metastasis of prostate cancer cells, in which TRAF5 is directly targeted for downregulation by the tumor suppressive miR-141-3p (223); and (5) TRAF5-MEK1/2-ERK1/2-Bcl2 in the survival and proliferation of melanoma cells, in which TRAF5 is directly targeted for downregulation by tumor suppressive MiR-26b (224). The above evidence supports the hypothesis that TRAF5 plays oncogenic roles in various human cancer cells primarily

by inducing NF-κB activation but also by activating the ERK1/2 pathway.

TRAF6

Landscape of Genetic Alterations

The frequency of genetic alterations of *TRAF6* is generally <7% in human cancers (**Figure 1A**) based on the TCGA and COSMIC datasets of sample size n > 120. The eight human cancers with relatively higher genetic alterations of *TRAF6* are breast cancer (6.9%) (225), uterine cancer (4.5%) (TCGA, PanCancer Atlas), stomach cancer (4.1%) (8), HNSCC (3.6%) (113), lung cancer (3.4%) (10), bladder cancer (3.1%) (226), sarcoma (3%) (TCGA, Provisional), and ovarian cancer (2.8%) (TCGA, Provisional). Although not listed in TCGA, *TRAF6* amplification is recognized as one of the most frequent genomic alterations in human lung cancer (9.2%, 24/261) (227) and OSCC (10%, 2/20) (228). Consistent with the frequent amplification of *TRAF6* in human cancers, *TRAF6* is overexpressed in many human cancers such as breast cancer, HCC, colon cancer, esophageal cancer, and melanoma (229–233). *TRAF6* overexpression is also identified as a prognostic factor for breast and esophageal cancers (229, 232). Together, the most common genetic alterations of *TRAF6* are mutation and amplification (**Figure 1A**). Deep deletion of *TRAF6* is less common but also detected in several different types of human cancers. Truncation and fusion of *TRAF6* are rare in human cancers.

Overview and Map of Recurrent Mutations

There are 178 different mutations of *TRAF6* detected in human cancers, comprising 85% (152/178) mutations that alter the protein sequence of *TRAF6* and 15% (26/178) coding silent mutations (**Table 1**). Only 27% (41/152) of the coding-altering mutations of *TRAF6* are recurrently detected in at least two cancer patients. Similar to *TRAF1*, *TRAF6* recurrent mutations also have the simplest composition and are almost exclusively missense mutations (93%, 38/41) except 2 truncations and 1 frameshift insertion (**Table 1** and **Figure 2**). These recurrent mutations occurred at 30 different amino acids that are distributed in all the major domains but are enriched in the coiled-coil and TRAF-C domains of the TRAF6 protein (**Figure 3**). Mutations of only two specific amino acids, R335 and P398, are detected in more than three patients (**Figure 3**). A truncation (R335*) and missense mutation (R335Q) at R335 within the coiled-coil domain of TRAF6 are detected in five patients with colon and uterine cancers (TCGA) (12, 234). Missense mutations at P398 (P398L or S) of the TRAF-C domain are identified in 4 patients with uterine, lung, and stomach cancers (TCGA) (8, 193, 235). Functional significance of these *TRAF6* recurrent mutations in cancer pathogenesis remains to be elucidated.

In vivo Evidence of Potential Roles for TRAF6 in Tumorigenesis

Causal roles of TRAF6 in tumorigenesis have not been directly demonstrated with *TRAF6* knockout or transgenic mice yet. However, available *in vivo* evidence supports potential contributions of TRAF6 dysregulation in tumorigenesis. Consistent with the genetic alterations (mainly amplification and mutation) and frequent overexpression of TRAF6 detected in human epithelial cancers such as breast cancer and uterine cancer (**Figure 1A**) (229–233), deletion of *TRAF6* in mice results in loss of NF-κB activity in epithelia and vasculature during mouse development (**Table 2**) (78). Corroborating initial evidence, intestinal epithelial cell-specific TRAF6$^{-/-}$ mice exhibit exacerbated DSS-induced colitis (87). In line with the *in vivo* data, knockdown of TRAF6 or inhibition of TRAF6 E3 ligase activity suppresses the survival, proliferation, migration, and metastasis of many human epithelial cancers, including breast, lung, liver, and colon cancers as well as HNSCC (230–232, 236–240). In the majority of these cases, the TRAF6-NF-κB axis is identified as the main oncogenic pathway, which is constitutively activated by TRAF6 overexpression or hyperactivated by upstream receptors such as TNFα, RANK, and TLR4/3 (236–240).

Similar findings have been obtained in the hematopoietic/lymphoid system. Hematopoietic-specific deletion of *TRAF6* in mice leads to decreased tonic IKKβ-NF-κB activation, impaired hematopoietic stem cell (HSC) self-renewal and loss of hematopoietic stem/progenitor cells (HSPCs) in the bone marrow (BM) (**Table 2**) (81). Knockdown of TRAF6 in human AML cell lines or patient samples results in rapid apoptosis and impaired malignant HSPC function as well as increased sensitivity to bortezomib (241). In the lymphoid lineage, *TRAF6* mutations have been detected in 2.1% human DLBCL (TCGA) and 2.4% human cutaneous T cell lymphoma (CTCL) (242). TRAF6$^{-/-}$ mice have reduced numbers of immature B cells in the BM and B cells from the null mice show defects in CD40 and LPS-induced proliferation and NF-κB activation (**Table 2**) (74, 75). B cell-specific TRAF6$^{-/-}$ mice (B-TRAF6$^{-/-}$) mice also exhibit reduced numbers of mature B cells in the BM and spleen as well as defective B1 B cell development (**Table 2**) (82). Knockdown of TRAF6 or inhibition of the TRAF6-NF-κB axis induces apoptosis and cell cycle arrest in DLBCL, and also inhibits the proliferation and bone resorption of MM (243, 244). Interestingly, T cell-specific TRAF6$^{-/-}$ mice (T-TRAF6$^{-/-}$) mice show multiorgan inflammation due to hyperactivation of the PI3K-Akt pathway in T cells (**Table 2**) (83). T-TRAF6$^{-/-}$ mice also exhibit increased Th17 differentiation due to enhanced sensitivity of CD4 T cells to TGFβ signaling (85), but have defects in generating CD8 memory T cells caused by defective AMPK activation in activated CD8 T cells (84). In addition, T-TRAF6$^{-/-}$ mice exhibit impaired OX40-induced CD4 Th9 differentiation, which requires TRAF6-mediated activation of the NIK-NF-κB2 pathway in CD4 T cells (**Table 2**) (86). In support of a role for TRAF6 in T cell tumorigenesis, inhibition of the TRAF6-NF-κB-c-Myc axis through miR-146a/b-mediated downregulation of TRAF6 delays PTEN$^{-/-}$ T cell lymphomagenesis in mice (245). Furthermore, inhibition of the IRAK1/4-TRAF6 axis sensitizes human T cell ALL (T-ALL) to chemotherapies (246). Collectively, the above evidence is consistent with the hypothesis that TRAF6 may serve as an oncoprotein in epithelial cancers and

hematopoietic/lymphoid neoplasms mainly through inducing aberrant NF-κB activation.

Interestingly, *in vivo* evidence also indicates the functional importance of TRAF6 in the brain and muscle (**Table 2**). TRAF6$^{-/-}$ mice show defective neural tube closure and exencephaly (80). Mechanistically, TRAF6 interacts with the p75 neurotrophin receptor (p75NTR), and thus is required for NGF-induced NF-κB activation and survival in Schwann cells as well as BDNF-induced JNK activation and apoptosis in superior cervical ganglia neurons (79). In skeletal muscle, TRAF6 deficiency prevents muscle loss and cancer cachexia in response to transplanted tumor growth, improves regeneration of myofibers upon injury and reduces skeletal muscle atrophy upon starvation through regulating NF-κB activation/ubiquitin-proteasome/autophagy-lysosomal systems, Akt/FoxO3a/AMPK activation and Notch signaling, respectively (88–90). In line with the mouse data, genetic alterations of *TRAF6*, including amplification, mutation and deletion, are detected in 1% of human glioblastoma (247, 248) and 3% of human sarcoma (TCGA) (9). It would be interesting to test potential causal roles of TRAF6 in brain and muscle tumorigenesis in future studies.

Key Signaling Pathways in Human Cancers

In addition to the TRAF6-NF-κB axis that has been verified in both human cancers and *in vivo* mouse models, numerous TRAF6-dependent oncogenic pathways have been reported with studies of patient samples, cultured human cancer cells or their xenografts in mice. Examples are: (1) the TRAF6-p53 crosstalk, in which TRAF6 promotes K63-linked ubiquitination of p53 and limits the tumor suppressive function of p53 in cancer development and resistance to chemotherapy and radiotherapy (249); (2) the Ras-TRAF6-NF-κB axis in the tumorigenesis of lung and pancreatic cancers (227, 250, 251); (3) the TRAF6-Akt axis in the tumorigenesis of glioblastoma, HNSCC, prostate cancer, oral cancer, and CRC (252–255); (4) the EGFR-TRAF6 axis in the growth, migration and metastasis of lung cancer and cutaneous SCC (256, 257); (5) the TRAF6-HIF1α axis in the tumorigenesis, angiogenesis, and metastasis of breast and colon cancers (258, 259); (6) the TGFβ-TβRI/II-TRAF6 axis in the proliferation, migration, and invasion of prostate cancer (260–263); (7) TRAF6-AEP-HSP90α in the invasion and metastasis of breast cancer (229); (8) nutrients-MEKK3-MEK3/6-p38δ-p62-TRAF6-mTORC1 in the growth of prostate cancer (264); (9) pVHL inactivation-CARD9-BCL10-TRAF6-TAK1-MKK4-JNK-AP1-Twist in the EMT of renal cell carcinoma (265); (10) ADAM10-p75NTR ICD-TRAF6 in the metastasis and chemoresistance of HNSCC and breast cancer (266); (11) TRAF6-DNMT1-DNA methylation in chemoresistance of breast cancer (267); and (12) IL-1β-TRAF6-TNFα and TRAF6-Cdc42-F-actin in the invasion of SCC (268). Interestingly, the importance of TRAF6-dependent oncogenic pathways in human cancers is also underscored by the findings that *TRAF6* mRNA is the direct target of tumor suppressive mi-RNAs, including miR-146a (269–272), miR-146b-5p (273, 274), and miR-141-3p (223). Thus, most evidence indicates that *TRAF6* is an oncogene in human cancers.

Intriguingly, several TRAF6-dependent tumor suppressive pathways have also been described for human cancers in the literature. Examples are: (1) TRAF6-p62-mediated inhibition of the HK2 glycolytic activity and the growth of liver cancer, in which TRAF6 catalyzes the K63-linked ubiquitination of HK2 and targets HK2 for p62-mediated autophagic degradation (275); (2) TRAF6-mediated suppression of the EZH2-H3K27me3 pathway and the progression of prostate cancer, in which TRAF6 mediates K63-linked ubiquitination and degradation of EZH2 (276); and (3) TRAF6-mediated decrease of the H3K4me3 level and thus the tumorigenesis of prostate cancer, in which TRAF6 mediates K63-linked ubiquitination of JARID1B to increase the demethylase activity of JARID1B on H3K4me3 (277). Taken together, both tumor suppressive and oncogenic roles of TRAF6 have been reported in human liver cancer and prostate cancer. As discussed for TRAF2, this phenomenon may be related to the mutational profile and malignant stage of the cancer cells as well as the nature of the environmental cue or treatment regimen.

TRAF7

Landscape of Genetic Alterations

TRAF7 lacks the TRAF homology domain and does not directly interact with any member of the TNFR superfamily, two defining features of the TRAF family (278, 279), and is therefore still controversial to be considered as a genuine member of the TRAF family. The frequency of genetic alterations of TRAF7 is generally <7% in human cancers (**Figure 1A**) according to the TCGA and COSMIC datasets of sample size n > 150. The eight human cancers with relatively higher genetic alterations of *TRAF7* are breast cancer (6%) (189), prostate cancer (5.1%) (280), stomach cancer (4.8%) (8), sarcoma (3.8%) (TCGA, Provisional), esophageal cancer (3.3%) (TCGA, PanCancer Atlas), uterine cancer (3.2%) (TCGA, PanCancer Atlas), melanoma (3.1%) (TCGA, PanCancer Atlas), and liver cancer (2.4%) (TCGA, PanCancer Atlas). However, it should be noted that although not yet cataloged in TCGA, the rate of *TRAF7* mutation is overwhelmingly high in patients with adenomatoid tumors of the male and female genital tracts (100%, 31/31) (281), secretory meningiomas (97%, 29/30) (282), intraneural perineuriomas (62.5%, 10/16) (283), and meningiomas 23% (182/775) (284) (**Figure 1B**). In particular, high frequencies (15–26%) of *TRAF7* mutations has been reproducibly detected in multiple studies (284–288), and knowledge of *TRAF7* mutations has contributed significantly to improving the diagnosis, classification, prognosis, and treatment of patients with meningiomas (282, 286, 289–291). Additionally, deletion of *TRAF7* is detected in 67% (18/27) of malignant mesothelioma patients' malignant cells in pleural fluids (292). The most common genetic alteration of *TRAF7* is mutation, followed by amplification and then deep deletion (**Figure 1**). Truncation and fusion of *TRAF7* are rarely detected in human cancers.

Overview and Map of Recurrent Mutations

In the *TRAF* family, *TRAF7* has the highest counts of total and recurrent mutations. There are 376 different mutations of *TRAF7*

detected in human cancers, including 87% (326/376) coding-altering mutations and 13% (50/376) coding silent mutations (**Table 1**). Over half (53%, 174/326) of the *TRAF7* coding-altering mutations are recurrently detected in at least two cancer patients. *TRAF7* recurrent mutations are mostly missense mutations (92%, 161/174). Small percentages of other recurrent mutations include 5 frameshifts, 3 truncations, 2 in frame deletions, 2 splice mutations, and 1 fusion (**Table 1** and **Figure 2**). These recurrent mutations occurred at 89 different amino acids covering different regions of the entire length but highly enriched in the last 4 WD40 repeats of the TRAF7 protein (**Figure 3**). Of particular interest, missense mutations of six specific amino acids located within the C-terminal WD40 repeats, N520, H521, G536, S561, K615, and R641, are identified as mutation hotspots of *TRAF7* detected in more than 15 cancer patients (**Figure 3**). N520 mutations (N520S, H, Y, or T) are found in 31 patients with meningioma, mesothelioma, sarcoma and colon cancer (12, 106, 282, 284, 285, 293, 294). Mutations of the next amino acid H521 (H521R or N) are identified in 15 patients with adenomatoid tumor, perineurioma, and meningioma (281, 283, 284). G536 mutations (G536S or V) are detected in 16 patients with meningioma, pancreatic cancer, mesothelioma and stomach cancer (106, 282, 284, 285, 293–295). S561 mutations (S561R, N or T) are identified in 19 patients with adenomatoid tumor, perineurioma and meningioma (281, 283, 284). K615E mutations are detected in 15 patients with meningioma and OSCC (284, 296). R641 mutations (R641H, C, P, or L) are detected in 24 patients with uterine, bile duct, colon, stomach and lung cancers and meningioma (TCGA, PanCancer Atlas) (8, 106, 282, 284, 285, 294, 297–299). Although the functional significance of most *TRAF7* mutations is currently unclear, the exceptionally high recurrence and clustering of missense mutations implicate TRAF7 malfunction as a critical pathogenic event in relevant human cancers.

Fusion

There are six different fusions of *TRAF7* and other genes detected in human cancers, including *TRAF7-LRRC1* in lung cancer, *GFER-TRAF7* in mesothelioma, *CORO7-TRAF7* in glioma, *TRAF7-MAPK8IP3* in bladder cancer, and *TRAF7-RAB26* and *E4F1-TRAF7* in ovarian cancer (TCGA) (106). Among these, only the *TRAF7-LRRC1* fusion is recurrently detected in two patients with lung cancer (TCGA). However, all the *TRAF7* gene

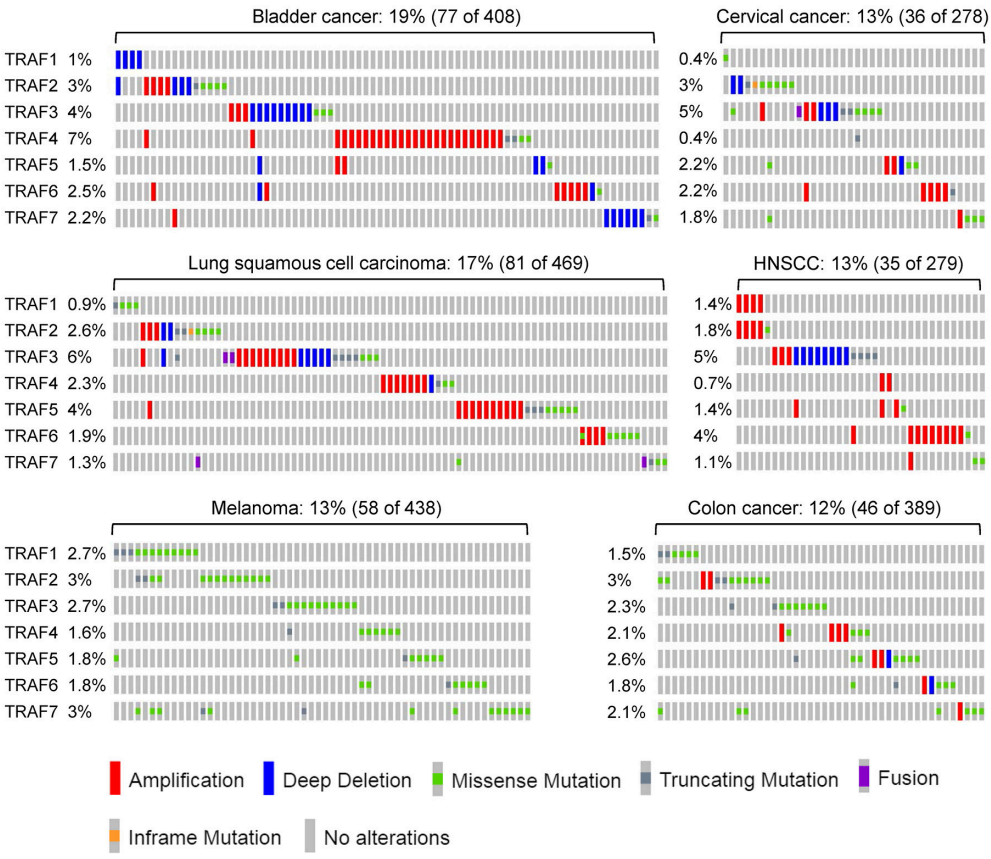


FIGURE 4 | Combined genetic alterations of the *TRAF* family in human cancers. Representative results of the combined genetic alterations of the *TRAF* family in individual human cancers are retrieved from TCGA, specifically bladder cancer, lung cancer, melanoma, cervical cancer, HNSCC, and colon cancer. The sample size and the number of patients containing genetic alterations of *TRAFs* as well as the frequency of each *TRAF* alteration are indicated for each type of cancer in the figure. The nature of *TRAF* genetic alteration identified in each patient is indicated by a mutation symbol as shown at the bottom legend of the figure.

fusions have not been verified at the protein level and their functional consequence is unknown.

In vivo Evidence of Potential Roles in Cancer Pathogenesis

No TRAF7$^{-/-}$ or TRAF7-tg mouse model has been published yet. Importantly however, Tokita et al. recently reported that *de novo* missense mutations in *TRAF7* cause developmental abnormalities and other clinical symptoms in seven unrelated patients, including developmental delay (5/5), congenital heart defects (6/7), limb and digital anomalies (7/7), and dysmorphic facial features (7/7) (300). *TRAF7* mutations identified in this study include a recurrent R655Q mutation found in four patients, and another 3 single mutations each identified in one patient, including K346E, R371G, and T601A (300). Interestingly, R371 recurrent mutations are also detected in human cancers (**Figure 3**). K346 is a ubiquitination site of TRAF7 (301). Both K346 and R371 are located in the coiled-coil domain of TRAF7 that is important for TRAF7 homodimerization (302, 303). The recurrent R655Q mutation has also been previously identified as a *de novo* event in an autism patient (304). Both T601 and R665 are located in the C-terminal WD40 repeats of TRAF7, which contain most mutation hotspots of *TRAF7* detected in human cancers (**Figure 3**) and are known to mediate the interaction of TRAF7 with MEKK3 or c-Myb (302, 305). Tokita et al. further revealed that transfection of the R665Q, T601A, or R371G mutants of *TRAF7* into HEK293T cells results in significantly reduced levels of ERK1/2 phosphorylation, both basal and in response to TNFα signaling (300). Consistent with this biochemical evidence, conditional ERK2$^{-/-}$ mice show a phenotype mirroring that observed in the seven patients with *TRAF7* mutations, including craniofacial abnormalities, cardiovascular malformations and limb defects (306). These highly interesting findings warrant further investigation of the *in vivo* functions of *TRAF7* mutations in cancer pathogenesis using animal models.

TRAF7-Mediated Signaling Pathways

Compared to other TRAF proteins, the signaling mechanisms of TRAF7 are understudied and remain poorly defined (289, 307). In addition to the above TRAF7-ERK1/2 pathway revealed by studying *TRAF7* mutants of patients with developmental defects, the following TRAF7 signaling pathways have been proposed based on *in vitro* studies. (1) Transfection of tumor-derived *TRAF7* mutants (H521R, Y538S, or S561R) but not WT *TRAF7* in 293T cells causes increased phosphorylation of RelA and expression of the NF-κB target gene L1CAM, which is also elevated in adenomatoid tumors (281). (2) Overexpression of *TRAF7* or TNFα induces caspase-dependent apoptosis in HEK293 and HeLa cells via the TRAF7-MEKK3-NF-κB/p38/JNK-AP1/CHOP pathway, in which TRAF7 interacts with MEKK3 and potentiates the kinase activity of MEKK3

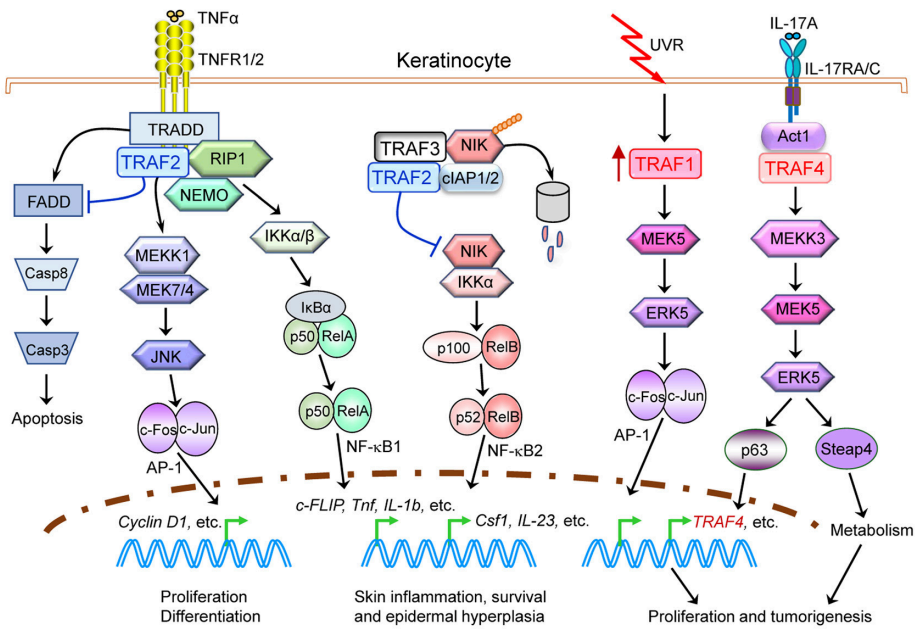


FIGURE 5 | Causal roles and signaling mechanisms of TRAF proteins in skin carcinogenesis. Evidence of both genetic alterations of *TRAFs* in human patients as well as *in vivo* TRAF$^{-/-}$ mouse models indicates that alterations of multiple TRAF proteins, specifically TRAF1, TRAF2, and TRAF4, play causal roles in skin carcinogenesis. Oncogenic TRAF proteins (TRAF1 and TRAF4) are depicted in red, while tumor suppressive TRAF2 proteins are depicted in blue. This figure depicts a simplified model of keratinocyte-intrinsic, TRAF-dependent signaling mechanisms in skin carcinogenesis. Only key TRAF-dependent receptors, TRAF-interacting proteins and downstream kinases and transcription factors that have been verified in both human cancers and *in vivo* mouse models are shown. Keratinocyte-extrinsic, indirect mechanisms of TRAF proteins in skin carcinogenesis are not depicted in the figure, including the known roles of TRAFs in tumor immunity, inflammation and bone resorption and thus their indirect contributions in tumorigenesis and metastasis.

(302, 303). (3) TRAF7 mediates TNFα-induced apoptosis in Jurkat and HeLa cells via promoting the K29-, K33-, and K63-linked ubiquitination and lysosomal degradation of c-FLIP, an inhibitor of caspase activation (308). (4) TRAF7 represses TNFα-induced NF-κB activation to enhance apoptosis in HEK293 cells by promoting K29-linked ubiquitination and lysosomal degradation of NEMO and RelA (309). Paradoxically, TRAF7 is identified as an activator of the NEMO-RelA-NF-κB-cyclin D1 pathway in mouse myoblasts and thus a suppressor of myoblast differentiation (310). (5) TRAF7 participates in TLR2-induced production of inflammatory cytokines (TNFα, IL-1β, and IL-8) in A549 and HeLa cells by acting in the TLR2-TRAF6/TRAF7-IKK1/2/NEMO-NF-κB and TLR2-TRAF6/TRAF7-MKK3/6-p38 pathways (311). (6) TRAF7 inhibits the transcriptional activity of the oncoprotein c-Myb in M1 mouse leukemia cells and DND39 human Burkitt's B lymphoma cells by directly interacting with c-Myb and stimulating the sumoylation of c-Myb to sequester c-Myb in the cytoplasm (305). (7) TRAF7 mediates K48-linked ubiquitination of p53 as demonstrated by an *in vitro* ubiquitination assay, which likely induces p53 degradation. Correspondingly, TRAF7 protein is downregulated and p53 protein is upregulated in a panel of breast cancer specimens, and TRAF7 downregulation correlates with poor prognosis in breast cancer (312). In summary, WT TRAF7 appears to be a tumor suppressor that promotes cell apoptosis. *TRAF7* mutations or downregulated protein levels may lead to aberrant NF-κB activation or altered signaling of ERK1/2, p38, JNK, c-FLIP, c-Myb, or p53 to drive tumorigenesis. Further studies are required to clarify the roles and mechanisms of *TRAF7* alterations in cancer pathogenesis.

COMBINED GENETIC ALTERATIONS OF ALL *TRAFS* IN THE SAME HUMAN CANCER

After analyzing the genetic alterations of each *TRAF* gene in human cancers individually, we next examined the combined genetic alterations of all *TRAFs* in the same type of human cancer using the TCGA tool. Although the frequency of the genetic alterations of each *TRAF* is generally low (usually <5%), their combined rate is substantially increased to 10–35% in many types of human cancers (**Figure 4**) (TCGA). For example, the combined frequency of gene amplification of all seven TRAFs is 35% (709/2015) in breast cancer (313, 314). The combined frequency of genetic alterations of all seven TRAFs is 23% (71/311) in ovarian cancer (TCGA, Provisional), 19% (77/408) in bladder cancer (152), 19% (45/240) in uterine cancer (193), 17% (81/469) in lung cancer (TCGA, PanCancer Atlas), 15% (41/265) in oesophageal cancer (315), 14% (48/353) in liver cancer (TCGA, PanCancer Atlas), 13% (35/279) in HNSCC (113), 13% (36/278) in cervical cancer (TCGA, PanCancer Atlas),

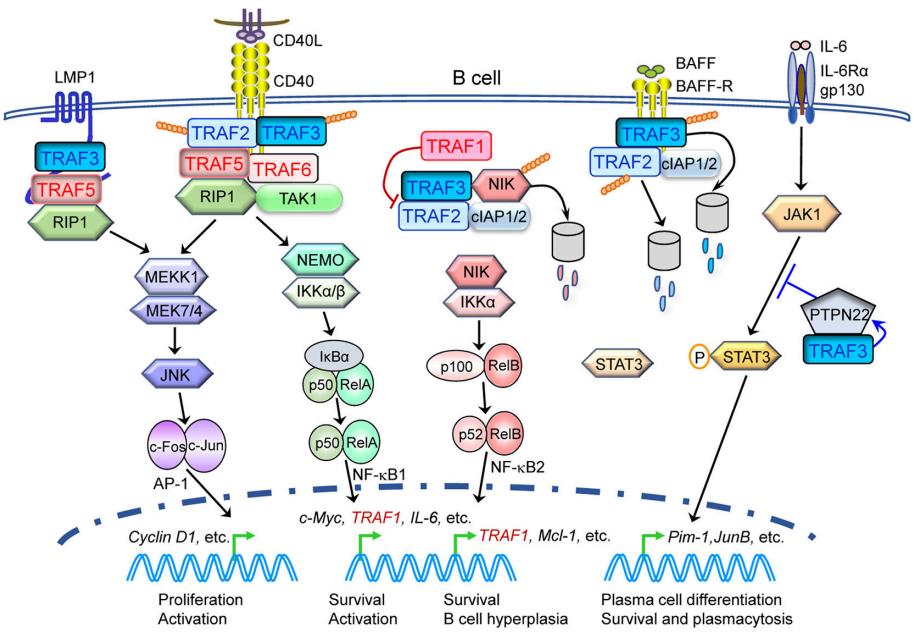


FIGURE 6 | Complex protective and pathogenic roles as well as signaling mechanisms of TRAF proteins in B cell malignancies. Evidence of both genetic alterations of *TRAFs* in human patients as well as *in vivo* TRAF knockout and transgenic mouse models indicates that alterations of multiple TRAF proteins, specifically TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6, play causal roles in the pathogenesis of B cell malignancies, such as B lymphomas and multiple myeloma. Tumor suppressive TRAF proteins (TRAF2 and TRAF3) are depicted in blue, while oncogenic TRAF proteins (TRAF1, TRAF5, and TRAF6) are depicted in red. This figure depicts a simplified model of B cell-intrinsic, TRAF-dependent signaling mechanisms in B cell malignancies. Only key TRAF-dependent receptors, TRAF-interacting proteins and downstream kinases and transcription factors that have been verified in both human cancers and *in vivo* mouse models are shown. Potential contribution of TRAF1, TRAF2, and TRAF6 in LMP1 signaling, TRAF6 in B cell receptor (BCR) signaling and TRAFs in CD40- and LMP1-induced activation of PI-3K-Akt to B cell tumorigenesis are not included in the figure.

TABLE 3 | Pathogen-encoded proteins that exploit or target TRAFs to induce carcinogenesis in humans.

Pathogen proteins	TRAFs	Mechanisms	Cancer type	References
BACTERIAL ONCOPROTEINS				
Cag PAI of *H. pylori*	TRAF1, 2, 6	Utilizes TRAF1, 2, and 6 to induce NF-κB activation and IL-8 secretion	Gastric cancer	(316, 319)
Tip-α of *H. pylori*	TRAF3	Induces TRAF3 protein and NF-κB activation by inhibiting miR-3178 expression, which targets TRAF3	Gastric cancer	(320)
VIRAL ONCOPROTEINS				
LMP1 of EBV	TRAF1, 2, 3, 5, 6	Sequesters cellular TRAF3, and usurps TRAF1, 2, 3, 5, and 6 to mimic constitutively activated CD40 signaling, induces NF-κB1 and NF-κB2 activation, and induces	B lymphomas	(1, 172, 173, 321)
		EGFR expression	Nasopharyngeal carcinoma	(322–324)
	TRAF5, 6	Recruits TRAF5 and 6 to activate p38 and suppress the replication of EBV, maintaining the lalent state of EBV	Burkitt's lymphoma	(325)
v-FLIP of KSHV	TRAF2, 3	Recruits TRAF2 and 3 to activate NF-κB and JNK, and to induce cell survival	Primary effusion lymphoma	(326)
pUL48 of HCMV	TRAF3, 6	Deubiquitinates TRAF3 and 6 to inhibit type I IFN production, enhances cellular metabolic activity and upregulates anti-apoptotic proteins	Breast cancer, glioma	(327)
E6 protein of HPV	TRAF3	Inhibits p53 and RB expression, but E6 protein levels are inhibited by TRAF3	HNSCC	(328)
Core protein of HCV	TRAF2, 5, 6	Interacts with TRAF2, 5, and 6 to activate NF-κB and induce inflammation	Hepatocellular carcinoma	(329, 330)
Tax of HTLV-1	TRAF3, 6	Interacts with TRAF3 and 6 to induce TBK1-IKKε activation, type I IFN production and Mcl-1 stabilization	T cell leukemia	(331, 332)
VIRAL TUMOR SUPPRESSORS				
E2 protein of HPV	TRAF5, 6	Interacts with TRAF5 and 6, promotes K63-linked ubiquitination of TRAF5, and potentiates TNFα-induced NF-κB activation by activating TRAF5	Cervical cancer, HNSCC	(333, 334)

13% (58/438) in melanoma (TCGA, PanCancer Atlas), 12% (46/389) in colon cancer (TCGA, PanCancer Atlas), and 10% (106/1013) in prostate cancer (98). It is interesting that genetic alterations of different *TRAFs* are often mutually exclusive in the same cancer patient and simultaneous genetic alterations of two or three different *TRAFs* in the same cancer patient are generally rare events (**Figure 4**) except for ovarian cancer (TCGA, Provisional), uterine cancer and melanoma (TCGA, PanCancer Atlas).

We summarize key oncogenic pathways that involve multiple TRAF proteins in skin carcinogenesis as depicted in **Figure 5** as well as in B cell malignancies as depicted in **Figure 6**, both of which have been verified by studies of human cancers and *in vivo* mouse models. However, we believe current understanding only represents "the tip of the iceberg" of oncogenic mechanisms involving TRAF proteins. Given the often mutually exclusive genetic alterations of different TRAFs in the same cancer, it is very likely that all seven TRAFs may have non-overlapping and distinct contributions to different aspects or at different stages of the initiation, progression and metastasis of the same cancer. These unanswered questions represent fascinating areas for future exploration.

TRAF PROTEINS IN PATHOGEN-INDUCED CARCINOGENESIS

The importance of TRAF proteins in cancer pathogenesis is strengthened by mounting evidence that demonstrates their involvement in pathogen-mediated carcinogenesis in certain human cancers. For example, chronic infection with the bacteria *Helicobacter pylori (H. pylori)* is a major cause of gastric cancer. *H. pylori* infection induces TRAF1 overexpression and the expression of the transcription factor Cdx2 in both human gastric epithelial cells and mice, which are mainly driven by NF-κB activation. TRAF1 overexpression plays an antiapoptotic role in *H. pylori*-infected gastric cells (316). Induction of Cdx2 contributes to intestinal metaplasia, a precursor event to gastric cancer. Interestingly, TRAF3 inhibits *H. pylori* infection-induced NF-κB activation and Cdx2 expression, and is required to resist the infection by acting in the NOD1-RIP2-TRAF3 pathway in gastric epithelial cells (317, 318). Furthermore, the oncoprotein *cag* PAI of *H. pylori* activates NF-κB and induces IL-8 secretion through the TRAF2/TRAF6-NIK-IKK pathway in gastric cancer cells (319). Another carcinogenic factor, Tip-α, of *H. pylori* activates NF-κB by inhibiting the

expression of miR-3178, which directly targets TRAF3 mRNA for downregulation, in gastric epithelial cells (320). Therefore, *H. pylori* chronic infection-induced gastric tumorigenesis involves activation of TRAF1, TRAF2, and *TRAF6* as well as inhibition of TRAF3 (**Table 3**).

A variety of viral infections have also been linked to cancer development. DNA viruses, such as Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), human cytomegalovirus (HCMV) and human papilloma virus (HPV), cause NPC, B lymphomas, breast cancer, glioma, cervical cancer, and HNSCC in the host (335, 336). RNA viruses, such as hepatitis C virus (HCV) and human T-cell leukemia virus type 1 (HTLV-1), may lead to HCC and T cell leukemia, respectively, in an infected individual (335, 336). Notably, oncogenic proteins of these viruses exploit or target one or multiple TRAF proteins for their signal transduction. These include the EBV-encoded oncoprotein LMP1, v-FLIP of KSHV, pUL48 of HCMV, E2 and E6 of HPV, Core protein of HCV and Tax protein of HTLV-1. In particular, consistent with the high frequency of TRAF3 deletions and mutations in HPV+ HNSCC, overexpression of TRAF3 inhibits the growth, migration and chemoresistance of HPV+ HNSCC by decreasing HPV E6 oncoprotein and increasing p53 and RB tumor suppressors (328). We briefly summarize the TRAF-dependent signaling mechanisms of pathogen-encoded proteins that contribute to human carcinogenesis as detailed in **Table 3**.

INDIRECT MECHANISMS OF TRAFS IN HUMAN CANCERS

Although beyond the scope of this review, we would like to point out that as critical regulators of adaptive immunity, innate immunity, and inflammation (1–4), TRAF proteins may indirectly contribute to the development, progression, and metastasis of various cancers by affecting tumor surveillance, tumor immunity, chronic inflammation and the tumor microenvironment. For example, disorders of innate antibacterial response are of fundamental importance in the development of gastrointestinal cancers, including pancreatic cancer, and increased expression of TRAF6, TLR4, and NOD1 are detected in peripheral blood leukocytes of pancreatic cancer patients (337). Specific deletion of TRAF3 from myeloid cells leads to development of B lymphomas and liver cancer in mice (56, 174). Similarly, lymphocyte-specific TRAF3 transgenic mice develop autoimmunity, inflammation and cancers (such as squamous cell carcinomas of the tongue, salivary gland tumors, and hepatoma) (55). TRAF2 regulates inflammatory cytokine production in tumor-associated macrophages, which facilitates tumor growth (46). TRAF4 promotes lung cancer aggressiveness by modulating the tumor microenvironment in normal fibroblasts via the TRAF4-NOX2/NOX4/p47-phox-ROS pathway (338). Importantly, TRAFs are also recognized as potential targets or modulators of cancer immunotherapy. For example, the immune adjuvants dsRNA such as Sendai Virus, poly-I:C, and rintatolimod all activate the TLR3-TRAF3-IRF3 axis to promote CD8 cytotoxic T lymphocytes chemotaxis to the tumor microenvironment in cancer immunotherapy (339). Anti-GITR immunotherapy-induced tumor-specific Th9 cells, which are highly effective in eradicating advanced tumors *in vivo*, display a unique hyperproliferative feature driven by the Pu.1-TRAF6-NF-κB axis (340, 341). Furthermore, TRAF3 and TRAF6 are crucial for osteoclast differentiation, and therefore can regulate bone metastasis of various cancers (4, 342). We shall witness rapid advancement in these exciting areas in the coming years.

CONCLUSIONS

In this article, we have analyzed the current evidence of genetic alterations of the *TRAF* family in human cancers. The results revealed that genetic alterations of all seven *TRAF* genes are present in various human cancers and that recurrent mutations of each *TRAF* gene have been detected in cancer patients. In particular, loss-of-function genetic alterations of *TRAF2* and *TRAF3* are frequently detected in B cell malignancies, and the rates of missense mutations of *TRAF7* are overwhelmingly high in adenomatoid tumors, secretory meningiomas and perineuriomas. Gain-of-function alterations (gene amplification and overexpression) are common for *TRAF1*, *TRAF4*, *TRAF5*, and *TRAF6* in human cancers, and are also identified for *TRAF2* in epithelial cancers. Corroborating human evidence, direct causal roles of *TRAF* genetic alterations (except *TRAF7*) in tumorigenesis have been demonstrated *in vivo* with genetically engineered mouse models that have each *TRAF* gene deleted or overexpressed in specific cell types. Importantly, however, the functional significance of most *TRAF* point mutations identified in human cancers remains to be assessed in future studies. A number of interesting TRAF-dependent oncogenic and tumor suppressive pathways have been elucidated from both *in vivo* and *in vitro* studies, although current understanding is still far from complete and further investigation is required. The significance of TRAFs in cancer pathogenesis is reinforced by the evidence that TRAF proteins also participate in pathogen-induced carcinogenesis, including bacteria and viruses. Furthermore, emerging evidence indicates that TRAF proteins can indirectly contribute to tumorigenesis and metastasis by affecting tumor immunity, chronic inflammation, bone resorption, and the tumor microenvironment. In conclusion, the information presented in this article provides a rationale for the development of novel immunotherapies and other strategies to manipulate TRAF proteins or TRAF-dependent signaling pathways in human cancers by precision medicine, which represents the next primary challenge in the field.

AUTHOR CONTRIBUTIONS

PX and SZ have taken the leading roles in designing and writing this manuscript, and all co-authors (JJ, SG, AL, HS, and JF) have also made significant contributions to writing this manuscript.

ACKNOWLEDGMENTS

This study was supported by the National Institutes of Health grants R01 CA158402 and R21 AI128264 (PX), the Department of Defense grant W81XWH-13-1-0242 (PX), a Busch Biomedical Grant (PX), and a Pilot Award from the Cancer Institute of New Jersey through Grant Number P30CA072720 from the National Cancer Institute (PX).

REFERENCES

1. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal.* (2013) 8:7. doi: 10.1186/1750-2187-8-7
2. So T, Nagashima H, Ishii N. TNF Receptor-Associated Factor (TRAF) signaling network in CD4(+) T-lymphocytes. *Tohoku J Exp Med.* (2015) 236:139–54. doi: 10.1620/tjem.236.139
3. Yang XD, Sun SC. Targeting signaling factors for degradation, an emerging mechanism for TRAF functions. *Immunol Rev.* (2015) 266:56–71. doi: 10.1111/imr.12311
4. Lalani AI, Zhu S, Gokhale S, Jin J, Xie P. TRAF molecules in inflammation and inflammatory diseases. *Curr Pharmacol Rep.* (2018) 4:64–90. doi: 10.1007/s40495-017-0117-y
5. Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet.* (2013) 45:1113–20. doi: 10.1038/ng.2764
6. Forbes SA, Bhamra G, Bamford S, Dawson E, Kok C, Clements J, et al. The Catalogue of Somatic Mutations in Cancer (COSMIC). *Curr Protoc Hum Genet.* (2008) Chapter 10:Unit 10.11. doi: 10.1002/0471142905.hg1011s57
7. Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun.* (2015) 6:6744. doi: 10.1038/ncomms7744
8. Network TCGAR. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* (2014) 513:202–9. doi: 10.1038/nature13480
9. Barretina J, Taylor BS, Banerji S, Ramos AH, Lagos-Quintana M, Decarolis PL, et al. Subtype-specific genomic alterations define new targets for soft-tissue sarcoma therapy. *Nat Genet.* (2010) 42:715–21. doi: 10.1038/ng.619
10. Network TCGAR. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* (2012) 489:519–25. doi: 10.1038/nature11404
11. Kim TM, Jung SH, Kim MS, Baek IP, Park SW, Lee SH, et al. The mutational burdens and evolutionary ages of early gastric cancers are comparable to those of advanced gastric cancers. *J Pathol.* (2014) 234:365–74. doi: 10.1002/path.4401
12. Network TCGA. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* (2012) 487:330–7. doi: 10.1038/nature11252
13. Sanz-Pamplona R, Lopez-Doriga A, Pare-Brunet L, Lazaro K, Bellido F, Alonso MH, et al. Exome sequencing reveals AMER1 as a frequently mutated gene in colorectal cancer. *Clin Cancer Res.* (2015) 21:4709–18. doi: 10.1158/1078-0432.CCR-15-0159
14. Van Allen EM, Wagle N, Sucker A, Treacy DJ, Johannessen CM, Goetz EM, et al. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov.* (2014) 4:94–109. doi: 10.1158/2159-8290.CD-13-0617
15. Ljungstrom V, Cortese D, Young E, Pandzic T, Mansouri L, Plevova K, et al. Whole-exome sequencing in relapsing chronic lymphocytic leukemia: clinical impact of recurrent RPS15 mutations. *Blood* (2016) 127:1007–16. doi: 10.1182/blood-2015-10-674572
16. Feldman AL, Vasmatzis G, Asmann YW, Davila J, Middha S, Eckloff BW, et al. Novel TRAF1-ALK fusion identified by deep RNA sequencing of anaplastic large cell lymphoma. *Genes Chromosomes Cancer* (2013) 52:1097–102. doi: 10.1002/gcc.22104
17. Takeoka K, Okumura A, Honjo G, Ohno H. Variant translocation partners of the anaplastic lymphoma kinase (ALK) gene in two cases of anaplastic large cell lymphoma, identified by inverse cDNA polymerase chain reaction. *J Clin Exp Hematop.* (2014) 54:225–35. doi: 10.3960/jslrt.54.225
18. Abate F, Todaro M, van der Krogt JA, Boi M, Landra I, Machiorlatti R, et al. A novel patient-derived tumorgraft model with TRAF1-ALK anaplastic large-cell lymphoma translocation. *Leukemia* (2015) 29:1390–401. doi: 10.1038/leu.2014.347
19. Lawrence K, Berry B, Handshoe J, Hout D, Mazzola R, Morris SW, et al. Detection of a TRAF1-ALK fusion in an anaplastic large cell lymphoma patient with chemotherapy and ALK inhibitor-resistant disease. *BMC Res Notes* (2015) 8:308. doi: 10.1186/s13104-015-1277-7
20. Yamamoto H, Ryu J, Min E, Oi N, Bai R, Zykova TA, et al. TRAF1 is critical for DMBA/Solar UVR-induced skin carcinogenesis. *J Invest Dermatol.* (2017) 137:1322–32. doi: 10.1016/j.jid.2016.12.026
21. Wen X WB, Feng T, Yuan W, Zhou J, Fang T. TNF receptor-associated factor 1 as a biomarker for assessment of non-small cell lung cancer metastasis and overall survival. *Clin Respir J.* (2018) 12:2197–203. doi: 10.1111/crj.12789
22. Durkop H, Hirsch B, Hahn C, Foss HD, Stein H. Differential expression and function of A20 and TRAF1 in Hodgkin lymphoma and anaplastic large cell lymphoma and their induction by CD30 stimulation. *J Pathol.* (2003) 200:229–39. doi: 10.1002/path.1351
23. Zapata JM, Krajewska M, Krajewski S, Kitada S, Welsh K, Monks A, et al. TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J Immunol.* (2000) 165:5084-96. doi: 10.4049/jimmunol.165.9.5084
24. Munzert G, Kirchner D, Stobbe H, Bergmann L, Schmid RM, Dohner H, et al. Tumor necrosis factor receptor-associated factor 1 gene overexpression in B-cell chronic lymphocytic leukemia: analysis of NF-kappa B/Rel-regulated inhibitors of apoptosis. *Blood* (2002) 100:3749–56. doi: 10.1182/blood.V100.10.3749
25. Savage KJ, Monti S, Kutok JL, Cattoretti G, Neuberg D, De Leval L, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. *Blood* (2003) 102:3871–9. doi: 10.1182/blood-2003-06-1841
26. Zapata JM, Reed JC. TRAF1: lord without a RING. Sci STKE (2002) 2002:pe27. doi: 10.1126/stke.2002.133.pe27
27. Zhang B, Wang Z, Li T, Tsitsikov EN, Ding HF. NF-kappaB2 mutation targets TRAF1 to induce lymphomagenesis. *Blood* (2007) 110:743–51. doi: 10.1182/blood-2006-11-058446
28. Slager SL, Kay NE, Fredericksen ZS, Wang AH, Liebow M, Cunningham JM, et al. Susceptibility genes and B-chronic lymphocytic leukaemia. *Br J Haematol.* (2007) 139:762–71. doi: 10.1111/j.1365-2141.2007.06872.x
29. Tsitsikov EN, Laouini D, Dunn IF, Sannikova TY, Davidson L, Alt FW, et al. TRAF1 is a negative regulator of TNF signaling. enhanced TNF signaling in TRAF1-deficient mice. *Immunity* (2001) 15:647–57. doi: 10.1016/S1074-7613(01)00207-2
30. Wang Q, Gao G, Zhang T, Yao K, Chen H, Park MH, et al. TRAF1 is critical for regulating the BRAF/MEK/ERK pathway in non-small cell lung carcinogenesis. *Cancer Res.* (2018) 78:3982–94. doi: 10.1158/0008-5472.CAN-18-0429
31. McPherson AJ, Snell LM, Mak TW, Watts TH. Opposing roles for TRAF1 in the alternative versus classical NF-κB pathway in T cells. *J Biol Chem.* (2012) 287:23010-9. doi: 10.1074/jbc.M112.350538
32. Sabbagh L, Srokowski CC, Pulle G, Snell LM, Sedgmen BJ, Liu Y, et al. A critical role for TNF receptor-associated factor 1 and Bim down-regulation in CD8 memory T cell survival. *Proc Natl Acad Sci USA.* (2006) 103:18703–8. doi: 10.1073/pnas.0602919103
33. Sabbagh L, Pulle G, Liu Y, Tsitsikov EN, Watts TH. ERK-dependent Bim modulation downstream of the 4-1BB-TRAF1 signaling axis is a critical mediator of CD8 T cell survival *in vivo*. *J Immunol.* (2008) 180:8093–101. doi: 10.4049/jimmunol.180.12.8093
34. Wang C, McPherson AJ, Jones RB, Kawamura KS, Lin GH, Lang PA, et al. Loss of the signaling adaptor TRAF1 causes CD8+ T cell dysregulation during human and murine chronic infection. *J Exp Med.* (2012) 209:77–91. doi: 10.1084/jem.20110675

35. Speiser DE, Lee SY, Wong B, Arron J, Santana A, Kong YY, et al. A regulatory role for TRAF1 in antigen-induced apoptosis of T cells. *J Exp Med.* (1997) 185:1777–83.
36. Yeh WC, Shahinian A, Speiser D, Kraunus J, Billia F, Wakeham A, et al. Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* (1997) 7:715–25.
37. Piao JH, Hasegawa M, Heissig B, Hattori K, Takeda K, Iwakura Y, et al. Tumor necrosis factor receptor-associated factor (TRAF) 2 controls homeostasis of the colon to prevent spontaneous development of murine inflammatory bowel disease. *J Biol Chem.* (2011) 286:17879–88. doi: 10.1074/jbc.M111.221853
38. MacLachlan TK, Giordano A. TRAF2 expression in differentiated muscle. *J Cell Biochem.* (1998) 71:461–6.
39. Gardam S, Sierro F, Basten A, Mackay F, Brink R. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. *Immunity* (2008) 28:391–401. doi: 10.1016/j.immuni.2008.01.009
40. Lee SY, Reichlin A, Santana A, Sokol KA, Nussenzweig MC, Choi Y. TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. *Immunity* (1997) 7:703–13.
41. Zapata JM, Krajewska M, Morse HC III, Choi Y, Reed JC. TNF receptor-associated factor (TRAF) domain and Bcl-2 cooperate to induce small B cell lymphoma/chronic lymphocytic leukemia in transgenic mice. *Proc Natl Acad Sci USA.* (2004) 101:16600–5. doi: 10.1073/pnas.0407541101
42. Perez-Chacon G, Llobet D, Pardo C, Pindado J, Choi Y, Reed JC, et al. TNFR-associated factor 2 deficiency in B lymphocytes predisposes to chronic lymphocytic leukemia/small lymphocytic lymphoma in mice. *J Immunol.* (2012) 189:1053–61. doi: 10.4049/jimmunol.1200814
43. Schneider AT, Gautheron J, Feoktistova M, Roderburg C, Loosen SH, Roy S, et al. RIPK1 suppresses a TRAF2-dependent pathway to liver cancer. *Cancer Cell* (2017) 31:94–109. doi: 10.1016/j.ccell.2016.11.009
44. Petersen SL, Chen TT, Lawrence DA, Marsters SA, Gonzalvez F, Ashkenazi A. TRAF2 is a biologically important necroptosis suppressor. *Cell Death Differ.* (2015) 22:1846–57. doi: 10.1038/cdd.2015.35
45. Etemadi N, Chopin M, Anderton H, Tanzer MC, Rickard JA, Abeysekera W, et al. TRAF2 regulates TNF and NF-κB signalling to suppress apoptosis and skin inflammation independently of Sphingosine kinase 1. *Elife* (2015) 4:e10592. doi: 10.7554/eLife.10592
46. Jin J, Xiao Y, Hu H, Zou Q, Li Y, Gao Y, et al. Proinflammatory TLR signalling is regulated by a TRAF2-dependent proteolysis mechanism in macrophages. *Nat Commun.* (2015) 6:5930. doi: 10.1038/ncomms6930
47. Villanueva JE, Malle EK, Gardam S, Silveira PA, Zammit NW, Walters SN, et al. TRAF2 regulates peripheral CD8(+) T-cell and NKT-cell homeostasis by modulating sensitivity to IL-15. *Eur J Immunol.* (2015) 45:1820–31. doi: 10.1002/eji.201445416
48. Zarnegar BJ, Wang Y, Mahoney DJ, Dempsey PW, Cheung HH, He J, et al. Noncanonical NF-kappaB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nat Immunol.* (2008) 9:1371–8. doi: 10.1038/ni.1676
49. Xu Y, Cheng G, Baltimore D. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity* (1996) 5:407–15.
50. He JQ, Zarnegar B, Oganesyan G, Saha SK, Yamazaki S, Doyle SE, et al. Rescue of TRAF3-null mice by p100 NF-kappa B deficiency. *J Exp Med.* (2006) 203:2413–8. doi: 10.1084/jem.20061166
51. Xie P, Stunz LL, Larison KD, Yang B, Bishop GA. Tumor necrosis factor receptor-associated factor 3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. *Immunity* (2007) 27:253–67. doi: 10.1016/j.immuni.2007.07.012
52. Moore CR, Liu Y, Shao CS, Covey LR, Morse HC, 3rd, Xie P. Specific deletion of TRAF3 in B lymphocytes leads to B lymphoma development in mice. *Leukemia* (2012) 26:1122–7. doi: 10.1038/leu.2011.309
53. Xie P, Poovassery J, Stunz LL, Smith SM, Schultz ML, Carlin LE, et al. Enhanced Toll-like receptor (TLR) responses of TNFR-associated factor 3 (TRAF3)-deficient B lymphocytes. *J Leukoc Biol.* (2011) 90:1149–57. doi: 10.1189/jlb.0111044
54. Gardam S, Turner VM, Anderton H, Limaye S, Basten A, Koentgen F, et al. Deletion of cIAP1 and cIAP2 in murine B lymphocytes constitutively activates cell survival pathways and inactivates the germinal center response. *Blood* (2011) 117:4041–51. doi: 10.1182/blood-2010-10-312793
55. Zapata JM, Llobet D, Krajewska M, Lefebvre S, Kress CL, Reed JC. Lymphocyte-specific TRAF3-transgenic mice have enhanced humoral responses and develop plasmacytosis, autoimmunity, inflammation, and cancer. *Blood* (2009) 113:4595–603. doi: 10.1182/blood-2008-07-165456
56. Lalani AI, Moore CR, Luo C, Kreider BZ, Liu Y, Morse HC, 3rd, et al. Myeloid cell TRAF3 regulates immune responses and inhibits inflammation and tumor development in mice. *J Immunol.* (2015) 194:334–48. doi: 10.4049/jimmunol.1401548
57. Xie P, Kraus ZJ, Stunz LL, Liu Y, Bishop GA. TNF receptor-associated factor 3 is required for T cell-mediated immunity and TCR/CD28 signaling. *J Immunol.* (2011) 186:143–55. doi: 10.4049/jimmunol.100029
58. Yi Z, Lin WW, Stunz LL, Bishop GA. The adaptor TRAF3 restrains the lineage determination of thymic regulatory T cells by modulating signaling via the receptor for IL-2. *Nat Immunol.* (2014) 15:866–74. doi: 10.1038/ni.2944
59. Yi Z, Stunz LL, Bishop GA. TNF receptor associated factor 3 plays a key role in development and function of invariant natural killer T cells. *J Exp Med.* (2013) 210:1079–86. doi: 10.1084/jem.20122135
60. Yi Z, Stunz LL, Lin WW, Bishop GA. TRAF3 regulates homeostasis of CD8+ central memory T cells. *PLoS ONE* (2014) 9:e102120. doi: 10.1371/journal.pone.0102120
61. Shiels H, Li X, Schumacker PT, Maltepe E, Padrid PA, Sperling A, et al. TRAF4 deficiency leads to tracheal malformation with resulting alterations in air flow to the lungs. *Am J Pathol.* (2000) 157:679–88. doi: 10.1016/S0002-9440(10)64578-6
62. Regnier CH, Masson R, Kedinger V, Textoris J, Stoll I, Chenard MP, et al. Impaired neural tube closure, axial skeleton malformations, and tracheal ring disruption in TRAF4-deficient mice. *Proc Natl Acad Sci USA.* (2002) 99:5585–90. doi: 10.1073/pnas.052124799
63. Blaise S, Kneib M, Rousseau A, Gambino F, Chenard MP, Messadeq N, et al. *in vivo* evidence that TRAF4 is required for central nervous system myelin homeostasis. *PLoS ONE* (2012) 7:e30917. doi: 10.1371/journal.pone.0030917
64. Cherfils-Vicini J, Vingert B, Varin A, Tartour E, Fridman WH, Sautes-Fridman C, et al. Characterization of immune functions in TRAF4-deficient mice. *Immunology* (2008) 124:562–74. doi: 10.1111/j.1365-2567.2008.02810.x
65. Wu L, Chen X, Zhao J, Martin B, Zepp JA, Ko JS, et al. A novel IL-17 signaling pathway controlling keratinocyte proliferation and tumorigenesis via the TRAF4-ERK5 axis. *J Exp Med.* (2015) 212:1571–87. doi: 10.1084/jem.20150204
66. Zepp JA, Wu L, Qian W, Ouyang W, Aronica M, Erzurum S, et al. TRAF4-SMURF2-mediated DAZAP2 degradation is critical for IL-25 signaling and allergic airway inflammation. *J Immunol.* (2015) 194:2826–37. doi: 10.4049/jimmunol.1402647
67. Nakano H, Sakon S, Koseki H, Takemori T, Tada K, Matsumoto M, et al. Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. *Proc Natl Acad Sci USA.* (1999) 96:9803–8.
68. Kraus ZJ, Haring JS, Bishop GA. TNF receptor-associated factor 5 is required for optimal T cell expansion and survival in response to infection. *J Immunol.* (2008) 181:7800–9. doi: 10.4049/jimmunol.181.11.7800
69. Esparza EM, Lindsten T, Stockhausen JM, Arch RH. Tumor necrosis factor receptor (TNFR)-associated factor 5 is a critical intermediate of costimulatory signaling pathways triggered by glucocorticoid-induced TNFR in T cells. *J Biol Chem.* (2006) 281:8559–64. doi: 10.1074/jbc.M512915200
70. So T, Salek-Ardakani S, Nakano H, Ware CF, Croft M. TNF receptor-associated factor 5 limits the induction of Th2 immune responses. *J Immunol.* (2004) 172:4292–7. doi: 10.4049/jimmunol.172.7.4292
71. Nagashima H, Okuyama Y, Asao A, Kawabe T, Yamaki S, Nakano H, et al. The adaptor TRAF5 limits the differentiation of inflammatory CD4(+) T cells by antagonizing signaling via the receptor for IL-6. *Nat Immunol.* (2014) 15:449–56. doi: 10.1038/ni.2863
72. Shang J, Li L, Wang X, Pan H, Liu S, He R, et al. Disruption of tumor necrosis factor receptor-associated factor 5 exacerbates murine experimental colitis

via regulating T helper cell-mediated inflammation. *Mediators Inflamm.* (2016) 2016:9453745. doi: 10.1155/2016/9453745
73. Kraus ZJ, Nakano H, Bishop GA. TRAF5 is a critical mediator of *in vitro* signals and *in vivo* functions of LMP1, the viral oncogenic mimic of CD40. *Proc Natl Acad Sci USA.* (2009) 106:17140–5. doi: 10.1073/pnas.0903786106
74. Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, et al. Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. *Genes Cells* (1999) 4:353–62.
75. Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, et al. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev.* (1999) 13:1015–24.
76. Kobayashi T, Walsh PT, Walsh MC, Speirs KM, Chiffoleau E, King CG, et al. TRAF6 is a critical factor for dendritic cell maturation and development. *Immunity* (2003) 19:353–63. doi: 10.1016/S1074-7613(03)00230-9
77. Shimo Y, Yanai H, Ohshima D, Qin J, Motegi H, Maruyama Y, et al. TRAF6 directs commitment to regulatory T cells in thymocytes. *Genes Cells* (2011) 16:437–47. doi: 10.1111/j.1365-2443.2011.01500.x
78. Dickson KM, Bhakar AL, Barker PA. TRAF6-dependent NF-kB transcriptional activity during mouse development. *Dev Dyn.* (2004) 231:122–7. doi: 10.1002/dvdy.20110
79. Yeiser EC, Rutkoski NJ, Naito A, Inoue J, Carter BD. Neurotrophin signaling through the p75 receptor is deficient in traf6$^{-/-}$ mice. *J Neurosci* (2004) 24:10521–9. doi: 10.1523/JNEUROSCI.1390-04.2004
80. Lomaga MA, Henderson JT, Elia AJ, Robertson J, Noyce RS, Yeh WC, et al. Tumor necrosis factor receptor-associated factor 6 (TRAF6) deficiency results in exencephaly and is required for apoptosis within the developing CNS. *J Neurosci.* (2000) 20:7384–93. doi: 10.1523/JNEUROSCI.20-19-07384.2000
81. Fang J, Muto T, Kleppe M, Bolanos LC, Hueneman KM, Walker CS, et al. TRAF6 mediates basal activation of NF-κB necessary for hematopoietic stem cell homeostasis. *Cell Rep.* (2018) 22:1250–62. doi: 10.1016/j.celrep.2018.01.013
82. Kobayashi T, Kim TS, Jacob A, Walsh MC, Kadono Y, Fuentes-Panana E, et al. TRAF6 is required for generation of the B-1a B cell compartment as well as T cell-dependent and -independent humoral immune responses. *PLoS ONE* (2009) 4:e4736.
83. King CG, Kobayashi T, Cejas PJ, Kim T, Yoon K, Kim GK, et al. TRAF6 is a T cell-intrinsic negative regulator required for the maintenance of immune homeostasis. *Nat Med.* (2006) 12:1088-92. doi: 10.1038/nm1449
84. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* (2009) 460:103–7. doi: 10.1038/nature08097
85. Cejas PJ, Walsh MC, Pearce EL, Han D, Harms GM, Artis D, et al. TRAF6 inhibits Th17 differentiation and TGF-beta-mediated suppression of IL-2. *Blood* (2010) 115:4750–7. doi: 10.1182/blood-2009-09-242768
86. Xiao X, Balasubramanian S, Liu W, Chu X, Wang H, Taparowsky EJ, et al. OX40 signaling favors the induction of T(H)9 cells and airway inflammation. *Nat Immunol.* (2012) 13:981–90. doi: 10.1038/ni.2390
87. Vlantis K, Polykratis A, Welz PS, van Loo G, Pasparakis M, Wullaert A. TLR-independent anti-inflammatory function of intestinal epithelial TRAF6 signalling prevents DSS-induced colitis in mice. *Gut* (2016) 65:935–43. doi: 10.1136/gutjnl-2014-308323
88. Paul PK, Gupta SK, Bhatnagar S, Panguluri SK, Darnay BG, Choi Y, et al. Targeted ablation of TRAF6 inhibits skeletal muscle wasting in mice. *J Cell Biol.* (2010) 191:1395–411. doi: 10.1083/jcb.201006098
89. Hindi SM, Paul PK, Dahiya S, Mishra V, Bhatnagar S, Kuang S, et al. Reciprocal interaction between TRAF6 and notch signaling regulates adult myofiber regeneration upon injury. *Mol Cell Biol.* (2012) 32:4833–45. doi: 10.1128/MCB.00717-12
90. Paul PK, Bhatnagar S, Mishra V, Srivastava S, Darnay BG, Choi Y, et al. The E3 ubiquitin ligase TRAF6 intercedes in starvation-induced skeletal muscle atrophy through multiple mechanisms. *Mol Cell Biol.* (2012) 32:1248–59. doi: 10.1128/MCB.06351-11
91. Guo F, Sun A, Wang W, He J, Hou J, Zhou P, et al. TRAF1 is involved in the classical NF-kappaB activation and CD30-induced alternative activity in Hodgkin's lymphoma cells. *Mol Immunol.* (2009) 46:2441–8. doi: 10.1016/j.molimm.2009.05.178
92. Wicovsky A, Henkler F, Salzmann S, Scheurich P, Kneitz C, Wajant H. Tumor necrosis factor receptor-associated factor-1 enhances proinflammatory TNF receptor-2 signaling and modifies TNFR1-TNFR2 cooperation. *Oncogene* (2009) 28:1769–81. doi: 10.1038/onc.2009.29
93. Deng J, Miller SA, Wang HY, Xia W, Wen Y, Zhou BP, et al. β-catenin interacts with and inhibits NF-κ B in human colon and breast cancer. *Cancer Cell* (2002) 2:323-34. doi: 10.1016/S1535-6108(02)00154-X
94. Du Q, Zhang X, Cardinal J, Cao Z, Guo Z, Shao L, et al. Wnt/beta-catenin signaling regulates cytokine-induced human inducible nitric oxide synthase expression by inhibiting nuclear factor-kappaB activation in cancer cells. *Cancer Res.* (2009) 69:3764–71. doi: 10.1158/0008-5472.CAN-09-0014
95. Carmona Arana JA, Seher A, Neumann M, Lang I, Siegmund D, Wajant H. TNF receptor-associated factor 1 is a major target of soluble TWEAK. *Front Immunol.* (2014) 5:63. doi: 10.3389/fimmu.2014.00063
96. Lassen UN, Meulendijks D, Siu LL, Karanikas V, Mau-Sorensen M, Schellens JH, et al. A phase I monotherapy study of RG7212, a first-in-class monoclonal antibody targeting TWEAK signaling in patients with advanced cancers. *Clin Cancer Res.* (2015) 21:258–66. doi: 10.1158/1078-0432.CCR-14-1334
97. Meulendijks D, Lassen UN, Siu LL, Huitema AD, Karanikas V, Mau-Sorensen M, et al. Exposure and tumor Fn14 expression as determinants of pharmacodynamics of the anti-TWEAK monoclonal antibody RG7212 in patients with Fn14-positive solid tumors. *Clin Cancer Res.* (2016) 22:858–67. doi: 10.1158/1078-0432.CCR-15-1506
98. Armenia J, Wankowicz SAM, Liu D, Gao J, Kundra R, Reznik E, et al. The long tail of oncogenic drivers in prostate cancer. *Nat Genet.* (2018) 50:645–51. doi: 10.1038/s41588-018-0078-z
99. Rahal R, Frick M, Romero R, Korn JM, Kridel R, Chan FC, et al. Pharmacological and genomic profiling identifies NF-κB-targeted treatment strategies for mantle cell lymphoma. *Nat Med.* (2014) 20:87–92. doi: 10.1038/nm.3435
100. Meissner B, Kridel R, Lim RS, Rogic S, Tse K, Scott DW, et al. The E3 ubiquitin ligase UBR5 is recurrently mutated in mantle cell lymphoma. *Blood* (2013) 121:3161–4. doi: 10.1182/blood-2013-01-478834
101. Saba N, Wiestner A. Do mantle cell lymphomas have an 'Achilles heel'? *Curr Opin Hematol.* (2014) 21:350–7. doi: 10.1097/MOH.0000000000000057
102. Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, Shen Q, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. *Nature* (2009) 459:717–21. doi: 10.1038/nature07968
103. Shen RR, Zhou AY, Kim E, O'Connell JT, Hagerstrand D, Beroukhim R, et al. TRAF2 is an NF-κB-activating oncogene in epithelial cancers. *Oncogene* (2015) 34:209–16. doi: 10.1038/onc.2013.543
104. Giannakis M, Hodis E, Jasmine Mu X, Yamauchi M, Rosenbluh J, Cibulskis K, et al. RNF43 is frequently mutated in colorectal and endometrial cancers. *Nat Genet.* (2014) 46:1264–6. doi: 10.1038/ng.3127
105. Yaeger R, Chatila WK, Lipsyc MD, Hechtman JF, Cercek A, Sanchez-Vega F, et al. Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. *Cancer Cell* (2018) 33:125–36.e3. doi: 10.1016/j.ccell.2017.12.004
106. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med.* (2017) 23:703–13. doi: 10.1038/nm.4333
107. Wang K, Yuen ST, Xu J, Lee SP, Yan HH, Shi ST, et al. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat Genet.* (2014) 46:573–82. doi: 10.1038/ng.2983
108. Giannakis M, Mu XJ, Shukla SA, Qian ZR, Cohen O, Nishihara R, et al. Genomic correlates of immune-cell infiltrates in colorectal carcinoma. *Cell Rep.* (2016) 15:857–65. doi: 10.1016/j.celrep.2016.03.075
109. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. *Cell* (2016) 164:550–63. doi: 10.1016/j.cell.2015.12.028
110. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med.* (2016) 22:298–305. doi: 10.1038/nm.4045
111. Jones S, Stransky N, McCord CL, Cerami E, Lagowski J, Kelly D, et al. Genomic analyses of gynaecologic carcinosarcomas reveal frequent

mutations in chromatin remodelling genes. *Nat Commun.* (2014) 5:5006. doi: 10.1038/ncomms6006
112. Chen K, Yang D, Li X, Sun B, Song F, Cao W, et al. Mutational landscape of gastric adenocarcinoma in Chinese: implications for prognosis and therapy. *Proc Natl Acad Sci USA.* (2015) 112:1107–12. doi: 10.1073/pnas.1422640112
113. Network TCGA. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* (2015) 517:576–82. doi: 10.1038/nature14129
114. Galante PA, Parmigiani RB, Zhao Q, Caballero OL, de Souza JE, Navarro FC, et al. Distinct patterns of somatic alterations in a lymphoblastoid and a tumor genome derived from the same individual. *Nucleic Acids Res.* (2011) 39:6056–68. doi: 10.1093/nar/gkr221
115. Lezcano C, Shoushtari AN, Ariyan C, Hollmann TJ, Busam KJ. Primary and metastatic melanoma with NTRK fusions. *Am J Surg Pathol.* (2018) 42:1052–8. doi: 10.1097/PAS.0000000000001070
116. Garrison JB, Samuel T, Reed JC. TRAF2-binding BIR1 domain of c-IAP2/MALT1 fusion protein is essential for activation of NF-kappaB. *Oncogene* (2009) 28:1584–93. doi: 10.1038/onc.2009.17
117. Grech AP, Amesbury M, Chan T, Gardam S, Basten A, Brink R. TRAF2 differentially regulates the canonical and noncanonical pathways of NF-kappaB activation in mature B cells. *Immunity* (2004) 21:629–42. doi: 10.1016/j.immuni.2004.09.011
118. Vallabhapurapu S, Matsuzawa A, Zhang W, Tseng PH, Keats JJ, Wang H, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling. *Nat Immunol.* (2008) 9:1364–70. doi: 10.1038/ni.1678
119. Ahn SM, Jang SJ, Shim JH, Kim D, Hong SM, Sung CO, et al. Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. *Hepatology* (2014) 60:1972–82. doi: 10.1002/hep.27198
120. Jackson-Bernitsas DG, Ichikawa H, Takada Y, Myers JN, Lin XL, Darnay BG, et al. Evidence that TNF-TNFR1-TRADD-TRAF2-RIP-TAK1-IKK pathway mediates constitutive NF-kappaB activation and proliferation in human head and neck squamous cell carcinoma. *Oncogene* (2007) 26:1385–97. doi: 10.1038/sj.onc.1209945
121. Tobin D, van Hogerlinden M, Toftgard R. UVB-induced association of tumor necrosis factor (TNF) receptor 1/TNF receptor-associated factor-2 mediates activation of Rel proteins. *Proc Natl Acad Sci USA.* (1998) 95:565–9.
122. Massoumi R, Chmielarska K, Hennecke K, Pfeifer A, Fassler R. Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-kappaB signaling. *Cell* (2006) 125:665–77. doi: 10.1016/j.cell.2006.03.041
123. Chen MB, Zhang Y, Wei MX, Shen W, Wu XY, Yao C, et al. Activation of AMP-activated protein kinase (AMPK) mediates plumbagin-induced apoptosis and growth inhibition in cultured human colon cancer cells. *Cell Signal.* (2013) 25:1993–2002. doi: 10.1016/j.cellsig.2013.05.026
124. Mhaidat NM, Thorne R, Zhang XD, Hersey P. Involvement of endoplasmic reticulum stress in Docetaxel-induced JNK-dependent apoptosis of human melanoma. *Apoptosis* (2008) 13:1505–12. doi: 10.1007/s10495-008-0276-8
125. Zhang J, Liang Y, Lin Y, Liu Y, YouYou, Yin W. IRE1α-TRAF2-ASK1 pathway is involved in CSTMP-induced apoptosis and ER stress in human non-small cell lung cancer A549 cells. *Biomed Pharmacother.* (2016) 82:281–9. doi: 10.1016/j.biopha.2016.04.050
126. Chen H, Yang H, Pan L, Wang W, Liu X, Ren X, et al. The molecular mechanisms of XBP-1 gene silencing on IRE1α-TRAF2-ASK1-JNK pathways in oral squamous cell carcinoma under endoplasmic reticulum stress. *Biomed Pharmacother.* (2016) 77:108–13. doi: 10.1016/j.biopha.2015.12.010
127. Lamkanfi M, D'Hondt K, Vande Walle L, van Gurp M, Denecker G, Demeulemeester J, et al. A novel caspase-2 complex containing TRAF2 and RIP1. *J Biol Chem.* (2005) 280:6923–32. doi: 10.1074/jbc.M411180200
128. Robeson AC, Lindblom KR, Wojton J, Kornbluth S, Matsuura K. Dimer-specific immunoprecipitation of active caspase-2 identifies TRAF proteins as novel activators. *EMBO J.* (2018) 37:e97072. doi: 10.15252/embj.201797072
129. Doppler H, Liou GY, Storz P. Downregulation of TRAF2 mediates NIK-induced pancreatic cancer cell proliferation and tumorigenicity. *PLoS ONE* (2013) 8:e53676. doi: 10.1371/journal.pone.0053676
130. Ohtsu N, Nakatani Y, Yamashita D, Ohue S, Ohnishi T, Kondo T. Eva1 maintains the stem-like character of glioblastoma-initiating cells by activating the noncanonical NF-κB signaling pathway. *Cancer Res.* (2016) 76:171–81. doi: 10.1158/0008-5472.CAN-15-0884
131. Wang B, Jie Z, Joo D, Ordureau A, Liu P, Gan W, et al. TRAF2 and OTUD7B govern a ubiquitin-dependent switch that regulates mTORC2 signalling. *Nature* (2017) 545:365–9. doi: 10.1038/nature22344
132. Trauzold A, Roder C, Sipos B, Karsten K, Arlt A, Jiang P, et al. CD95 and TRAF2 promote invasiveness of pancreatic cancer cells. *FASEB J.* (2005) 19:620–2. doi: 10.1096/fj.04-2984fje
133. Wei B, Ruan J, Mi Y, Hu J, Zhang J, Wang Z, et al. Knockdown of TNF receptor-associated factor 2 (TRAF2) modulates *in vitro* growth of TRAIL-treated prostate cancer cells. *Biomed Pharmacother.* (2017) 93:462–9. doi: 10.1016/j.biopha.2017.05.145
134. Zheng M, Morgan-Lappe SE, Yang J, Bockbrader KM, Pamarthy D, Thomas D, et al. Growth inhibition and radiosensitization of glioblastoma and lung cancer cells by small interfering RNA silencing of tumor necrosis factor receptor-associated factor 2. *Cancer Res.* (2008) 68:7570–8. doi: 10.1158/0008-5472.CAN-08-0632
135. Zhao J, Li H, Min L, Han X, Shu P, Yang Y, et al. High expression of tumor necrosis factor receptor-associated factor 2 promotes tumor metastasis and is associated with unfavorable prognosis in gastric cancer. *J Gastroenterol Hepatol.* (2018) 33:431–42. doi: 10.1111/jgh.13818
136. Peng C, Zhu F, Wen W, Yao K, Li S, Zykova T, et al. Tumor necrosis factor receptor-associated factor family protein 2 is a key mediator of the epidermal growth factor-induced ribosomal S6 kinase 2/cAMP-responsive element-binding protein/Fos protein signaling pathway. *J Biol Chem.* (2012) 287:25881–92. doi: 10.1074/jbc.M112.359521
137. Zhang W, Sun Y, Liu L, Li Z. Prognostic significance of TNFR-associated factor 1 and 2 (TRAF1 and TRAF2) in glioblastoma. *Med Sci Monit.* (2017) 23:4506–12. doi: 10.12659/MSM.903397
138. Peramuhendige P, Marino S, Bishop RT, de Ridder D, Khogeer A, Baldini I, et al. TRAF2 in osteotropic breast cancer cells enhances skeletal tumour growth and promotes osteolysis. *Sci Rep.* (2018) 8:39. doi: 10.1038/s41598-017-18327-5
139. Yu C, Chen S, Guo Y, Sun C. Oncogenic TRIM31 confers gemcitabine resistance in pancreatic cancer via activating the NF-κB signaling pathway. *Theranostics* (2018) 8:3224–36. doi: 10.7150/thno.23259
140. Chiche J, Pommier S, Beneteau M, Mondragon L, Meynet O, Zunino B, et al. GAPDH enhances the aggressiveness and the vascularization of non-Hodgkin's B lymphomas via NF-κB-dependent induction of HIF-1α. *Leukemia* (2015) 29:1163–76. doi: 10.1038/leu.2014.324
141. Blakely CM, Pazarentzos E, Olivas V, Asthana S, Yan JJ, Tan I, et al. NF-κB-activating complex engaged in response to EGFR oncogene inhibition drives tumor cell survival and residual disease in lung cancer. *Cell Rep.* (2015) 11:98–110. doi: 10.1016/j.celrep.2015.03.012
142. Zhou AY, Shen RR, Kim E, Lock YJ, Xu M, Chen ZJ, et al. IKKε-mediated tumorigenesis requires K63-linked polyubiquitination by a cIAP1/cIAP2/TRAF2 E3 ubiquitin ligase complex. *Cell Rep.* (2013) 3:724–33. doi: 10.1016/j.celrep.2013.01.031
143. Shen RR, Zhou AY, Kim E, Lim E, Habelhah H, Hahn WC. IκB kinase ε phosphorylates TRAF2 to promote mammary epithelial cell transformation. *Mol Cell Biol.* (2012) 32:4756–68. doi: 10.1128/MCB.00468-12
144. Martinez-Forero I, Rouzaut A, Palazon A, Dubrot J, Melero I. Lysine 63 polyubiquitination in immunotherapy and in cancer-promoting inflammation. *Clin Cancer Res.* (2009) 15:6751–7. doi: 10.1158/1078-0432.CCR-09-1225
145. Gonzalvez F, Lawrence D, Yang B, Yee S, Pitti R, Marsters S, et al. TRAF2 Sets a threshold for extrinsic apoptosis by tagging caspase-8 with a ubiquitin shutoff timer. *Mol Cell* (2012) 48:888–99. doi: 10.1016/j.molcel.2012.09.031
146. Oh YT, Yue P, Wang D, Tong JS, Chen ZG, Khuri FR, et al. Suppression of death receptor 5 enhances cancer cell invasion and metastasis through activation of caspase-8/TRAF2-mediated signaling. *Oncotarget* (2015) 6:41324–38. doi: 10.18632/oncotarget.5847
147. He W, Wang Q, Xu J, Xu X, Padilla MT, Ren G, et al. Attenuation of TNFSF10/TRAIL-induced apoptosis by an autophagic survival pathway involving TRAF2- and RIPK1/RIP1-mediated MAPK8/JNK activation. *Autophagy* (2012) 8:1811–21. doi: 10.4161/auto.22145

148. Xu L, Zhang Y, Qu X, Che X, Guo T, Li C, et al. DR5-Cbl-b/c-Cbl-TRAF2 complex inhibits TRAIL-induced apoptosis by promoting TRAF2-mediated polyubiquitination of caspase-8 in gastric cancer cells. *Mol Oncol.* (2017) 11:1733–51. doi: 10.1002/1878-0261.12140
149. Hibino T, Sakaguchi M, Miyamoto S, Yamamoto M, Motoyama A, Hosoi J, et al. S100A9 is a novel ligand of EMMPRIN that promotes melanoma metastasis. *Cancer Res.* (2013) 73:172–83. doi: 10.1158/0008-5472.CAN-11-3843
150. Basudhar D, Glynn SA, Greer M, Somasundaram V, No JH, Scheiblin DA, et al. Coexpression of NOS2 and COX2 accelerates tumor growth and reduces survival in estrogen receptor-negative breast cancer. *Proc Natl Acad Sci USA*. (2017) 114:13030–5. doi: 10.1073/pnas.1709119114
151. Fortin Ensign SP, Mathews IT, Eschbacher JM, Loftus JC, Symons MH, Tran NL. The Src homology 3 domain-containing guanine nucleotide exchange factor is overexpressed in high-grade gliomas and promotes tumor necrosis factor-like weak inducer of apoptosis-fibroblast growth factor-inducible 14-induced cell migration and invasion via tumor necrosis factor receptor-associated factor 2. *J Biol Chem.* (2013) 288:21887–97. doi: 10.1074/jbc.M113.468686
152. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. *Cell* (2017) 171:540–56.e25. doi: 10.1016/j.cell.2017.09.007
153. Moore CR, Edwards SK, Xie P. Targeting TRAF3 downstream signaling pathways in b cell neoplasms. *J Cancer Sci Ther.* (2015) 7:67–74. doi: 10.4172/1948-5956.1000327
154. Hyeon J, Lee B, Shin SH, Yoo HY, Kim SJ, Kim WS, et al. Targeted deep sequencing of gastric marginal zone lymphoma identified alterations of TRAF3 and TNFAIP3 that were mutually exclusive for MALT1 rearrangement. *Mod Pathol.* (2018) 31:1418–28. doi: 10.1038/s41379-018-0064-0
155. Keats JJ, Fonseca R, Chesi M, Schop R, Baker A, Chng WJ, et al. Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. *Cancer Cell* (2007) 12:131–44. doi: 10.1016/j.ccr.2007.07.003
156. Walker BA, Boyle EM, Wardell CP, Murison A, Begum DB, Dahir NM, et al. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. *J Clin Oncol.* (2015) 33:3911–20. doi: 10.1200/JCO.2014.59.1503
157. Otto C, Giefing M, Massow A, Vater I, Gesk S, Schlesner M, et al. Genetic lesions of the TRAF3 and MAP3K14 genes in classical Hodgkin lymphoma. *Br J Haematol.* (2012) 157:702–8. doi: 10.1111/j.1365-2141.2012.09113.x
158. Zhang B, Calado DP, Wang Z, Frohler S, Kochert K, Qian Y, et al. An oncogenic role for alternative NF-κB signaling in DLBCL revealed upon deregulated BCL6 expression. *Cell Rep.* (2015) 11:715–26. doi: 10.1016/j.celrep.2015.03.059
159. Rossi D, Deaglio S, Dominguez-Sola D, Rasi S, Vaisitti T, Agostinelli C, et al. Alteration of BIRC3 and multiple other NF-κB pathway genes in splenic marginal zone lymphoma. *Blood* (2011) 118:4930–4. doi: 10.1182/blood-2011-06-359166
160. Braggio E, Keats JJ, Leleu X, Van Wier S, Jimenez-Zepeda VH, Valdez R, et al. Identification of copy number abnormalities and inactivating mutations in two negative regulators of nuclear factor-kappaB signaling pathways in Waldenstrom's macroglobulinemia. *Cancer Res.* (2009) 69:3579–88. doi: 10.1158/0008-5472.CAN-08-3701
161. Li YY, Chung GT, Lui VW, To KF, Ma BB, Chow C, et al. Exome and genome sequencing of nasopharynx cancer identifies NF-κB pathway activating mutations. *Nat Commun.* (2017) 8:14121. doi: 10.1038/ncomms14121
162. Martin D, Abba MC, Molinolo AA, Vitale-Cross L, Wang Z, Zaida M, et al. The head and neck cancer cell oncogenome: a platform for the development of precision molecular therapies. *Oncotarget* (2014) 5:8906–23. doi: 10.18632/oncotarget.2417
163. Rossi D, Trifonov V, Fangazio M, Bruscaggin A, Rasi S, Spina V, et al. The coding genome of splenic marginal zone lymphoma: activation of NOTCH2 and other pathways regulating marginal zone development. *J Exp Med.* (2012) 209:1537–51. doi: 10.1084/jem.20120904
164. Seiwert TY, Zuo Z, Keck MK, Khattri A, Pedamallu CS, Stricker T, et al. Integrative and comparative genomic analysis of HPV-positive and HPV-negative head and neck squamous cell carcinomas. *Clin Cancer Res.* (2015) 21:632–41. doi: 10.1158/1078-0432.CCR-13-3310
165. Clipson A, Wang M, de Leval L, Ashton-Key M, Wotherspoon A, Vassiliou G, et al. KLF2 mutation is the most frequent somatic change in splenic marginal zone lymphoma and identifies a subset with distinct genotype. *Leukemia* (2014) 29:1177–85. doi: 10.1038/leu.2014.330
166. Annunziata CM, Davis RE, Demchenko Y, Bellamy W, Gabrea A, Zhan F, et al. Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* (2007) 12:115–30. doi: 10.1016/j.ccr.2007.07.004
167. Puente XS, Bea S, Valdes-Mas R, Villamor N, Gutierrez-Abril J, Martin-Subero JI, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature* (2015) 526:519–24. doi: 10.1038/nature14666
168. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. *Nat Genet.* (2011) 43:830–7. doi: 10.1038/ng.892
169. South AP, Purdie KJ, Watt SA, Haldenby S, den Breems N, Dimon M, et al. NOTCH1 mutations occur early during cutaneous squamous cell carcinogenesis. *J Invest Dermatol.* (2014) 134:2630–8. doi: 10.1038/jid.2014.154
170. Demchenko YN, Glebov OK, Zingone A, Keats JJ, Bergsagel PL, Kuehl WM. Classical and/or alternative NF-kappaB pathway activation in multiple myeloma. *Blood* (2010) 115:3541–52. doi: 10.1182/blood-2009-09-243535
171. Lin WW, Yi Z, Stunz LL, Maine CJ, Sherman LA, Bishop GA. The adaptor protein TRAF3 inhibits interleukin-6 receptor signaling in B cells to limit plasma cell development. *Sci Signal.* (2015) 8:ra88. doi: 10.1126/scisignal.aaa5157
172. Xie P, Hostager BS, Bishop GA. Requirement for TRAF3 in signaling by LMP1 But not CD40 in B lymphocytes. *J Exp Med.* (2004) 199:661–71. doi: 10.1084/jem.20031255
173. Bangalore-Prakash P, Stunz LL, Mambetsariev N, Whillock AL, Hostager BS, Bishop GA. The oncogenic membrane protein LMP1 sequesters TRAF3 in B-cell lymphoma cells to produce functional TRAF3 deficiency. *Blood Adv.* (2017) 1:2712–23. doi: 10.1182/bloodadvances.2017009670
174. Lalani AI, Luo C, Han Y, Xie P. TRAF3: a novel tumor suppressor gene in macrophages. *Macrophage* (2015) 2:e1009. doi: 10.14800/macrophage.1009
175. Mashima R, Honda K, Yang Y, Morita Y, Inoue A, Arimura S, et al. Mice lacking Dok-1, Dok-2, and Dok-3 succumb to aggressive histiocytic sarcoma. *Lab Invest.* (2010) 90:1357–64. doi: 10.1038/labinvest.2010.121
176. Snyder JM, Treuting PM, Nagy L, Yam C, Yi J, Brasfield A, et al. Humanized TLR7/8 expression drives proliferative multisystemic histiocytosis in C57BL/6 mice. *PLoS ONE* (2014) 9:e107257. doi: 10.1371/journal.pone.0107257
177. Kim SS, Lee KG, Chin CS, Ng SK, Pereira NA, Xu S, et al. DOK3 is required for IFN-β production by enabling TRAF3/TBK1 complex formation and IRF3 activation. *J Immunol.* (2014) 193:840–8. doi: 10.4049/jimmunol.1301601
178. Carrasco DR, Fenton T, Sukhdeo K, Protopopova M, Enos M, You MJ, et al. The PTEN and INK4A/ARF tumor suppressors maintain myelolymphoid homeostasis and cooperate to constrain histiocytic sarcoma development in humans. *Cancer Cell* (2006) 9:379–90. doi: 10.1016/j.ccr.2006.03.028
179. Durham BH, Diamond EL, Abdel-Wahab O. Histiocytic neoplasms in the era of personalized genomic medicine. *Curr Opin Hematol.* (2016) 23:416–25. doi: 10.1097/MOH.0000000000000256
180. Allen IC, Wilson JE, Schneider M, Lich JD, Roberts RA, Arthur JC, et al. NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-κB signaling. *Immunity* (2012) 36:742–54. doi: 10.1016/j.immuni.2012.03.012
181. Malcomson FC, Willis ND, McCallum I, Xie L, Lagerwaard B, Kelly S, et al. Non-digestible carbohydrates supplementation increases miR-32 expression in the healthy human colorectal epithelium: a randomized controlled trial. *Mol Carcinog.* (2017) 56:2104–11. doi: 10.1002/mc.22666
182. Muro I, Fang G, Gardella KA, Mahajan IM, Wright CW. The TRAF3 adaptor protein drives proliferation of anaplastic large cell lymphoma cells by regulating multiple signaling pathways. *Cell Cycle* (2014) 13:1918–27. doi: 10.4161/cc.28895
183. Newman AC, Kemp AJ, Drabsch Y, Behrends C, Wilkinson S. Autophagy acts through TRAF3 and RELB to regulate gene expression via antagonism of SMAD proteins. *Nat Commun.* (2017) 8:1537. doi: 10.1038/s41467-017-00859-z

184. Chen MC, Hwang MJ, Chou YC, Chen WH, Cheng G, Nakano H, et al. The role of apoptosis signal-regulating kinase 1 in lymphotoxin-beta receptor-mediated cell death. *J Biol Chem.* (2003) 278:16073–81. doi: 10.1074/jbc.M208661200
185. Georgopoulos NT, Steele LP, Thomson MJ, Selby PJ, Southgate J, Trejdosiewicz LK. A novel mechanism of CD40-induced apoptosis of carcinoma cells involving TRAF3 and JNK/AP-1 activation. *Cell Death Differ.* (2006) 13:1789–801. doi: 10.1038/sj.cdd.4401859
186. Elmetwali T, Young LS, Palmer DH. CD40 ligand-induced carcinoma cell death: a balance between activation of TNFR-associated factor (TRAF) 3-dependent death signals and suppression of TRAF6-dependent survival signals. *J Immunol.* (2010) 184:1111–20. doi: 10.4049/jimmunol.0900528
187. Dunnill CJ, Ibraheem K, Mohamed A, Southgate J, Georgopoulos NT. A redox state-dictated signalling pathway deciphers the malignant cell specificity of CD40-mediated apoptosis. *Oncogene* (2017) 36:2515–28. doi: 10.1038/onc.2016.401
188. Cai X, Yang Y, Xia W, Kong H, Wang M, Fu W, et al. RIP2 promotes glioma cell growth by regulating TRAF3 and activating the NFkappaB and p38 signaling pathways. *Oncol Rep.* (2018) 39:2915–23. doi: 10.3892/or.2018.6397
189. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, et al. Comprehensive molecular portraits of invasive lobular breast cancer. *Cell* (2015) 163:506–19. doi: 10.1016/j.cell.2015.09.033
190. Network TCGAR. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* (2014) 511:543–50. doi: 10.1038/nature13385
191. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. *Cell* (2012) 150:251–63. doi: 10.1016/j.cell.2012.06.024
192. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. *Nature* (2012) 487:239–43. doi: 10.1038/nature11125
193. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. *Nature* (2013) 497:67–73. doi: 10.1038/nature12113
194. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science* (2011) 333:1154–7. doi: 10.1126/science.1206923
195. Pickering CR, Zhang J, Yoo SY, Bengtsson L, Moorthy S, Neskey DM, et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov.* (2013) 3:770–81. doi: 10.1158/2159-8290.CD-12-0537
196. Bieche I, Tomasetto C, Regnier CH, Moog-Lutz C, Rio MC, Lidereau R. Two distinct amplified regions at 17q11-q21 involved in human primary breast cancer. *Cancer Res.* (1996) 56:3886–90.
197. Krajewska M, Krajewski S, Zapata JM, Van Arsdale T, Gascoyne RD, Berern K, et al. TRAF-4 expression in epithelial progenitor cells. Analysis in normal adult, fetal, and tumor tissues. *Am J Pathol.* (1998) 152:1549–61.
198. Camilleri-Broet S, Cremer I, Marmey B, Comperat E, Viguie F, Audouin J, et al. TRAF4 overexpression is a common characteristic of human carcinomas. *Oncogene* (2007) 26:142–7. doi: 10.1038/sj.onc.1209762
199. Li W, Peng C, Lee MH, Lim D, Zhu F, Fu Y, et al. TRAF4 is a critical molecule for Akt activation in lung cancer. *Cancer Res.* (2013) 73:6938–50. doi: 10.1158/0008-5472.CAN-13-0913
200. Zhang L, Zhou F, Garcia de Vinuesa A, de Kruijf EM, Mesker WE, Hui L, et al. TRAF4 promotes TGF-β receptor signaling and drives breast cancer metastasis. *Mol Cell* (2013) 51:559–72. doi: 10.1016/j.molcel.2013.07.014
201. Yang K, Wang F, Han JJ. TRAF4 promotes the growth and invasion of colon cancer through the Wnt/β-catenin pathway. *Int J Clin Exp Pathol.* (2015) 8:1419–26.
202. Yao W, Wang X, Cai Q, Gao S, Wang J, Zhang P. TRAF4 enhances osteosarcoma cell proliferation and invasion by Akt signaling pathway. *Oncol Res.* (2014) 22:21–8. doi: 10.3727/096504014X14077751730351
203. Liu K, Wu X, Zang X, Huang Z, Lin Z, Tan W, et al. TRAF4 regulates migration, invasion, and epithelial-mesenchymal transition via PI3K/AKT signaling in hepatocellular carcinoma. *Oncol Res.* (2017) 25:1329–40. doi: 10.3727/096504017X14876227286564
204. Singh R, Karri D, Shen H, Shao J, Dasgupta S, Huang S, et al. TRAF4-mediated ubiquitination of NGF receptor TrkA regulates prostate cancer metastasis. *J Clin Invest.* (2018) 128:3129–43. doi: 10.1172/JCI96060
205. Kedinger V, Rio MC. TRAF4, the unique family member. *Adv Exp Med Biol.* (2007) 597:60–71. doi: 10.1007/978-0-387-70630-6_5
206. Zhu L, Zhang S, Huan X, Mei Y, Yang H. Down-regulation of TRAF4 targeting RSK4 inhibits proliferation, invasion and metastasis in breast cancer xenografts. *Biochem Biophys Res Commun.* (2018) 500:810–6. doi: 10.1016/j.bbrc.2018.04.164
207. Wang X, Jin C, Tang Y, Tang LY, Zhang YE. Ubiquitination of tumor necrosis factor receptor-associated factor 4 (TRAF4) by Smad ubiquitination regulatory factor 1 (Smurf1) regulates motility of breast epithelial and cancer cells. *J Biol Chem.* (2013) 288:21784–92. doi: 10.1074/jbc.M113.472704
208. Yi P, Xia W, Wu RC, Lonard DM, Hung MC, O'Malley BW. SRC-3 coactivator regulates cell resistance to cytotoxic stress via TRAF4-mediated p53 destabilization. *Genes Dev.* (2013) 27:274–87. doi: 10.1101/gad.203760.112
209. Zhang X, Wen Z, Sun L, Wang J, Song M, Wang E, et al. TRAF2 regulates the cytoplasmic/nuclear distribution of TRAF4 and its biological function in breast cancer cells. *Biochem Biophys Res Commun.* (2013) 436:344–8. doi: 10.1016/j.bbrc.2013.05.107
210. Yang J, Wei D, Wang W, Shen B, Xu S, Cao Y. TRAF4 enhances oral squamous cell carcinoma cell growth, invasion and migration by Wnt-β-catenin signaling pathway. *Int J Clin Exp Pathol.* (2015) 8:11837–46.
211. Wang A, Wang J, Ren H, Yang F, Sun L, Diao K, et al. TRAF4 participates in Wnt/β-catenin signaling in breast cancer by upregulating β-catenin and mediating its translocation to the nucleus. *Mol Cell Biochem.* (2014) 395:211–9. doi: 10.1007/s11010-014-2127-y
212. Ren HY, Wang J, Yang F, Zhang XL, Wang AL, Sun LL, et al. Cytoplasmic TRAF4 contributes to the activation of p70s6k signaling pathway in breast cancer. *Oncotarget* (2015) 6:4080–96. doi: 10.18632/oncotarget.2977
213. Rousseau A, McEwen AG, Poussin-Courmontagne P, Rognan D, Nomine Y, Rio MC, et al. TRAF4 is a novel phosphoinositide-binding protein modulating tight junctions and favoring cell migration. *PLoS Biol.* (2013) 11:e1001726. doi: 10.1371/journal.pbio.1001726
214. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* (2015) 161:1215–28. doi: 10.1016/j.cell.2015.05.001
215. Morin RD, Assouline S, Alcaide M, Mohajeri A, Johnston RL, Chong L, et al. Genetic landscapes of relapsed and refractory diffuse large B-cell lymphomas. *Clin Cancer Res.* (2016) 22:2290–300. doi: 10.1158/1078-0432.CCR-15-2123
216. Ruiz-Ballesteros E, Mollejo M, Rodriguez A, Camacho FI, Algara P, Martinez N, et al. Splenic marginal zone lymphoma: proposal of new diagnostic and prognostic markers identified after tissue and cDNA microarray analysis. *Blood* (2005) 106:1831–8. doi: 10.1182/blood-2004-10-3898
217. Weston VJ, Austen B, Wei W, Marston E, Alvi A, Lawson S, et al. Apoptotic resistance to ionizing radiation in pediatric B-precursor acute lymphoblastic leukemia frequently involves increased NF-κB survival pathway signaling. *Blood* (2004) 104:1465–73. doi: 10.1182/blood-2003-11-4039
218. Stunz LL, Busch LK, Munroe ME, Sigmund CD, Tygrett LT, Waldschmidt TJ, et al. Expression of the cytoplasmic tail of LMP1 in mice induces hyperactivation of B lymphocytes and disordered lymphoid architecture. *Immunity* (2004) 21:255–66. doi: 10.1016/j.immuni.2004.07.008
219. Horie R, Watanabe T, Morishita Y, Ito K, Ishida T, Kanegae Y, et al. Ligand-independent signaling by overexpressed CD30 drives NF-kappaB activation in Hodgkin-Reed-Sternberg cells. *Oncogene* (2002) 21:2493–503. doi: 10.1038/sj.onc.1205337
220. Horie R, Watanabe T, Ito K, Morisita Y, Watanabe M, Ishida T, et al. Cytoplasmic aggregation of TRAF2 and TRAF5 proteins in the Hodgkin-Reed-Sternberg cells. *Am J Pathol.* (2002) 160:1647–54. doi: 10.1016/S0002-9440(10)61112-1
221. Horie R, Gattei V, Ito K, Imajo-Ohmi S, Tange T, Miyauchi J, et al. Frequent expression of the variant CD30 in human malignant myeloid and lymphoid neoplasms. *Am J Pathol.* (1999) 155:2029–41.

222. Tao T, Cheng C, Ji Y, Xu G, Zhang J, Zhang L, et al. Numbl inhibits glioma cell migration and invasion by suppressing TRAF5-mediated NF-κB activation. *Mol Biol Cell* (2012) 23:2635–44. doi: 10.1091/mbc.E11-09-0805
223. Huang S, Wa Q, Pan J, Peng X, Ren D, Huang Y, et al. Downregulation of miR-141-3p promotes bone metastasis via activating NF-κB signaling in prostate cancer. *J Exp Clin Cancer Res.* (2017) 36:173. doi: 10.1186/s13046-017-0645-7
224. Li M, Long C, Yang G, Luo Y, Du H. MiR-26b inhibits melanoma cell proliferation and enhances apoptosis by suppressing TRAF5-mediated MAPK activation. *Biochem Biophys Res Commun.* (2016) 471:361–7. doi: 10.1016/j.bbrc.2016.02.021
225. Lefebvre C, Bachelot T, Filleron T, Pedrero M, Campone M, Soria JC, et al. Mutational profile of metastatic breast cancers: a retrospective analysis. *PLoS Med.* (2016) 13:e1002201. doi: 10.1371/journal.pmed.1002201
226. Network TCGAR. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* (2014) 507:315–22. doi: 10.1038/nature12965
227. Starczynowski DT, Lockwood WW, Delehouzee S, Chari R, Wegrzyn J, Fuller M, et al. TRAF6 is an amplified oncogene bridging the RAS and NF-κB pathways in human lung cancer. *J Clin Invest.* (2011) 121:4095–105. doi: 10.1172/JCI58818
228. Al-Hebshi NN, Li S, Nasher AT, El-Setouhy M, Alsanosi R, Blancato J, et al. Exome sequencing of oral squamous cell carcinoma in users of Arabian snuff reveals novel candidates for driver genes. *Int J Cancer* (2016) 139:363–72. doi: 10.1002/ijc.30068
229. Lin Y, Qiu Y, Xu C, Liu Q, Peng B, Kaufmann GF, et al. Functional role of asparaginyl endopeptidase ubiquitination by TRAF6 in tumor invasion and metastasis. *J Natl Cancer Inst.* (2014) 106:dju012. doi: 10.1093/jnci/dju012
230. Li JJ, Luo J, Lu JN, Liang XN, Luo YH, Liu YR, et al. Relationship between TRAF6 and deterioration of HCC: an immunohistochemical and *in vitro* study. *Cancer Cell Int.* (2016) 16:76. doi: 10.1186/s12935-016-0352-z
231. Sun H, Li X, Fan L, Wu G, Li M, Fang J. TRAF6 is upregulated in colon cancer and promotes proliferation of colon cancer cells. *Int J Biochem Cell Biol.* (2014) 53:195–201. doi: 10.1016/j.biocel.2014.04.010
232. Liu X, Wang Z, Zhang G, Zhu Q, Zeng H, Wang T, et al. High TRAF6 expression is associated with esophageal carcinoma recurrence and prompts cancer cell invasion. *Oncol Res.* (2017) 25:485–93. doi: 10.3727/096504016X14749340314441
233. Luo Z, Zhang X, Zeng W, Su J, Yang K, Lu L, et al. TRAF6 regulates melanoma invasion and metastasis through ubiquitination of Basigin. *Oncotarget* (2016) 7:7179–92. doi: 10.18632/oncotarget.6886
234. Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, Conboy CB, et al. Recurrent R-spondin fusions in colon cancer. *Nature* (2012) 488:660–4. doi: 10.1038/nature11282
235. Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh TJ, Hodis E, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell* (2012) 150:1107–20. doi: 10.1016/j.cell.2012.08.029
236. Min Y, Kim MJ, Lee S, Chun E, Lee KY. Inhibition of TRAF6 ubiquitin-ligase activity by PRDX1 leads to inhibition of NFKB activation and autophagy activation. *Autophagy* (2018) 14:1347–58. doi: 10.1080/15548627.2018.1474995
237. Wang Y, Xu H, Jiao H, Wang S, Xiao Z, Zhao Y, et al. STX2 promotes colorectal cancer metastasis through a positive feedback loop that activates the NF-κB pathway. *Cell Death Dis.* (2018) 9:664. doi: 10.1038/s41419-018-0675-x
238. Chen L, Li YC, Wu L, Yu GT, Zhang WF, Huang CF, et al. TRAF6 regulates tumour metastasis through EMT and CSC phenotypes in head and neck squamous cell carcinoma. *J Cell Mol Med.* (2018) 22:1337–49. doi: 10.1111/jcmm.13439
239. Zhan Z, Xie X, Cao H, Zhou X, Zhang XD, Fan H, et al. Autophagy facilitates TLR4- and TLR3-triggered migration and invasion of lung cancer cells through the promotion of TRAF6 ubiquitination. *Autophagy* (2014) 10:257–68. doi: 10.4161/auto.27162
240. Sambandam Y, Ethiraj P, Hathaway-Schrader JD, Novince CM, Panneerselvam E, Sundaram K, et al. Autoregulation of RANK ligand in oral squamous cell carcinoma tumor cells. *J Cell Physiol.* (2018) 233:6125–34. doi: 10.1002/jcp.26456
241. Fang J, Rhyasen G, Bolanos L, Rasch C, Varney M, Wunderlich M, et al. Cytotoxic effects of bortezomib in myelodysplastic syndrome/acute myeloid leukemia depend on autophagy-mediated lysosomal degradation of TRAF6 and repression of PSMA1. *Blood* (2012) 120:858–67. doi: 10.1182/blood-2012-02-407999
242. da Silva Almeida AC, Abate F, Khiabanian H, Martinez-Escala E, Guitart J, Tensen CP, et al. The mutational landscape of cutaneous T cell lymphoma and Sezary syndrome. *Nat Genet.* (2015) 47:1465–70. doi: 10.1038/ng.3442
243. Dai YH, Hung LY, Chen RY, Lai CH, Chang KC. ON 01910.Na inhibits growth of diffuse large B-cell lymphoma by cytoplasmic sequestration of sumoylated C-MYB/TRAF6 complex. *Transl Res.* (2016) 175:129–43.e13. doi: 10.1016/j.trsl.2016.04.001
244. Chen H, Li M, Sanchez E, Wang CS, Lee T, Soof CM, et al. Combined TRAF6 targeting and proteasome blockade has anti-myeloma and anti-bone resorptive effects. *Mol Cancer Res.* (2017) 15:598–609. doi: 10.1158/1541-7786.MCR-16-0293
245. Burger ML, Xue L, Sun Y, Kang C, Winoto A. Premalignant PTEN-deficient thymocytes activate microRNAs miR-146a and miR-146b as a cellular defense against malignant transformation. *Blood* (2014) 123:4089–100. doi: 10.1182/blood-2013-11-539411
246. Li Z, Younger K, Gartenhaus R, Joseph AM, Hu F, Baer MR, et al. Inhibition of IRAK1/4 sensitizes T cell acute lymphoblastic leukemia to chemotherapies. *J Clin Invest.* (2015) 125:1081–97. doi: 10.1172/JCI75821
247. Network CGAR. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* (2008) 455:1061–8. doi: 10.1038/nature07385
248. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. *Cell* (2013) 155:462–77. doi: 10.1016/j.cell.2013.09.034
249. Zhang X, Li CF, Zhang L, Wu CY, Han L, Jin G, et al. TRAF6 restricts p53 mitochondrial translocation, apoptosis, and tumor suppression. *Mol Cell* (2016) 64:803–14. doi: 10.1016/j.molcel.2016.10.002
250. Duran A, Linares JF, Galvez AS, Wikenheiser K, Flores JM, Diaz-Meco MT, et al. The signaling adaptor p62 is an important NF-kappaB mediator in tumorigenesis. *Cancer Cell* (2008) 13:343–54. doi: 10.1016/j.ccr.2008.02.001
251. Chiu HW, Lin SW, Lin LC, Hsu YH, Lin YF, Ho SY, et al. Synergistic antitumor effects of radiation and proteasome inhibitor treatment in pancreatic cancer through the induction of autophagy and the downregulation of TRAF6. *Cancer Lett.* (2015) 365:229–39. doi: 10.1016/j.canlet.2015.05.025
252. Yang WL, Wang J, Chan CH, Lee SW, Campos AD, Lamothe B, et al. The E3 ligase TRAF6 regulates Akt ubiquitination and activation. *Science* (2009) 325:1134–8. doi: 10.1126/science.1175065
253. Feng H, Lopez GY, Kim CK, Alvarez A, Duncan CG, Nishikawa R, et al. EGFR phosphorylation of DCBLD2 recruits TRAF6 and stimulates AKT-promoted tumorigenesis. *J Clin Invest.* (2014) 124:3741–56. doi: 10.1172/JCI73093
254. Shi YF, Yu DJ, Jiang CY, Wang XJ, Zhu YP, Zhao RZ, et al. TRAF6 regulates proliferation of stromal cells in the transition and peripheral zones of benign prostatic hyperplasia via Akt/mTOR signaling. *Prostate* (2018) 78:193–201. doi: 10.1002/pros.23456
255. Wu YH, Wu WS, Lin LC, Liu CS, Ho SY, Wang BJ, et al. Bortezomib enhances radiosensitivity in oral cancer through inducing autophagy-mediated TRAF6 oncoprotein degradation. *J Exp Clin Cancer Res.* (2018) 37:91. doi: 10.1186/s13046-018-0760-0
256. Pan D, Jiang C, Ma Z, Blonska M, You MJ, Lin X. MALT1 is required for EGFR-induced NF-κB activation and contributes to EGFR-driven lung cancer progression. *Oncogene* (2016) 35:919–28. doi: 10.1038/onc.2015.146
257. Zhang X, Wu L, Xiao T, Tang L, Jia X, Guo Y, et al. TRAF6 regulates EGF-induced cell transformation and cSCC malignant phenotype through CD147/EGFR. *Oncogenesis* (2018) 7:17. doi: 10.1038/s41389-018-0030-1
258. Rezaeian AH, Li CF, Wu CY, Zhang X, Delacerda J, You MJ, et al. A hypoxia-responsive TRAF6-ATM-H2AX signalling axis promotes HIF1α activation, tumorigenesis and metastasis. *Nat Cell Biol.* (2017) 19:38–51. doi: 10.1038/ncb3445
259. Sun H, Li XB, Meng Y, Fan L, Li M, Fang J. TRAF6 upregulates expression of HIF-1α and promotes tumor angiogenesis. *Cancer Res.* (2013) 73:4950–9. doi: 10.1158/0008-5472.CAN-13-0370
260. Mu Y, Sundar R, Thakur N, Ekman M, Gudey SK, Yakymovych M, et al. TRAF6 ubiquitinates TGFβ type I receptor to promote its

cleavage and nuclear translocation in cancer. *Nat Commun.* (2011) 2:330. doi: 10.1038/ncomms1332
261. Sundar R, Gudey SK, Heldin CH, Landstrom M. TRAF6 promotes TGFβ-induced invasion and cell-cycle regulation via Lys63-linked polyubiquitination of Lys178 in TGFβ type I receptor. *Cell Cycle* (2015) 14:554–65. doi: 10.4161/15384101.2014.990302
262. Gudey SK, Sundar R, Mu Y, Wallenius A, Zang G, Bergh A, et al. TRAF6 stimulates the tumor-promoting effects of TGFβ type I receptor through polyubiquitination and activation of presenilin 1. *Sci Signal.* (2014) 7:ra2. doi: 10.1126/scisignal.2004207
263. Hamidi A, Song J, Thakur N, Itoh S, Marcusson A, Bergh A, et al. TGF-β promotes PI3K-AKT signaling and prostate cancer cell migration through the TRAF6-mediated ubiquitylation of p85α. *Sci Signal.* (2017) 10:eaal4186. doi: 10.1126/scisignal.aal4186
264. Linares JF, Duran A, Reina-Campos M, Aza-Blanc P, Campos A, Moscat J, et al. Amino acid activation of mTORC1 by a PB1-domain-driven kinase complex cascade. *Cell Rep.* (2015) 12:1339–52. doi: 10.1016/j.celrep.2015.07.045
265. An J, Liu H, Magyar CE, Guo Y, Veena MS, Srivatsan ES, et al. Hyperactivated JNK is a therapeutic target in pVHL-deficient renal cell carcinoma. *Cancer Res.* (2013) 73:1374–85. doi: 10.1158/0008-5472.CAN-12-2362
266. Bao X, Shi J, Xie F, Liu Z, Yu J, Chen W, et al. Proteolytic release of the p75(NTR) intracellular domain by ADAM10 promotes metastasis and resistance to anoikis. *Cancer Res.* (2018) 78:2262–76. doi: 10.1158/0008-5472.CAN-17-2789
267. Yu J, Qin B, Moyer AM, Nowsheen S, Liu T, Qin S, et al. DNA methyltransferase expression in triple-negative breast cancer predicts sensitivity to decitabine. *J Clin Invest.* (2018) 128:2376–88. doi: 10.1172/JCI97924
268. Chaudhry SI, Hooper S, Nye E, Williamson P, Harrington K, Sahai E. Autocrine IL-1β-TRAF6 signalling promotes squamous cell carcinoma invasion through paracrine TNFα signalling to carcinoma-associated fibroblasts. *Oncogene* (2013) 32:747–58. doi: 10.1038/onc.2012.91
269. Fang J, Barker B, Bolanos L, Liu X, Jerez A, Makishima H, et al. Myeloid malignancies with chromosome 5q deletions acquire a dependency on an intrachromosomal NF-κB gene network. *Cell Rep.* (2014) 8:1328–38. doi: 10.1016/j.celrep.2014.07.062
270. Liu R, Liu C, Chen D, Yang WH, Liu X, Liu CG, et al. FOXP3 Controls an miR-146/NF-κB negative feedback loop that inhibits apoptosis in breast cancer cells. *Cancer Res.* (2015) 75:1703–13. doi: 10.1158/0008-5472.CAN-14-2108
271. Liu R, Yi B, Wei S, Yang WH, Hart KM, Chauhan P, et al. FOXP3-miR-146-NF-κB axis and therapy for precancerous lesions in prostate. *Cancer Res.* (2015) 75:1714–24. doi: 10.1158/0008-5472.CAN-14-2109
272. Zu Y, Yang Y, Zhu J, Bo X, Hou S, Zhang B, et al. MiR-146a suppresses hepatocellular carcinoma by downregulating TRAF6. *Am J Cancer Res.* (2016) 6:2502–13.
273. Liu J, Xu J, Li H, Sun C, Yu L, Li Y, et al. miR-146b-5p functions as a tumor suppressor by targeting TRAF6 and predicts the prognosis of human gliomas. *Oncotarget* (2015) 6:29129–42. doi: 10.18632/oncotarget.4895
274. Li C, Miao R, Liu S, Wan Y, Zhang S, Deng Y, et al. Down-regulation of miR-146b-5p by long noncoding RNA MALAT1 in hepatocellular carcinoma promotes cancer growth and metastasis. *Oncotarget* (2017) 8:28683–95. doi: 10.18632/oncotarget.15640
275. Jiao L, Zhang HL, Li DD, Yang KL, Tang J, Li X, et al. Regulation of glycolytic metabolism by autophagy in liver cancer involves selective autophagic degradation of HK2 (hexokinase 2). *Autophagy* (2018) 14:671–84. doi: 10.1080/15548627.2017.1381804
276. Lu W, Liu S, Li B, Xie Y, Izban MG, Ballard BR, et al. SKP2 loss destabilizes EZH2 by promoting TRAF6-mediated ubiquitination to suppress prostate cancer. *Oncogene* (2017) 36:1364–73. doi: 10.1038/onc.2016.300
277. Lu W, Liu S, Li B, Xie Y, Adhiambo C, Yang Q, et al. SKP2 inactivation suppresses prostate tumorigenesis by mediating JARID1B ubiquitination. *Oncotarget* (2015) 6:771–88. doi: 10.18632/oncotarget.2718
278. Zapata JM, Pawlowski K, Haas E, Ware CF, Godzik A, Reed JC. A diverse family of proteins containing tumor necrosis factor receptor-associated factor domains. *J Biol Chem.* (2001) 276:24242–52. doi: 10.1074/jbc.M100354200
279. Zapata JM, Martinez-Garcia V, Lefebvre S. Phylogeny of the TRAF/MATH domain. *Adv Exp Med Biol.* (2007) 597:1–24. doi: 10.1007/978-0-387-70630-6_1
280. Kumar A, Coleman I, Morrissey C, Zhang X, True LD, Gulati R, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. *Nat Med.* (2016) 22:369–78. doi: 10.1038/nm.4053
281. Goode B, Joseph NM, Stevers M, Van Ziffle J, Onodera C, Talevich E, et al. Adenomatoid tumors of the male and female genital tract are defined by TRAF7 mutations that drive aberrant NF-kB pathway activation. *Mod Pathol.* (2018) 31:660–73. doi: 10.1038/modpathol.2017.153
282. Reuss DE, Piro RM, Jones DT, Simon M, Ketter R, Kool M, et al. Secretory meningiomas are defined by combined KLF4 K409Q and TRAF7 mutations. *Acta Neuropathol.* (2013) 125:351–8. doi: 10.1007/s00401-013-1093-x
283. Klein CJ, Wu Y, Jentoft ME, Mer G, Spinner RJ, Dyck PJ, et al. Genomic analysis reveals frequent TRAF7 mutations in intraneural perineuriomas. *Ann Neurol.* (2017) 81:316–21. doi: 10.1002/ana.24854
284. Clark VE, Harmanci AS, Bai H, Youngblood MW, Lee TI, Baranoski JF, et al. Recurrent somatic mutations in POLR2A define a distinct subset of meningiomas. *Nat Genet.* (2016) 48:1253–9. doi: 10.1038/ng.3651
285. Clark VE, Erson-Omay EZ, Serin A, Yin J, Cotney J, Ozduman K, et al. Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. *Science* (2013) 339:1077–80. doi: 10.1126/science.1233009
286. Yuzawa S, Nishihara H, Yamaguchi S, Mohri H, Wang L, Kimura T, et al. Clinical impact of targeted amplicon sequencing for meningioma as a practical clinical-sequencing system. *Mod Pathol.* (2016) 29:708–16. doi: 10.1038/modpathol.2016.81
287. Abedalthagafi M, Bi WL, Aizer AA, Merrill PH, Brewster R, Agarwalla PK, et al. Oncogenic PI3K mutations are as common as AKT1 and SMO mutations in meningioma. *Neuro Oncol.* (2016) 18:649–55. doi: 10.1093/neuonc/nov316
288. Peyre M, Gaillard S, de Marcellus C, Giry M, Bielle F, Villa C, et al. Progestin-associated shift of meningioma mutational landscape. *Ann Oncol.* (2018) 29:681–6. doi: 10.1093/annonc/mdx763
289. Zotti T, Scudiero I, Vito P, Stilo R. The emerging role of TRAF7 in tumor development. *J Cell Physiol.* (2017) 232:1233–8. doi: 10.1002/jcp.25676
290. Karsy M, Azab MA, Abou-Al-Shaar H, Guan J, Eli I, Jensen RL, et al. Clinical potential of meningioma genomic insights: a practical review for neurosurgeons. *Neurosurg Focus* (2018) 44:E10. doi: 10.3171/2018.2.FOCUS1849
291. Apra C, Peyre M, Kalamarides M. Current treatment options for meningioma. *Expert Rev Neurother.* (2018) 18:241–9. doi: 10.1080/14737175.2018.1429920
292. Sneddon S, Dick I, Lee YCG, Musk AWB, Patch AM, Pearson JV, et al. Malignant cells from pleural fluids in malignant mesothelioma patients reveal novel mutations. *Lung Cancer* (2018) 119:64–70. doi: 10.1016/j.lungcan.2018.03.009
293. Bueno R, Stawiski EW, Goldstein LD, Durinck S, De Rienzo A, Modrusan Z, et al. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. *Nat Genet.* (2016) 48:407–16. doi: 10.1038/ng.3520
294. Yuzawa S, Nishihara H, Tanaka S. Genetic landscape of meningioma. *Brain Tumor Pathol.* (2016) 33:237–47. doi: 10.1007/s10014-016-0271-7
295. Peyre M, Feuvret L, Sanson M, Navarro S, Boch AL, Loiseau H, et al. Diffuse midline skull base meningiomas: identification of a rare and aggressive subgroup of meningiomas. *J Neurooncol.* (2017) 133:633–9. doi: 10.1007/s11060-017-2480-2
296. Fadlullah MZ, Chiang IK, Dionne KR, Yee PS, Gan CP, Sam KK, et al. Genetically-defined novel oral squamous cell carcinoma cell lines for the development of molecular therapies. *Oncotarget* (2016) 7:27802–18. doi: 10.18632/oncotarget.8533
297. Zhang X, Jia H, Lu Y, Dong C, Hou J, Wang Z, et al. Exome sequencing on malignant meningiomas identified mutations in neurofibromatosis type 2 (NF2) and meningioma 1 (MN1) genes. *Discov Med.* (2014) 18:301–11.
298. Kuhn E, Wu RC, Guan B, Wu G, Zhang J, Wang Y, et al. Identification of molecular pathway aberrations in uterine serous carcinoma by genome-wide analyses. *J Natl Cancer Inst.* (2012) 104:1503–13. doi: 10.1093/jnci/djs345

299. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* (2015) 348:124–8. doi: 10.1126/science.aaa1348
300. Tokita MJ, Chen CA, Chitayat D, Macnamara E, Rosenfeld JA, Hanchard N, et al. *De novo* missense variants in TRAF7 cause developmental delay, congenital anomalies, and dysmorphic features. *Am J Hum Genet.* (2018) 103:154–62. doi: 10.1016/j.ajhg.2018.06.005
301. Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, et al. Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol Cell* (2011) 44:325–40. doi: 10.1016/j.molcel.2011.08.025
302. Xu LG, Li LY, Shu HB. TRAF7 potentiates MEKK3-induced AP1 and CHOP activation and induces apoptosis. *J Biol Chem.* (2004) 279:17278–82. doi: 10.1074/jbc.C400063200
303. Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, et al. A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. *Nat Cell Biol.* (2004) 6:97–105. doi: 10.1038/ncb1086
304. Krumm N, Turner TN, Baker C, Vives L, Mohajeri K, Witherspoon K, et al. Excess of rare, inherited truncating mutations in autism. *Nat Genet.* (2015) 47:582–8. doi: 10.1038/ng.3303
305. Morita Y, Kanei-Ishii C, Nomura T, Ishii S. TRAF7 sequesters c-Myb to the cytoplasm by stimulating its sumoylation. *Mol Biol Cell* (2005) 16:5433–44. doi: 10.1091/mbc.e05-08-0731
306. Fremin C, Saba-El-Leil MK, Levesque K, Ang SL, Meloche S. Functional redundancy of ERK1 and ERK2 MAP kinases during development. *Cell Rep.* (2015) 12:913–21. doi: 10.1016/j.celrep.2015.07.011
307. Zotti T, Vito P, Stilo R. The seventh ring: exploring TRAF7 functions. *J Cell Physiol.* (2012) 227:1280–4. doi: 10.1002/jcp.24011
308. Scudiero I, Zotti T, Ferravante A, Vessichelli M, Reale C, Masone MC, et al. Tumor necrosis factor (TNF) receptor-associated factor 7 is required for TNFα-induced Jun NH2-terminal kinase activation and promotes cell death by regulating polyubiquitination and lysosomal degradation of c-FLIP protein. *J Biol Chem.* (2012) 287:6053–61. doi: 10.1074/jbc.M111.300137
309. Zotti T, Uva A, Ferravante A, Vessichelli M, Scudiero I, Ceccarelli M, et al. TRAF7 protein promotes Lys-29-linked polyubiquitination of IkappaB kinase (IKKgamma)/NF-kappaB essential modulator (NEMO) and p65/RelA protein and represses NF-kappaB activation. *J Biol Chem.* (2011) 286:22924–33. doi: 10.1074/jbc.M110.215426
310. Tsikitis M, Acosta-Alvear D, Blais A, Campos EI, Lane WS, Sanchez I, et al. Traf7, a MyoD1 transcriptional target, regulates nuclear factor-κB activity during myogenesis. *EMBO Rep.* (2010) 11:969–76. doi: 10.1038/embor.2010.154
311. Yoshida H, Jono H, Kai H, Li JD. The tumor suppressor cylindromatosis (CYLD) acts as a negative regulator for toll-like receptor 2 signaling via negative cross-talk with TRAF6 AND TRAF7. *J Biol Chem.* (2005) 280:41111–21. doi: 10.1074/jbc.M509526200
312. Wang L, Zhang S, Qu G, Zhang D, Li S, Liu S. Downregulation of ubiquitin E3 ligase TNF receptor-associated factor 7 leads to stabilization of p53 in breast cancer. *Oncol Rep.* (2013) 29:283–7. doi: 10.3892/or.2012.2121
313. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* (2012) 486:346–52. doi: 10.1038/nature10983
314. Pereira B, Chin SF, Rueda OM, Vollan HK, Provenzano E, Bardwell HA, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. *Nat Commun.* (2016) 7:11479. doi: 10.1038/ncomms11479
315. Network TCGAR. Integrated genomic characterization of oesophageal carcinoma. *Nature* (2017) 541:169–75. doi: 10.1038/nature20805
316. Wan XK, Yuan SL, Tao HX, Diao LP, Wang YC, Cao C, et al. The upregulation of TRAF1 induced by helicobacter pylori plays an antiapoptotic effect on the infected cells. *Helicobacter* (2016) 21:554–64. doi: 10.1111/hel.12311
317. Asano N, Imatani A, Watanabe T, Fushiya J, Kondo Y, Jin X, et al. Cdx2 expression and intestinal metaplasia induced by *H. pylori* infection of gastric cells is regulated by NOD1-mediated innate immune responses. *Cancer Res.* (2016) 76:1135–45. doi: 10.1158/0008-5472.CAN-15-2272
318. Watanabe T, Asano N, Fichtner-Feigl S, Gorelick PL, Tsuji Y, Matsumoto Y, et al. NOD1 contributes to mouse host defense against Helicobacter pylori via induction of type I IFN and activation of the ISGF3 signaling pathway. *J Clin Invest.* (2010) 120:1645–62. doi: 10.1172/JCI39481
319. Maeda S, Yoshida H, Ogura K, Mitsuno Y, Hirata Y, Yamaji Y, et al. *H. pylori* activates NF-κB through a signaling pathway involving IκB kinases, NF-κB-inducing kinase, TRAF2, and TRAF6 in gastric cancer cells. *Gastroenterology* (2000) 119:97–108. doi: 10.1053/gast.2000.8540
320. Zou M, Wang F, Jiang A, Xia A, Kong S, Gong C, et al. MicroRNA-3178 ameliorates inflammation and gastric carcinogenesis promoted by Helicobacter pylori new toxin, Tip-α, by targeting TRAF3. *Helicobacter* (2017) 22:e12348. doi: 10.1111/hel.12348
321. Greenfeld H, Takasaki K, Walsh MJ, Ersing I, Bernhardt K, Ma Y, et al. TRAF1 Coordinates polyubiquitin signaling to enhance epstein-barr virus LMP1-mediated growth and survival pathway activation. *PLoS Pathog.* (2015) 11:e1004890. doi: 10.1371/journal.ppat.1004890
322. Siegler G, Meyer B, Dawson C, Brachtel E, Lennerz J, Koch C, et al. Expression of tumor necrosis factor receptor-associated factor 1 in nasopharyngeal carcinoma: possible upregulation by Epstein-Barr virus latent membrane protein 1. *Int J Cancer* (2004) 112:265–72. doi: 10.1002/ijc.20367
323. Kung CP, Raab-Traub N. Epstein-Barr virus latent membrane protein 1 modulates distinctive NF-κB pathways through C-terminus-activating region 1 to regulate epidermal growth factor receptor expression. *J Virol.* (2010) 84:6605–14. doi: 10.1128/JVI.00344-10
324. Schnetzke U, Fischer M, Spies-Weisshart B, Zirm E, Hochhaus A, Muller JP, et al. The E3 ubiquitin ligase TRAF2 can contribute to TNF-α resistance in FLT3-ITD-positive AML cells. *Leuk Res.* (2013) 37:1557–64. doi: 10.1016/j.leukres.2013.08.004
325. Shirakata M, Imadome KI, Okazaki K, Hirai K. Activation of TRAF5 and TRAF6 signal cascades negatively regulates the latent replication origin of Epstein-Barr virus through p38 mitogen-activated protein kinase. *J Virol.* (2001) 75:5059–68. doi: 10.1128/JVI.75.11.5059-5068.2001
326. Guasparri I, Wu H, Cesarman E. The KSHV oncoprotein vFLIP contains a TRAF-interacting motif and requires TRAF2 and TRAF3 for signalling. *EMBO Rep.* (2006) 7:114–9. doi: 10.1038/sj.embor.7400580
327. Kumari P, Saha I, Narayanan A, Narayanan S, Takaoka A, Kumar NS, et al. Essential role of HCMV deubiquitinase in promoting oncogenesis by targeting anti-viral innate immune signaling pathways. *Cell Death Dis.* (2017) 8:e3078. doi: 10.1038/cddis.2017.461
328. Zhang J, Chen T, Yang X, Cheng H, Spath SS, Clavijo PE, et al. Attenuated TRAF3 fosters alternative activation of NF-κB and reduced expression of anti-viral interferon, TP53, and RB to promote HPV-positive head and neck cancers. *Cancer Res.* (2018) 78:4613–26. doi: 10.1158/0008-5472.CAN-17-0642
329. Yoshida H, Kato N, Shiratori Y, Otsuka M, Maeda S, Kato J, et al. Hepatitis C virus core protein activates nuclear factor kappa B-dependent signaling through tumor necrosis factor receptor-associated factor. *J Biol Chem.* (2001) 276:16399–405. doi: 10.1074/jbc.M006671200
330. Khan KA, Abbas W, Varin A, Kumar A, Di Martino V, Dichamp I, et al. HIV-1 Nef interacts with HCV Core, recruits TRAF2, TRAF5 and TRAF6, and stimulates HIV-1 replication in macrophages. *J Innate Immun.* (2013) 5:639–56. doi: 10.1159/000350517
331. Diani E, Avesani F, Bergamo E, Cremonese G, Bertazzoni U, Romanelli MG. HTLV-1 Tax protein recruitment into IKKepsilon and TBK1 kinase complexes enhances IFN-I expression. *Virology* (2015) 476:92–9. doi: 10.1016/j.virol.2014.12.005
332. Choi YB, Harhaj EW. HTLV-1 tax stabilizes MCL-1 via TRAF6-dependent K63-linked polyubiquitination to promote cell survival and transformation. *PLoS Pathog.* (2014) 10:e1004458. doi: 10.1371/journal.ppat.1004458
333. Boulabiar M, Boubaker S, Favre M, Demeret C. Keratinocyte sensitization to tumour necrosis factor-induced nuclear factor kappa B activation by the E2 regulatory protein of human papillomaviruses. *J Gen Virol.* (2011) 92(Pt 10):2422–7. doi: 10.1099/vir.0.032466-0
334. Bellanger S, Tan CL, Xue YZ, Teissier S, Thierry F. Tumor suppressor or oncogene? A critical role of the human papillomavirus (HPV) E2 protein in cervical cancer progression. *Am J Cancer Res.* (2011) 1:373–89.

335. McLaughlin-Drubin ME, Munger K. Viruses associated with human cancer. *Biochim Biophys Acta* (2008) 1782:127–50. doi: 10.1016/j.bbadis.2007.12.005
336. Morales-Sanchez A, Fuentes-Panana EM. Human viruses and cancer. *Viruses* (2014) 6:4047–79. doi: 10.3390/v6104047
337. Slotwinski R, Slotwinska SM. Dysregulation of signaling pathways associated with innate antibacterial immunity in patients with pancreatic cancer. *Cent Eur J Immunol* (2016) 41:404-18. doi: 10.5114/ceji.2016.65140
338. Kim E, Kim W, Lee S, Chun J, Kang J, Park G, et al. TRAF4 promotes lung cancer aggressiveness by modulating tumor microenvironment in normal fibroblasts. *Sci Rep.* (2017) 7:8923. doi: 10.1038/s41598-017-09447-z
339. Theodoraki MN, Yerneni S, Sarkar SN, Orr B, Muthuswamy R, Voyten J, et al. Helicase-driven activation of NFκB-COX2 mediates the immunosuppressive component of dsRNA-driven inflammation in the human tumor microenvironment. *Cancer Res.* (2018) 78:4292–302. doi: 10.1158/0008-5472.CAN-17-3985
340. Snell LM, Lin GH, McPherson AJ, Moraes TJ, Watts TH. T-cell intrinsic effects of GITR and 4-1BB during viral infection and cancer immunotherapy. *Immunol Rev.* (2011) 244:197–217. doi: 10.1111/j.1600-065X.2011.01063.x
341. Lu Y, Wang Q, Xue G, Bi E, Ma X, Wang A, et al. Th9 cells represent a unique subset of CD4(+) T cells endowed with the ability to eradicate advanced tumors. *Cancer Cell* (2018) 33:1048–60.e7. doi: 10.1016/j.ccell.2018.05.004
342. Liu J, Li D, Dang L, Liang C, Guo B, Lu C, et al. Osteoclastic miR-214 targets TRAF3 to contribute to osteolytic bone metastasis of breast cancer. *Sci Rep.* (2017) 7:40487. doi: 10.1038/srep40487

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 11 January 2019
doi: 10.3389/fimmu.2018.03114

Dysregulated TRAF3 and BCL2 Expression Promotes Multiple Classes of Mature Non-hodgkin B Cell Lymphoma in Mice

Gema Perez-Chacon[1,2], Magdalena Adrados[3], Maria T. Vallejo-Cremades[2], Sophie Lefebvre[4†], John C. Reed[4†] and Juan M. Zapata[1,2]*

[1] Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC-UAM, Madrid, Spain, [2] Instituto de Investigación Hospital Universitario La Paz, Madrid, Spain, [3] Instituto de Investigación del Hospital Universitario de La Princesa, Madrid, Spain, [4] Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, United States

OPEN ACCESS

Edited by:
Ali Abdul-Sater, York University, Canada

Reviewed by:
Yi Hao, Huazhong University of Science and Technology, China
Matthew Aaron Pettengill, Thomas Jefferson University, United States

***Correspondence:**
Juan M. Zapata
jmzapata@iib.uam.es

†Present Address:
Sophie Lefebvre, Departements d'urgence et d'imagerie médicale, Montpellier University Hospital, Montpellier, France
John C. Reed, Sanofi, Pharmaceuticals R&D, 640 Memorial Drive, Boston, MA, United States

Specialty section:
This article was submitted to B Cell Biology, a section of the journal Frontiers in Immunology

Received: *01 September 2018*
Accepted: *17 December 2018*
Published: *11 January 2019*

Citation:
Perez-Chacon G, Adrados M, Vallejo-Cremades MT, Lefebvre S, Reed JC and Zapata JM (2019) Dysregulated TRAF3 and BCL2 Expression Promotes Multiple Classes of Mature Non-hodgkin B Cell Lymphoma in Mice. Front. Immunol. 9:3114. doi: 10.3389/fimmu.2018.03114

TNF-Receptor Associated Factor (TRAF)-3 is a master regulator of B cell homeostasis and function. TRAF3 has been shown to bind and regulate various proteins involved in the control of innate and adaptive immune responses. Previous studies showed that TRAF3 overexpression renders B cells hyper-reactive to antigens and Toll-like receptor (TLR) agonists, while TRAF3 deficiency has been implicated in the development of a variety of B cell neoplasms. In this report, we show that transgenic mice overexpressing TRAF3 and BCL2 in B cells develop with high incidence severe lymphadenopathy, splenomegaly and lymphoid infiltrations into tissues and organs, which is the result of the growth of monoclonal and oligoclonal B cell neoplasms, as demonstrated by analysis of V_HDJ_H gene rearrangement. FACS and immunohistochemical analyses show that different types of mature B cell neoplasms arise in *TRAF3/BCL2* double-transgenic (tg) mice, all of which are characterized by the loss of surface IgM and IgD expression. However, two types of lymphomas are predominant: (1) mature B cell neoplasms consistent with diffuse large B cell lymphoma and (2) plasma cell neoplasms. The Ig isotypes expressed by the expanded B-cell clones included IgA, IgG, and IgM, with most having undergone somatic hypermutation. In contrast, mouse littermates representing all the other genotypes (*TRAF3-/BCL2-*; *TRAF3+/BCL2-*, and *TRAF3-/BCL2+*) did not develop significant lymphadenopathy or clonal B cell expansions within the observation period of 20 months. Interestingly, a large representation of the HCDR3 sequences expressed in the *TRAF3*-tg and *TRAF3/BCL2*-double-tg B cells are highly similar to those recognizing pathogen-associated molecular patterns and damage-associated molecular patterns, strongly suggesting a role for TRAF3 in promoting B cell differentiation in response to these antigens. Finally, allotransplantation of either splenocytes or cell-containing ascites from lymphoma-bearing TRAF3/BCL2 mice into SCID/NOD immunodeficient mice showed efficient transfer of the parental expanded B-cell clones. Altogether, these results indicate that TRAF3, perhaps by promoting exacerbated B cell responses to certain antigens, and BCL2, presumably by supporting survival of these clones, cooperate to induce mature B cell neoplasms in transgenic mice.

Keywords: TRAF3, BCL2, DLBCL—diffuse large B cell lymphoma, plasma cell neoplasms, pathogen recognition receptors (PRRs), B cell lymphoma

INTRODUCTION

Tumor Necrosis Factor Receptor (TNFR)-associated factors (TRAFs) constitute a family of scaffold proteins that interact with the cytoplasmic regions of various members of the TNFR superfamily upon their activation. TRAFs act as docking molecules for kinases, ubiquitin-ligases, ubiquitin-proteases and other effector proteins to comprise and modulate TNFR-signalosomes. In this regard, TRAFs regulate both the subcellular localization of the receptor-ligand complexes and the extent of the signaling response by controlling the composition and post-translational modification of proteins within these receptor signaling complexes. Additionally, some members of the TRAF family also regulate signaling and function of pattern recognition receptors (PRRs) and some interleukin-family receptors (1).

The role of TRAF-family proteins in regulating lymphocyte physiology and function is incompletely understood. This gap in knowledge is particularly relevant for TRAF3, which reportedly modulates multiple signaling pathways and plays a critical role in regulating B cell survival, activation and differentiation (2, 3). With regards to adaptive immunity, for example, TRAF3 binds and regulates signaling by different TNFR family members in B-lymphocytes, including CD40, B-cell activating factor receptor (BAFFR), transmembrane activator and CAML interactor (TACI) and B-cell maturation antigen (BCMA), which are critical regulators of B cell proliferation, differentiation and survival (4, 5). Additionally, TRAF3 regulates Toll-like receptors (TLRs) through its interaction with myeloid differentiation primary response 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF) (6), thereby participating in innate immune responses against pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (7, 8). TRAF3 is also involved in the regulation of nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and retinoic acid-inducible gene (RIG)-1-like Receptors (RLRs) through its interaction with receptor interacting protein (RIP)-2 (9) and mitochondrial antiviral-signaling (MAVS) protein (10), respectively, which are intracellular sensors of bacteria and virus products (11). Moreover, TRAF3 has also been shown to regulate IL17R signaling (12). Thus, TRAF3 is a pleiotropic protein controlling multiple pathways involved in the regulation of B cell proliferation, differentiation, survival with broad relevance to both adaptive, and innate immunity.

Probably because of its pleiotropic effects, TRAF3 has seemingly opposite functions in some cellular contexts. This is well-illustrated by analysis of B cell-specific *Traf3*-deficient mice (13) and lymphocyte-specific *TRAF3*-tg mice (14). B cell-specific *Traf3*-deficient mice develop B cell hyperplasia and have high Ig titers in serum, suggesting that endogenous TRAF3 might suppress B-cell expansion. In this regard, *Traf3* deficiency in B cells results in nuclear factor κB (NF-κB)-2 activation due to a role of endogenous TRAF3 in recruiting ubiquitin ligases that promote degradation of NK-kB-inducing kinase (NIK) (15), although the actual mechanism involved in TRAF3-mediated NIK regulation in B cells remains controversial (16). One of the consequences of TRAF3 deficiency (presumably attributed to the NF-kB2 over-activation) is the expansion of marginal zone (MZ) B cells (13, 17), which might explain the hyperreactivity to TLR ligands (18) and the systemic lupus erythematosus (SLE)-like autoimmunity observed in these mice (13). MZ B cells do not normally express or have very reduced levels of TRAF3 expression (19) and are naturally overreactive to TLR ligands (11, 20). In contrast, lymphocyte-specific *TRAF3*-tg mice develop progressive plasmacytosis and hypergammaglobulinemia, show exacerbated TLR responses as well as increased IgG production in response to T-I and T-D antigens, and develop systemic inflammation and SLE-like autoimmunity (14). This phenotype suggests that TRAF3 over-expression also causes excessive B-cell function that can manifest as SLE-like autoimmunity, in this case perhaps by driving B cell differentiation to produce abundant antibody-secreting cells (ASCs) via a process that might speculatively be PRR-dependent.

TRAF3 has been proposed as a tumor suppressor protein since a number of biallelic deletions or inactivating mutations has been identified in human B cell malignancies, including B-chronic lymphocytic leukemia (CLL), splenic marginal zone lymphoma (SMZL), mantle cell lymphoma, diffuse large B-cell lymphoma (DLBCL), and multiple myeloma (MM), as well as in Waldenström's macroglobulinemia (21–27). In agreement with these results, B-cell-specific *Traf3*-deficient mice were reported to develop clonal SMZL or B1a lymphomas (28). These results are consistent with the hypothesis that TRAF3 inactivating mutations (resulting in constitutive NF-κB2 activation in B cells) predispose to malignant transformation irrespective of the B cell maturation state.

Previously, we have shown that lymphocyte-specific *TRAF3*-tg mice have extra-nodal infiltration of B-cells into many organs and these animals experience an increased incidence of inflammation-driven solid tumors, including squamous cell carcinomas, lung carcinomas and hepatomas. However, these mice did not show formal evidence of B-lymphoid malignancy. Interestingly, intraperitoneal (I.P.) inoculation of pristane, a natural saturated terpenoid alkane known to promote autoimmune diseases and plasmacytoma in mice (29, 30), into *TRAF3*-tg mice resulted in increased tertiary organs formation and exacerbated autoimmunity, but other than a few cases of plasmacytoma, this treatment failed to promote manifest development of myeloma or other B-cell neoplasms (14). Taken together, these results suggest that TRAF3 upregulation causes severe alterations in B cell differentiation but is not sufficient to promote B cell transformation by itself.

Previously, we reported that mice with the combination of upregulated BCL2 and deficient TRAF2 signaling in B cells develop small B cell lymphoma (SBL)/CLL with high incidence, while neither deficient TRAF2 function nor BCL2 upregulation alone were sufficient to induce CLL or other malignancies in mice (31, 32). BCL2 overexpression is a landmark of CLL, follicular lymphoma (FL) and other B cell malignancies (33), including DLBCL (34). In this report, we show that the combination of TRAF3 and BCL2 overexpression in B cells leads over time to severe lymphadenopathy, splenomegaly and extranodal lymphoid infiltrations in tissues and organs in the mice, which is not observed in mice with mono-transgenic TRAF3 or BCL2.

This dysplasia is the result of monoclonal and oligoclonal B cell neoplasms (as demonstrated by the analysis of rearranged V_HDJ_H genes). In addition, we show that TRAF3 upregulation favors the production of V_HDJ_H rearrangements producing HCDR3 sequences similar to those recognizing PAMPs and DAMPs.

MATERIALS AND METHODS

Transgenic Mice

Lymphocyte-specific *TRAF3*-tg (14) and B cell-specific *BCL2*-tg mice mimicking the t(14;18) (q32;21) chromosomal translocation involving *BCL2* and *IgH* found in human FLs (35) have been previously described. *TRAF3*-tg (FVB/N) and *BCL2*-tg (BALB/c) heterozygous mice were bred to produce F1 litters with progeny of the four possible genotypes [(wild-type –/–; *TRAF3*-tg (single-positive +/–); *BCL2*-tg (single-positive –/+); and *TRAF3/BCL2* (double-positive +/+)] expressed on FVB/N x BALB/c mixed background. Analysis of the transgenic mouse genotypes was performed by polymerase chain reaction (PCR) using primers specific for human TRAF3 (forward 5′-TCGAGTTTGCCACCATGG-3′ and reverse 5′-GCGCGATCATCGGAACC-3′) and BCL2 (forward 5′-TTAGAGAGTTGCTTTACGTGGCCTG-3′ and reverse 5′-ACCTGAGGAGACGGTGACC-3′). The animal protocols were approved by the Institutional Animal Care and Use Committees of the Sanford Burnham Prebys Medical Discovery Institute and by the Bioethics Committee of the Consejo Superior de Investigaciones Científicas. Mice showing symptoms of distress and pain (heavy breath, weight loss, lethargy, etc.) were euthanized. All transgenic mice in the study were heterozygotes for each transgene.

Antibodies

Antibodies against human TRAF3 (19) and BCL2 (36) were previously described. TRAF3 (C-20), CD10 (F-4), BCL6 (N-3), PCNA (FL-261), and ERK2 (C-14) were from Santa Cruz Biotechnologies. MUM-1 (ABIN721195, antibodies online), CD45R/B220 (14-0452-81, Thermofisher scientific), Ki67 (Ab15580, Abcam), cIAP1/2 (R&D systems) and pre-adsorbed HRP-conjugated anti-mouse IgG (Sigma-Aldrich) and anti-mouse IgA (Novus biologicals) were used for western blot and/or immunohistochemistry analysis. Anti-rabbit and anti-mouse HRP-conjugated secondary antibodies were from Santa Cruz Biotechnologies or from Sigma-Aldrich. For flow cytometry analysis FITC- PE- and APC-labeled antibodies against mouse CD45R/B220, CD19, CD21, CD23, CD5, CD43, CD138/Syndecan-1, IgM, IgD, IgG (all from BD Biosciences) were used.

Isolation of Mononuclear and B Cells

Spleens, lymph nodes and blood from tg mice and WT littermates were collected and mononuclear cells were isolated by Ficoll density centrifugation (Lympholyte-M; Cedarlane Laboratories, Burlington, NC). B cells were isolated by negative magnetic selection using the StemSep mouse B cells enrichment kit (StemCells Technologies, Vancouver, CA), following the manufacturer's specifications.

Flow Cytometry Analysis

Mononuclear cells isolated as described earlier were incubated with 50 μg/ml human γ-globulin for 10 min at 4°C. Then, 10^6 cells were incubated with a combination of FITC-, PE-, or APC-conjugated antibodies recognizing various surface markers. After 40 min of incubation at 4°C, cells were washed with PBS and analyzed by flow cytometry in a FACS Canto II cytofluorimeter and the FlowJo (LLC) and FACSDiVa 6.1.2 (BD Biosciences) cytometry analysis softwares. Intracellular IgG expression was determined using a commercial fixation/permeabilization kit (Fitx&Perm; Invitrogen Life Technologies), following the manufacturer's instructions.

Immunohistochemistry

Tissues and organs from transgenic mice were fixed in 10% formalin (Sigma-Aldrich), embedded in paraffin. Tissue sections (5 μm) were deparaffinized and antigen retrieval was then performed in citrate buffer solution pH 6 (Dako). Sections were then rinsed with distilled water, treated 10 min at room temperature with peroxidase blocking solution (10% H_2O_2 in methanol) and then washed with TBS. After blocking with a TBS buffer containing normal goat serum for 1 h at room temperature, the corresponding primary antibodies were applied to the sections over night at 4°C. After washing with TBS, a HRP conjugated secondary antibody (Sigma Aldrich) was used to detect the primary antibody. Color was developed using a diaminobenzidine-based detection method (Vector Laboratories, Burlingame, CA), and sections were then counterstained with hematoxylin, dehydrated, and mounted in DPX (Fluka). Tissue sections were stained with hematoxylin and eosin (H&E).

Immunoblots

Cells from different mouse tissues were lysed in modified Laemmli buffer (0.125 M Tris pH 6.8, 4% SDS, and 20% glycerol) and sonicated. Protein concentration was determined by the bicinchoninic acid method (Pierce, Rockford, IL). Protein samples (8–15 μg/sample) were supplemented with 2.5% 2-mercaptoethanol and 0.004% bromophenol blue, and subjected to SDS-PAGE analysis and immunoblotting, using the indicated primary antibodies and horseradish peroxidase-conjugated secondary antibodies. Specific bands were detected using enhanced chemiluminescence and exposure on film. ERK2 expression was used as an internal loading control.

V_HDJ_H Analysis

Tissues and cells from *TRAF3xBCL2* mice representing all different genotypic combinations (–/–; +/–; –/+; and +/+) were extracted and total RNA was isolated using TRI_{ZOL} reagent and the PureLink™ RNA mini kit (Life Technologies, Carlsbad, CA), following the manufacturer's instructions. Then, RNA was reverse transcribed into cDNA using 2 U Superscript II reverse transcriptase (Life Technologies). The *IGHV* regions were amplified using the following primers, VH primer (forward) 5′-SARGTBMAGCTGSAGSAGTCWGG-3′; CHμ primer (reverse) 5′-CAGATCTCTGTTTTTGCCTCGTA-3′; CHγ primer (reverse) 5′-ATGCAAGGCTTACACCACAATCC-3′; and CHα primer (reverse) 5′-TAATAGGAGGAGGAGGAGTAGGAC-3′

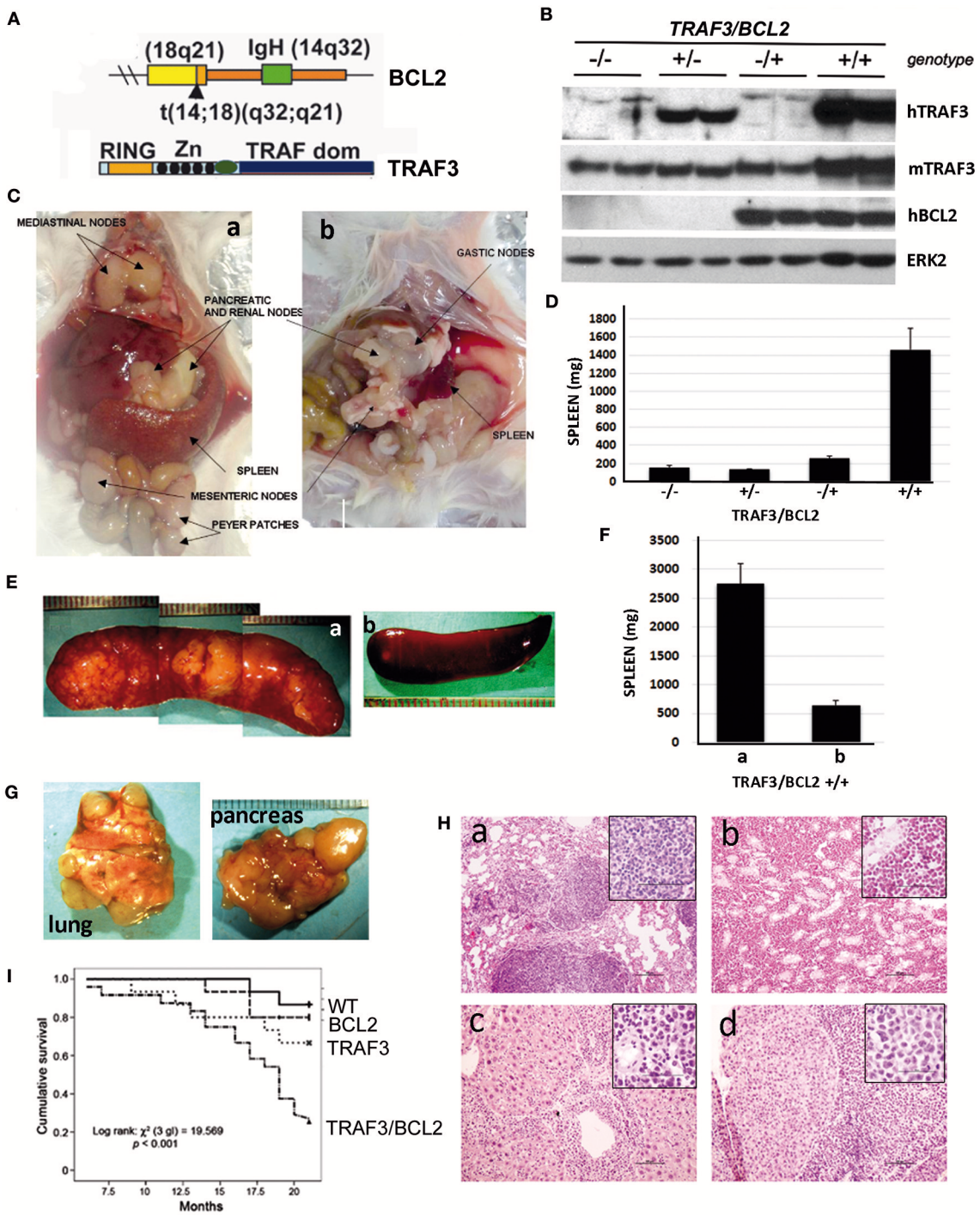


FIGURE 1 | *TRAF3/BCL2* double-tg mice develop severe lymphoid dysplasias and have a reduced lifespan. **(A)** Schematic representation of the expressed transgenes. *BCL2* gene mimics the t(14;18)(q32;q21) translocation involving BCL2 and IgH found in human FLs resulting in BCL2 overexpression. *TRAF3* is under the control of the IgH promoter and the μ enhancer. **(B)** Representative examples of the TRAF3 and BCL2 expression in spleen extracts from mice with different *TRAF3/BCL2* genotypes. Expression of ERK2 is used as loading control. **(C)** Representative examples of *TRAF3/BCL2* double-tg mice with lymphoid dyscrasias. Mice usually develop two types of lymphadenopathies, group 1 characterized by massive splenomegaly and disseminated lymphadenopathy **(a)** and group 2 with moderate splenomegaly and disseminated lymphadenopathy **(b)**. **(D)** Weight of the spleens of mouse littermates with the different *TRAF3/BCL2* genotypes (–/–, $n = 7$; +/–, $n = 10$; –/+, $n = 10$; +/+, $n = 32$). Mice were euthanized when +/+ mice developed lymphoid dyscrasias. Data represent mean ± SEM. **(E)** Representative examples of the enlarged spleens developed by diseased *TRAF3/BCL2*-double-tg mice. The pictures illustrate the differences in aspect and morphology of group 1 **(a)** and group 2 **(b)** spleens. **(F)** Weight of the spleens of group 1 [**(a)**, $n = 13$] and group 2 [**(b)**, $n = 19$]. Mice were euthanized when +/+ mice developed lymphoid

(Continued)

FIGURE 1 | dyscrasias. Data represent mean ± SEM. **(G)** Representative examples of lungs (left) and pancreas (right) showing prominent lymphoid metastasis from *TRAF3/BCL2*-double-tg mice. **(H)** H & E staining of tissues from representative *TRAF3/BCL2*-double-tg mice showing lymphoid infiltrations. Figure shows lungs **(a)**, kidney **(b)**, liver **(c)**, and pancreas **(d)**. Magnification is 100 x. The square inside shows a 400 x magnification capture of the infiltrating lymphocytes. Scale bars are shown. **(I)** Kaplan–Meier analysis of survival of mice with the different *TRAF3/BCL2* genotypes (–/–, $n = 15$; +/–, $n = 15$; –/+, $n = 15$; +/+, $n = 24$). Survival analysis was performed by using the nonparametric model of Kaplan–Meier. Log-rank test analysis of *TRAF3/BCL2* double-tg mice survival compared to the other groups demonstrated statistical significance (vs. wild-type, 0.0005; vs. TRAF3-tg, 0.029; vs. BCL2-tg, 0.002).

(Life Technologies). After denaturing DNA at 94°C for 10 min, the PCR conditions entailed 38 cycles of denaturing at 94°C for 1 min, annealing at 52°C for 1 min and extension at 68°C for 1 min, with a final extension step at 68°C for 10 min. The PCR products were then analyzed by gel electrophoresis in a 2% agarose gel, excised and purified (Qiagen). Purified products were cloned using the pGEM®-T Vector System (Promega, Madison, WI, USA) and transformed into bacteria, following the manufacturer's instructions. From 5 to 15 bacterial colonies of each sample were grown in culture overnight and the plasmids were extracted using the Wizard® Plus SV Minipreps DNA Purification System (Promega). Sequencing was performed by GATC Biotech (Konstanz, Germany). Nucleotide sequences were analyzed by means of Chromas 2.4.3 software (Technelysium, Queensland, Australia) and compared with those mouse germ-line sequences available in the IMGT/V-QUEST databases (37, 38). Sequences with <97.5% identity to the corresponding germ-line *IGHV* sequence were considered as mutated. Isolectric point (pI) of HCDR3 region was calculated with the Compute pI/Mw tool (ExPASy Bioinformatics Resource Portal, http://web.expasy.org/compute_pi/). HCDR3 analysis was carried out comparing the sequence in the protein BLAST database (restricted to *Mus musculus* sequences). Only sequences with at least 75% identity were considered.

Adoptive Transfer

Splenocytes or lymphocytes isolated from lymph nodes, ascites or pleural effusion ($40–60 \times 10^6$) from representative *TRAF3/BCL2* double-tg mice with lymphoma were I.P. allo-transplanted into 8–12 weeks-old non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Animals were euthanized when the mice develop sign of illness (distended belly, respiratory distress, lethargy, etc).

Statistical Analysis

Survival analyses were performed using the Kaplan-Meier method and the log-rank test. Differences were regarded as significant when $p < 0.05$.

RESULTS

TRAF3/BCL2 Double-tg Mice Develop Severe Lymphoid Dysplasia and Have a Reduced Lifespan

To assess whether TRAF3 upregulation might contribute to B cell transformation, we crossed lymphocyte-specific *TRAF3*-tg mice (14) with B cell-specific *BCL2*-tg mice (35). A schematic representation of the expressed transgenes is shown in **Figure 1A**. *TRAF3*-tg (FVB/N background) and *BCL2*-tg (BALB/c background) mice were crossed to produce F1 litters with mice harboring the different transgene combinations, *TRAF3/BCL2* –/–, +/–, –/+, and +/+. Immunoblot analysis of spleen extracts from mice bearing the *TRAF3* and *BCL2* transgenes readily demonstrated the expression of TRAF3 and BCL2. Moreover, hTRAF3 did not alter the expression of endogenous mTRAF3 (**Figure 1B**). Young *TRAF3/BCL2* double-tg mice did not show any significant alteration other than modest splenomegaly, which was similar to that of the *BCL2*-tg mice (31). However, when *TRAF3/BCL2* double-tg mice became older they began developing severe lymphoid dyscrasias, characterized by massive splenomegaly, and overt disseminated lymphadenopathy (**Figure 1C**). Some of the mice also develop pleural effusions and ascites. In contrast, these pathologies were not found in littermates of the other genotypes as they aged (**Figure 1D** and data not shown).

Interestingly, we observed a pattern in the size and shape of the spleens of the *TRAF3/BCL2* double-tg mice that developed lymphoid pathologies. Many spleens were extremely large, ranging from 1.5 to 5.8 g, with a pale appearance suggestive of an accumulation of white blood cells disproportionally to red blood cells. The spleens grossly had a lumpy surface and patchy decolorized zones suggestive of large lymphoid nodules (**Figures 1E,Fa**). Alternatively, spleens from some animals were larger than normal (0.3–1.2 g) but with a grossly normal appearance (**Figures 1E,Fb**).

Lymphadenopathy could be found in the double transgenic mice irrespective of gross splenic morphology (**Figure 1C**). In addition, diseased *TRAF3/BCL2* double-tg mice show massive lymphoid infiltrations in a variety of organs that often could be seen on gross pathological examination (**Figure 1G**). Histopathology studies confirmed the severe lymphoid infiltration of various tissues and organs (**Figure 1H**), including lung (**Figure 1Ha**), kidney (**Figure 1Hb**), liver (**Figure 1Hc**), and pancreas (**Figure 1Hd**). Consistent with the lymphoproliferative pathology observed in the *TRAF3/BCL2* double-tg mice, these animals also have a significantly shorter lifespan than their littermates with wild-type, *BCL2*-tg, and *TRAF3*-tg genotypes (**Figure 1I**).

DLBCL and Plasma Cell Neoplasms Are the Most Common Types of B Cell Dyscrasias Developed by *TRAF3/BCL2* Double-tg Mice

Flow cytometry analysis of the lymphoid populations from lymphoid tissues of diseased *TRAF3/BCL2* double-tg mice, including spleen, nodes, blood, as well as ascites, and pleural effusion when found in the mice, showed that they were

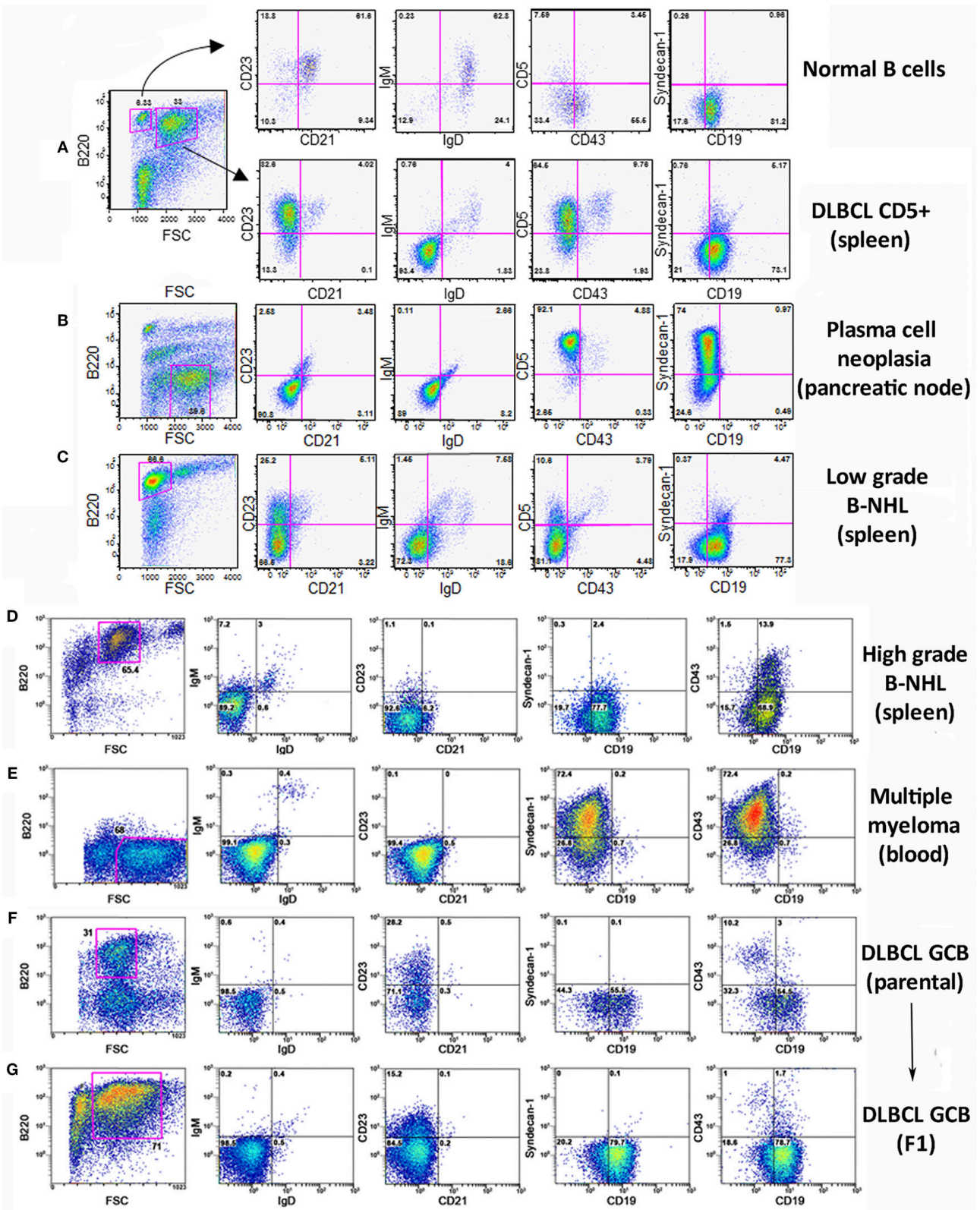


FIGURE 2 | Analysis of the B lymphocyte populations expanded in *TRAF3/BCL2* double-tg mice with lymphoid dyscrasias. Three-color flow-cytometry analysis was performed to determine the phenotype of expanded B lymphocyte populations. Gating of the expanded population was based on the CD45R/B220 and FSC plot of

(Continued)

FIGURE 2 | each sample analyzed and is indicated in the figure. The surface molecules analyzed are indicated in the plots, as well as the percentage of cells found in each quadrant. The quadrants settings were selected based on the staining of isotype-controls (not shown). The tissue source where the analyzed lymphocytes were extracted from and the type of B cell malignancy developed by the *TRAF3/BCL2* double-tg mice, according to the flow-cytometry and immunohistochemical analysis, is indicated in the figure.

consistently composed by B cell expansions with distinct surface marker expression but all indicative of a mature B cell phenotype. Representative examples are shown in **Figure 2**. Our results indicated that the vast majority of these B cell populations could be allocated into two major groups. The first group was characterized by large cells (FSC^H) expressing CD45R/B220 and CD19, but having lost surface IgM, IgD, and CD21 expression (**Figures 2A,D,F,G**). The other group was composed by large cells lacking CD45R/B220, CD19, CD21, CD23, IgM, and IgD on their surface but expressing syndecan-1 (CD138) (**Figures 2B,E, 4A**), which is indicative of plasma cell lineage. In addition, a few mice developed a type of lymphoid expansion composed by small B cells expressing CD45R/B220 and CD19 and lacking the expression of CD21, CD23, IgM, and IgD on their surface (**Figure 2C**). For comparison, **Figure 2A**, top, shows the surface markers expression analysis of the remaining normal B cell population present in the spleen of that mouse.

Sequencing of the V_HDJ_H region of the heavy chain (*IgH*) gene locus (deduced from the transcriptome) showed that both the large B-cell and the plasma cell types of lymphoid expansions were either monoclonal or oligoclonal (**Table 1**), thus indicating that the *TRAF3/BCL2* double-tg mice develop lymphoid neoplasms. Further characterization of the B cell neoplasms developed by these mice was accomplished by immunohistochemistry. These results further confirm the expression in these neoplasms of TRAF3 (which was more often located in the nucleus than in the cytoplasm), and BCL2 (which was present in the cytosol) (**Figure 3** and **Supplementary Figures 1–3**). Moreover, based on the differential expression of MUM-1, BCL6, and CD10, we conclude that most of the lymphoid neoplasms characterized by very large spleens were consistent with DLBCL. **Figure 3** shows a DLBCL expressing $BCL6^+$, $MUM\text{-}1^{null}$ and $CD10^{null}$, representative of the GC B cell cluster. Other examples of DLBCL are shown in **Supplementary Figure 1**. In the DLBCL group, which is characterized by large cells (FSC^H) expressing CD45R/B220 and CD19, we also found examples of mice with B cell neoplasms showing a prominent starry sky pattern, positive staining for BCL6, MUM-1, and CD10 and a high proliferation index, as shown by Ki67 staining, consistent with a high-grade B-non Hodgkin lymphoma (NHL) (**Supplementary Figure 2**). Of note is that this later lymphoma also has c-MYC overexpression (not shown). Interestingly, one mouse developed a B cell neoplasm consistent with a rare type of DLBCL expressing CD5 (**Figure 2A**). Immunohistochemical analysis of representative examples of the plasmacytoid neoplasms confirmed expression of either cytosolic IgG or IgA and showed a high Ki67 proliferation index (>50%), consistent with a plasma cell neoplasia (**Figure 3** and **Supplementary Figure 3**). Consistent with this diagnosis, some neoplasms of this group also express cytosolic IgG as demonstrated by FACS and immunoblotting of protein extracts of lymphoid tissues from representative *TRAF3/BCL2* double-tg mice with this type of lymphoid expansions (**Figure 4**). A diagram representing the frequency of the different B cell neoplasms found in the *TRAF3/BCL2* double-tg mice is provided in **Figure 3**.

TRAF3/BCL2 Double-tg Mice Develop Clonal B Cell Expansions

As indicated above, the analysis of the V_HDJ_H rearrangements confirmed the existence of clonal B cell expansions in the *TRAF3/BCL2* double-tg mice observed with aging (**Table 1**). The expanded clones were mono- or oligoclonal and the Ig subtypes of these clones varied (IgA, IgG, and IgM were observed). In addition, somatic hypermutation (SHM) took place in approximately half of the IgM clones and most (75–80%) of the IgA and IgG clones. The fact that most of these clones have experienced Ig class switching and SHM suggests that these neoplasms arise from antigen-activated B cells that have undergone differentiation in germinal centers (GCs), although extra-follicular differentiation is also a possibility, in particular for those clones expressing IgM. In some instances, distinct clonal expansions were found in different lymphoid tissues of the same mouse, as indicated by the Ig subclass and the HCDR3 sequence of the expanded clones (**Table 1**).

As one hallmark of cancer is the ability of tumor cells to grow into immunodeficient recipients after transplantation, we used splenocytes or lymphocytes from either ascitic fluid or pleural effusion for allo-transplantation into SCID/NOD immunodeficient mice (**Table 2**). For these experiments we used lymphocytes from mice representing three of the most characteristic of the B cell neoplasms developed by the *TRAF3/BCL2* double-tg mice. One of the donor mice (#3) developed splenomegaly (1,200 mg), severe diffuse lymphadenopathy (5,000 mg) and ascitic fluid. FACS analysis (**Figure 2D**) and immunohistochemistry (**Supplementary Figure 2**) of the spleen was consistent with a high-grade B-NHL. A major IgA clone was found in spleen and ascites, although other polyclonal IgM, IgG, and IgA populations were also found, in particular in ascitic fluid (**Supplementary Figure 4**). In addition, the analysis of a node from this mouse showed the existence of an expanded clone (IgM) different to that found in spleen and ascites (**Table 1**). Allo-transplantation of ascitic lymphocytes from mouse #3 resulted in efficient implantation, taking only 3–4 weeks for the development of overt lymphoma in recipient mice. Necropsies showed that recipient SCID/NOD mice had massive lymphadenopathy and ascitic fluid but fairly normal spleens (**Table 2**). A similar result was obtained when ascites from one F1 transplanted mouse was transferred to another SCID/NOD recipient (F2). The analysis of the IgM, IgG, and IgA populations in the recipient mice showed a striking enrichment of the

TABLE 1 | TRAF3/BCL2 double-tg mice develop clonal B cell expansions.

Mouse ID	Tissue	Ig class	IGHV family	IGHD family	IGHJ gene	% Homology	SHMs	Clone (%)	Funcionality	HCDR3	pI	Tumor type
TRAF3/BCL2 3 Parental	Ascitis	IgA	VH3	D2	JH2	93.8	M	62	Productive	ASRYGLFDY	5.88	HIGH GRADE B-NHL
	Node	IgM	VH12	D2	JH1	97.6	NM	40	Productive	AGDSDGYWYFDV	3.42	Not determined
TRAF3/BCL2 11	Node+	IgM	VH1	D2	JH4	94.4	M	100	Productive	AREPYGDYDAMDY	3.84	Plasma cell neoplasia
TRAF3/BCL2 22	Node	IgA	VH1	D2	JH3	91.3	M	100	Productive	AREDYYAWFAY	4.37	Plasma cell neoplasia
TRAF3/BCL2 25	Spleen	IgG	VH1	D2	JH4	86.1	M	80	Productive	ARSRSGVTYFYYTLDF	8.54	DLBCL
TRAF3/BCL2 39	Node	IgA	VH2	D2	JH4	96.14	M	100	Productive	AKHRYDAMDY	6.79	Plasma cell neoplasia
TRAF3/BCL2 53a	Spleen	IgG	VH1	D6	JH4	91.7	M	78	Productive	TRQSHYAMDY	6.41	DLBCL
	Node	IgG	VH1	D2	JH4	95.8	M	100	Productive	ARRGYDGAMDY	6	Not determined
TRAF3/BCL2 73	Blood	IgM	VH2	D3	JH3	97.5	NM	100	Productive	ASQGY	5.57	Multiple myeloma
TRAF3/BCL2 79	Node	IgG	VH3	D1	JH4	97.22	M	100	Productive	ALRGDY	5.88	plasma cell neoplasia
TRAF3/BCL2 20	Spleen	IgM	VH5	D4	JH1	96.9	M	100	Productive	ARLDWYFDV	4.21	DLBCL
	Pleural effusion	IgM	VH5	D4	JH1	96.2	M	100	Productive	ARLDWYFDV	4.21	
TRAF3/BCL2 26	Node	IgG	VH14	D2	JH2	90.97	M	80	Productive	ASDYDCLGF	3.56	Not determined
TRAF3/BCL2 32	Node	IgG	VH1	D1	JH3	98.26	NM	80	Productive	TRSDFYGPWFAY	5.5	DLBCL
TRAF3/BCL2 36	Node	IgA	VH5	D1	JH4	98.61	NM	100	Productive	ARHKKAMDY	9.7	DLBCL CD5+
TRAF3/BCL2 40	Node	IgG	VH1	D1	JH1	88.2	M	40	Productive	GRVGHYYGFHLFFDV	6.92	DLBCL
TRAF3/BCL2 53b	Spleen	IgM	VH3	D2	JH2	95.8	M	43	Productive	AREKDTDGYYFDY	4.23	DLBCL

Representative examples of the expanded B cell clones found in TRAF3/BCL2 double-tg mice. In the table is indicated the ID number of the mouse, the source of the mRNA sample, the Ig class of the expanded clone is also indicated and highlighted (IgM, green; IgG, blue; IgA, red). The IGHV, IGHD, and IGHJ families that were reorganized in the clone are indicated, according to IMGT/V-QUEST database. The percentage of sequence homology of the VDJ sequence of each clone compared to that of the germinal sequence (GC) is also indicated (% homology) and whether the clone meets the criteria of having been subjected to somatic hypermutation (SHMs) (non-mutated (NM) ≥ 97.5 identities with the GC sequence; mutated (M) < 97.5% identities with the GC sequence). The percentage of the VDJ sequences analyzed from a given sample corresponding to the expanded clone is indicated (% clone). Functionality refers to whether the expanded clone encoded a productive Ig or contained internal stop codons (unproductive). The HCDR3 sequence is also provided. Basic (red) and acid (green) amino acids are highlighted and the isoelectric point (pI) of the HCDR3 sequence is shown. The B cell neoplasia developed by these representative TRAF3/BCL2 double-tg mice is indicated. The diagnostic was based on macroscopic and microscopic features, flow cytometry and immunohistochemical analysis. Mouse 53a and 53b come from two different and independent colonies and have different parents.

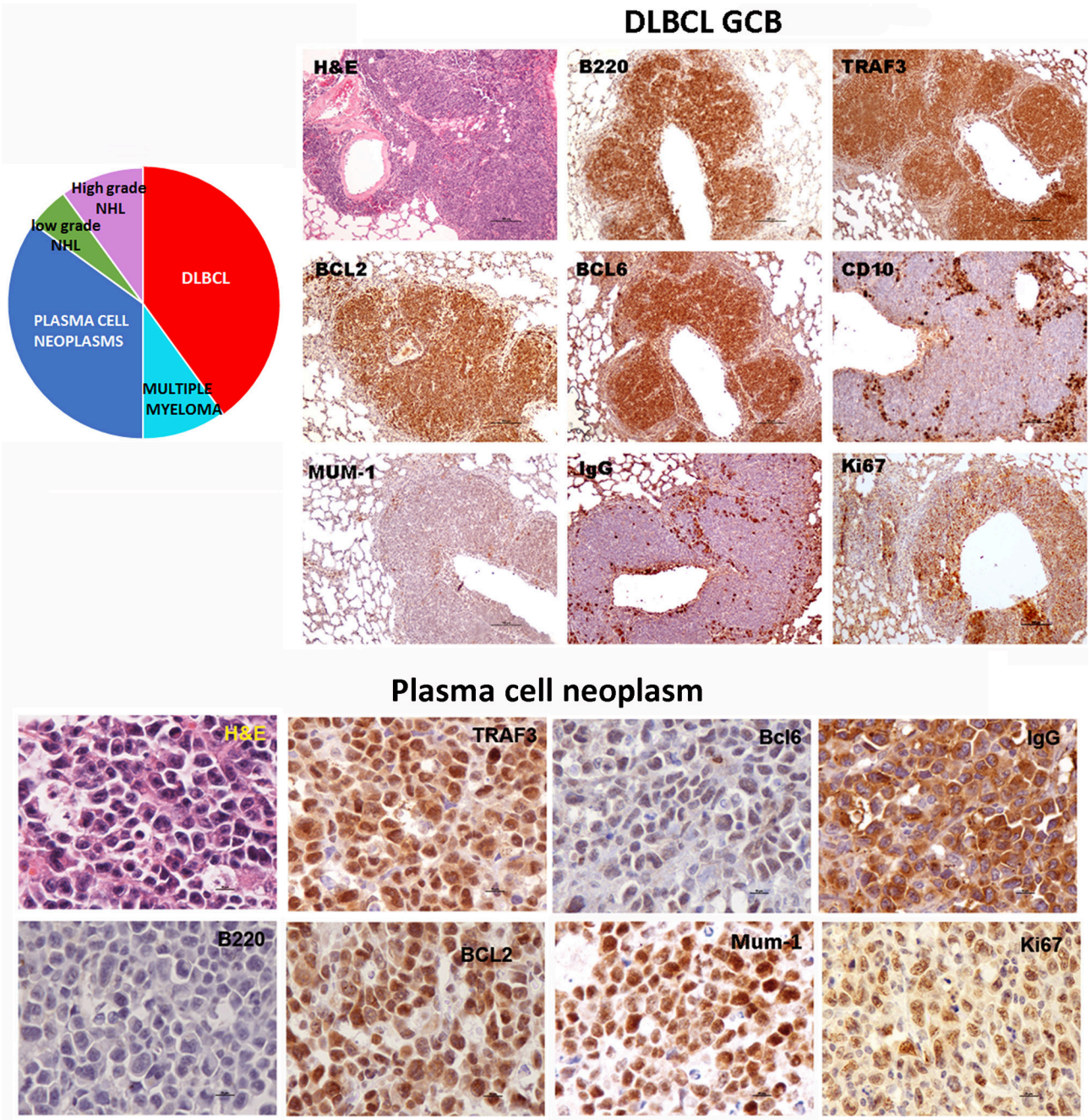


FIGURE 3 | *TRAF3/BCL2* double-tg mice develop different types of mature B cell neoplasias, but predominantly DLBCL and plasma cell neoplasias. Immunohistochemical analysis of representative examples of a DLBCL GCB type and a plasma cell neoplasia developed by the *TRAF3/BCL2* double-tg mice. Tissue slides were stained either with H&E or with antibodies specific for human TRAF3 and BCL2 and for mouse CD45B220, BCL6, MUM-1, CD10, IgG and Ki67, as indicated. Scale bars are 100 μm (DLBCL) and 10 μm (plasma cell neoplasia). A diagram indicating the frequency of the different lymphoid neoplasias found in the *TRAF3/BCL2* double-tg mice is shown ($n = 18$).

IgA population in the lymphoid tissues from F1 and F2 mice (**Supplementary Figure 4**). The analysis of the rearranged $V_H DJ_H$ sequences showed that the major parental expanded clone (IgA) found in spleen and ascites of the donor mouse was the only clone detected in the F1 and F2 allotransplanted mice (**Table 2**). A similar result was obtained when lymphocytes from mouse #20 were used for allo-transplantation. Mouse #20 developed a monoclonal IgM neoplasia consistent with DLBCL. The transfer of splenocytes or ascitic lymphocytes from this mouse into recipient SCID/NOD mice resulted in the expansion

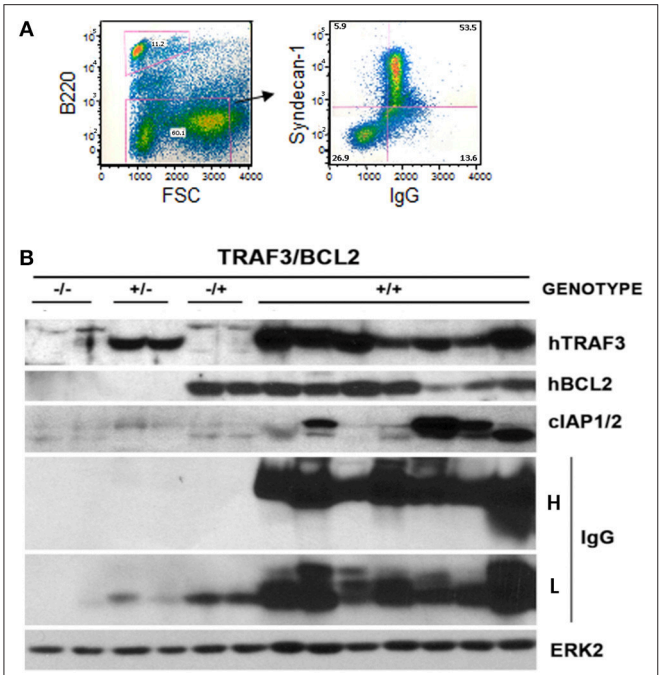


FIGURE 4 | Development of plasmablastic B cell neoplasms by *TRAF3/BCL2* double-tg mice. **(A)** Flow cytometry analysis of a representative *TRAF3/BCL2* double-tg mouse showing the expansion of an FSC^{high} $CD45B220^{null}$ lymphoid population with surface syndecan-1 (CD138) and cytosolic IgG expression. **(B)** Western blot analysis of lymphoid extracts (spleens and nodes) of representative examples of TRAF3/BCL2 double-tg (+/+) mice that have developed plasma cell neoplasms. For comparison, 2 representative examples of spleen extract from wild-type (–/–), *TRAF3*-tg (+/–), and *BCL2*-tg (–/+) mice is also shown. The expression of human TRAF3 and BCL2, and mouse cIAP1/2 and IgG heavy H and light L chains is shown. ERK2 expression is shown as loading control.

of the parental expanded clone (**Table 2**) and the development of a lymphoma that recapitulated the characteristics of the parental neoplasm (**Figures 2F,G**). Finally, we also allo-transplanted lymphocytes of mouse #39, which developed lymphadenopathy and ascites, consistent with a plasma cell neoplasia caused by the expansion of an IgA clone (**Supplementary Figure 3**). Although in this case the tumor implantation took longer, the recipient immunodeficient mouse developed the same neoplasm and clonal IgA expansion than the donor mouse (**Table 2**).

TRAF3-tg and *TRAF3/BCL2* Double-tg Mice Have a Large Representation of B Cells With Rearranged HCDR3 With Similarities to Those Recognizing PAMPS and DAMPs

We analyzed the HCDR3 sequences obtained from the *TRAF3/BCL2* double-tg mice in an effort to determine the potential antigens recognized by these clones (39), making comparisons with HCDR3 sequences obtained from littermates of the other genotypes (wild-type, *TRAF3*-tg, and *BCL2*-tg). Only HCDR3 sequences with ≥75% identities to antigen-matched HCDR3 sequences were considered for these analyses.

As shown in **Figure 5**, *TRAF3*-tg and *TRAF3/BCL2*-double-tg mice have a remarkable percentage of V_HDJ_H rearrangements producing HCDR3 potentially recognizing DAMPs (including nuclear antigens, DNA), and PAMPs (including phosphatidylcholine, lipoteichoic acid and other bacteria, mite and virus antigens). In contrast, the representation of HCDR3 sequences recognizing these types of antigens is much reduced in wild-type and *BCL2*-tg littermates, thus underscoring the key role of TRAF3 in promoting humoral responses against these antigens. Remarkably, a highly represented group of HCDR3 sequences found in the *TRAF3*-tg and *TRAF3/BCL2* double-tg mice (12.5 and 9.5% of the clones, respectively) had high similarities to HCDR3 recognizing anti-nuclear antigens. The presence of clones expressing autoreactive Ig, such as anti-nuclear antibodies (ANAs) is also consistent with the involvement of TRAF3 in the development of autoimmune disorders and confirms previous results showing the existence of ANAs in the serum of the *TRAF3*-tg mice (14). The expanded *TRAF3/BCL2* double-tg clones maintained this trend and have HCDR3 sequences similar to those recognizing viral antigens, nuclear antigens, DNA and phosphatidylcholine (**Figure 5**). Consistent with the role of some of these antibodies in autoimmunity, *TRAF3/BCL2* double-tg mice also develop autoimmune lesions, such as IgG depositions in glomeruli and tertiary lymphoid organs formation (**Figure 6**).

DISCUSSION

In this report, we show that TRAF3 and BCL2 cooperate to promote development of a variety of mature B cell lymphomas arising from antigen-challenged B cells. In this process, TRAF3 seems to promote antigen-dependent B cell differentiation toward ASCs, and BCL2 seems to provide the survival tools required to facilitate B cell transformation and survival of the expanded clones. Neither TRAF3 nor BCL2 alone have the capacity to support B cell transformation to the same extent that is achieved when both TRAF3 and BCL2 act in combination. Indeed, *BCL2*-tg mice have been shown to develop FL with advanced aging at an approximately 15% incidence (35), although these mice are otherwise healthy and have a normal life-span. In contrast, *TRAF3*-tg mice develop several pathologies associated to inflammation and autoimmunity, including inflammation-driven solid tumors, but very rarely develop lymphoid malignancies (14).

As shown in this report, lymphoid-specific *TRAF3/BCL2* double-tg mice develop B cell neoplasms, mostly DLBCL and plasma cell neoplasia, with high incidence (approximately 80% of the mice). However, the fact that these B cell malignancies arise in mice well over 1 year old suggests that TRAF3 and BCL2 might be necessary but are not sufficient for B cell transformation, and that additional transforming events are required. Nevertheless, given the high incidence of B cell tumors developed by the *TRAF3/BCL2* double-tg mice, the

TABLE 2 | TRAF3/BCL2 +/+ B cell neoplasms can be transferred to and survive in immunodeficient mice.

Mouse ID	Source of implanted lymphocytes	Days after implant	Spleen (mg)	Nodes (mg)	Other lymphoid anomalies	EXPANDED CLONE							
						mRNA source	Ig subclass	VDJ families	Homology %	SHM	Clone %	HCDR3	Pathology assessment
TRAF3/BCL2 3	–	–	1200	5000	Ascites	Ascites	IgA	VH3/D2/JH2	93.8	M	62	ASRYGLFDY	HIGH GRADE B-NHL
						Spleen	IgA	VH3/D2/JH2	93.8	M	80	ASRYGLFDY	
						node	IgM	VH12/D2/JH2	97.6	NM	40	AGDSDGYWYFDV	Not determined
3 F1 #1	Ascites frozen (60×10^6)	25	200	2000	Ascites	Ascites	IgA	VH3/D2/JH2	94.4	M	100	ASRYGLFDY	HIGH GRADE B-NHL
						Node	IgA	VH3/D2/JH2	94.4	M	100	ASRYGLFDY	
3 F1 #2	Ascites frozen (60×10^6)	30	100	4500	Ascites	Ascites	IgA	VH3/D2/JH2	94.4	M	100	ASRYGLFDY	
						Node	IgA	VH3/D2/JH2	94.4	M	100	ASRYGLFDY	
3 F2	Ascites 3F1 #1 fresh (60×10^6)	14	200	3000	Ascites	Ascites	IgA	VH3/D2/JH2	94.4	M	100	ASRYGLFDY	
						Node	IgA	VH3/D2/JH2	94.4	M	100	ASRYGLFDY	
TRAF3/BCL2 20	–	–	2470	975	Ascites, pleural effusion	Spleen	IgM	VH5/D4/JH1	96.9	M	100	ARLDWYFDV	DLBCL
						Pleural effusion	IgM	VH5/D4/JH1	96.2	M	100	ARLDWYFDV	
20 F1 #1	Spleen frozen (50×10^6)	153	1800	500	Ascites, pleural effusion	Spleen	IgM	VH5/D4/JH1	96.5	M	100	ARLDWYFDV	
						Node	IgM	VH5/D4/JH1	96.5	M	100	ARLDWYFDV	
						Ascites	Ig	VH5/D4/JH1	96.2	M	100	ARLDWYFDV	
20 F1 #2	Pleural effusion frozen (40×10^6)	160	1500	800	Ascites, pleural effusion	Spleen	IgM	VH5/D4/JH1	96.5	M	100	ARLDWYFDV	
						Pleural effusion	IgM	VH5/D4/JH1	96.5	M	100	ARLDWYFDV	
TRAF3/BCL2 39	–	–	375	800	Ascites	Nodes	IgA	VH3/D2/JH4	96.14	M	100	AKHRYDAMDY	PLASMA CELL NEOPLASM
						Ascites	IgA	VH3/D2/JH4	96.14	M	100	AKHRYDAMDY	
39 F1 #1	Nodes (mesenteric) frozen (60×10^6)	232	220	900	Ascites	Nodes	IgA	VH3/D2/JH4	96.14	M	100	AKHRYDAMDY	
						Ascites	IgA	VH3/D2/JH4	96.14	M	100	AKHRYDAMDY	

Lymphocytes from representative TRAF3/BCL2 double-tg mice were used to assess whether the expanded B cell clones of these mice could be allotransplanted to immunodeficient SCID/NOD mice. Table indicates the mouse ID, the source of the lymphoid tissues used for implantation and how many days took the recipient mice to develop evidence of disease. Table also show the weight of spleen and lymphoid nodes, and whether ascites and pleural effusion was found. The source of the mRNA sample, the Ig class of the expanded clone is also indicated and highlighted (IgM, green; IgA, red). The IGHV, IGHD and IGHJ families that were reorganized in the clone are indicated, according to IMGT/V-QUEST database. The percentage of sequence homology of the VDJ sequence of each clone compared to that of the germinal sequence (GC) is also indicated (% homology) and whether the clone meets the criteria of having been subjected to somatic hypermutation (SHMs) (non-mutated (NM) ≥ 97.5 identities with the GC sequence; mutated (M) < 97.5% identities with the GC sequence). The percentage of the VDJ sequences analyzed from a given sample corresponding to the expanded clone is indicated (% clone). The HCDR3 of the main clone of mouse #3 (yellow), mouse #39 (pink), and mouse #20 (blue) are highlighted. The B cell neoplasia developed by these representative TRAF3/BCL2 double-tg mice is indicated. The diagnostic was based on macroscopic and microscopic features, flow cytometry and immunohistochemical analysis.

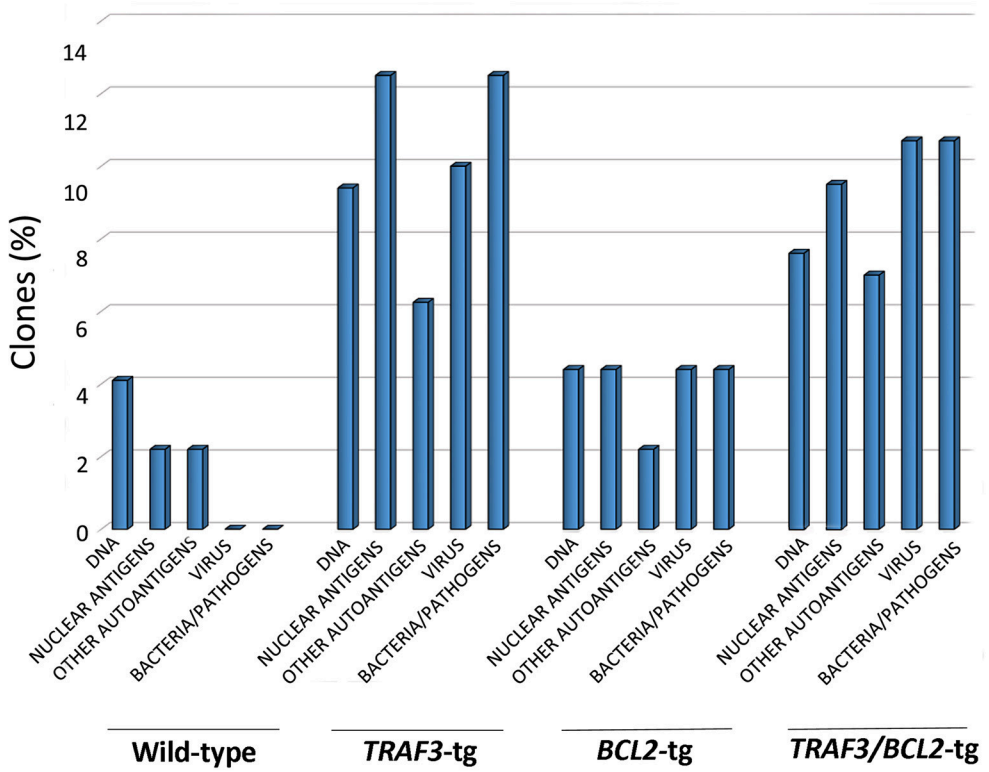


FIGURE 5 | TRAF3 upregulation increases the incidence of immunoglobulins with HCDR3 sequences potentially reactive to autoantigens and PAMPs. HCDR3 sequences were analyzed by blastp (non-redundant protein sequences from *Mus musculus*) and those showing ≥75% identity to described sequences recognizing DNA, ANAs, other autoantigens, and PAMPs (virus, bacteria, and other pathogens) were selected. Data represents the percentage of HCDR3 sequences similar to those recognizing the indicated antigens.

overexpression of both transgenes might favor the occurrence of these additional transforming events. In this regard, upregulation of c-MYC expression has been observed in two *TRAF3/BCL2* double-tg mice that have developed high grade B NHL.

It is noteworthy that many of the B cell lymphomas arising in the *TRAF3/BCL2* double-tg mice show a nuclear localization of TRAF3. Recently, studies showed that TRAF3 can traffic into the nucleus where it associates with and inhibits the transcriptional regulator cAMP response element binding protein (CREB) (35). CREB-binding protein (CREBBP) is a key coactivator of CREB transcriptional function (40) and this gene is frequently mutated in FL and DLBCL (41). Remarkably, mice deficient in *Crebbp* have reduced B cell numbers affecting different B cell subsets. However, BCL2 can overcome these deficiencies and collaborate with *Crebbp* loss to promote DLBCL development, as shown in mice where both *Crebbp* gene inactivation and BCL2 over-expression in B cells were combined (42). Interestingly, c-MYC expression is upregulated and seems to play a crucial role in the B cell transformation process in this mouse model, thus underlining some similarities to the high-grade B NHLs developed by some *TRAF3/BCL2* double-tg mice.

Our results indicate that both lymphocyte-specific *TRAF3*-tg and *TRAF3/BCL2* double-tg mice have a large representation of V_HDJ_H rearrangements producing HCDR3 sequences highly similar to those recognizing PAMPs and DAMPs, including DNA, nuclear antigens, and other autoantigens (platelet glycoproteins, hemoglobin and myosin, among others), bacteria antigens (including phosphatidylcholine and lipoteichoic acid), virus, and other parasite antigens. In contrast, wild-type and *BCL2*-tg littermates sharing cages with the *TRAF3*-tg and *TRAF3/BCL2* double-tg and therefore being exposed to the same antigens have significantly fewer of these HCDR3 sequences, thus underscoring TRAF3 involvement in this process. These results are consistent with the participation of TRAF3 in the regulation of several PRRs involved in the innate immune responses to PAMPs and DAMPs. Indeed, a role for TRAF3 in controlling TLR and RLR-mediated interferon (IFN) responses against virus is well-documented (43, 44) and many examples of viral proteins have been identified that subvert TRAF3 antiviral function by targeting it or by out-competing TRAF3 binding to its signaling partners (45, 46). Furthermore, TRAF3 overexpression in B cells induced exacerbated TLR-mediated antibody responses (14). This is consistent with the role of TLRs in humoral responses against bacteria and other pathogens (47) and with the involvement of TRAF3-binding partner MyD88 in promoting robust TLR-mediated B cell humoral responses to virus (48). However, TLR hyper-responsiveness have been also shown in *Traf3*-deficient B cells (18). These seemingly opposite results

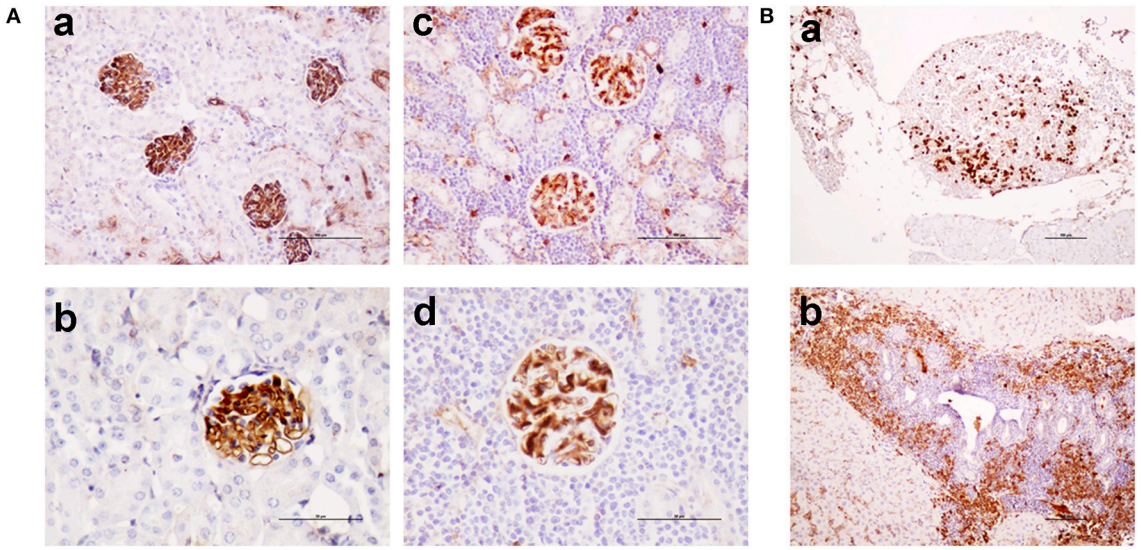


FIGURE 6 | *TRAF3/BCL2* double-tg mice develop autoimmune features similar to the *TRAF3*-tg mice. **(A)** IgG depositions in glomeruli of *TRAF3/BCL2* double-tg mice in samples showing an otherwise normal kidney architecture [**(a)**, 100 x and **(b)**, 200 x] or showing heavy lymphocyte infiltration [**(c)**, 100 x and **(d)**, 200 x]. Microphotographs are from 4 representative *TRAF3/BCL2* double-tg mice. Staining was performed with anti-mouse IgG-HRP. Scale bars are shown [**(a,c)**, 100 μm; **(b,d)**, 50 μm] **(B)**. Tertiary lymphoid organs formation in the *TRAF3/BCL2* double-tg mice. Two representative examples of tertiary lymphoid organs developed in two mice are shown. Panel **(a)** shows a tertiary lymphoid organ in the omentum. Plasma cells are shown by staining with anti-mouse IgG-HRP. Panel **(b)** shows lymphoid neogenesis in the liver, with a prominent presence of plasma cells. Staining was performed with anti-mouse IgG-HRP. Magnification was 100 x. Scale bars are shown (100 μm).

might underline different TRAF3 requirements to activate the immune response in distinct B cell types. Of note is that, as we previously reported (14), TRAF3 overexpression does not seems to alter the initiation of the humoral response, since the IgM response to TI and TD antigens is similar in *TRAF3*-tg and wild-type mice. Instead, TRAF3 seems to control later stages of B cell differentiation, such as class switching and SHM and/or the duration of antibody responses, as indicated by the elevated IgG serum levels in the *TRAF3*-tg mice and the increased IgG production seen upon antigen challenge. Indeed, in further support of this idea, *TRAF3*-tg, and *TRAF3/BCL2* double-tg B cells with V_HDJ_H rearrangements recognizing typical TI antigens, such as DNA and phosphatidylcholine, have gone through class switching and SHM (not shown). Furthermore, most of the DLBCL and plasma cell neoplasms developed by the *TRAF3/BCL-2* double-tg mice are composed by expanded transformed clones that have also undergone class switching and SHM. This is true even for half of the expanded clones expressing IgM, which also show SHM. In contrast, the B cell neoplasms developed by the B cell-specific *Traf3*-deficient mice (28) were consistent with SBL/CLL and MZL, with over 86% of the expanded cloned having non-mutated V_HDJ_H regions (applying the 97.5% identity to the germ line criteria that we have used in our analyses).

Altogether, the present evidence allows speculation about whether TRAF3 overexpression might drive TI-antigen activated B cells through an ASC differentiation program that enforces the production of high-affinity, SHM, class-switched antibodies (49). In this scenario, TRAF3 might facilitate antigen-challenged B cells to escape from the B tolerance surveillance mechanisms resulting in the production of autoreactive Ig clones (49). Indeed, it has been shown that high-affinity SHM IgG autoantibodies exacerbate SLE symptoms compared to IgM autoantibodies (50, 51), which is consistent with the presence of IgG depositions in the renal glomeruli of the *TRAF3/BCL2* double-tg mice. Most interestingly, recent results suggest that B cell intrinsic type 1 IFN keeps BCR signaling beyond the threshold required for effective tolerance (52). As a result, type 1 IFN would contribute to the loss of B cell tolerance and the development of autoreactive B cells into the GC and extra-follicular pathways. Thus, considering the key role of TRAF3 in the promotion of efficient type-1 IFN production in response to pathogen challenges, these results may underlie the role of TRAF3 in the development of the SLE (14). Altogether, these results further emphasize the differences between *Traf3*-defficiency and TRAF3 overexpression in B cell pathophysiology and underscore the need of keeping TRAF3 expression tightly regulated to assure normal B cell homeostasis and humoral responses to antigens.

Finally, while ample evidence exists about the role of deleterious *TRAF3* mutations in the development of human B cell neoplasia [which presumably is the result, at least in part, of the activation of NF-κB2-mediated transcriptional programs (21–28)], little is known about whether TRAF3 upregulation also plays a role in human lymphoid tumorigenesis (53). While genomic analysis has not revealed *TRAF3* gene amplification in lymphoid malignancies, epigenetic mechanisms could contribute to elevated TRAF3 expression. Alternatively, a gain of TRAF3 protein function could instead be caused by

modifications of either the expression or the activity of any of the abundant proteins involved in TRAF3 regulation (54, 55). Besides, as shown in this article, TRAF3 overexpression in B cells is not sufficient to induced B cell transformation and requires additional partners to facilitate B cell transformation. In summary, the results presented herein are consistent with a scenario in which TRAF3 overexpression or gain-of-function causes the anomalous selection and differentiation of PRR-co-stimulated B cell clones that in combination with BCL2 over-expression predisposes to malignant B cell transformation.

AUTHOR CONTRIBUTIONS

JZ and GP-C designed research; GP-C, SL, MV-C, and JZ performed experiments; GP-C, MA, and JZ analyzed data; JR provided essential reagents; JZ, GP-C, MA, and JR interpreted and discussed the data; JZ wrote the paper.

FUNDING

Funding was from Instituto de Salud Carlos III (ISCIII) PI080170, PI12/01135, and PI16/00895 to JZ and from the National Institutes of Health (AI070859 to JZ and CA163743 to JR). The cost of this publication was paid in part by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI) and by funds from the European Fund for Economic and Regional Development (FEDER).

ACKNOWLEDGMENTS

We are indebted to the excellent technical support of Maria G. Gonzalez-Bueno, Christina L Kress, and Andrea de Andres. We are grateful to the personnel of the Comparative Medicine, Genomics and Histology facilities at Instituto de Investigaciones Biomedicas Alberto Sols (Madrid), to the personnel of the Animal and Histology facilities at Sanford Burnham Prevys Medical Discovery Institute (La Jolla, CA), to the personnel of the Immunohistochemistry laboratory at IdiPaz (Madrid) and to the personnel of the Flow Cytometry Service at SIDI (UAM, Madrid). We also thank Dr. Paloma Perez-Aciego (LAIR Foundation, Madrid, Spain) and Dr. Ping Xie (Rutgers Cancer Institute, NJ) for kindly providing reagents and for helpful discussions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2018.03114/full#supplementary-material

REFERENCES

1. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal.* (2013) 8:7. doi: 10.1186/1750-2187-8-7
2. Zapata JM, Lefebvre S, Reed JC. Targeting TRAFs for therapeutic intervention. *Adv Exp Med Biol.* (2007) 597:188–201. doi: 10.1007/978-0-387-70630-6_15
3. Hildebrand JM, Yi Z, Buchta CM, Poovassery J, Stunz LL, Bishop GA. Roles of tumor necrosis factor receptor associated factor 3 (TRAF3) and TRAF5 in immune cell functions. *Immunol Rev.* (2011) 244:55–74. doi: 10.1111/j.1600-065X.2011.01055.x
4. Yi Z, Lin WW, Stunz LL, Bishop GA. Roles for TNF-receptor associated factor 3 (TRAF3) in lymphocyte functions. *Cytokine Growth Factor Rev.* (2014) 25:147–56. doi: 10.1016/j.cytogfr.2013.12.002
5. Rickert RC, Jellusova J, Miletic AV. Signaling by the tumor necrosis factor receptor superfamily in B-cell biology and disease. *Immunol Rev.* (2011) 244:115–33. doi: 10.1111/j.1600-065X.2011.01067.x
6. Tseng PH, Matsuzawa A, Zhang W, Mino T, Vignali DA, Karin M. Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. *Nat Immunol.* (2010) 11:70–5. doi: 10.1038/ni.1819
7. Oganesyan G, Saha SK, Guo B, He JQ, Shahangian A, Zarnegar B, et al. Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. *Nature* (2006) 439:208–11. doi: 10.1038/nature04374
8. Hacker H, Redecke V, Blagoev B, Kratchmarova I, Hsu LC, Wang GG, et al. Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. *Nature* (2005) 439:204–7. doi: 10.1038/nature04369
9. Cai X, Du J, Liu Y, Xia W, Liu J, Zou M, et al. Identification and characterization of receptor-interacting protein 2 as a TNFR-associated factor 3 binding partner. *Gene* (2013) 517:205–11. doi: 10.1016/j.gene.2012.12.026
10. Guan K, Wei C, Zheng Z, Song T, Wu F, Zhang Y, et al. MAVS Promotes inflammasome activation by targeting ASC for K63-linked ubiquitination via the E3 ligase TRAF3. *J Immunol.* (2015) 194:4880–90. doi: 10.4049/jimmunol.1402851
11. Saleh M. The machinery of Nod-like receptors: refining the paths to immunity and cell death. *Immunol Rev.* (2011) 243:235–46. doi: 10.1111/j.1600-065X.2011.01045.x
12. Zhu S, Pan W, Shi P, Gao H, Zhao F, Song X, et al. Modulation of experimental autoimmune encephalomyelitis through TRAF3-mediated suppression of interleukin 17 receptor signaling. *J Exp Med.* (2010) 207:2647–62. doi: 10.1084/jem.20100703
13. Xie P, Stunz LL, Larison KD, Yang B, Bishop GA. Tumor necrosis factor receptor-associated factor 3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. *Immunity* (2007) 27:253–67. doi: 10.1016/j.immuni.2007.07.012
14. Zapata JM, Llobet D, Krajewska M, Lefebvre S, Kress CL, Reed JC. Lymphocyte-specific TRAF3 transgenic mice have enhanced humoral responses and develop plasmacytosis, autoimmunity, inflammation, and cancer. *Blood* (2009) 113:4595–603. doi: 10.1182/blood-2008-07-165456
15. Sun SC. Non-canonical NF-kappaB signaling pathway. *Cell Res.* (2011) 21:71–85. doi: 10.1038/cr.2010.177
16. Lin WW, Hildebrand JM, Bishop GA. A Complex Relationship between TRAF3 and Non-Canonical NF-kappaB2 Activation in B Lymphocytes. *Front Immunol.* (2013) 4:477. doi: 10.3389/fimmu.2013.00477
17. Gardam S, Sierro F, Basten A, Mackay F, Brink R. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. *Immunity* (2008) 28:391–401. doi: 10.1016/j.immuni.2008.01.009
18. Xie P, Poovassery J, Stunz LL, Smith SM, Schultz ML, Carlin LE, et al. Enhanced Toll-like receptor (TLR) responses of TNFR-associated factor 3 (TRAF3)-deficient B lymphocytes. *J Leukoc Biol.* (2011) 90:1149–57. doi: 10.1189/jlb.0111044
19. Krajewski S, Zapata JM, Krajewska M, VanArsdale T, Shabaik A, Gascoyne RD, et al. Immunohistochemical analysis of *in vivo* patterns of TRAF-3 expression, a member of the TNF receptor-associated factor family. *J Immunol.* (1997) 159:5841–52.

20. Lopes-Carvalho T, Kearney JF. Marginal zone B cell physiology and disease. *Curr Dir Autoimmun.* (2005) 8:91–123. doi: 10.1159/000082100
21. Puente XS, Bea S, Valdes-Mas R, Villamor N, Gutierrez-Abril J, Martin-Subero JI, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature* (2015) 526:519–24. doi: 10.1038/nature14666
22. Rossi D, Deaglio S, Dominguez-Sola D, Rasi S, Vaisitti T, Agostinelli C, et al. Alteration of BIRC3 and multiple other NF-kappaB pathway genes in splenic marginal zone lymphoma. *Blood* (2011) 118:4930–4. doi: 10.1182/blood-2011-06-359166
23. Keats JJ, Fonseca R, Chesi M, Schop R, Baker A, Chng WJ, et al. Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. *Cancer Cell* (2007) 12:131–44. doi: 10.1016/j.ccr.2007.07.003
24. Annunziata CM, Davis RE, Demchenko Y, Bellamy W, Gabrea A, Zhan F, et al. Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* (2007) 12:115–30. doi: 10.1016/j.ccr.2007.07.004
25. Braggio E, Keats JJ, Leleu X, Van Wier S, Jimenez-Zepeda VH, Valdez R, et al. Identification of copy number abnormalities and inactivating mutations in two negative regulators of nuclear factor-kappaB signaling pathways in Waldenstrom's macroglobulinemia. *Cancer Res.* (2009) 69:3579–88. doi: 10.1158/0008-5472.CAN-08-3701
26. Nagel I, Bug S, Tonnies H, Ammerpohl O, Richter J, Vater I, et al. Biallelic inactivation of TRAF3 in a subset of B-cell lymphomas with interstitial del(14)(q24.1q32.33). *Leukemia* (2009) 23:2153–5. doi: 10.1038/leu.2009.149
27. Bushell KR, Kim Y, Chan FC, Ben-Neriah S, Jenks A, Alcaide M, et al. Genetic inactivation of TRAF3 in canine and human B-cell lymphoma. *Blood* (2015) 125:999–1005. doi: 10.1182/blood-2014-10-602714
28. Moore CR, Liu Y, Shao C, Covey LR, Morse HC III, Xie P. Specific deletion of TRAF3 in B lymphocytes leads to B-lymphoma development in mice. *Leukemia* (2012) 26:1122–7. doi: 10.1038/leu.2011.309
29. Reeves WH, Lee PY, Weinstein JS, Satoh M, Lu L. Induction of autoimmunity by pristane and other naturally occurring hydrocarbons. *Trends Immunol.* (2009) 30:455–64. doi: 10.1016/j.it.2009.06.003
30. Gado K, Silva S, Paloczi K, Domjan G, Falus A. Mouse plasmacytoma: an experimental model of human multiple myeloma. *Haematologica* (2001) 86:227–36.
31. Zapata JM, Krajewska M, Morse HC III, Choi Y, Reed JC. TNF receptor-associated factor (TRAF) domain and Bcl-2 cooperate to induce small B cell lymphoma/chronic lymphocytic leukemia in transgenic mice. *Proc Natl Acad Sci USA.* (2004) 101:16600–5. doi: 10.1073/pnas.0407541101
32. Perez-Chacon G, Llobet D, Pardo C, Pindado J, Choi Y, Reed JC, et al. TNFR-associated factor 2 deficiency in B lymphocytes predisposes to chronic lymphocytic leukemia/small lymphocytic lymphoma in mice. *J Immunol.* (2012) 189:1053–61. doi: 10.4049/jimmunol.1200814
33. Huang J, Fairbrother W, Reed JC. Therapeutic targeting of Bcl-2 family for treatment of B-cell malignancies. *Expert Rev Hematol.* (2015) 8:283–97. doi: 10.1586/17474086.2015.1026321
34. Schuetz JM, Johnson NA, Morin RD, Scott DW, Tan K, Ben-Nierah S, et al. BCL2 mutations in diffuse large B-cell lymphoma. *Leukemia* (2012) 26:1383–90. doi: 10.1038/leu.2011.378
35. Katsumata M, Siegel RM, Louie DC, Miyashita T, Tsujimoto Y, Nowell PC, et al. Differential effects of Bcl-2 on B and T lymphocytes in transgenic mice. *Proc Natl Acad Sci USA* (1992) 89:11376–80. doi: 10.1073/pnas.89.23.11376
36. Reed J, Meister L, Cuddy M, Geyer C, Pleasure D. Differential expression of the Bcl-2 proto-oncogene in neuroblastoma and other human tumor cell lines of neural origin. *Cancer Res.* (1991) 5l:6529–38.
37. Brochet X, Lefranc MP, Giudicelli V. IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. *Nucleic Acids Res.* (2008) 36:W503–8. doi: 10.1093/nar/gkn316
38. Giudicelli V, Brochet X, Lefranc MP. IMGT/V-QUEST: IMGT standardized analysis of the immunoglobulin (IG) and T cell receptor (TR) nucleotide sequences. *Cold Spring Harbor Protocol.* (2011) 2011:695–715. doi: 10.1101/pdb.prot5633
39. Yan XJ, Albesiano E, Zanesi N, Yancopoulos S, Sawyer A, Romano E, et al. B cell receptors in TCL1 transgenic mice resemble those of aggressive, treatment-resistant human chronic lymphocytic leukemia. *Proc Natl Acad Sci USA.* (2006) 103:11713–8. doi: 10.1073/pnas.0604564103
40. Johannessen M, Delghandi MP, Moens U. What turns CREB on? *Cell Signal.* (2004) 16:1211–27. doi: 10.1016/j.cellsig.2004.05.001
41. Pasqualucci L, Dalla-Favera R. Genetics of diffuse large B-cell lymphoma. *Blood* (2018) 131:2307–19. doi: 10.1182/blood-2017-11-764332
42. Garcia-Ramirez I, Tadros S, Gonzalez-Herrero I, Martin-Lorenzo A, Rodriguez-Hernandez G, Moore D, et al. Crebbp loss cooperates with Bcl2 overexpression to promote lymphoma in mice. *Blood* (2017) 129:2645–56. doi: 10.1182/blood-2016-08-733469
43. Hacker H, Tseng PH, Karin M. Expanding TRAF function: TRAF3 as a tri-faced immune regulator. *Nat Rev Immunol.* (2011) 11:457–68. doi: 10.1038/nri2998
44. van Zuylen WJ, Doyon P, Clement JF, Khan KA, D'Ambrosio LM, Do F, et al. Proteomic profiling of the TRAF3 interactome network reveals a new role for the ER-to-Golgi transport compartments in innate immunity. *PLoS Pathog.* (2012) 8:e1002747. doi: 10.1371/journal.ppat.1002747
45. Guven-Maiorov E, Keskin O, Gursoy A, VanWaes C, Chen Z, Tsai CJ, et al. TRAF3 signaling: competitive binding and evolvability of adaptive viral molecular mimicry. *Biochim Biophys Acta* (2016) 1860(11 Pt B):2646–55. doi: 10.1016/j.bbagen.2016.05.021
46. Bangalore-Prakash P, Stunz LL, Mambetsariev N, Whillock AL, Hostager BS, Bishop GA. The oncogenic membrane protein LMP1 sequesters TRAF3 in B-cell lymphoma cells to produce functional TRAF3 deficiency. *Blood Adv.* (2017) 1:2712–23. doi: 10.1182/bloodadvances.2017009670
47. DeFranco AL, Rookhuizen DC, Hou B. Contribution of Toll-like receptor signaling to germinal center antibody responses. *Immunol Rev.* (2012) 247:64–72. doi: 10.1111/j.1600-065X.2012.01115.x
48. Hou B, Saudan P, Ott G, Wheeler ML, Ji M, Kuzmich L, et al. Selective utilization of Toll-like receptor and MyD88 signaling in B cells for enhancement of the antiviral germinal center response. *Immunity* (2011) 34:375–84. doi: 10.1016/j.immuni.2011.01.011
49. Brink R, Phan TG. Self-reactive B cells in the germinal center reaction. *Annu Rev Immunol.* (2018) 36:339–57. doi: 10.1146/annurev-immunol-051116-052510
50. Jackson SW, Kolhatkar NS, Rawlings DJ. B cells take the front seat: dysregulated B cell signals orchestrate loss of tolerance and autoantibody production. *Curr Opin Immunol.* (2015) 33:70–7. doi: 10.1016/j.coi.2015.01.018
51. Rawlings DJ, Metzler G, Wray-Dutra M, Jackson SW. Altered B cell signalling in autoimmunity. *Nat Rev Immunol.* (2017) 17:421–36. doi: 10.1038/nri.2017.24
52. Domeier PP, Chodisetti SB, Schell SL, Kawasawa YI, Fasnacht MJ, Soni C, et al. B-cell-intrinsic type 1 interferon signaling is crucial for loss of tolerance and the development of autoreactive B cells. *Cell Reports* (2018) 24:406–18. doi: 10.1016/j.celrep.2018.06.046
53. Muro I, Fang G, Gardella KA, Mahajan IM, Wright CW. The TRAF3 adaptor protein drives proliferation of anaplastic large cell lymphoma cells by regulating multiple signaling pathways. *Cell Cycle* (2014) 13:1918–27. doi: 10.4161/cc.28895
54. Lin WW, Hostager BS, Bishop GA. TRAF3, ubiquitination, and B-lymphocyte regulation. *Immunol Rev.* (2015) 266:46–55. doi: 10.1111/imr.12299
55. Lin D, Zhang M, Zhang MX, Ren Y, Jin J, Zhao Q, et al. Induction of USP25 by viral infection promotes innate antiviral responses by mediating the stabilization of TRAF3 and TRAF6. *Proc Natl Acad Sci USA.* (2015) 112:11324–9. doi: 10.1073/pnas.1509968112

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 22 October 2018
doi: 10.3389/fimmu.2018.02412

Role of TRAFs in Signaling Pathways Controlling T Follicular Helper Cell Differentiation and T Cell-Dependent Antibody Responses

Christophe Pedros, Amnon Altman and Kok-Fai Kong**

Division of Cell Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States

Follicular helper T (T_{FH}) cells represent a highly specialized $CD4^+$ T cell subpopulation that supports the generation of germinal centers (GC) and provides B cells with critical signals promoting antibody class switching, generation of high affinity antibodies, and memory formation. T_{FH} cells are characterized by the expression of the chemokine receptor CXCR5, the transcription factor Bcl-6, costimulatory molecules ICOS, and PD-1, and the production of cytokine IL-21. The acquisition of a T_{FH} phenotype is a complex and multistep process that involves signals received through engagement of the TCR along with a multitude of costimulatory molecules and cytokines receptors. Members of the Tumor necrosis factor Receptor Associated Factors (TRAF) represent one of the major classes of signaling mediators involved in the differentiation and functions of T_{FH} cells. TRAF molecules are the canonical adaptor molecules that physically interact with members of the Tumor Necrosis Factor Receptor Superfamily (TNFRSF) and actively modulate their downstream signaling cascades through their adaptor function and/or E3 ubiquitin ligase activity. OX-40, GITR, and 4-1BB are the TRAF-dependent TNFRSF members that have been implicated in the differentiation and functions of T_{FH} cells. On the other hand, emerging data demonstrate that TRAF proteins also participate in signaling from the TCR and CD28, which deliver critical signals leading to the differentiation of T_{FH} cells. More intriguingly, we recently showed that the cytoplasmic tail of ICOS contains a conserved TANK-binding kinase 1 (TBK1)-binding motif that is shared with TBK1-binding TRAF proteins. The presence of this TRAF-mimicking signaling module downstream of ICOS is required to mediate the maturation step during T_{FH} differentiation. In addition, JAK-STAT pathways emanating from IL-2, IL-6, IL-21, and IL-27 cytokine receptors affect T_{FH} development, and crosstalk between TRAF-mediated pathways and the JAK-STAT pathways can contribute to generate integrated signals required to drive and sustain T_{FH} differentiation. In this review, we will introduce the molecular interactions and the major signaling pathways controlling the differentiation of T_{FH} cells. In each case, we will highlight the contributions of TRAF proteins to these signaling pathways. Finally, we will discuss the role of individual TRAF proteins in the regulation of T cell-dependent humoral responses.

Keywords: TRAF, follicular helper T cell, antibody response, TCR signaling, costimulation signaling, cytokine signaling, NF-κB

OPEN ACCESS

Edited by:
Tania H. Watts,
University of Toronto, Canada

Reviewed by:
Andreas Hutloff,
Deutsches Rheuma-Forschungszentrum (DRFZ), Germany
Woong-Kyung Suh,
Institut de Recherches Cliniques de Montreal (IRCM), Canada

****Correspondence:***
Christophe Pedros
cpedros@lji.org
Kok-Fai Kong
kokfaikong@gmail.com

Specialty section:
This article was submitted to T Cell Biology, a section of the journal Frontiers in Immunology

Received: *04 September 2018*
Accepted: *28 September 2018*
Published: *22 October 2018*

Citation:
Pedros C, Altman A and Kong KF (2018) Role of TRAFs in Signaling Pathways Controlling T Follicular Helper Cell Differentiation and T Cell-Dependent Antibody Responses. Front. Immunol. 9:2412. doi: 10.3389/fimmu.2018.02412

INTRODUCTION

Production of high-affinity immunoglobulins (Ig) by B cells represents an essential component of protective immunity against pathogens. Antibodies (Abs) function through various mechanisms including specific binding and neutralization of pathogens or toxins, activation of the classical complement pathway, opsonization of pathogens through phagocytosis by innate immune cells, and induction of antibody-dependent cell cytotoxicity (1). The initial activation of naïve B cell leads to the production of secreted IgM and cell surface-bound IgD. After activation, B cells undergo class-switch and acquire the capacity to produce Abs belonging to the IgA, IgE, or IgG subclasses, depending on environmental cues. These Ig subclasses, which differ in their heavy chains, function through different mechanisms and provide adaptability in response to the diverse forms of foreign antigens. Activated B cells can also undergo somatic hypermutations in the complementarity determining regions of the antigen-binding fragment (Fab), leading to the generation and selection of Ab-forming B cells expressing high-affinity Ig (1). B cells which lose affinity for their target or acquire autoreactivity during this process are eliminated. These B cell maturation events occur in specialized zones of the secondary lymphoid organs, dubbed the germinal centers (GC). GC B cells can differentiate into long-lived plasma cells, providing long lasting memory, and protection. The initial activation of a naïve B cell is T cell-independent, but the maturation events that lead to the generation of high affinity and long lasting protective Ab responses is critically dependent on help signals delivered by a specific $CD4^+$ T cell population, known as follicular helper T (T_{FH}) cells. T_{FH} cells are characterized by the expression of the transcription factor Bcl6, the chemokine receptor CXCR5, ICOS and PD-1. They provide B cells with essential maturation signals, promote GC formation and reactions, and govern the development of high-affinity Abs (2–4). Expression of the costimulatory molecule CD40L by T_{FH} cells plays a critical role in B cell activation and maturation, and the production of IL-21 and other cytokines by GC T_{FH} cells influence B cell proliferation, survival and isotype switch.

Deficiency of T_{FH} cells, such as in humans suffering from the X-linked lymphoproliferative disease (XLP) or in *Bcl6*$^{fl/fl}$ *Cd4*^{Cre} mice, results in disruption of GC responses, impaired Ab production, and defective memory formation following immunization or infection (5, 6). In humans, several genetic mutations that affect T_{FH} cell differentiation or function have been associated with primary immunodeficiencies characterized by failure to develop protective antibody responses such as the XLP, hyper-IgM syndrome, and common variable immunodeficiency (CVID) [reviewed in (7)]. On the other hand, dysregulated T_{FH} responses, and uncontrolled GC reactions can lead to the production of autoantibodies implicated in the pathogenesis of several autoimmune diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis (MS) [reviewed in (7)]. Dysregulated T_{FH} responses can also contribute to allergic responses (8), favor the development of B cell malignancies such as follicular lymphomas (9, 10), and even give rise to several subsets of T cell lymphomas such as angioimmunoblastic T-cell lymphoma, follicular T cell lymphoma, and nodal peripheral lymphoma with T_{FH} phenotype (11, 12). Among the mechanisms that dampen GC reactions and Ab responses, follicular regulatory T (T_{FR}) cells represent a highly specialized subpopulation of $Foxp3^+$ regulatory T cells (Tregs) that co-express Bcl6 and CXCR5. T_{FR} cells have the ability inhibit T_{FH} and B cell responses occurring in the GC [reviewed in (13)]. In *Bcl6*$^{fl/fl}$ *Foxp3*^{Cre} mice, T_{FR} deficiency leads to the development of late onset spontaneous autoimmune diseases and enhanced susceptibility to Ab-mediated autoimmunity (14). The involvement of T_{FR} cells in the pathogenesis of human autoimmune diseases remains speculative, but alteration of the T_{FR}:T_{FH} ratio is observed in the blood of patients suffering from several autoimmune diseases [reviewed in (15)].

In light of the key contributions of T_{FH} cells to immune responses, strategies aimed at promoting T_{FH} responses have the potential to improve protective Ab responses against pathogens and vaccines efficacy. On the other hand, inhibiting T_{FH} development or function could be of use for the treatment of immune-mediated diseases or malignancies where increased T_{FH} and GC activity contribute to the disease development or severity such as myasthenia gravis, autoimmune thyroid disease, SLE or RA. Understanding the mechanisms and intracellular signaling pathways that control T_{FH} differentiation and functions is therefore of paramount importance.

In this review, we will first chronicle the spatiotemporal cellular interactions during the multistage T_{FH} differentiation process. Then, we will review the molecular interactions and the intracellular signaling pathways of the T cell receptor (TCR), costimulatory molecules of the immunoglobulin superfamily (IgSF), and tumor necrosis factor receptor superfamily (TNFRSF), and cytokine signaling that play major roles in the differentiation, maintenance, and functions of T_{FH} cells. In each case, we will discuss the known contribution of the tumor necrosis factor receptor associated factors (TRAF) in these signaling pathways. Members of the TRAF family of proteins (TRAF1–6) have been initially identified for their modulation of signaling cascades downstream of TNFRSF members through their adaptor function and/or E3 ubiquitin ligase activity (16). The TRAF-dependent TNFRSF OX-40 (17, 18), GITR (19), and 4-1BB (20, 21) are implicated in the differentiation and functions of T_{FH} cells. TRAF proteins can also participate in signaling from the TCR and the costimulatory receptor CD28 (22–28), which deliver critical signals leading to the differentiation of T_{FH} cells. Engagement of the CD28-related costimulatory receptor ICOS is critical for T_{FH} differentiation (29–31). ICOS plays an important role in T_{FH} differentiation by recruiting phosphatidylinositol 3-kinase (PI3K) (31). Interestingly, ICOS does not recruit TRAFs directly but its cytoplasmic tail contains a binding motif for the TRAF family member-associated NF-κB activator (TANK)-binding kinase 1 (TBK1). This TBK1-binding motif is also present in TRAF2, 3 and 5, the TRAF proteins known to bind TBK1 (32). The presence of this motif in ICOS and the expression of TBK1 are required for the late step of T_{FH} differentiation (32). Furthermore, TRAF proteins can also interfere with the JAK-STAT pathways that are activated downstream of the IL-2, IL-7, IL-6, IL-21, and IL-27 cytokine

receptors (33–36) and might therefore affect T_{FH} development by modulating cytokine signaling.

Following discussion of the surface receptors regulating T_{FH} development, we will summarize the TRAF-dependent canonical and non-canonical NF-κB pathways that lead to the differentiation and functions of T-dependent Ab responses. Finally, we will focus on the role of individual TRAF proteins in the regulation of T cell-dependent humoral responses, and discuss their potential contributions at the mechanistic level based on their involvement in the multiple signaling pathways that affect humoral responses.

Cellular Interactions in T_{FH} Differentiation

Differentiation of T_{FH} cells is a complex multistep process. It involves sequential interactions between $CD4^+$ T cells and professional antigen-presenting cells (APC), namely, dendritic cells (DCs), and B cells. Using traceable immunization and pathogen infection models, the T_{FH} differentiation process can be divided into three spatiotemporal phases: (1) Initiation of T_{FH} differentiation by DC priming of naïve $CD4^+$ T cells in the T cell zone of the secondary lymphoid organs; (2) T_{FH} maturation induced by interactions with cognate B cells at the T-B border; and (3) the functional/maintenance phase, within the GC [reviewed in (37, 38)]. The antigen-specific interactions between developing T_{FH} and B cells provide a bidirectional communication that is critical for the maturation of both adaptive immune cells.

During the first few days (days 1–3) following immunization or viral infection, DCs, which are activated at the inflammatory site, enter secondary lymphoid organs and present the engulfed foreign peptides to naïve T cells at the interfollicular and paracortical T cell zones (39). Naïve T cells recognizing the peptide-MHC complex are activated and primed, leading to the induction of the transcription factor Bcl6 (40, 41). Bcl6, the master regulator of T_{FH} cells, is a transcriptional repressor that antagonizes the expression of other lineage-specific transcription factors (42) and microRNAs (43). Bcl6 represses CCR7, the chemokine receptor for the chemokine CCL19 and CCL21 predominantly expressed in the T cell zone, and indirectly promotes the expression of the chemokine receptor CXCR5, the receptor for CXCL13 produced within the B cell zones. As a result of this shift in surface chemokine receptors, these $Bcl6^+CXCR5^+$ pre-T_{FH} cells are no longer retained in the T cell zones, but are attracted along the CXCL13 chemokine gradient toward the T-B border (44). Several costimulatory molecules, such as ICOS, OX40, and CD40L, are also upregulated at the priming stage, regulating the migration, differentiation, and commitment to the T_{FH} cell fate.

During the following few days (day 4–6), the second step of T_{FH} differentiation begins at the T-B border, where pre-T_{FH} cells seek out and interact with cognate B cells. Successful interactions with B cells provide pre-T_{FH} cells with critical signals that ensure the continuation of T_{FH} differentiation programming. During this stage, the expression of Bcl6 and CXCR5 continues to rise, promoting the migration of T_{FH} cells deeper into the B cell follicles, and acquisition of the capacity to help B cells (45). In turn, B cells receive reciprocal signals from differentiating T_{FH} cells, promoting their maturation and entry into the B cell follicles. Only stable T-B conjugates further migrate into the GC (46).

The third phase (day 7 and beyond following immunization or infection) occurs within the GC. Fully differentiated T_{FH} cells localized in the B cell follicles, dubbed GC T_{FH} cells, are characterized by the highest expression of CXCR5 and Bcl6 as well as high expression of PD-1 (44). Through their high expression of CD40L and production of the cytokines IL-4 and IL-21, GC T_{FH} cells control GC B cell proliferation and survival, and drive affinity maturation and the generation of memory B cells. GC T_{FH} can express IL-21 or IL-4 alone or in combination. IL-21-producing T_{FH} cells are efficient in promoting somatic hypermutation, whereas IL-4-producing GC T_{FH} have higher CD40L expression and are able to induce isotype switching and plasma cell differentiation (47). GC T_{FH} cells require continuous antigenic stimulation for their maintenance. In the presence of further antigenic stimulation, long-lived memory T_{FH} cells can persist and rapidly recall the T_{FH} program upon reactivation (48).

The aforementioned cellular interactions between T cells and APCs influence T_{FH} differentiation through a variety of signals delivered through engagement of the TCR, costimulatory molecules, and cytokine receptors. We will first discuss the molecules at play, and then review the implication of TRAF proteins in their signaling pathways.

MOLECULAR INTERACTIONS IN T_{FH} DIFFERENTIATION AND FUNCTIONS

Upon sequential interactions with DCs and B cells, the T_{FH} differentiation program is initiated and maintained through integration of multiple signals received from the TCR, costimulatory and coinhibitory receptors, and cytokine receptors. In this part, we will review the role of these signals in T_{FH} differentiation and the contribution of TRAFs in the signaling pathways that they trigger (summarized in **Figure 1**).

TCR Signaling

Engagement of the TCR is the initial and central event that triggers naïve T cell activation and differentiation. Together with other factors, including engagement of costimulatory or inhibitory receptors and cytokine signaling, the strength and duration of TCR signals impact the outcome of T cell activation and differentiation.

Using TCR-transgenic T cells with varying binding affinities to a pigeon cytochrome C peptide, it was revealed that T cells with a high-affinity TCR preferentially develop into $CXCR5^+$ T_{FH} (49). Concomitantly, a knock-in mouse strain expressing a mutated, non-signaling CD3ζ chain showed a selective defect in the generation of T_{FH} cells (50). However, a high-affinity TCR does not appear to be an absolute prerequisite for T_{FH} differentiation as T_{FH} cells can also be generated after priming with intermediate and low affinity antigens (51). In the latter cases, B cells appear to play a key role in driving the differentiation of T_{FH} cells with low TCR affinity (51). Additionally, experiments with different doses of antigen reveal that, for a given TCR affinity, increasing

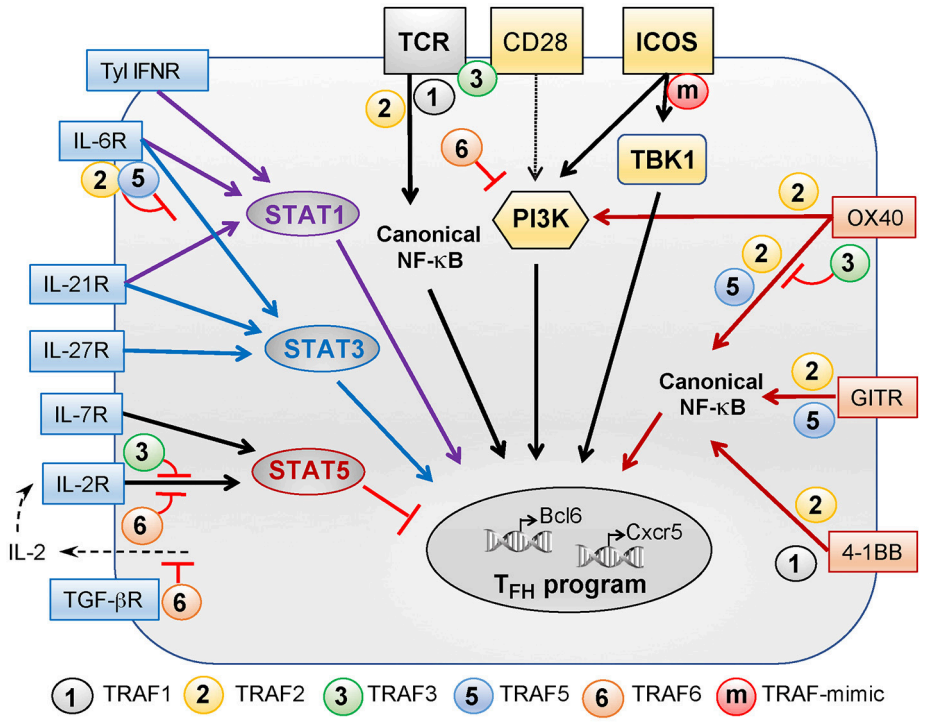


FIGURE 1 | Role of TRAFs in TCR-, costimulatory receptor-, and cytokine receptor-associated signaling pathways controlling T_{FH} differentiation. T_{FH} differentiation depends on a variety of signals received through the TCR, costimulatory receptors of the Ig superfamily (yellow boxes), costimulatory proteins of the TNF receptor superfamily (orange boxes), and cytokine receptors (blue boxes). TRAF1, −2, −3, and −6 regulate TCR signals but TRAF3 activation depends on additional CD28 signaling. TRAF2 contributes to activation of the canonical NF-κB pathway that is critical for T_{FH} differentiation. CD28 and ICOS plays a key role in T_{FH} differentiation and can both activate PI3K. PI3K activation by ICOS is critical for T_{FH} differentiation as compared to CD28-induced PI3K signals. Recently, we revealed that the TANK-binding kinase TBK1 functions downstream of ICOS to promote T_{FH} differentiation. TRAFs are not recruited to ICOS but the presence of a TRAF-like motif in the intracellular tail of ICOS bypasses the need for TRAFs to recruit TBK1. The TNFR family members OX40, GITR, and 4-1BB signal through TRAFs and also contribute to T_{FH} differentiation. TRAF2 is involved in recruitment and activation of PI3K by OX40. TRAF2 and−5 promote NF-κB pathway activation downstream OX40 and GITR while TRAF1 and−2 associate with 4-1BB to promote this pathway. TRAF3 plays a regulatory role in OX40 signaling. Signals through type 1 IFN, IL-6, and IL-21 receptors converge through STAT1 activation, positively regulating T_{FH} differentiation. Signaling through IL-6, IL-21, and IL-27 receptors activates STAT3 to promote T_{FH} differentiation. TRAF2 and−5 can both inhibit IL-6 mediated activation of STAT3. STAT5, a negative regulator of T_{FH} differentiation, can be activated by signals through the IL-2 and IL-7 receptors. TRAF3 and−6 both negatively regulate IL-2R-induced signaling. Finally, TRAF6 is involved in the suppression of IL-2 production in the presence of TGF-β and, thus, could indirectly promote early T_{FH} differentiation by limiting signals received through the IL-2R.

the amount of antigen available (45, 52) or a second peptide immunization that prolong antigen presentation (53) favors T_{FH} differentiation. In contrast, another group demonstrated that the differentiation of T_{FH} cells is reduced upon immunization with high doses of strong agonist peptide, as compared to lower doses (54). Differences in the inflammatory environment generated by the different antigen delivery systems might therefore influence the strength of TCR signals in favoring or antagonizing T_{FH} differentiation. Taken together, our current understanding is that strong and sustained TCR–DC interactions promote T_{FH} differentiation. Indeed, intravital imaging analysis reveals that sustained T-DC interactions promote T_{FH} differentiation (52, 55).

TRAF1, 2, 3, or 6 can positively or negatively modulate signaling downstream of the TCR-CD3 complex (**Figure 1**). For example, *Traf1*$^{-/-}$ CD8^{+} T cells exhibit increased levels of active p52 after anti-CD3 stimulation, indicating that TRAF1 restrains the activation of the non-canonical NF-κB pathway in the absence of costimulation (23). As a result, *Traf1*$^{-/-}$ T cells hyperproliferate in response to stimulation with anti-CD3 Ab (22, 23). In contrast, *Traf2*$^{-/-}$ CD4^{+} T cells show reduced proliferation and activation after *in vitro* anti-CD3 stimulation (24). TRAF2 plays a positive role in the regulation of NF-κB signaling as *Traf2*$^{-/-}$*Tnf*$^{-/-}$ T cells display a constitutively active non-canonical NF-κB pathway (56). In the absence of TRAF3, T cells exhibit reduced proliferation and cytokine production following costimulation with anti-CD3/CD28 Abs, reflecting an impaired activation of TCR signaling molecules Zap70, LAT, Erk, and PLCγ1 (25). Furthermore, TRAF3 has been shown to sequester the membrane localization of the kinase Csk and the phosphatase PTPN22, two known inhibitors of the TCR signaling, thereby reducing the threshold of T cell activation (26). On the other hand, *Traf6*$^{-/-}$ T cells hyperproliferate *in vitro* in response to stimulation with anti-CD3 Ab alone, bypassing the requirement for costimulation. Interestingly, the NF-κB pathway is independent of TRAF6. Instead, *Traf6*$^{-/-}$ T cells

exhibit constitutive activation of phosphatidyl-inositol 3-kinase (PI3K), demonstrating that TRAF6 negatively regulates PI3K signaling following TCR engagement (27). In addition, TRAF6 can also be recruited to the T cell immunological synapse through the adaptor molecule LAT, promoting its ubiquitination and phosphorylation and positively regulating the activation of the calcium-sensing transcription factor, nuclear factor of activated T cells (NFAT) (28). Hence, it is becoming increasingly apparent that TRAF1, 2, 3, and 6 can influence the quality and intensity of TCR signaling through various mechanisms. However, it remains to be determined whether this TRAF-dependent modulation of TCR signaling is necessary and/or sufficient to significantly impact the differentiation of T_{FH} cells.

Costimulatory Signaling

CD28 Signaling

Activated DCs present pathogen-derived peptide antigens associated with MHC class II molecules and upregulate the costimulatory ligands CD80 and 86, which interact with the costimulatory receptor CD28 on T cells. Interestingly, there is a selective preference for CD86 over CD80 to induce the formation of T_{FH} cells (57, 58). This reflects the fact that CD86 is a higher affinity ligand of CD28 (59). As a result, the CD86-CD28 interaction is less likely to be attenuated by the competing CD86-CTLA-4 interaction, and, therefore, could deliver a more sustained stimulatory signal than CD80.

Signals elicited through CD28 are essential for the activation of naïve T cells and their development into all effector T cell subsets. The differentiation of T_{FH} cells is no exception to this rule. The importance of CD28 for T-dependent Ab responses has been demonstrated using two different genetic models. First, *Cd28*$^{-/-}$ mice are deficient in GC formation and exhibit a delay in serum IgG titers following immunization with the hapten nitrophenol (NP) conjugated to chicken γ-globulin (NP-CGG) (60). Lack of CD28 costimulation in *Cd28*$^{-/-}$ T cells intrinsically inhibits the upregulation of the T_{FH} master transcription factor Bcl-6 and, thus, all subsequent T_{FH} differentiation steps are abrogated (61). Second, using a transgenic mouse strain ectopically expressing the soluble CD28 competitor, CTLA4–IgG fusion protein that blocks the interaction between CD28 and CD80/86, the T cell-dependent GC responses and antigen-specific T_{FH} cells are dramatically attenuated (62, 63). However, this defect can be compensated by the coinjection of the NP-CGG antigen and an agonistic anti-CD28 Ab (63), because the latter bypasses the inhibitory effect of CTLA4-Ig. On the contrary, GC reactions in CTLA4-Ig mice are not restored when the agonistic anti-CD28 Ab is administered 10 days after immunization (64). Similarly, blocking CD28 by injection of CTLA4-Ig in wt mice 6–7 days post-immunization does not negatively impact T_{FH} differentiation (61). Altogether, these results suggest that CD28 plays a key role during early T cell priming but not during the later phase of T_{FH} maturation or maintenance in the GC. Consistent with this notion, the absence of CD80 specifically on DCs abolishes T_{FH} differentiation whereas the absence of CD80 expression on B cells does not (65).

The signaling events that mediate CD28 function have been extensively studied, and signaling molecules that bind to specific motifs within the cytoplasmic tail of CD28 have been identified. The proximal tyrosine motif (YMNM) binds and activates the p85α subunit of PI3K as well as other adaptor proteins, including Grb2 and GADS. The distal proline-rich motif (PYAP) binds and activates Src family kinases and, indirectly, protein kinase C-θ (PKCθ) (66, 67). Using knock-in mouse strains expressing CD28 with mutations in either the proximal tyrosine motif or the distal proline-rich motif, it was demonstrated that the formation of GC and isotype switching are dependent on the PYAP motif, whereas the PI3K-binding YMNM sequence is dispensable (68). These results imply that CD28-mediated Lck and PKCθ signaling are critical for T_{FH} differentiation. However, PI3K signaling mediated by CD28 is less important than PI3K signaling emanating from ICOS (see below).

ICOS Signaling

In humans, ICOS deficiency results in severe impairment of germinal center formation and inability to mount antibody responses against infection or vaccination (69, 70). Since its initial characterization (71), it has been established that ICOS is a major driver of T-dependent Ab responses and GC reactions. *Icos*$^{-/-}$ mice have defective GCs, impaired humoral response to antigens, and lack immunological memory (72–74). Similarly, ICOS ligand (ICOSL) deficiency or blockade of ICOS-ICOSL interaction using an anti-ICOSL Ab strongly reduces T_{FH} development (29, 30). However, the temporal requirement for ICOS signals during the complex T_{FH} differentiation process appears to vary depending on the experimental model. In an acute infection model, ICOS is required for the early CXCR5^{+}Bcl6^{+} T_{FH} differentiation of antigen-specific T cells as early as 3 days following infection with lymphocytic choriomeningitis virus (LCMV) (32, 75). Consistent with ICOS signaling during the early DC-T cell engagement favoring T_{FH} differentiation through Bcl6 induction, ICOSL expression on CD8α^{-} DCs favors the initiation of CXCR5^{+}Bcl6^{+} T_{FH} differentiation (76). In stark contrast, the early expression of Bcl6 by ovalbumin-specific OT-II CD4^{+} T cells is not affected by ICOS deficiency 3 days following NP-OVA immunization (61). Similarly, *Icos*$^{-/-}$ mice show intact T_{FH} differentiation for as long as 6 days following infection with the non-lethal strain of malaria, *Plasmodium chabaudi* (77), indicating that early T_{FH} differentiation can occur in an ICOS-independent manner in some models.

In addition to the priming stage, the ICOS-ICOSL interaction between T_{FH} and B cells is also required for the maturation of developing T_{FH} cells. Administration of an anti-ICOSL blocking Ab drastically curtails the T_{FH} cell population in various infection models (61, 75, 77). Similarly, the expression of ICOSL by B cells is required for the generation of TFH cells (78). Additionally, ICOS is required for close contacts between T and B cells in the GC, promoting the expression of CD40L at the T cell surface and delivery of contact-dependent help to B cells (79).

ICOS-mediated activation of PI3K

PI3K signaling has been implicated as an important mediator downstream of several T cell molecules (TCR, CD28, CTLA-4, and ICOS). PI3K is a heterodimer consisting of a p110 catalytic subunit (of either the α, β, γ, or δ isoform) and a

regulatory subunit, which can be p85α, p55α, p50α, p85β, or p55γ. The relevance of the ICOS-mediated PI3K signaling in the differentiation of T_{FH} cells has been elegantly demonstrated using a knock-in mouse strain expressing an ICOS mutant incapable of binding PI3K (ICOS-YF). Similar to *Icos*$^{-/-}$ mice, ICOS-YF knock-in mice fail to generate T_{FH} cells and GC reactions (31). The phenotype of ICOS-YF mice is in stark contrast to the CD28-YF mice, which are capable of mounting T cell-dependent Ab responses (68). This is consistent with the fact that ICOS delivers a more potent PI3K signaling than CD28 in T cells (80).

Although the PI3K-binding motifs of CD28 and ICOS differ by a single amino acid, i.e., YMNM in CD28 and YMFM in ICOS, the resulting difference in hydrophobicity property of these motifs confers a significant alteration in T cell signaling (81). ICOS triggering not only promotes the physical interaction between ICOS and the PI3K regulatory subunits p85α and p50α in activated T cells, but also promotes their recruitment to CD28 "in trans," in the absence of CD28 ligation (80). Because p50α is the most potent isoform in regulating the kinase activity of PI3K (82, 83), ICOS ligation induces a higher PI3K activity as compared with CD28 ligation and delivers a more potent costimulatory signal favorable for the differentiation of T_{FH} cells.

To understand the role of PI3K in the generation of humoral responses, several complementary approaches have been used. First, Ab responses, including isotype switching, GC formation, and GC B cells, are severely impaired in *p110δ*$^{-/-}$ mice following hapten-induced T-dependent and T-independent challenges (84). Second, using a mouse strain expressing a catalytically inactive form of p110δ (p110δ^{D910A}), but intact (active) p110α, and p110β isoforms, the abrogation of p110δ lipid kinase activity alone was sufficient to result in a near complete absence of GC and a profound reduction of serum IgG titers following immunization with T-dependent or T-independent antigens (85). However, these initial observations are confounded by the combined functional defects in T and B cell compartments. Third, a T cell-specific deletion of the p110δ catalytic subunit in *p110δ*$^{fl/fl}$ *Cd4*^{Cre} mice results in a nearly absence of CXCR5^{+}PD-1^{+} GC T_{FH} cells, and a significant reduction of GC B cells, GC reactions, and Ab affinity maturation following immunization with NP conjugated to keyhole limpet hemocyanin (KLH) (86). These findings reveal the non-redundant and T cell-intrinsic role of p110δ in T_{FH} cell development. Fourth, *p110δ*$^{fl/fl}$ *Ox40*^{Cre} mice show similar defects in humoral responses following immunization (86). Since OX40 is expressed following TCR- and CD28-mediated T cell activation, ablation of p110δ at this later time point indicates that this catalytic subunit is crucial for T-dependent Ab responses after the initial activation of naïve T cells (86). Fifth, the magnitude and output from GC reactions are unperturbed in immunized *p110δ*$^{fl/fl}$ *Cd19*^{Cre} mice (86), implying that p110δ is dispensable in B cells, and/or that other PI3K catalytic subunits may contribute in a redundant manner to the GC reactions. Sixth, combined deletion of genes that encode four PI3K regulatory isoforms normally expressed in T cells (p85α, p55α, p50α, p85β) results in a drastic deficiency in T cell help to B cells *in vivo*. These mice display a significant reduction in GC numbers and size, as well as the production of class-switched Abs following immunization (87). Taken together, these data indicate that the ICOS-mediated PI3K pathway is crucial for T-dependent Ab responses.

Importance of PI3K-independent ICOS signaling

The PI3K-binding YMFM motif is a crucial feature of ICOS signaling in mediating the differentiation and functions of T_{FH} cells. However, the knock-in ICOS-YF mouse strain, in which the association between ICOS and PI3K is selectively lost, is not a true phenocopy of *Icos*$^{-/-}$ mice (31). For example, in a model of respiratory infection with *Chlamydia muridarum*, ICOS-YF mice develop a much milder disease as compared to *Icos*$^{-/-}$ mice, albeit they are still not fully protected (88). Th17 responses negatively correlate with disease severity and are strongly reduced in *Icos*$^{-/-}$ mice but partially retained in ICOS-YF mice. Similarly, the severity of graft-vs.-host disease in ICOS-YF mice is intermediate between wild-type (wt) and *Icos*$^{-/-}$ mice, in a model of MHC-mismatched bone marrow transplantation (89). Interestingly, in this model, CD8^{+} T cells from ICOS-YF mice induce a disease indistinguishable from that induced by wt CD8^{+} T cells, whereas ICOS-YF and *Icos*$^{-/-}$ CD4^{+} T cells behave similarly. *In vitro*, ligation of ICOS induces T cell activation, calcium flux and proliferation of CD8$^{\pm}$ T cells in a PI3K-independent manner (89). Similarly, the PI3K-independent role of ICOS in activating calcium flux was demonstrated in CD4$^{\pm}$ T cells (31). Altogether, these data strongly evince the presence of important PI3K-independent pathway(s) downstream of ICOS.

TRAF-mimicking ICOS signaling

The aforementioned studies also pose a conundrum because other than its PI3K-binding motif, the cytoplasmic tail of ICOS lacks canonical motifs for protein-protein interactions. To resolve this issue, we looked for potential evolutionarily conserved sequence(s) in the cytoplasmic tail of ICOS (32). Remarkably, in addition to the YMFM motif, we found two additional highly conserved motifs in the intracellular domain of ICOS. They are the IProx motif (SSSVHDPNGE) and a more distal motif (AVNTAKK). Using an unbiased proteomics approach, TANK-binding kinase 1 (TBK1), a non-canonical member of the inhibitor of transcription factor NF-κB kinase (IKK) family, was unexpectedly found to interact with the serine-rich IProx motif. Mutation of this specific motif abrogated TBK1 binding to ICOS, but did not affect ICOS ability to recruit PI3K (32).

Similar to the mutation of the PI3K-binding motif (ICOS-YF), *Icos*$^{-/-}$ CD4^{+} T cells reconstituted with a mutated IProx motif (mIProx) displayed impaired CXCR5^{+}PD-1^{+} GC T_{FH} differentiation, GC formation, and IgG responses. Moreover, TBK1 knockdown in T cells resulted in defective humoral responses in response to acute LCMV infection (32). Although *Icos*$^{-/-}$ CD4^{+} T cells reconstituted with an ICOS-YF mutant fail to generate nascent CXCR5^{+} Bcl6^{+} T_{FH} cells, this initial step of T_{FH} development was not compromised in T cells expressing mIProx (32). Consistently, TBK1 was also dispensable for the development of nascent T_{FH} cells, indicating that signals mediated by TBK1 binding to the ICOS IProx motif 'license' nascent T_{FH} cells to enter the GC phase of T_{FH} cell

development. In agreement with our findings, it has recently been demonstrated that therapeutic inhibition of TBK1 reduced the number of GC T_{FH} and their expression of *Bcl6*, caused a reduction in GC size, diminished the anti-collagen Ab levels and alleviated the progression of established collagen induced arthritis (90).

In contrast to the interaction between ICOS and PI3K, which can be induced by anti-CD3 or anti-ICOS stimulation alone, TBK1 coimmunoprecipitates with ICOS only when T cells are stimulated with a combination of anti-CD3 plus anti-ICOS Abs (32). These combined stimuli are physiologically provided by the strong cognate interaction that occurs *in vivo* between T cells and APCs. Hence, the requirement for activation of ICOS-TBK1 signaling is more stringent than that for the ICOS-PI3K pathway.

We further found that despite the known ability of TRAF2, 3, and 5 to physically interact with TBK1 (91–93), these TRAFs proteins were not corecruited with TBK1 to ICOS upon stimulation (32). Unexpectedly, the serine-rich IProx motif in ICOS turned out to be highly homologous with a region of TRAF2 and TRAF3 known as the "serine tongs," which consists of the sequence SSSxxxPxGD/E (where 'S' is serine, "x" is any amino acid, "P" is proline, "G" is glycine and "D/E" indicates aspartic acid or glutamic acid). Substitution of this region in TRAF2 and TRAF3 with a string of alanines abolished their ability to bind TBK1. Thus, this sequence, which is also present in a similar form in the cytoplasmic region of ICOS, represents a previously unknown consensus TBK1-binding motif. The presence of this motif in ICOS therefore allows it to directly recruit TBK1, obviating the need for TRAF proteins as intermediary partners for TBK1 activation (**Figure 1**).

ICOS-dependent calcium signaling

The ability of ICOS to potentiate TCR-induced calcium flux is conserved in *Icos*$^{-/-}$ CD4$^{\pm}$ T cells expressing an ICOS mutant where most of the cytoplasmic tail is truncated, including the PI3K and TBK1 binding motifs (94), demonstrating that the ICOS-triggering calcium flux is independent of PI3K and TBK1. Interestingly, a short membrane anchoring sequence consisting of the sequence KKKY (where "K" is lysine and "Y" is tryptophan) is present in this mutant. Mutation of the KKKY motif in full-length ICOS dampens the calcium response in T cells, showing that this motif is both necessary and sufficient for calcium flux (94). This motif in ICOS is likely to positively regulate T_{FH} responses since ICOS engagement and calcium flux promote CD40L surface expression (79), a critical requirement for T_{FH} to provide B cells with contact dependent help signals.

OX40 Signaling

CD28 costimulation induces the expression of OX40, a TNFRSF member, on T cells (63). OX40 stimulation is involved in upregulation of *Cxcr5* mRNAs (95), and higher expression of OX40 has been reported on T_{FH} cells (49). However, the degree to which OX40 influences the development of T_{FH} cells and Ab responses is highly context-dependent. *Ox40*$^{-/-}$ mice are able to mount effective humoral responses against acute LCMV, vesicular stomatitis virus (VSV), and influenza A virus infections, suggesting a non-essential role for OX40 in T_{FH} development (96). Similarly, the absence of OX40 does not affect the expression of CXCR5 on antigen-specific CD4^{+} T cells and the development of IgG1 responses after infection with the rodent roundworm *Heligmosomoides polygyrus* (97). In stark contrast, OX40 is required to mount an efficient T_{FH} and humoral response against chronic infection with the Clone 13 strain of LCMV. *Ox40*$^{-/-}$ mice are not able to control viral replication (17). In a *Vaccinia* virus infection model, *Ox40*$^{-/-}$ mice also exhibit a dramatically reduced T_{FH} differentiation and Ab response. Blocking experiments using an anti-OX40L Ab showed that the OX40-OX40L interaction is required for both T_{FH} generation and maintenance in this model (18). Variations in the expression of OX40 by CXCR5^{+} CD4 T cells in different mouse strains might account for the differential impact of OX40L Ab treatment on T_{FH} generation and GC responses (29).

OX40 signaling can induce the expression of multiple T_{FH} molecules, including CXCR5 and IL-21, by human T cells, and likely contributes to the pathogenic role of T_{FH} cells in SLE (98). Mechanistically, TRAF2, 3, and 5 are recruited to the cytoplasmic tail of OX40 [**Figure 1**; (99, 100)]. However, TRAF2 plays a more important role in OX40 signaling by promoting the recruitment of PI3K, AKT, PKCθ, and IKKα, β, and γ, which trigger the mTOR and the canonical NF-κB pathways (99–101).

GITR Signaling

GITR expression is induced late during the maturation phase of GC T_{FH} cells (102). The number of T_{FH} cells is not affected in *Gitr*$^{-/-}$ mice during the first week of chronic infection with LCMV Clone 13. This is consistent with findings that the initial production of LCMV-specific IgG is not affected. However, Ab titers do not increase in *Gitr*$^{-/-}$ mice beyond the first week of infection. This defect is associated with an increase in the proportion of splenic Foxp3^{+} CXCR5^{+}PD-1^{+} T_{FR} cells, and a reduction in Foxp3^{-} CXCR5^{+}PD-1^{+} T_{FH} cell numbers, suggesting that GITR plays a role in regulating the ratio between T_{FR} and T_{FH} cells. This GITR-mediated function is T-cell intrinsic because in mixed bone marrow chimera experiments, the T_{FH} cell population is diminished in *Gitr*$^{-/-}$ CD4^{+} T cells, as compared to the wt CD4^{+} T_{FH} cells following chronic LCMV infection (102). Consistent with a role for GITR signals in promoting humoral responses, administration of a recombinant GITR ligand protein enhanced the frequency of CXCR5^{+}ICOS^{+} T_{FH} cells and the expression of Bcl6 and IL-21 in a model of collagen-induced arthritis (19). Conversely, blocking GITR signals using a GITR-Fc fusion protein reduced the frequency of T_{FH} cells, IgG production, and disease severity (19).

As a member of the TNFRSF, several TRAF molecules interact with the cytoplasmic tail of GITR. In CD8^{+} T cells, TRAF2 and 5 are involved in activation of the canonical NF-κB pathway triggered by GITR stimulation [**Figure 1**; (103)]. Additionally, the GITR-TRAF5 axis is known to activate the MAP kinase signaling pathways because *Traf5*$^{-/-}$ CD4^{+} T cells are defective in the activation of p38 and ERK kinases (104). On the other hand, TRAF3 has been demonstrated to inhibit the activation of the non-canonical NF-κB pathway triggered by GITR engagement (105). However, it is unclear which of these

TRAF molecule(s) in the GITR signaling pathway plays a more prominent role in the maintenance of T_{FH} cells.

CD40L Signaling

CD40L is rapidly upregulated upon TCR and costimulatory receptor ligation, and/or cytokine signaling (106). CD40L expressed on T cells is the ligand for the TNFRSF member CD40 expressed on B cells. CD40-CD40L signaling is essential for the development of T cell-dependent humoral responses. *Cd40*$^{-/-}$ or *Cd40l*$^{-/-}$ mice are severely defective in their ability to generate GC or develop IgG responses (107, 108). Similarly, individuals deficient for CD40 or CD40L suffer from hyper-IgM syndrome characterized by elevated IgM level, disrupted GC formation and reduced IgG, IgA and IgE levels (7). The requirement for CD40L signals received by T cells appears to be dispensable for early T_{FH} differentiation during the DC priming phase (75), consistent with the fact that CD40L is highly expressed after the priming phase. Similarly, CD40 expression by DC is dispensable for an efficient T_{FH} and IgG response, whereas CD40 expression on B cells is absolutely required for the generation of GC and T_{FH} development (65). Interestingly, the intrinsic role of CD40L signaling in T cells does not appear to be critical for T_{FH} differentiation as wt and *Cd40l*$^{-/-}$ antigen-specific T cells expand and differentiate into T_{FH} cells to a comparable extent in a cotransfer experiment (65).

Very little is known about CD40L signaling in T cells. However, engagement of B cell-expressed CD40 by CD40L directly or indirectly recruits TRAF1, −2, −3, −5, and −6 to its cytoplasmic domain [reviewed in (109)]. The persistent TRAF-dependent CD40 signaling in B cells, delivered by CD40L expressed by T cells is considered to be one of the most potent signals in mediating different aspects of B cell biology, including differentiation, survival, proliferation, expression of costimulatory molecules, and cytokines, maturation of GC B cells, isotype switching, somatic hypermutation, and formation of long-lived plasma cells and memory B cells.

4-1BB Signaling

4-1BB (CD137 or TNFRSF9) is highly expressed on human T_{FH} cells (110). However, *4-1bb*$^{-/-}$ mice show no impairment of IgG production following VSV infection (111). Similarly, the absence of 4-1BB ligand in *4-1bbl*$^{-/-}$ mice does not affect the T cell-dependent Ab responses (112). These data imply that 4-1BB and its ligand might be dispensable for the generation of T cell-dependent humoral responses in rodents. However, *in vivo* treatment with an agonistic anti-4-1BB Ab inhibits T cell-dependent Ab responses in various mouse models (20, 21, 113). *In vivo* administration of an agonistic anti-4-1BB Ab at the time of priming strongly reduces the development of Ab responses to T cell-dependent antigens (20). Additionally, treatment with an agonistic anti-4-1BB Ab suppresses the ongoing $CD4^+$ T cell-dependent autoantibody production in the NZB × NZW mouse model of SLE (21). Therefore, excessive 4-1BB signals during both the initiation of Ab responses and their maintenance could negatively modulate T_{FH} differentiation and/or functions. The exact mechanisms by which these agonistic Abs influence T cell-dependent humoral responses warrants careful interpretation because the expression of 4-1BB is not restricted to T cells. For instance, one study suggested that anti-4-1BB Ab treatment blocks GC formation by downregulating the follicular dendritic cell (FDC) network (114), a specialized subset of follicle-residing cells that support the GC reaction.

Cytoplasmic TRAF1 and TRAF2 are recruited to 4-1BB upon stimulation [**Figure 1**; (100, 115)]. TRAF1 is required for the activation of the classical NF-κB pathway following 4-1BB engagement (23). Following stimulation with an agonistic anti-4-1BB Ab, 4-1BB is internalized to an endosomal compartment. TRAF2, and its K63 polyubiquitination activity, colocalizes with 4-1BB in endosomes. The TRAF2-associated E3 ubiquitin ligase activity and K63 polyubiquitination are required for the 4-1BB-mediated activation of the classical NF-κB pathway (116). Additionally, TRAF2 mediates the p38 MAP kinase pathway downstream of 4-1BB as T cells expressing a dominant negative form of TRAF2 lose the ability to signal via the p38 cascade (117).

Coinhibitory Signaling

CTLA-4 Signaling

The inhibitory receptor CTLA-4 is constitutively expressed on Tregs and highly expressed on T_{FR} cells (118). CTLA-4 plays a key role in the suppressive functions of Tregs (119) and *Ctla4*$^{-/-}$ mice develop systemic immune dysregulation, including increased T_{FH} and GC B cell responses (58). Short-term blockade with anti-CTLA-4 Ab or Treg-specific deletion of CTLA-4 increases T_{FH} and GC B cell responses *in vivo* (58, 120, 121) and reduces the ability of T_{FR} cells to inhibit B cell activation *in vitro* upon coculture with T_{FH} cells (120). CTLA-4 expressed by Tregs/T_{FR} cells therefore has a major influence on T_{FH} responses. Similar to the findings in mice, heterozygous, deleterious mutations in the human *CTLA4* gene manifest an immune dysregulation disorder, characterized by lymphocytic infiltration of multiple non-lymphoid organs. These individuals exhibit increased frequency of circulating $CXCR5^{\pm}PD1^{\pm}$ T_{FH} cells, which is normalized in response to treatment with CTLA4-Ig therapy (122).

CTLA-4 is also expressed by T_{FH} cells, although at a lower level than in T_{FR} cells (120). The cell-intrinsic role of CTLA-4 in T_{FH} differentiation and functions is far less defined than its cell-extrinsic role through Tregs and T_{FR} cells. One study reported that late deletion of CTLA-4 from *in vivo* differentiated T_{FH} cells using an inducible Cre/lox system increased their ability to induce isotype class switching and IgG production upon coculture with B cells (120). T_{FH}-expressed CTLA-4 might therefore function to limit the B cell-stimulating activity of T_{FH} cells in a cell-intrinsic manner. Its contribution to T_{FH} differentiation during interactions between nascent T_{FH} cells and B cells is currently unknown.

CTLA-4 delivers its negative signaling via multiple mechanisms. At the cell surface, CTLA-4 competes with CD28 for access to the CD80/86 ligands. Through a process called trogocytosis, CTLA-4 removes CD80/86 ligands from the surface of APCs, further limiting the availability of these ligands for CD28 (123). Intracellularly, the tyrosine-phosphorylated cytoplasmic domain of CTLA-4 can interact with the phosphatases SHP-2 and PP2A (124, 125). Altogether, these

CTLA-4-mediated cell-extrinsic and cell-intrinsic mechanisms dampen signaling downstream of the TCR and CD28. As such, it is conceivable that TRAF molecules, which can modulate TCR and CD28 signaling (see above), may potentially influence the CTLA-4-mediated regulatory pathway to modulate T cell signaling during an immune response. On the other hand, in Foxp3$^+$ Tregs, CTLA-4 recruits the kinase PKCη to potentiate its suppressive functions *in vitro* and *in vivo* (126, 127). The CTLA-4-PKCη complex promotes the activation of the canonical NF-κB pathway in Tregs, representing a unique positive signaling event (126). It remains, however, to be determined whether and how the CTLA-4-PKCη axis regulates the activity of the T_{FR} subset. Although two other members of the novel PKC family, PKCδ and PKCε, have been shown to promote TRAF2 phosphorylation, IKK, and NF-κB activation in response to TNFα (128), it is unknown whether TRAFs are involved in the CTLA4-PKCη signal transduction pathway.

PD-1 Signaling

GC T_{FH} cells express high levels of PD-1, consistent with this immunomodulatory protein being upregulated following chronic TCR stimulation, such as in the case of persistent interaction between T and B cells, which occurs during T_{FH} differentiation. The PD-1 ligands, PD-L1, and PD-L2, are also highly expressed by GC B cells (129). PD-1 inhibits T cell activation by suppressing CD28 costimulatory signaling (130). In the absence of PD-1, early T_{FH} differentiation is not affected, but the GC T_{FH} cell population is enriched at later time points (129). Similar studies investigating the role of PD-1-PD-L1 interaction in T_{FH} responses consistently report an expansion of T_{FH} cells in *Pdl1*$^{-/-}$ mice and PD-1-deficient (*Pdcd1*$^{-/-}$) mice, respectively, following protein immunization and viral infection (131–133). These findings reveal that PD-1 signaling can limit the proliferation of T_{FH} cells (134).

Surprisingly, the absence of PD-1 signals leads to a reduction of B cell responses in some studies, despite an expansion of the T_{FH} cell population (129, 131, 133). In one study, the increased T_{FH} cell numbers observed in *Pdcd1*$^{-/-}$ mice is associated with a reduced synthesis of *Il4* and *Il21* mRNA by these cells (129), potentially explaining the reduced GC B cell responses. The discrepancy between the increased T_{FH} cell numbers and the reduced B cell responses could also result, in part, from the contributions of PD-1 to the T_{FR} cell population. In one study, *Pdcd1*$^{-/-}$ mice have elevated numbers of T_{FR} cells that display enhanced suppressive activity following immunization with NP-OVA (135). The contribution of this suppressive population has not been assessed in other studies. It is possible that PD-1 affects the ratio between T_{FH} and T_{FR} cells differently in various models.

The ability of PD-1 to inhibit T cell activation depends on the recruitment of phosphatases SHP-1 and SHP-2 to the cytoplasmic domain of PD-1 (136, 137). More importantly, CD28 costimulatory signaling is distinctively sensitive to the PD-1-associated phosphatase activity (130). The recruitment of p85α and the phosphorylation of the CD28-associated kinases, Lck and PKCθ, are attenuated by the PD-1-SHP complex (130, 136). Interestingly, TRAF6 interacts with SHP-1, and this molecular complex restrains the phosphorylation of the p85α subunit of PI3K and the activation of the canonical NF-κB pathway (138), suggesting that TRAF6 might interfere with the co-inhibitory signaling of PD-1.

BTLA Signaling

BTLA, the ligand of the TNFRSF member HVEM, is highly expressed on T_{FH} cells. *Btla*$^{-/-}$ mice have elevated level of IgG in response to the T-cell dependent NP-KLH antigen (139). Moreover, *Btla*$^{-/-}$ mice produce autoantibodies spontaneously (140), indicating that BTLA acts as a negative regulator of the humoral response. Upon immunization, T_{FH} generation is not affected in *Btla*$^{-/-}$ mice, but the number of GC B cells is elevated (141). BTLA acts in a T cell-intrinsic fashion as *Btla*$^{-/-}$ CD4$^+$ T cells activated *in vitro* in presence of IL-6 increase the production of IL-21, and promote IgG2a and IgG2b Ab responses upon *in vivo* transfer.

Similar to PD-1, BTLA relies on dual tyrosine phosphorylation motifs in its cytoplasmic tail to recruit SHP-1 and SHP-2 in T cells (142). Because TRAF6 interacts with SHP-1 (138), it is conceivable that TRAF6 might affect BTLA signaling.

Cytokines and STAT Signaling

In addition to the TCR and costimulatory receptors, interactions of autocrine or paracrine cytokines with their cognate receptors provide essential signals that regulate the differentiation and function of T_{FH} cells. The signal transducer and activator of transcription (STAT) proteins are critical integrators of cytokine signals. Multiple STAT molecules can be activated simultaneously by one or more cytokines (143). The differentiation of T_{FH} cells is positively or negatively modulated by STAT3-dependent cytokines (IL-6, IL-21, IL-27) and STAT5-dependent cytokines (IL-2 and IL-7), respectively. Interestingly, several TRAF molecules are involved in these cytokine/STAT signaling cascades.

IL-6

IL-6 is a pleiotropic cytokine that plays a major role in inflammation. Several studies have independently demonstrated the importance of IL-6 in T_{FH} generation (78, 144, 145). Indeed, *Il6*$^{-/-}$ mice display reduced GC formation and humoral responses (146). An initial spike of IL-6 production is detected on days 1–3 in both acute and chronic LCMV infection models (145). At the T cell priming stage, conventional DC secrete large amounts of IL-6 upon activation. IL-6 can transiently induce the expression of the transcription factor Bcl6 and cytokine IL-21 (42, 78), creating a positive feedback loop for enforcing the T_{FH} cell fate. Hence, the early programming of T_{FH} cells is abated in the absence of IL-6 (144). However, during chronic infection with LCMV Clone 13, a second wave of IL-6 expression is observed 3 weeks post-infection. FDC are responsible for the production of IL-6 at this late phase of viral infection (145). Administration of an IL-6-neutralizing Ab or IL-6R-blocking Ab 20 days after infection reduces *Bcl6* expression, T_{FH} and GC B cells (145). Interference with IL-6 functions also impairs the host's ability to clear the virus, indicating a late, but critical, role of IL-6 in maintaining an intact humoral response. However, other studies demonstrate that the differentiation of T_{FH} cells

is not compromised in *Il6*$^{-/-}$ mice or upon IL-6 neutralization (147–149), indicating that other signals, including IL-21, may compensate for the absence of IL-6 (see below).

Interestingly, the impact of T cell-specific deletion of IL-6Rα is less profound than the systemic deletion of IL-6 (144, 149, 150). The proportion of CXCR5^{+}PD-1^{+} T_{FH} cells is moderately reduced in *Il6ra*$^{fl/fl}$ *Cd4*^{Cre} mice following antigen immunization. Antigen-specific T_{FH} cells generated *in vivo* in the absence of IL-6Rα show reduced expression of Bcl-6 and IL-21 (150). However, there is a significant reduction in the fraction of GC B cells and plasma cells, implying that IL-6Rα signaling is indispensable for T_{FH} cell functions. At the molecular level, the IL-6 receptor is composed of IL-6Rα and the glycoprotein 130 (gp130), a signal transducer common to IL-6 receptor family members. TRAF2 and TRAF5 constitutively associate with gp130 (35, 36). This interaction suppresses the recruitment of STAT3 to the IL-6R complex, because they compete for the same binding site on gp130 (35, 36). Therefore, TRAF2 and TRAF5 are negative regulators of the IL-6R signaling pathway that could potentially limit the induction and functions of T_{FH} cells (**Figure 1**).

IL-21

IL-21 is a member of the common γ-chain family of cytokines produced by activated T and B cells. Its cognate receptor, IL-21R, is also highly expressed on T_{FH} cells (151) and GC B cells (152). Interestingly, lack of IL-21 or IL-21R does not affect the initial differentiation and expansion of T_{FH} cells (153, 154). However, the contraction of CXCR5^{+} PD-1^{+} GC T_{FH} cells occurs at a faster rate in *Il21*$^{-/-}$ or *Il21r*$^{-/-}$ mice after the first week of antigen challenge (153, 154). Although T cells are found in the GCs, these T cells are not able to support GC reactions in *Il21*$^{-/-}$ or *Il21r*$^{-/-}$ mice, leading to diminished levels of GC B cells, plasma cells, and serum IgG. Taken together, these data suggest that the IL-21-IL-21R axis is required for the T_{FH} cell persistence and functions.

Despite supportive evidence, the T-cell intrinsic role of IL-21 in the generation of humoral responses is hotly contested. Studies using *Il21*$^{-/-}$ and *Il21r*$^{-/-}$ mice show that the *in vivo* generation of CXCR5^{+}PD-1^{+} T_{FH} cells in these mice is as robust as in wt mice following NP-KLH (152) or NP-CGG (147) immunization, or infection with LCMV (148) or Influenza (149). Several additional studies provide potential insights into this discrepancy: First, while the loss of either IL-6 or IL-21 alone has only a marginal effect on T_{FH} development and GC formation in response to acute viral infection, the simultaneous loss of both cytokines in *Il6*$^{-/-}$*Il21*$^{-/-}$ mice (149) or the neutralization of IL-6 in *Il21*$^{-/-}$ mice (148), significantly blunts the antiviral Ab responses. These results indicate that IL-6 and IL-21 can act redundantly or complementarily to promote T_{FH} development. This is mechanistically conceivable because IL-6 and IL-21 signal predominantly through the same intracellular signal transducer, STAT3 (see below). Second, even in the presence of an intact T_{FH} cell population, *Il21*$^{-/-}$ and *Il21r*$^{-/-}$ mice are severely defective in mounting Ab responses. Mixed bone marrow chimera experiments revealed that IL-21 acts directly on B cells (152). In the absence of IL-21, the proliferation of GC B cells is significantly curtailed at the later stage of viral infection. However, as mentioned earlier, T_{FH} and B cells in GCs are mutually dependent on each other. The absence of either IL-21 or IL-21R on either T or B cells could lead to similar defects. Therefore, the impaired T cell-dependent humoral responses in intact *Il21*$^{-/-}$ and *Il21r*$^{-/-}$ mice do not reveal whether IL-21 acts in an autocrine fashion or, alternatively, whether T and/or B cells respond to IL-21 in a paracrine fashion. Transfer of wt or *Il21r*$^{-/-}$ T cells and B cells into irradiated recipient mice shows that the presence of IL-21R on both T and B cells is required for the optimal production of high-affinity Abs in response to LCMV infection (154). Nonetheless, owing to the essential and yet complicated roles of IL-21 in T-dependent humoral responses, a "cleaner" experimental setup, based on the inducible Cre/lox system, would be required to rigorously dissect the spatiotemporal functions of IL-21 and/or IL-21R in T and B cells.

TRAF5 acts a negative regulator of IL-21 production. *Traf5*$^{-/-}$ CD4^{+} T cells secrete significantly elevated amount of IL-21 upon CD3 plus CD28 costimulation in the presence of IL-6 and TGF-β (35). This effect is dependent on the presence of IL-6 as the binding of TRAF5 to the IL-6R complex restricts the activation of STAT3 (**Figure 1**).

IL-27

IL-27 is a member of the IL-6/IL-12 family of cytokines, which binds to a heterodimeric receptor consisting of IL-27Rα and gp130 subunits. *Il27ra*$^{-/-}$ mice display defective development of CXCR5^{+}PD-1^{+} GC T_{FH} cells (155). Stimulation with recombinant IL-27 *in vitro* enhances ICOS expression and IL-21 production from naïve CD4^{+} T cells (155, 156). Additionally, IL-27 is required to promote the maturation of GC B cells (157). *In vivo*, IL-27 promotes T-dependent Ab responses through a combination of T- and B cell-intrinsic mechanisms (157). Because IL-6 and IL-27 share the gp130 subunit, it is possible that TRAF2 and TRAF5 could similarly modulate the signaling events downstream of IL-27R, and hence, alter the differentiation and functions of T_{FH} cells.

Type I IFN

The dependence on STAT1 for the early stage of T_{FH} differentiation (144) implies a role for type I IFNs (IFNα/β) in this process, because STAT1 is the key transcription regulator downstream of the type I IFN signaling pathway. IFNα/β are ubiquitous cytokines produced by innate immune cells during the early phase of viral infection. An early report demonstrates that exogenous administration of IFNα/β strongly promotes the production of IgG in a dose-dependent manner following antigen immunization (158). Conversely, in the absence of the IFNα/β receptor (IFNAR) subunit IFNAR1, the differentiation of T_{FH} cells, migration of T_{FH} cells into the GC, and B cell responses are impaired following immunization (159–161). Mechanistically, IFNα/β signaling in DC induces the production of IL-6, which in turn promotes T_{FH} differentiation *in vivo* (159). *In vitro* treatment of CD4 T cells with IFNα/β induces the expression of Bcl6, CXCR5 and PD-1, but not the production of IL-21, suggesting that T cell-intrinsic IFNα/β signaling can positively contribute to the T_{FH} differentiation. In agreement, in mixed

bone marrow chimera experiments, the T_{FH} differentiation of *Ifnar*$^{-/-}$ T cells is compromised, compared to wt T cells in the same recipients, demonstrating a T cell-intrinsic role of IFNα/β in the T_{FH} differentiation following immunization (160, 161). Paradoxically, in the context of experimental *Plasmodium* infection, the differentiation of T_{FH} cells, GC B cells, and Ab responses are significantly enhanced in *Ifnar1*$^{-/-}$ mice or upon anti-IFNAR1 Ab neutralization (162, 163), implying a negative role of IFNα/β signaling in parasitic infections. The contrasting roles of IFNα/β in the differentiation and functions of T_{FH} cells might reflect the differential requirement of IFNα/β and its signaling in viral *vs.* parasitic infections.

Upon stimulation with IFNβ, TRAF2 coimmunoprecipitates with the IFNAR1 subunit of the IFN receptor complex (164). Analysis of *Traf2*$^{-/-}$ mouse embryonic fibroblasts (MEF) shows that the formation of the p52-p65 complex in the non-canonical NF-κB signaling pathway is absent upon stimulation with IFNβ. However, the IFN-induced activation of the canonical NF-κB pathway, and the phosphorylation of STAT1, STAT2, and STAT3 are indistinguishable between WT and *Traf2*$^{-/-}$ MEFs. Therefore, in lieu of TRAF2, other TRAF molecules might regulate these latter signaling cascades in response to IFNα stimulation (see below).

STAT1 and STAT3

As described above, IL-6,−21, and−27 promote the differentiation, persistence and functions of T_{FH} cells in a T cell-intrinsic manner. A common feature among these T_{FH}-inducing cytokines is their signaling via the transcription factors STAT1 and STAT3. Not surprisingly, STAT1 or STAT3 deficiency affects the generation of T-dependent B cell memory and high affinity Ab-secreting cells. The lack of STAT3 leads to profound defects in the acquisition of B cell help functions. T-cell specific deletion of STAT3 significantly impairs the number of CXCR5$^+$ T_{FH} cells, GC B cells, and IgG levels in mice following challenge with antigen plus adjuvant or LCMV infection (78, 165, 166). In humans, individuals with missense mutations or short deletions of *STAT3* suffer from Hyper-IgE syndrome, a primary immunodeficiency characterized by heightened susceptibility to *Staphylococcus aureus* and *Candida albicans*. T cells from these STAT3 mutated individuals fail to upregulate IL-21 and provide help to B cells upon *in vitro* culture and a reduction of circulating CXCR5$^{\pm}$ CD4$^{\pm}$ T cells is observed in patients suffering from hyper IgE syndrome resulting from STAT3 mutations (167).

In addition, type I interferon also mediates its signaling through STAT1. Knockdown of STAT1 in mouse T cells results in defective generation of early CXCR5$^+$Bcl6$^+$ T_{FH} cells 2 days after infection. This defect is more pronounced when both STAT1 and STAT3 are absent, suggesting a redundant role of these transcriptional regulators as downstream mediators of IL-6, −21, −27, and type I interferon (144).

The crosstalk between TRAF proteins and STATs has only been studied in recent years. As aforementioned, gp130, which mediates signaling downstream of IL-6 and IL-21, interacts with TRAF2 and TRAF5. *Traf5*$^{-/-}$ CD4$^+$ T cells exhibit an elevated phosphorylation of JAK1 kinase upon stimulation with IL-6, suggesting that the recruitment of these TRAFs to the IL-6R and IL-21R complexes limits the phosphorylation of JAK1 in T cells (168).

In addition, TRAF6 associates with, and mediates the ubiquitination of STAT3 in fibroblasts (169). This interaction represses the transcriptional activity of STAT3 and downregulates the expression of STAT3-regulated genes upon stimulation with IFNα (169), suggesting that TRAF6 acts as a negative signaling mediator of STAT3 downstream of Type I interferon signaling. TRAF3 inhibits STAT3 activation downstream of IL-6R signaling in B cells (170). Moreover, TRAF3 is required for the association of the phosphatase PTPN22 with JAK1, which in turn inhibits STAT3 phosphorylation (170). TRAF3 and−6 might negatively regulate STAT3 activity in T cells via similar mechanisms.

IL-12

IL-12 is well known for its key role in inducing Th1 differentiation in both mouse and humans. However, in rodents, stimulation of naïve mouse CD4 T cells *in vitro* in the presence of IL-12 induces the expression of both the T_{FH} transcription factor Bcl6 and the Th1 transcription factor T-bet (171). IL-21$^{\pm}$, IFNγ$^{\pm}$, and IL-21$^{\pm}$ IFNγ$^{\pm}$ cells are simultaneously present in the *in vitro* culture. However, the percentage of IL-21 producing cells declines rapidly over time in favor of IFNγ$^{\pm}$ cells, coinciding with reduction of Bcl6 expression in favor of T-bet (171).

IL-12 has been shown to mediate the differentiation of human T_{FH} cells. Activated DC can induce naïve human CD4 T cells to produce IL-21 in an IL-12 dependent manner (172), and conversely, naïve human T cells primed with IL-12 can induce B cells to produce Ig *in vitro* (172). *In vitro* stimulation of naïve human CD4 T cells in the presence of IL-12 also induces the expression of CXCR5, Bcl6, and ICOS (172–174). The role of IL-12 signals for T_{FH} generation is also important for *in vivo* responses as individuals deficient in the IL12-receptor subunit IL-12Rβ1 display less circulating CXCR5$^{\pm}$ CD4 T cells, altered GC responses, and reduced numbers of memory B cells (174). Induction of IL-21 and Bcl6 by IL-12 depends on the transcription factor STAT4 (171, 172). GC T_{FH} in human tonsils show high levels of activated STAT4, suggesting that they could be actively receiving IL-12 signals *in vivo* (174). Concomitantly, the generation of T_{FH} and GC B cells is impaired in *Stat4*$^{-/-}$ mice 4 days following immunization, but not at later stages (171). To date, no TRAF activity has been identified in the IL-12R or STAT4 signaling.

IL-2

IL-2 acts primarily on T cells via the IL-2R, consisting of the α, β, and the common γ subunits. The high-affinity IL-2Rα, CD25, is differentially expressed in T_{FH} and non-T_{FH} cells. CD25 is downregulated in Bcl6$^+$CXCR5$^+$ T_{FH} cells, whereas CD25$^+$ T cells express the transcription factor Blimp1, which is antagonistic to Bcl6 (75). These findings support the notion that T_{FH} cells do not require IL-2 signaling for their differentiation and functions. In fact, the expression of Bcl6 is elevated under limiting IL-2 conditions. The accumulated Bcl6 proteins in turn bind to DNA and repress its direct target *Prdm1* (which encodes the transcriptional repressor Blimp-1) (175). Reduction of IL-2 signaling results in increased T_{FH} cell differentiation during the

early DC priming phase in *Il2ra*$^{+/--}$ mice (176) or upon anti-IL-2 Ab-mediated neutralization. Correspondingly, treatment with recombinant IL-2 impairs T_{FH} differentiation and suppresses GC B cell responses (177).

TRAF3 and 6 are both negative regulators of IL-2 signaling (**Figure 1**). TRAF3 is recruited to the IL-2 receptor complex and promotes the recruitment of the phosphatase TCPTP. *Traf3*$^{fl/fl}$ *Cd4*^{Cre} T cells show enhanced phosphorylation of Jak1, Jak3, and STAT5 upon IL-2 stimulation (33). Additionally, TRAF6 coimmunoprecipitates with IL-2Rβ in 293T cells coexpressing those two proteins, and preactivated *Traf6*$^{-/-}$ CD4^{+} T cells display enhanced phosphorylation and activation of Jak1 and Erk in response to IL-2 (34). TRAF3 and TRAF6 could therefore contribute to the control of T_{FH} differentiation by modulating IL-2 signals.

IL-7

IL-7, a member of the IL-2 cytokine family, is important for T and B cell survival, proliferation and development. Similar to CD25, IL-7Rα expression is strongly downregulated during T_{FH} differentiation, as early as 3 days following LCMV infection (178). IL-7Rα is then progressively reexpressed and GC T_{FH} cells express high IL-7Rα levels (178), consistent with a role for IL-7 in the long-term survival of memory T cells. The early downregulation of IL-7Rα suggests a negative role for IL-7 signals in the differentiation of T_{FH} cells. Indeed, Bcl6 represses IL-7R (179) and, reciprocally, T_{FH} exposure to IL-7 represses the expression of the key T_{FH} genes, *Bcl6* and *Cxcr5* (180). Consistent with these findings, administration of anti-IL7Rα Ab enhances T_{FH} development and GC reactions, whereas transgenic expression of IL-7Rα by CD4^{+} T cells reduces their T_{FH} differentiation (179). Intriguingly, one study showed the opposite, i.e., positive role of IL-7 on T_{FH} cells. The administration of exogenous Fc-fused IL-7 significantly increases both CD4^{+} and CD8^{+} T cell responses induced by a DNA vaccine (181). The enhancement of CD4^{+} T cell responses was accompanied by the expansion of T_{FH} cells, GC B cells, and GC reactions (181). The enhanced development of T_{FH} cells in this experimental model is not dramatically affected by IL-6 and IL-21 neutralization, suggesting an independent role of IL-7 in T_{FH} differentiation. No TRAF activity has been associated with IL-7R.

STAT5

IL-2 and IL-7, which negatively regulate T_{FH} generation, signal through STAT5. STAT5 acts as a transcriptional repressor for the expression of Bcl6 (182). Inhibition of Bcl6 expression correlates with the enhanced binding of STAT5 to the *Bcl6* promoter region in Th1 cells stimulated with IL-2 *in vitro* (175). Similarly, in IL-7 stimulated cells, there is an increase of STAT5 binding to the *Bcl6* gene promoter, leading to a reduction in Bcl6 expression (180). Accordingly, T cell-specific deletion of STAT5 increases T_{FH} cell development, GC B cell numbers, and Ab levels following immunization (183). On the other hand, the presence of a constitutively active STAT5 mutant in antigen-specific T cells blocks the differentiation of T_{FH} cells following LCMV infection (176).

TRAF3 and 6 are recruited to the IL-2R and negatively regulate its signaling activity (33, 34). After IL-2 stimulation, activation of STAT5 is enhanced in *Traf3*$^{fl/fl}$ *Cd4*^{Cre} T cells, suggesting that TRAF3 acts as a negative regulator of STAT5 (33).

TGF-β

In the human immune system, TGF-β alone is insufficient to induce expression of the T_{FH} cell phenotype (184). *In vitro* TGF-β stimulation in combination with IL-12 or IL-23 optimally promotes the expression of T_{FH}-associated molecules Bcl6, CXCR5, ICOS and IL-21, and antagonizes Blimp1 expression, in naïve human CD4^{+} T cells (184). Elevated phosphorylation of Smad2, a downstream effector of TGF-β signaling, is found in T cells localized close to the GC in tonsils, suggesting that TGF-β signaling is likely to participate in human T_{FH} differentiation (184). However, the requirement of TGF-β for T_{FH} differentiation appears to be species-specific as *in vitro* stimulation of murine T cells with TGF-β inhibits the induction of Bcl6, IL-21 and ICOS (42, 184, 185). In contrast, experiments using adoptive transfer of antigen-specific *Tgfbr2*$^{-/-}$ T cells revealed that T cell-intrinsic TGF-β signaling is required for the differentiation of CXCR5^{+}PD-1^{+} T_{FH} cells and the generation of GC B cell and Ab responses *in vivo* following LCMV infection (186). TGF-β suppresses the expression of CD25. The absence of IL-2 signaling, in turn, is beneficial for the early induction of T_{FH} cells.

In vitro stimulation of *Traf6*$^{fl/fl}$ *Cd4*^{cre} murine T cells in the presence of TGF-β shows enhanced and sustained Smad2 and Smad3 phosphorylation. This sustained TGF-β signaling results in lower *Il2* mRNA and protein levels (187). Therefore, TRAF6 acts as a negative regulator of Smad-mediated TGF-β signaling in T cells, and thus, may influence the differentiation and functions of T_{FH} cells (**Figure 1**).

TRAF-MEDIATED CANONICAL AND NON-CANONICAL NF-κB SIGNALING IN T_{FH} CELL DIFFERENTIATION

TRAF family members are critical signal transducers that relay signals between stimulus-sensing surface receptors and transcription regulators, ultimately leading to a change in gene expression. Many studies using different cell types and stimuli reveal that TRAF family members are involved in the activation of the transcription factors of the NF-κB family. NF-κB can be activated via two major pathways: the canonical and non-canonical signaling pathways [reviewed in (188, 189)]. Briefly, the canonical NF-κB pathway is controlled by TAK1 kinase activation that leads to the ubiquitination and proteasomal degradation of IκB family members, resulting in the release and nuclear translocation of the NF-κB1/p50–RelA/p65 and NF-κB1/p50–c-Rel dimers. On the other hand, activation of the non-canonical NF-κB pathway depends on the NF-κB-inducing kinase NIK. NIK can phosphorylate and activate IKKα, which in turn promotes p100 processing to generate NF-κB2/p52 and allow its nuclear translocation together with RelB. In the absence of activating signals, constitutive ubiquitination and degradation

of NIK ensures the repression of the non-canonical NF-κB pathway. Herein, we will focus on the role of TRAF proteins in the canonical and non-canonical NF-κB signaling pathways (summarized in **Figure 2**) and discuss how TRAF-mediated NF-κB signaling can contribute to T_{FH} differentiation and T-dependent humoral responses. Readers are advised to refer to other chapters in this volume to gain a broader perspective of TRAF-mediated canonical and non-canonical NF-κB pathways in the immune system.

TRAF-Mediated Canonical NF-κB Signaling in T_{FH} Cells

Several studies demonstrate the T cell-intrinsic requirement for canonical NF-κB signaling in T_{FH} differentiation. First, genetic ablation of the transcriptional subunit, NF-κB1/p50, in OT-II $CD4^+$ T cells selectively impairs the upregulation of CXCR5 following immunization, leading to a severe defect in the generation of $CXCR5^+PD1^+$ GC T_{FH} cells and GC B cell responses (190). Second, because the NF-κB1/p50 subunit dimerizes with RelA/p65 or c-Rel, $Rel^{-/-}$ mice (deficient for c-Rel) display defects in T cell-dependent humoral immunity (191). Subsequently, it was demonstrated that the mRNA and protein levels of IL-21 are reduced in $Rel^{-/-}$ T cells, indicating that c-Rel positively regulates the expression of IL-21 in T cells (192). Moreover, the expression of c-Rel is regulated by a microRNA, miR-155 (193). T cell-specific ablation of miR-155 promotes the degradation of c-Rel, which impedes the upregulation of CD40L in $mir155^{-/-}$ T cells, and severely impairs T_{FH} differentiation and B cell Ab responses *in vivo* (193). This defect can be restored by over-expression of c-Rel in $mir155^{-/-}$ T cells, pointing to a T cell-intrinsic role for c-Rel in the control of T_{FH} differentiation. Interestingly, the other NF-κB1/p50 partner, RelA/p65, is dispensable for T_{FH} differentiation (194).

TRAF1, 2, 5, and 6 can positively regulate the activation of the canonical NF-κB pathway (**Figure 2**). $Traf1^{-/-}$ T cells show reduced IκB degradation upon stimulation with anti-4-1BB Ab (23). TRAF2 knockdown impairs the canonical NF-κB activation induced by anti-CD3/CD28 stimulation in Jurkat T cells (195), by anti-OX40 stimulation in T cell hybridomas (101), or by anti-4-1BB in HEK293T fibroblasts (116). Preactivated $Traf5^{-/-}$ T cells also show reduced canonical NF-κB activation upon treatment with anti-GITR Ab (104). In addition, knockdown of TRAF6 hinders the activation of the canonical NF-κB pathway in Jurkat T cells stimulated with anti-CD3/CD28 Ab (195), and the degradation of IκB is delayed in $Traf6^{-/-}$ T cells (27). Hence, TRAF1, 2, 5, and 6 can contribute to T_{FH} differentiation by positively regulating the canonical NF-κB pathway.

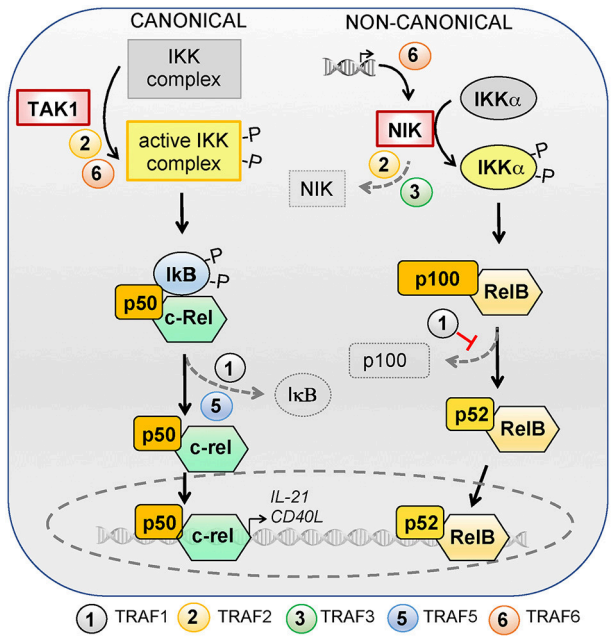


FIGURE 2 | Role of TRAFs in the canonical and non-canonical NF-κB signaling pathways. NF-κB can be activated via the canonical or non-canonical signaling pathways. The canonical pathway is controlled by TAK1 kinase activation, which activates the IKK complex and leads to ubiquitylation and proteasomal degradation of IκB family members, resulting in the release and nuclear translocation of NF-κB1/p50–RelA/p65 and NF-κB1/p50–c-Rel dimers. RelA/p65 is dispensable for T_{FH} differentiation but c-Rel regulates the expression of IL-21 and CD40L and is required for T_{FH} differentiation. TRAF2 and−6 favor IKK complex activation by TAK1 and TRAF1 and−5 are required for optimal IκB degradation. The activation of the non-canonical NF-κB pathway depends on the NF-κB-inducing kinase NIK. NIK can phosphorylate and activate IKKα, which in turn promotes p100 processing to generate NF-κB2/p52 and allows nuclear translocation of NF-κB2/p52 and RelB. In the absence of activating signals, constitutive ubiquitylation and degradation of NIK ensures repression of the non-canonical NF-κB pathway. NIK deficiency in T cells does not impact T_{FH} differentiation. TRAFs regulate the non-canonical NF-κB pathway by modulating NIK expression levels: TRAF6 is involved in transcriptional regulation of *Nik* whereas TRAF2 and−3 contribute to its degradation. TRAF1 restrains the non-canonical NF-κB pathway activation by inhibiting p100 processing.

TRAF-Mediated Non-canonical NF-κB Signaling in T_{FH} Cells

The role of non-canonical NF-κB signaling in T cell-dependent Ab responses has been extensively studied in $Nik^{-/-}$ mice, which display an impaired development of $CXCR5^+$ $PD\text{-}1^+$ GC T_{FH} cells (196). However, T_{FH} differentiation was not affected when NIK deficiency was restricted to T cells using an adoptive transfer model, implying that the role of NIK in T_{FH} differentiation is not T cell-intrinsic (196). Instead, the expression of NIK in B cells is required for the optimal expression of ICOSL, and, thus, the promotion of T_{FH} differentiation. These findings suggest that the non-canonical NF-κB signaling pathway in B cells, but not in T cells, is required for humoral responses. Although the deletion of either RelB or NF-κB2/p52 does not affect B cell responses, genetic ablation of both RelB and NF-κB2/p52 in GC B cells dramatically impedes GC reactions (197). Therefore, the non-canonical NF-κB signaling is more important in B cells, which in turn could affect the differentiation and maintenance of GC T_{FH} cells.

TRAF2 and TRAF3 play a negative role in the control of the non-canonical NF-κB pathway (**Figure 2**). The absence of either TRAF2 or TRAF3 results in the constitutive activation of this pathway in T cells (25, 198). TRAF2 and TRAF3 form a complex

with cIAP1 and cIAP2, which are E3 ubiquitin ligases responsible for NIK ubiquitination and degradation. In the absence of TRAF2 or TRAF3, the cIAP-TRAF complex is disrupted, allowing an increase of NIK protein level and aberrant activation of the non-canonical NF-κB pathway (199–201). In a similar fashion, TRAF1 has also been found to restrain the non-canonical NF-κB pathway in response to stimulation with anti-CD3 Ab (23). On the other hand, TRAF6 acts as a positive regulator by inducting the expression of NIK, resulting in activation of the non-canonical NF-κB pathway in the presence of OX40 ligation (202).

As T cell-specific *Nik* deficiency did not affect T_{FH} differentiation (196), modulation of the non-canonical NF-κB pathway by TRAF2, 3, and 6 is not likely to directly impact on T_{FH} differentiation. However, because the non-canonical NF-κB pathway is important for B cell maturation, which in turn is required to maintain T_{FH} cells, TRAF1, 2, 3, and 6 might contribute to the overall T-dependent and T-independent Ab responses.

T CELL- AND B CELL-INTRINSIC ROLES OF TRAFS IN HUMORAL RESPONSES

In this part, we will review the contribution of individual TRAF proteins to the development of T cell-dependent humoral responses and discuss whether each TRAF member influences humoral responses through T cell-intrinsic or B cell-intrinsic pathways. In humans, single nucleotide polymorphisms of several members of the TRAF family are associated with the development of SLE and RA (16), two autoimmune disorders with excessive T_{FH} responses and GC reactions (7). However, the mechanisms by which TRAFs contribute to disease susceptibility or development are unknown. In this part we will infer the potential contributions of each TRAF family member to the differentiation of T_{FH} cells at the mechanistic level, in light of the known role of TRAFs in the signaling pathways controlling T_{FH} differentiation reviewed in the preceding sections.

TRAF1

Traf1$^{-/-}$ mice display normal T cell and B cell lymphocyte development (22). Increased T cell proliferation of *Traf1*$^{-/-}$ T cells is observed in response to anti-CD3 Ab (22, 23) or antigen stimulation (203). *Traf1*$^{-/-}$ CD4^{+} T cells express higher levels of the Th2 cytokines IL-4, IL-5, and IL-13 upon *in vitro* stimulation. Accordingly, transfer of OVA-stimulated *Traf1*$^{-/-}$ CD4^{+} T cells into naïve wt recipients trigger an enhanced asthmatic response following aerosol inhalation with ovalbumin as compared to the transfer of OVA-stimulated wt CD4^{+} T cells (203). TRAF1 has been reported to associate with CD40 (204). However, the proliferation of *Traf1*$^{-/-}$ B cells is not affected upon *in vitro* stimulation with anti-IgM or anti-CD40 Abs, or *in vivo* challenge with T cell-independent antigens (22). *Traf1*$^{-/-}$ mice display normal IgG1, IgG2a, and IgE anti-ovalbumin responses, suggestive of an intact B cell isotype switching and T cell help (22). These data suggest that TRAF1 is dispensable for the development of T cell-dependent humoral responses.

TRAF2

Traf2$^{-/-}$ mice are embryonic lethal as a result of excessive TNFα production. Simultaneous deletion of the TNFα-TNFR1 axis results in partial rescue of the *Traf2*$^{-/-}$*Tnf*$^{-/-}$ or *Traf2*$^{-/-}$*Tnfr1*$^{-/-}$ animals (205). *Traf2*$^{-/-}$*Tnf*$^{-/-}$ mice display normal IgM levels in response to VSV infection, but the IgG responses are abrogated. B cells from *Traf2*$^{-/-}$*Tnfr1*$^{-/-}$ mice fail to proliferate and activate NF-κB in response to *in vitro* anti-CD40 stimulation (205). These data are consistent with the fact that TRAF2 interacts with CD40 (204), and that this interaction is essential for isotype switching (206). However, the fact that these models lack TRAF2 and TNFα-TNFR1 signaling confounds the interpretation regarding the actual role of TRAF2 in the development of T cell-dependent Ab responses.

To better define the roles of TRAF2 in B cells, *Traf2*$^{fl/fl}$ *Mx1*^{Cre} and *Traf2*$^{fl/fl}$ *Cd19*^{Cre} mice were generated, resulting in B cell-specific TRAF2 deletion (198, 207). Unexpectedly, B cell specific TRAF2 deficiency resulted in increased B cell numbers in the secondary lymphoid organs. *Traf2*$^{-/-}$ B cells display enhanced survival, increased cell size and constitutive activation of the non-canonical NF-κB pathway. Nevertheless, the CD40-mediated activation of the canonical NF-κB pathway and B cell proliferation are impaired in the absence of TRAF2, implying a positive regulatory role of TRAF2 in CD40 signaling. Similar to the *Traf2*$^{fl/fl}$ *Mx1*^{Cre} and *Traf2*$^{fl/fl}$ *Cd19*^{Cre} mice, mice expressing a TRAF2 dominant negative transgene (TRAF2-DN) devoid of the N-terminal RING and zinc finger domains display splenomegaly and lymphadenopathy (208). Surprisingly, the canonical NF-κB pathway is unperturbed in B cells isolated from TRAF2-DN mice in response to CD40L or TNFα stimulation. Instead, the activation of the JNK pathway is dependent on TRAF2 following CD40L or TNFα stimulation (208). The functional discrepancy between *Traf2*$^{-/-}$ and TRAF2-DN-expressing B cells could be explained by the conservation of TRAF2-mediated protein-protein interactions in TRAF2-DN-expressing B cells.

The contribution of TRAF2 to T cell functions and its implication in the regulation of Ab responses is under-explored. T cells from TRAF2-DN (208, 209) and *Traf2*$^{fl/fl}$ *Lck*^{Cre} mice (24) show defective *in vitro* T cell proliferation in response to anti-TCR stimulation or allogenic APCs. *Traf2*$^{-/-}$ T cells show a propensity to skew into the Th2 lineage upon *in vitro* polarization and their Th17 differentiation is impaired (24). This is associated with reduced JNK and canonical NF-κB pathway activation following stimulation with TNFα. To date, a T cell-intrinsic role for TRAF2 in T_{FH} differentiation has not been reported. However, because TRAF2 is involved in recruitment of PI3K to OX40 (210) and activation of the classical NF-κB pathway downstream of OX40 (101) and GITR (103), two key molecules promoting T_{FH} differentiation, TRAF2 could potentially play a positive regulatory role in the differentiation of T_{FH} cells. On the other hand, TRAF2 has been shown to restrain IL-6 signaling, suggestive of a negative role in T_{FH} differentiation [**Figures 1**, **2**; (36)]. Therefore, the overall role of TRAF2 in T-dependent Ab responses awaits further exploration.

TRAF3

Traf3$^{-/-}$ mice die within 10 days of birth. To assess the contribution of TRAF3 to T cell-dependent Ab responses, fetal liver cells were used to reconstitute the hematopoietic system of sublethally irradiated recipients (211). *Traf3*$^{-/-}$ fetal liver cells could reconstitute the T cell, B cell, granulocytic, and erythroid lineages, and reconstituted recipients survived longer than 6 months. Using this chimeric system, recipient mice reconstituted with *Traf3*$^{-/-}$ cells failed to produce antigen-specific IgG in response to T cell-dependent antigens. However, the proliferation of *Traf3*$^{-/-}$ B cells in response to stimuli such as anti-IgM Ab and CD40L was normal. Because the T cell recall response after *in vivo* immunization is dramatically reduced in the absence of TRAF3, it was concluded that TRAF3 is required for T cell help (211).

To circumvent the early postnatal lethality of *Traf3*$^{-/-}$ mice, *Traf3*$^{fl/fl}$ *Cd19*^{Cre} mice with B cell-specific TRAF3 deletion were generated (198, 212). These mice exhibited splenomegaly and lymphoadenopathy, with a concomitant elevation of follicular B cells, spontaneous GC formation, hyperimmunoglobulinemia, T cell-independent Ab responses, and exacerbated autoimmune manifestations (212). At the molecular level, *Traf3*$^{-/-}$ B cells exhibit constitutive activation of the non-canonical NF-κB pathway, supporting the survival of B cells. In spite of all these B cell defects, the development of GC B cells in *Traf3*$^{fl/fl}$ *Cd19*^{Cre} mice following immunization remained intact (213). Paradoxically, B cell-specific overexpression of TRAF3 in a transgenic mouse strain induced excessive systemic inflammation, autoimmunity, and hyperimmunoglobulinemia at an old age (214). These transgenic mice are hyperresponsive to T-dependent and T-independent antigen challenges, despite the fact that the over-expression of TRAF3 does not alter the CD40-mediated NF-κB and MAP kinase pathways in B cells. Altogether, these results indicate an important, yet complicated, role of TRAF3 in regulating B cell homeostasis.

To understand the roles of TRAF3 in T cell biology, *Traf3*$^{fl/fl}$ *Cd4*^{Cre} mice were generated. These *Traf3*$^{fl/fl}$ *CD4*^{Cre} mice are born at the expected Mendelian ratio, and they survive and breed normally (25). Following immunization with a T-dependent antigen, the antigen-specific IgG1 Abs are nearly absent in these mice, indicative of a T cell-intrinsic role of TRAF3 (25). In a *Listeria monocytogenes* infection model, *Traf3*$^{fl/fl}$ *Cd4*^{Cre} mice are much more sensitive to bacterial challenge, displaying a higher bacterial load and lower numbers of IFNγ-producing T cells in the liver, demonstrating that *Traf3*$^{-/-}$ T cells are compromised (25). Mechanistically, TRAF3 is recruited to the TCR-CD28 complex and it participates in the activation of proximal TCR signaling (**Figure 1**). In its absence, proliferation and cytokine production are impaired in stimulated $CD4^+$ and $CD8^+$ T cells.

Interestingly, Treg-selective TRAF3 ablation in *Traf3*$^{fl/fl}$ *Foxp3*^{Cre} mice leads to a marked reduction in T_{FR} cell induction following immunization, resulting in increased expression of *Bcl6*, *Cxcr5* and the cytokines genes *Il-4*, *Il-10*, *Il-17,* and *Ifng* by T_{FH} cells, coupled with sustained GC reactions and production of high-affinity IgG Abs (215). The expression of ICOS is reduced in *Traf3*$^{-/-}$ T_{FR} cells because of the inactivation of TRAF3-dependent ERK and AP-1 signaling pathways (215). However, whether TRAF3 influences T_{FH} differentiation in a cell-intrinsic way remains to be determined. Interestingly, alternative splicing of TRAF3 can generates a TRAF3 isoform that mediates activation of the non-canonical NF-κB pathway and the production of CXCL13 by T cells (216). Although the relevance of TRAF3-controlled CXCL13 production *in vivo* in T cell-dependent Ab responses remains to be elucidated, these data suggest a positive role for TRAF3 in GC formation by favoring T_{FH} cells migration into the GC. TRAF3 might also positively influence T_{FH} differentiation by enhancing TCR- and CD28-induced signaling (25, 26) and restraining IL-2R signals [**Figure 1**; (33)]. Conversely, TRAF3 can inhibit IL-6R signaling in B cells (170) and negatively regulate OX40-induced NF-κB signaling in HEK293T cells (217). It is currently unknown whether a similar TRAF3-mediated regulation of IL-6R and NF-κB signaling occurs in primary T cells.

TRAF4

Traf4$^{-/-}$ mice show normal T and B cell differentiation. The T cell-dependent IgG response to OVA immunization is unaffected in *Traf4*$^{-/-}$ mice (218). Although the T_{FH} cell population has not been investigated in this study, this finding suggests that TRAF4 is dispensable for the differentiation and functions of T_{FH} cells. No other studies to date have demonstrated a role for TRAF4 in primary T cell functions.

TRAF5

Traf5$^{-/-}$ mice show unaltered development of T and B cell lineages (219, 220). *Traf5*$^{-/-}$ mice produce similar titers of IgG1 Ab than wt controls following antigen immunization, but there is a slight reduction in Ab affinity maturation (219). *Traf5*$^{-/-}$ T cells produce increased amounts of IL-4 and IL-5 in response to OX40 stimulation *in vitro*, and develop a more severe Th2-driven allergic lung inflammation following antigen immunization and airway challenge (220). In this model, *Traf5*$^{-/-}$ mice produce enhanced levels of OVA-specific IgE. Altogether, these data suggest that the T cell-dependent class switching and production of Ab *in vivo* are not dramatically affected by TRAF5 deficiency.

Notably, *Traf5*$^{-/-}$ T cells display impaired GITR signaling, with decreased canonical NF-κB, Erk, and p38 activation, and exhibit reduced proliferation and IL-2 production upon stimulation in presence of anti-GITR Ab (104). TRAF5 constitutively associates with the gp130 subunit of the IL-6R and negatively regulates IL-6R signaling by suppressing the recruitment of STAT3 to the IL-6R complex (35, 36). The role of TRAF5 in the development and maintenance of T_{FH} responses *in vivo* remains to be investigated. Because GITR signaling (19), activation of the canonical NF-κB pathway (190, 191), and IL-6 signaling (78, 144, 145) are all important for T_{FH} development, one could predict that TRAF5 is also involved in modulation of T_{FH} differentiation by integrating these signals (**Figures 1**, **2**).

TRAF6

Traf6$^{-/-}$ mice die prematurely within 17–19 days after birth, displaying severe osteopetrosis, splenomegaly, thymic atrophy, and defects in lymph node organogenesis (221, 222). B cells

isolated from these mice fail to proliferate in response to anti-CD40 stimulation, indicating that TRAF6 is a mediator of CD40 signaling (222). These *ex vivo* data are consistent with an *in vitro* study showing that a B cell line expressing a CD40 mutant incapable of binding TRAF6 fails to secrete IL-6 and Ig following anti-CD40 stimulation (223). Similarly, in transgenic mice expressing a CD40 mutant incapable of TRAF6 recruitment, the generation of plasma cells, IgG production, and affinity maturation are severely compromised upon antigen challenge (224). B cells from *Traf6*$^{fl/fl}$ *Cd19*^{Cre} mice display defects in proliferation, IL-6 production and phosphorylation of p38 MAP kinase upon stimulation with anti-CD40 Ab (225). *In vivo* antigen challenge also reveals the requirement of TRAF6 in T-dependent production of IgG, generation of long-lived plasma cells, isotype switching, and affinity maturation (225).

In T cells, TRAF6 acts as a negative rheostat. *Traf6*$^{fl/fl}$ *Lck*^{Cre} mice develop a systemic inflammatory disease with increased production of Th2 cytokines, significant expansion of the B cell compartment and elevated serum Ab titers, including anti double-stranded DNA Abs (27). *Traf6*$^{-/-}$ T cells are hyperproliferative and display constitutively active PI3K-AKT signaling. These hyperreactive T cells are refractory to Treg-mediated suppression (27). Additionally, *Traf6*$^{-/-}$ T cells exhibit enhanced Th17 differentiation *in vivo* and *in vitro* in the presence of TGF-β (187). This effect was due to increased responsiveness of *Traf6*$^{-/-}$ T cells to TGF-β as TRAF6 impedes the production of IL-2 (187), which is a known inhibitor of Th17 differentiation (226). TRAF6 can bind to IL-2Rβ and inhibit Jak1 activation induced by IL-2 (34). Modulation of PI3K, TGF-β, and IL-2 signaling pathways by TRAF6 could directly affect T_{FH} differentiation (**Figure 1**).

SUMMARY

The differentiation of T_{FH} cells is a complex process controlled by the integration of multiple signals. Many studies support the conclusion that TRAF proteins are important modulators of T-dependent and T-independent humoral responses. TRAFs act through a variety of mechanisms: modulation of TCR signals and integration of costimulatory and cytokine signals. As detailed above, TRAF2, 3, 5, and 6 are the most relevant ones involved in many T_{FH}-inducing and T_{FH}-antagonistic signaling pathways. However, the exact mechanisms of how each of these TRAF family members contributes to T_{FH} differentiation remain elusive. Precise elucidation of the relevant mechanisms has been challenging for several reasons. First, TRAFs have dual functions as E3 ubiquitin ligases as well as molecular adaptors for protein-protein interactions; second, TRAFs are ubiquitously expressed in innate and adaptive immune cells as well as in non-immune cells; and finally, TRAFs are involved in a variety of signaling pathways that reinforce and/or neutralize each other. All these factors confound the interpretation of results derived from systemic deletion of TRAF proteins *in vivo*.

Future studies addressing TRAF-related mechanisms will be facilitated by the modern genome editing tools that simplify the generation of knock-in or cell type-specific knockout mice. For example, the E3 ligase activity of TRAF proteins could be specifically attenuated using the CRISPR-Cas system to differentiate the enzymatic vs. adaptor function of these proteins in various signaling pathways. Other genomic technologies such as single-cell RNA-Seq and CyTOF could be incorporated into various experiments to simultaneously profile the gene and protein expression alterations of different immune and non-immune cell populations. These approaches will provide a broader perspective of the role of TRAFs in different cell types during an immune response.

Studies on the role of TRAFs in T_{FH} differentiation and B cell responses are of therapeutic interest as modulation of T_{FH} differentiation has the potential to either reduce pathological Ab production in autoimmune and inflammatory diseases, or favor the development of long-lasting and high affinity humoral responses in the context of vaccination or treatment of infectious diseases.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

This is publication number #3228 from the La Jolla Institute for Allergy and Immunology. CP is a Cancer Research Institute Irvington Fellow supported by the Cancer Research Institute.

REFERENCES

1. Schroeder HW, Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immun.* (2010) 125:S41–52. doi: 10.1016/j.jaci.2009.09.046
2. Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med.* (2000) 192:1545–52. doi: 10.1084/jem.192.11.1545
3. Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med.* (2000) 192:1553–62. doi: 10.1084/jem.192.11.1553
4. Kim CH, Rott JS, Clark-Lewis I, Campbell DJ, Wu L, Butcher EC. Subspecialization of CXCR5^{+} T cells: B helper activity is focused in a germinal center-localized subset of CXCR5^{+} T cells. *J Exp Med.* (2001) 193:1373–81. doi: 10.1084/jem.193.12.1373
5. Kaji T, Ishige A, Hikida M, Taka J, Hijikata A, Kubo M, et al. Distinct cellular pathways select germline-encoded and somatically mutated antibodies into immunological memory. *J Exp Med.* (2012) 209:2079–97. doi: 10.1084/jem.20120127
6. Hollister K, Kusam S, Wu H, Clegg N, Mondal A, Sawant DV, et al. Insights into the role of Bcl6 in follicular Th cells using a new conditional mutant mouse model. *J Immunol.* (2013) 191:3705–11. doi: 10.4049/jimmunol.1300378
7. Ma CS, Deenick EK. Human T follicular helper (Tfh) cells and disease. *Immunol Cell Biol.* (2014) 92:64–71. doi: 10.1038/icb.2013.55
8. Ballesteros-Tato A, Randall TD, Lund FE, Spolski R, Leonard WJ, Leon B. T follicular helper cell plasticity shapes pathogenic T helper 2 cell-mediated

immunity to inhaled house dust mite. *Immunity* (2016) 44:259–73. doi: 10.1016/j.immuni.2015.11.017
9. Ame-Thomas P, Le Priol J, Yssel H, Caron G, Pangault C, Jean R, et al. Characterization of intratumoral follicular helper T cells in follicular lymphoma: role in the survival of malignant B cells. *Leukemia* (2012) 26:1053–63. doi: 10.1038/leu.2011.301
10. Rawal S, Chu FL, Zhang M, Park HJ, Nattamai D, Kannan S, et al. Cross talk between follicular Th cells and tumor cells in human follicular lymphoma promotes immune evasion in the tumor microenvironment. *J Immunol.* (2013) 190:6681–93. doi: 10.4049/jimmunol.1201363
11. Gaulard P, de Leval L. Follicular helper T cells: implications in neoplastic hematopathology. *Semin Diagn Pathol.* (2011) 28:202–13. doi: 10.1053/j.semdp.2011.03.003
12. Fujisawa M, Chiba S, Sakata-Yanagimoto M. Recent progress in the understanding of angioimmunoblastic T-cell lymphoma. *J Clin Exp Hematop.* (2017) 57:109–19. doi: 10.3960/jslrt.17019
13. Sage PT, Sharpe AH. T follicular regulatory cells. *Immunol Rev.* (2016) 271:246–59. doi: 10.1111/imr.12411
14. Fu W, Liu X, Lin X, Feng H, Sun L, Li S, et al. Deficiency in T follicular regulatory cells promotes autoimmunity. *J Exp Med.* (2018) 215:815–25. doi: 10.1084/jem.20170901
15. Gong Y, Tong J, Wang S. Are follicular regulatory T cells involved in autoimmune diseases? *Front Immunol.* (2017) 8:1790. doi: 10.3389/fimmu.2017.01790
16. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal.* 8:7.
17. Boettler T, Moeckel F, Cheng Y, Heeg M, Salek-Ardakani S, Crotty S, et al. OX40 facilitates control of a persistent virus infection. *PLoS Pathog.* (2012)8:e1002913. doi: 10.1371/journal.ppat.1002913
18. Tahiliani V, Hutchinson TE, Abboud G, Croft M, Salek-Ardakani S. OX40 cooperates with ICOS to amplify follicular Th cell development and germinal center reactions during infection. *J Immunol.* (2017) 198:218–28. doi: 10.4049/jimmunol.1601356
19. Ma J, Feng D, Wei Y, Tian J, Tang X, Rui K, et al. Blockade of glucocorticoid-induced tumor necrosis factor-receptor-related protein signaling ameliorates murine collagen-induced arthritis by modulating follicular helper T cells. *Am J Pathol.* (2016) 186:1559–67. doi: 10.1016/j.ajpath.2016.02.010
20. Mittler RS, Bailey TS, Klussman K, Trailsmith MD, Hoffmann MK. Anti-4-1BB monoclonal antibodies abrogate T cell-dependent humoral immune responses *in vivo* through the induction of helper T cell anergy. *J Exp Med.* (1999) 190:1535–40. doi: 10.1084/jem.190.10.1535
21. Foell J, Strahotin S, O'Neil SP, McCausland MM, Suwyn C, Haber M, et al. CD137 costimulatory T cell receptor engagement reverses acute disease in lupus-prone NZB x NZW F1 mice. *J Clin Invest.* (2003) 111:1505–18. doi: 10.1172/JCI200317662
22. Tsitsikov EN, Laouini D, Dunn IF, Sannikova TY, Davidson L, Alt FW, et al. TRAF1 is a negative regulator of TNF signaling. Enhanced TNF signaling in TRAF1-deficient mice. *Immunity* (2001) 15:647–57. doi: 10.1016/S1074-7613(01)00207-2
23. McPherson AJ, Snell LM, Mak TW, Watts TH. Opposing roles for TRAF1 in the alternative versus classical NF-kappaB pathway in T cells. *J Biol Chem.* (2012) 287:23010–9. doi: 10.1074/jbc.M112.350538
24. Villanueva JE, Walters SN, Saito M, Malle EK, Zammit NW, Watson KA, et al. Targeted deletion of Traf2 allows immunosuppression-free islet allograft survival in mice. *Diabetologia* (2017) 60:679–89. doi: 10.1007/s00125-016-4198-7
25. Xie P, Kraus ZJ, Stunz LL, Liu Y, Bishop GA. TNF receptor-associated factor 3 is required for T cell-mediated immunity and TCR/CD28 signaling. *J Immunol.* (2011) 186:143–55. doi: 10.4049/jimmunol.1000290
26. Wallis AM, Wallace EC, Hostager BS, Yi ZJ,Houtman CD, Bishop GA. TRAF3 enhances TCR signaling by regulating the inhibitors Csk and PTPN22. *Sci Rep.* (2017) 7:2081. doi: 10.1038/s41598-017-02280-4
27. King CG, Kobayashi T, Cejas PJ, Kim T, Yoon K, Kim GK, et al. TRAF6 is a T cell-intrinsic negative regulator required for the maintenance of immune homeostasis. *Nat Med.* (2006) 12:1088–92. doi: 10.1038/nm1449
28. Xie JJ, Liang JQ, Diao LH, Altman A, Li Y. TNFR-associated factor 6 regulates TCR signaling via interaction with and modification of LAT adapter. *J Immunol.* (2013) 190:4027–36. doi: 10.4049/jimmunol.1202742
29. Akiba H, Takeda K, Kojima Y, Usui Y, Harada N, Yamazaki T, et al. The role of ICOS in the $CXCR5^+$ follicular B helper T cell maintenance *in vivo*. *J Immunol.* (2005) 175:2340–8. doi: 10.4049/jimmunol.175.4.2340
30. Hu YL, Metz DP, Chung J, Siu G, Zhang M. B7RP-1 blockade ameliorates autoimmunity through regulation of follicular helper T cells. *J Immunol.* (2009) 182:1421–8. doi: 10.4049/jimmunol.182.3.1421
31. Gigoux M, Shang J, Pak Y, Xu M, Choe J, Mak TW, et al. Inducible costimulator promotes helper T-cell differentiation through phosphoinositide 3-kinase. *Proc Nat Acad Sci USA.* (2009) 106:20371–6. doi: 10.1073/pnas.0911573106
32. Pedros C, Zhang Y, Hu JK, Choi YS, Canonigo-Balancio AJ, Yates JR III, et al. A TRAF-like motif of the inducible costimulator ICOS controls development of germinal center TFH cells via the kinase TBK1. *Nat Immunol.* (2016) 17:825–33. doi: 10.1038/ni.3463
33. Yi Z, Lin WW, Stunz LL, Bishop GA. The adaptor TRAF3 restrains the lineage determination of thymic regulatory T cells by modulating signaling via the receptor for IL-2. *Nat Immunol.* (2014) 15:866–74. doi: 10.1038/ni.2944
34. Motegi H, Shimo Y, Akiyama T, Inoue J-I. TRAF6 negatively regulates the Jak1-Erk pathway in interleukin-2 signaling. *Genes Cells* (2011) 16:179–89. doi: 10.1111/j.1365-2443.2010.01474.x
35. Nagashima H, Okuyama Y, Asao A, Kawabe T, Yamaki S, Nakano H, et al. The adaptor TRAF5 limits the differentiation of inflammatory $CD4^+$ T cells by antagonizing signaling via the receptor for IL-6. *Nat Immunol.* (2014) 15:449–56. doi: 10.1038/ni.2863
36. Nagashima H, Okuyama Y, Hayashi T, Ishii N, So T. TNFR-associated factors 2 and 5 differentially regulate the instructive IL-6 receptor signaling required for Th17 development. *J Immunol.* (2016) 196:4082–9. doi: 10.4049/jimmunol.1501610
37. Crotty S. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol.* (2011) 29:621–63. doi: 10.1146/annurev-immunol-031210-101400
38. Crotty S. T follicular helper cell differentiation, function, and roles in disease. *Immunity* (2014) 41:529–42. doi: 10.1016/j.immuni.2014.10.004
39. Kerfoot SM, Yaari G, Patel JR, Johnson KL, Gonzalez DG, Kleinstein SH, et al. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. *Immunity* (2011) 34:947–60. doi: 10.1016/j.immuni.2011.03.024
40. Baumjohann D, Okada T, Ansel KM. Cutting edge: distinct waves of BCL6 expression during T follicular helper cell development. *J Immunol.* (2011) 187:2089–92. doi: 10.4049/jimmunol.1101393
41. Kitano M, Moriyama S, Ando Y, Hikida M, Mori Y, Kurosaki T, et al. Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. *Immunity* (2011) 34:961–72. doi: 10.1016/j.immuni.2011.03.025
42. Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, et al. Bcl6 mediates the development of T follicular helper cells. *Science* (2009) 325:1001–5. doi: 10.1126/science.1176676
43. Yu D, Rao S, Tsai LM, Lee SK, He Y, Sutcliffe EL, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* (2009) 31:457–68. doi: 10.1016/j.immuni.2009.07.002
44. Haynes NM, Allen CDC, Lesley R, Ansel KM, Killeen N, Cyster JG. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1high germinal center-associated subpopulation. *J Immunol.* (2007) 179:5099–108. doi: 10.4049/jimmunol.179.8.5099
45. Baumjohann D, Preite S, Reboldi A, Ronchi F, Ansel KM, Lanzavecchia A, et al. Persistent antigen and germinal center B cells sustain T follicular helper cell responses and phenotype. *Immunity* (2013) 38:596–605. doi: 10.1016/j.immuni.2012.11.020
46. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature* (2008) 455:764–9. doi: 10.1038/nature07345
47. Weinstein JS, Herman EI, Lainez B, Licona-Limon P, Esplugues E, Flavell R, et al. TFH cells progressively differentiate to regulate the germinal center response. *Nat Immunol.* (2016) 17:1197–205. doi: 10.1038/ni.3554
48. Hale JS, Ahmed R. Memory T follicular helper CD4 T cells. *Front Immunol.* (2015) 6:16. doi: 10.3389/fimmu.2015.00016

49. Fazilleau N, McHeyzer-Williams LJ, Rosen H, McHeyzer-Williams MG. The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. *Nat Immunol.* (2009) 10:375–84. doi: 10.1038/ni.1704
50. Hwang S, Palin AC, Li L, Song KD, Lee J, Love PE. (2015). TCR ITAM multiplicity is required for the generation of follicular helper T-cells. *Nat Commun.* 6:6982. doi: 10.1038/ncomms7982
51. Keck S, Schmaler M, Ganter S, Wyss L, Oberle S, Huseby ES, et al. Antigen affinity and antigen dose exert distinct influences on CD4 T-cell differentiation. *Proc Nat Acad Sci USA.* (2014) 111:14852–7. doi: 10.1073/pnas.1403271111
52. Tubo NJ, Pagan AJ, Taylor JJ, Nelson RW, Linehan JL, Ertelt JM, et al. Single naive $CD4^+$ T cells from a diverse repertoire produce different effector cell types during infection. *Cell* (2013) 153:785–96. doi: 10.1016/j.cell.2013.04.007
53. Deenick EK, Chan A, Ma CS, Gatto D, Schwartzberg PL, Brink R, et al. Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity* (2010) 33:241–53. doi: 10.1016/j.immuni.2010.07.015
54. Krishnamoorthy V, Kannanganat S, Maienschein-Cline M, Cook SL, Chen JJ, Bahroos N, et al. The IRF4 gene regulatory module functions as a read-write integrator to dynamically coordinate t helper cell fate. *Immunity* (2017) 47:481–97.e7. doi: 10.1016/j.immuni.2017.09.001
55. Benson RA, MacLeod MKL, Hale BG, Patakas A, Garside P, Brewer JM. Antigen presentation kinetics control T cell/dendritic cell interactions and follicular helper T cell generation *in vivo*. *Elife* (2015) 4:e06994. doi: 10.7554/eLife.06994
56. Lin WJ, Su YW, Lu YC, Hao Z, Chio IIC, Chen NJ, et al. Crucial role for TNF receptor-associated factor 2 (TRAF2) in regulating NFkappaB2 signaling that contributes to autoimmunity. *Proc Nat Acad Sci USA.* (2011) 108:18354–9. doi: 10.1073/pnas.1109427108
57. Salek-Ardakani S, Choi YS, Rafii-El-Idrissi Benhnia M, Flynn R, Arens R, Shoenberger S. B cell-specific expression of B7-2 is required for follicular Th cell function in response to vaccinia virus. *J Immunol.* (2011) 186:5294–303. doi: 10.4049/jimmunol.1100406
58. Wang CJ, Heuts F, Ovcinnikovs V, Wardzinski L, Bowers C, Schmidt EM, et al. CTLA-4 controls follicular helper T-cell differentiation by regulating the strength of CD28 engagement. *Proc Nat Acad Sci USA.* (2015) 112:524–9. doi: 10.1073/pnas.1414576112
59. Collins AV, Brodie DW, Gilbert RJ, Iaboni A, Manso-Sancho R, Walse B, et al. The interaction properties of costimulatory molecules revisited. *Immunity* (2002) 17:201–10. doi: 10.1016/S1074-7613(02)00362-X
60. Ferguson SE, Han S, Kelsoe G, Thompson CB. CD28 is required for germinal center formation. *J Immunol.* (1996) 156:4576–81.
61. Weber JP, Fuhrmann F, Feist RK, Lahmann A, Al Baz MS, Gentz LJ, et al. ICOS maintains the T follicular helper cell phenotype by down-regulating Kruppel-like factor 2. *J Exp Med.* (2015) 212:217–33. doi: 10.1084/jem.20141432
62. Lane P, Burdet C, Hubele S, Scheidegger D, Muller U, McConnell F, et al. B cell function in mice transgenic for mCTLA4-H gamma 1: lack of germinal centers correlated with poor affinity maturation and class switching despite normal priming of $CD4^+$ T cells. *J Exp Med.* (1994) 179:819–30. doi: 10.1084/jem.179.3.819
63. Walker LS, Gulbranson-Judge A, Flynn S, Brocker T, Raykundalia C, Goodall M, et al. Compromised OX40 function in CD28-deficient mice is linked with failure to develop CXC chemokine receptor 5-positive CD4 cells and germinal centers. *J Exp Med.* (1999) 190:1115–22. doi: 10.1084/jem.190.8.1115
64. Walker LSK, Wiggett HE, Gaspal FMC, Raykundalia CR, Goodall MD, Toellner K-M, et al. Established T cell-driven germinal center B cell proliferation is independent of CD28 signaling but is tightly regulated through CTLA-4. *J Immunol.* (2003) 170:91–8. doi: 10.4049/jimmunol.170.1.91
65. Watanabe M, Fujihara C, Radtke AJ, Chiang YJ, Bhatia S, Germain RN, et al. Co-stimulatory function in primary germinal center responses: CD40 and B7 are required on distinct antigen-presenting cells. *J Exp Med.* (2017) 214:2795–810. doi: 10.1084/jem.20161955
66. Kong KF, Yokosuka T, Canonigo-Balancio AJ, Isakov N, Saito T, Altman A. A motif in the V3 domain of the kinase PKC-theta determines its localization in the immunological synapse and functions in T cells via association with CD28. *Nat Immunol.* (2011) 12:1105–12. doi: 10.1038/ni.2120
67. Altman A, Kong KF. Protein kinase C enzymes in the hematopoietic and immune systems. *Annu Rev Immunol.* (2016) 34:511–38. doi: 10.1146/annurev-immunol-041015-055347
68. Dodson LF, Boomer JS, Deppong CM, Shah DD, Sim J, Bricker TL, et al. Targeted knock-in mice expressing mutations of CD28 reveal an essential pathway for costimulation. *Mol Cell Biol.* (2009) 29:3710–21. doi: 10.1128/MCB.01869-08
69. Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Drager R, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat Immunol.* (2003) 4:261–8. doi: 10.1038/ni902
70. Yong PF, Salzer U, Grimbacher B. The role of costimulation in antibody deficiencies: ICOS and common variable immunodeficiency. *Immunol Rev.* (2009) 229:101–13. doi: 10.1111/j.1600-065X.2009.00764.x
71. Hutloff A, Dittrich AM, Beier KC, Eljaschewitsch B, Kraft R. Anagnostopoulos, I., and Kroczek, R. A., ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* (1999) 397:263–6. doi: 10.1038/16717
72. Dong C, Juedes AE, Temann UA, Shresta S, Allison JP, Ruddle NH, et al. ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* (2001) 409:97–101. doi: 10.1038/35051100
73. Tafuri A, Shahinian A, Bladt F, Yoshinaga SK, Jordana M, Wakeham A, et al. ICOS is essential for effective T-helper-cell responses. *Nature* (2001) 409:105–9. doi: 10.1038/35051113
74. McAdam AJ, Greenwald RJ, Levin MA, Chernova T, Malenkovich N, Ling V, et al. ICOS is critical for CD40-mediated antibody class switching. *Nature* (2001) 409:102–5. doi: 10.1038/35051107
75. Choi YS, Kageyama R, Eto D, Escobar TC, Johnston RJ, Monticelli L, et al. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. *Immunity* (2011) 34:932–46. doi: 10.1016/j.immuni.2011.03.023
76. Shin C, Han J-A, Koh H, Choi B, Cho Y, Jeong H, et al. $CD8alpha^-$ dendritic cells induce antigen-specific T follicular helper cells generating efficient humoral immune responses. *Cell Rep.* (2015) 11:1929–40. doi: 10.1016/j.celrep.2015.05.042
77. Wikenheiser DJ, Ghosh D, Kennedy B, Stumhofer JS. The costimulatory molecule ICOS regulates host Th1 and follicular Th cell differentiation in response to *Plasmodium chabaudi chabaudi* AS infection. *J Immunol.* (2016) 196:778–91. doi: 10.4049/jimmunol.1403206
78. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* (2008) 29:138–49. doi: 10.1016/j.immuni.2008.05.009
79. Liu D, Xu H, Shih C, Wan Z, Ma X, Ma W, et al. T-B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. *Nature* (2015) 517:214–8. doi: 10.1038/nature13803
80. Fos C, Salles A, Lang V, Carrette F, Audebert S, Pastor S, et al. ICOS ligation recruits the p50alpha PI3K regulatory subunit to the immunological synapse. *J Immunol.* (2008) 181:1969–77. doi: 10.4049/jimmunol.181.3.1969
81. Watanabe R, Harada Y, Takeda K, Takahashi J, Ohnuki K, Ogawa S, et al. Grb2 and Gads exhibit different interactions with CD28 and play distinct roles in CD28-mediated costimulation. *J Immunol.* (2006) 177:1085–91. doi: 10.4049/jimmunol.177.2.1085
82. Inukai K, Funaki M, Ogihara T, Katagiri H, Kanda A, Anai M, et al. p85alpha gene generates three isoforms of regulatory subunit for phosphatidylinositol 3-kinase (PI 3-Kinase), p50alpha, p55alpha, and p85alpha, with different PI 3-kinase activity elevating responses to insulin. *J Biol Chem.* (1997) 272:7873–82. doi: 10.1074/jbc.272.12.7873
83. Inukai I, Funaki M, Anai M, Ogihara T, Katagiri H, Fukushima Y, et al. Five isoforms of the phosphatidylinositol 3-kinase regulatory subunit exhibit different associations with receptor tyrosine kinases and their tyrosine phosphorylations. *Febs Lett.* (2001) 490:32–8. doi: 10.1016/S0014-5793(01)02132-9
84. Clayton E, Bardi G, Bell SE, Chantry D, Downes CP, Gray A, et al. A crucial role for the p110delta subunit of phosphatidylinositol 3-kinase

in B cell development and activation. *J Exp Med.* (2002) 196:753–63. doi: 10.1084/jem.20020805
85. Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, et al. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* (2002) 297:1031–4. doi: 10.1126/science.1073560
86. Rolf J, Bell SE, Kovesdi D, Janas ML, Soond DR, Webb LMC, et al. Phosphoinositide 3-kinase activity in T cells regulates the magnitude of the germinal center reaction. *J Immunol.* (2010) 185:4042–52. doi: 10.4049/jimmunol.1001730
87. Deane JA, Kharas MG, Oak JS, Stiles LN, Luo J, Moore TI, et al. T-cell function is partially maintained in the absence of class IA phosphoinositide 3-kinase signaling. *Blood* (2007) 109:2894–902.
88. Gao XL, Gigoux M, Yang J, Leconte J, Yang X, Suh WK. Anti-chlamydial Th17 responses are controlled by the inducible costimulator partially through phosphoinositide 3-kinase signaling. *PLoS ONE* (2012) 7–12:e52657. doi: 10.1371/journal.pone.0052657
89. Li J, Heinrichs J, Leconte J, Haarberg K, Semple K, Liu C, et al. Phosphatidylinositol 3-kinase-independent signaling pathways contribute to ICOS-mediated T cell costimulation in acute graft-versus-host disease in mice. *J Immunol.* (2013) 191:200–7. doi: 10.4049/jimmunol.1203485
90. Louis C, Ngo D, D'Silva DB, Hansen J, Phillipson L, Jousset H, et al. Therapeutic effects of a TBK1 kinase inhibitor in germinal center-driven, autoantibody-mediated inflammatory arthritis. *Arthritis Rheumatol.* (2018). doi: 10.1002/art.40670
91. Pomerantz JL, Baltimore D. NF-kappaB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. *EMBO J.* (1999) 18:6694–704. doi: 10.1093/emboj/18.23.6694
92. Oganesyan G, Saha SK, Guo B, He JQ, Shahangian A, Zarnegar B, et al. Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. *Nature* (2006) 439:208–11. doi: 10.1038/nature04374
93. Fang R, Jiang QF, Zhou X, Wang CG, Guan YK, Tao JL, et al. MAVS activates TBK1 and IKK epsilon through TRAFs in NEMO dependent and independent manner. *PLoS Pathog.* (2017) 13:e1006720. doi: 10.1371/journal.ppat.1006720
94. Leconte J, Bagherzadeh Yazdchi S, Panneton V, Suh WK. Inducible costimulator (ICOS) potentiates TCR-induced calcium flux by augmenting PLCgamma1 activation and actin remodeling. *Mol Immunol.* (2016) 79:38–46. doi: 10.1016/j.molimm.2016.09.022
95. Flynn S, Toellner KM, Raykundalia C, Goodall M, Lane P. CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. *J Exp Med.* (1998) 188:297–304. doi: 10.1084/jem.188.2.297
96. Kopf M, Ruedl C, Schmitz N, Gallimore A, Lefrang K, Ecabert B, et al. OX40-deficient mice are defective in Th cell proliferation but are competent in generating B cell and CTL Responses after virus infection. *Immunity* (1999) 11:699–708. doi: 10.1016/S1074-7613(00)80144-2
97. Ekkens MJ, Liu Z, Liu Q, Whitmire J, Xiao S, Foster A, et al. The role of OX40 ligand interactions in the development of the Th2 response to the gastrointestinal nematode parasite Heligmosomoides polygyrus. *J Immunol.* (2003) 170:384–93. doi: 10.4049/jimmunol.170.1.384
98. Jacquemin C, Schmitt N, Contin-Bordes C, Liu Y, Narayanan P, Seneschal J, et al. OX40 ligand contributes to human lupus pathogenesis by promoting T follicular helper response. *Immunity* (2015) 42:1159–70. doi: 10.1016/j.immuni.2015.05.012
99. Kawamata S, Hori T, Imura A, Takaori-Kondo A, Uchiyama T. Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2- and TRAF5-mediated NF-kappaB activation. *J Biol Chem.* (1998) 273:5808–14. doi: 10.1074/jbc.273.10.5808
100. Arch RH, Thompson CB. 4-1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclear factor kappaB. *Mol Cell Biol.* (1998) 18:558–65. doi: 10.1128/MCB.18.1.558
101. So T, Soroosh P, Eun SY, Altman A, Croft M. Antigen-independent signalosome of CARMA1, PKCtheta, and TNF receptor-associated factor 2 (TRAF2) determines NF-kappaB signaling in T cells. *Proc Nat Acad Sci USA.* (2011) 108:2903–8. doi: 10.1073/pnas.1008765108
102. Clouthier DL, Zhou AC, Wortzman ME, Luft O, Levy GA, Watts TH. GITR intrinsically sustains early type 1 and late follicular helper CD4 T cell accumulation to control a chronic viral infection. *PLoS Pathog.* (2015) 11:e1004517. doi: 10.1371/journal.ppat.1004517
103. Snell LM, McPherson AJ, Lin GHY, Sakaguchi S, Pandolfi PP, Riccardi C, et al. CD8 T cell-intrinsic GITR is required for T cell clonal expansion and mouse survival following severe influenza infection. *J Immunol.* (2010) 185:7223–34. doi: 10.4049/jimmunol.1001912
104. Esparza EM, Lindsten T, Stockhausen JM, Arch RH. Tumor necrosis factor receptor (TNFR)-associated factor 5 is a critical intermediate of costimulatory signaling pathways triggered by glucocorticoid-induced TNFR in T cells. *J Biol Chem.* (2006) 281:8559–64. doi: 10.1074/jbc.M512915200
105. Hauer J, Puschner S, Ramakrishnan P, Simon U, Bongers M, Federle C, et al. TNF receptor (TNFR)-associated factor (TRAF) 3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF-kappaB pathway by TRAF-binding TNFRs. *Proc Nat Acad Sci USA.* (2005) 102:2874–9. doi: 10.1073/pnas.0500187102
106. de Boer M, Kasran A, Kwekkeboom J, Walter H, Vandenberghe P, Ceuppens JL. Ligation of B7 with CD28/CTLA-4 on T cells results in CD40 ligand expression, interleukin-4 secretion and efficient help for antibody production by B cells. *Eur J Immunol.* (1993) 23:3120–5. doi: 10.1002/eji.1830231212
107. van Essen D, Kikutani H, Gray D. CD40 ligand-transduced co-stimulation of T cells in the development of helper function. *Nature* (1995) 378:620–3. doi: 10.1038/378620a0
108. Grewal IS, Xu J, Flavell RA. Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. *Nature* (1995) 378:617–20. doi: 10.1038/378617a0
109. Bishop GA, Moore CR, Xie P, Stunz LL, Kraus ZJ. TRAF proteins in CD40 signaling. *Adv Exp Med Biol.* (2007) 597:131–51. doi: 10.1007/978-0-387-70630-6_11
110. Alfaro C, Echeveste JI, Rodriguez-Ruiz ME, Solorzano JL, Perez-Gracia JL, Idoate MA, et al. Functional expression of CD137 (4-1BB) on T helper follicular cells. *Oncoimmunology* (2015) 4:e1054597. doi: 10.1080/2162402X.2015.1054597
111. Kwon BS, Hurtado JC, Lee ZH, Kwack KB, Seo SK, Choi BK, et al. Immune responses in 4-1BB (CD137)-deficient mice. *J Immunol.* (2002) 168:5483–90. doi: 10.4049/jimmunol.168.11.5483
112. DeBenedette MA, Wen T, Bachmann MF, Ohashi PS, Barber BH, Stocking KL, et al. Analysis of 4-1BB ligand (4-1BBL)-deficient mice and of mice lacking both 4-1BBL and CD28 reveals a role for 4-1BBL in skin allograft rejection and in the cytotoxic T cell response to influenza virus. *J Immunol.* (1999) 163:4833–41.
113. Hong HJ, Lee JW, Park SS, Kang YJ, Chang SY, Kim KM, et al. A humanized anti−4-1BB monoclonal antibody suppresses antigen-induced humoral immune response in nonhuman primates. *J Immunother.* (2000) 23:613–21. doi: 10.1097/00002371-200011000-00002
114. Sun Y, Blink SE, Chen JH, Fu YX. (2005). Regulation of follicular dendritic cell networks by activated T cells: the role of CD137 signaling. *J Immunol.* 175:884–90. doi: 10.4049/jimmunol.175.2.884
115. Saoulli K, Lee SY, Cannons JL, Yeh WC, Santana A, Goldstein MD, et al. CD28-independent, TRAF2-dependent costimulation of resting T cells by 4-1BB ligand. *J Exp Med.* (1998) 187:1849–62. doi: 10.1084/jem.187.11.1849
116. Martinez-Forero I, Azpilikueta A, Bolanos-Mateo E, Nistal-Villan E, Palazon A, Teijeira A, et al. T cell costimulation with anti-CD137 monoclonal antibodies is mediated by K63-polyubiquitin-dependent signals from endosomes. *J Immunol.* (2013) 190:6694–706. doi: 10.4049/jimmunol.1203010
117. Cannons JL, Choi Y, Watts TH. Role of TNF receptor-associated factor 2 and p38 mitogen-activated protein kinase activation during 4-1BB-dependent immune response. *J Immunol.* (2000) 165:6193–204. doi: 10.4049/jimmunol.165.11.6193
118. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, et al. Foxp3$^+$ follicular regulatory T cells control the germinal center response. *Nat Med.* (2011) 17:975–82. doi: 10.1038/nm.2425
119. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3$^+$ regulatory T cell function. *Science* (2008) 322:271–5. doi: 10.1126/science.1160062
120. Sage PT, Paterson AM, Lovitch SB, Sharpe AH. The coinhibitory receptor CTLA-4 controls B cell responses by modulating T follicular helper, T

follicular regulatory, and T regulatory cells. *Immunity* (2014) 41:1026–39. doi: 10.1016/j.immuni.2014.12.005
121. Wing JB, Ise W, Kurosaki T, Sakaguchi S. Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4. *Immunity* (2014) 41:1013–25. doi: 10.1016/j.immuni.2014.12.006
122. Alroqi FJ, Charbonnier LM, Baris S, Kiykim A, Chou J, Platt CD, et al. (2018). Exaggerated follicular helper T-cell responses in patients with LRBA deficiency caused by failure of CTLA4-mediated regulation. *J Allergy Clin Immun.* 141:1050–9.e10. doi: 10.1016/j.jaci.2017.05.022
123. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* (2011) 332:600–3. doi: 10.1126/science.1202947
124. Marengere LE, Waterhouse P, Duncan GS, Mittrucker HW, Feng GS, Mak TW. Regulation of T cell receptor signaling by tyrosine phosphatase SYP association with CTLA-4. *Science* (1996) 272:1170–3. doi: 10.1126/science.272.5265.1170
125. Chuang E, Fisher TS, Morgan RW, Robbins MD, Duerr JM, Vander Heiden MG, et al. The CD28 and CTLA-4 receptors associate with the serine/threonine phosphatase PP2A. *Immunity* (2000) 13:313–22. doi: 10.1016/S1074-7613(00)00031-5
126. Kong KF, Fu G, Zhang Y, Yokosuka T, Casas J, Canonigo-Balancio AJ, et al. Protein kinase C-eta controls CTLA-4-mediated regulatory T cell function. *Nat Immunol.* (2014) 15:465–72. doi: 10.1038/ni.2866
127. Pedros C, Canonigo-Balancio AJ, Kong KF, Altman A. Requirement of Treg-intrinsic CTLA4/PKCη signaling pathway for suppressing tumor immunity. *JCI Insight* (2017) 2:e95692. doi: 10.1172/jci.insight.95692
128. Li S, Wang L, Dorf ME. PKC phosphorylation of TRAF2 mediates IKKalpha/beta recruitment and K63-linked polyubiquitination. *Mol Cell* (2009) 33:30–42. doi: 10.1016/j.molcel.2008.11.023
129. Good-Jacobson KL, Szumilas CG, Chen L, Sharpe AH, Tomayko MM, Shlomchik MJ. PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nat Immunol.* (2010) 11:535–42. doi: 10.1038/ni.1877
130. Hui EF, Cheung J, Zhu J, Su XL, Taylor MJ, Wallweber HA, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science* (2017) 355:1428–33. doi: 10.1126/science.aaf1292
131. Hamel KM, Cao Y, Wang Y, Rodeghero R, Kobezda T, Chen L, et al. B7-H1 expression on non-B and non-T cells promotes distinct effects on T- and B-cell responses in autoimmune arthritis. *Eur J Immunol.* (2010) 40:3117–27. doi: 10.1002/eji.201040690
132. Hams E, McCarron MJ, Amu S, Yagita H, Azuma M, Chen L, et al. Blockade of B7-H1 (programmed death ligand 1) enhances humoral immunity by positively regulating the generation of T follicular helper cells. *J Immunol.* (2011) 186:5648–55. doi: 10.4049/jimmunol.1003161
133. Kawamoto S, Tran TH, Maruya M, Suzuki K, Doi Y, Tsutsui Y, et al. The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. *Science* (2012) 336:485–9. doi: 10.1126/science.1217718
134. Cubas RA, Mudd JC, Savoye AL, Perreau M, van Grevenynghe J, Metcalf T, et al. Inadequate T follicular cell help impairs B cell immunity during HIV infection. *Nat Med.* (2013) 19:494–9. doi: 10.1038/nm.3109
135. Sage PT, Francisco LM, Carman CV, Sharpe AH. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. *Nat Immunol.* (2013) 14:152–61. doi: 10.1038/ni.2496
136. Sheppard KA, Fitz LJ, Lee JM, Benander C, George JA, Wooters J, et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. *Febs Lett.* (2004) 574:37–41. doi: 10.1016/j.febslet.2004.07.083
137. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J Immunol.* (2004) 173:945–54. doi: 10.4049/jimmunol.173.2.945
138. Zhang ZY, Jimi E, Bothwell ALM. Receptor activator of NF-kappa B ligand stimulates recruitment of SHP-1 to the complex containing TNFR-associated factor 6 that regulates osteoclastogenesis. *J Immunol.* (2003) 171:3620–6. doi: 10.4049/jimmunol.171.7.3620
139. Watanabe N, Gavrieli M, Sedy JR, Yang JF, Fallarino F, Loftin SK, et al. BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat Immunol.* (2003) 4:670–9. doi: 10.1038/ni944
140. Oya Y, Watanabe N, Owada T, Oki M, Hirose K, Suto A, et al. Development of autoimmune hepatitis-like disease and production of autoantibodies to nuclear antigens in mice lacking B and T lymphocyte attenuator. *Arthritis Rheum.* (2008) 58:2498–510. doi: 10.1002/art.23674
141. Kashiwakuma D, Suto A, Hiramatsu Y, Ikeda K, Takatori H, Suzuki K, et al. B and T lymphocyte attenuator suppresses IL-21 production from follicular Th cells and subsequent humoral immune responses. *J Immunol.* (2010) 185:2730–6. doi: 10.4049/jimmunol.0903839
142. Gavrieli M, Watanabe N, Loftin SK, Murphy TL, Murphy KM. Characterization of phosphotyrosine binding motifs in the cytoplasmic domain of B and T lymphocyte attenuator required for association with protein tyrosine phosphatases SHP-1 and SHP-2. *Biochem Biophys Res Commun.* (2003) 312:1236–43. doi: 10.1016/j.bbrc.2003.11.070
143. Villarino AV, Kanno Y, O'Shea JJ. Mechanisms and consequences of Jak-STAT signaling in the immune system. *Nat Immunol.* (2017) 18:374–84. doi: 10.1038/ni.3691
144. Choi YS, Eto D, Yang JA, Lao C, Crotty, S. Cutting edge: STAT1 is required for IL-6-mediated Bcl6 induction for early follicular helper cell differentiation. *J Immunol.* (2013) 190:3049–53. doi: 10.4049/jimmunol.1203032
145. Harker JA, Lewis GM, Mack L, Zuniga EI. Late interleukin-6 escalates T follicular helper cell responses and controls a chronic viral infection. *Science* (2011) 334:825–9. doi: 10.1126/science.1208421
146. Kopf M, Herren S, Wiles MV, Pepys MB, Kosco-Vilbois MH. Interleukin 6 influences germinal center development and antibody production via a contribution of C3 complement component. *J Exp Med.* (1998) 188:1895–906. doi: 10.1084/jem.188.10.1895
147. Poholek AC, Hansen K, Hernandez SG, Eto D, Chandele A, Weinstein JS, et al. *In vivo* regulation of Bcl6 and T follicular helper cell development. *J Immunol.* (2010) 185:313–26. doi: 10.4049/jimmunol.0904023
148. Eto D, Lao C, DiToro D, Barnett B, Escobar TC, Kageyama R, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. *PLoS ONE* (2011) 6:e17739. doi: 10.1371/journal.pone.0017739
149. Karnowski A, Chevrier S, Belz GT, Mount A, Emslie D, D'Costa K, et al. B and T cells collaborate in antiviral responses via IL-6, IL-21, and transcriptional activator and coactivator, Oct2 and OBF-1. *J Exp Med.* (2012) 209:2049–64. doi: 10.1084/jem.20111504
150. Nish SA, Schenten D, Wunderlich FT, Pope SD, Gao Y, Hoshi N, et al. T cell-intrinsic role of IL-6 signaling in primary and memory responses. *Elife* (2014) 3:e01949. doi: 10.7554/eLife.01949
151. Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity* (2008) 29:127–37. doi: 10.1016/j.immuni.2008.06.001
152. Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A., et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J Exp Med.* (2010) 207:365–78. doi: 10.1084/jem.20091777
153. Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *J Exp Med.* (2010) 207:353–63. doi: 10.1084/jem.20091738
154. Rasheed MA, Latner DR, Aubert RD, Gourley T, Spolski R, Davis CW, et al. Interleukin-21 is a critical cytokine for the generation of virus-specific long-lived plasma cells. *J Virol.* (2013) 87:7737–46. doi: 10.1128/JVI.00063-13
155. Batten M, Ramamoorthi N, Kljavin NM, Ma CS, Cox JH, Dengler HS, et al. IL-27 supports germinal center function by enhancing IL-21 production and the function of T follicular helper cells. *J Exp Med.* (2010) 207:2895–906. doi: 10.1084/jem.20100064
156. Pot C, Jin H, Awasthi A, Liu SM, Lai C-Y, Madan R, et al. Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. *J Immunol.* (2009) 183:797–801. doi: 10.4049/jimmunol.0901233
157. Vijayan D, Mohd Redzwan N, Avery DT, Wirasinha RC, Brink R, Walters G, et al. IL-27 directly enhances germinal center B cell activity and

potentiates lupus in sanroque mice. *J Immunol.* (2016) 197:3008–17. doi: 10.4049/jimmunol.1600652
158. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type i interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells *in vivo*. *Immunity* (2001) 14:461–70. doi: 10.1016/S1074-7613(01)00126-1
159. Cucak H, Yrlid U, Reizis B, Kalinke U, Johansson-Lindbom B. Type I interferon signaling in dendritic cells stimulates the development of lymph-node-resident T follicular helper cells. *Immunity* (2009) 31:491–501. doi: 10.1016/j.immuni.2009.07.005
160. Riteau N, Radtke AJ, Shenderov K, Mittereder L, Oland SD, Hieny S, et al. Water-in-oil-only adjuvants selectively promote T follicular helper cell polarization through a type I IFN and IL-6-dependent pathway. *J Immunol.* (2016) 197:3884–93. doi: 10.4049/jimmunol.1600883
161. Loetsch C, Warren J, Laskowski A, Vazquez-Lombardi R, Jandl C, Langley DB, et al. Cytosolic recognition of RNA drives the immune response to heterologous erythrocytes. *Cell Rep.* (2017) 21:1624–38. doi: 10.1016/j.celrep.2017.10.044
162. Sebina I, James KR, Soon MS, Fogg LG, Best SE, Labastida Rivera F, et al. IFNAR1-Signalling obstructs ICOS-mediated humoral immunity during non-lethal blood-stage plasmodium infection. *PLoS Pathog.* (2016) 12:e1005999. doi: 10.1371/journal.ppat.1005999
163. Zander RA, Guthmiller JJ, Graham AC, Pope RL, Burke BE, Carr DJ, et al. Type I interferons induce T regulatory 1 responses and restrict humoral immunity during experimental malaria. *PLoS Pathog.* (2016) 12:e1005945. doi: 10.1371/journal.ppat.1005945
164. Yang CH, Murti A, Pfeffer SR, Fan M, Du Z, Pfeffer LM. The role of TRAF2 binding to the type I interferon receptor in alternative NF kappaB activation and antiviral response. *J Biol Chem.* (2008) 283:14309–16. doi: 10.1074/jbc.M708895200
165. Eddahri F, Denanglaire S, Bureau F, Spolski R, Leonard WJ, Leo O, et al. Interleukin-6/STAT3 signaling regulates the ability of naive T cells to acquire B-cell help capacities. *Blood* (2009) 113:2426–33. doi: 10.1182/blood-2008-04-154682
166. Ray JP, Marshall HD, Laidlaw BJ, Staron MM, Kaech SM, Craft J. Transcription factor STAT3 and type I interferons are corepressive insulators for differentiation of follicular helper and T helper 1 cells. *Immunity* (2014) 40:367–77. doi: 10.1016/j.immuni.2014.02.005
167. Ma CS, Avery DT, Chan A, Batten M, Bustamante J, Boisson-Dupuis S, et al. Functional STAT3 deficiency compromises the generation of human T follicular helper cells. *Blood* (2012) 119:3997–4008. doi: 10.1182/blood-2011-11-392985
168. Kimura M, Nagashima H, Okuyama Y, Ishii N, So T. TRAF2 and TRAF5 associated with the signal transducing receptor gp130 limit IL-6-driven transphosphorylation of JAK1 through the inhibition of proximal JAK-JAK interaction. *Int Immunol.* (2018) 30:291–9. doi: 10.1093/intimm/dxy029
169. Wei J, Yuan Y, Jin C, Chen H, Leng L, He F, et al. The ubiquitin ligase TRAF6 negatively regulates the JAK-STAT signaling pathway by binding to STAT3 and mediating its ubiquitination. *PLoS ONE* (2012) 7:e49567. doi: 10.1371/journal.pone.0049567
170. Lin WW, Yi Z, Stunz LL, Maine CJ, Sherman LA, Bishop GA. The adaptor protein TRAF3 inhibits interleukin-6 receptor signaling in B cells to limit plasma cell development. *Sci Signal.* (2015) 8:ra88. doi: 10.1126/scisignal.aaa5157
171. Nakayamada S, Kanno Y, Takahashi H, Jankovic D, Lu KT, Johnson TA, et al. Early Th1 cell differentiation is marked by a Tfh cell-like transition. *Immunity* (2011) 35:919–31. doi: 10.1016/j.immuni.2011.11.012
172. Schmitt N, Morita R, Bourdery L, Bentebibel SE, Zurawski SM, Banchereau J, et al. Human dendritic cells induce the differentiation of interleukin-21-producing T follicular helper-like cells through interleukin-12. *Immunity* (2009) 31:158–69. doi: 10.1016/j.immuni.2009.04.016
173. Ma CS, Suryani S, Avery DT, Chan A, Nanan R, Santner-Nanan B, et al. Early commitment of naive human $CD4^+$ T cells to the T follicular helper (T(FH)) cell lineage is induced by IL-12. *Immunol Cell Biol.* (2009) 87:590–600. doi: 10.1038/icb.2009.64
174. Schmitt N, Bustamante J, Bourdery L, Bentebibel SE, Boisson-Dupuis S, Hamlin F, et al. IL-12 receptor beta1 deficiency alters *in vivo* T follicular helper cell response in humans. *Blood* (2013) 121:3375–85. doi: 10.1182/blood-2012-08-448902
175. Oestreich KJ, Mohn SE, Weinmann AS. Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a TFH-like gene profile. *Nat Immunol.* (2012) 13:405–11. doi: 10.1038/ni.2242
176. Johnston RJ, Choi YS, Diamond JA, Yang JA, Crotty S. STAT5 is a potent negative regulator of TFH cell differentiation. *J Exp Med.* (2012) 209:243–50. doi: 10.1084/jem.20111174
177. Ballesteros-Tato A, Leon B, Graf BA, Moquin A, Adams PS, Lund FE, et al. Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation. *Immunity* (2012) 36:847–56. doi: 10.1016/j.immuni.2012.02.012
178. Choi YS, Yang JA, Yusuf I, Johnston RJ, Greenbaum J, Peters B, et al. Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. *J Immunol.* (2013) 190:4014–26. doi: 10.4049/jimmunol.1202963
179. Liu X, Lu H, Chen T, Nallaparaju KC, Yan X, Tanaka S, et al. Genome-wide analysis identifies Bcl6-controlled regulatory networks during T follicular helper cell differentiation. *Cell Rep.* (2016) 14:1735–47. doi: 10.1016/j.celrep.2016.01.038
180. McDonald PW, Read KA, Baker CE, Anderson AE, Powell MD, Ballesteros-Tato A, et al. IL-7 signalling represses Bcl-6 and the TFH gene program. *Nat Commun.* (2016) 7:10285. doi: 10.1038/ncomms10285
181. Seo YB, Im SJ, Namkoong H, Kim SW, Choi YW, Kang MC, et al. Crucial roles of interleukin-7 in the development of T follicular helper cells and in the induction of humoral immunity. *J Virol.* (2014) 88:8998–9009. doi: 10.1128/JVI.00534-14
182. Walker SR, Nelson EA, Frank DA. STAT5 represses BCL6 expression by binding to a regulatory region frequently mutated in lymphomas. *Oncogene* (2007) 26:224–33. doi: 10.1038/sj.onc.1209775
183. Nurieva RI, Podd A, Chen Y, Alekseev AM, Yu M, Qi X, et al. STAT5 protein negatively regulates T follicular helper (Tfh) cell generation and function. *J Biol Chem.* (2012) 287:11234–9. doi: 10.1074/jbc.M111.324046
184. Schmitt N, Liu Y, Bentebibel SE, Munagala I, Bourdery L, Venuprasad K, et al. The cytokine TGF-beta co-opts signaling via STAT3-STAT4 to promote the differentiation of human TFH cells. *Nat Immunol.* (2014) 15:856–65. doi: 10.1038/ni.2947
185. Suto A, Kashiwakuma D, Kagami S-I, Hirose K, Watanabe N, Yokote K, et al. Development and characterization of IL-21-producing $CD4^+$ T cells. *J Exp Med.* (2008) 205:1369–79. doi: 10.1084/jem.20072057
186. Marshall HD, Ray JP, Laidlaw BJ, Zhang N, Gawande D, Staron MM, et al. The transforming growth factor beta signaling pathway is critical for the formation of CD4 T follicular helper cells and isotype-switched antibody responses in the lung mucosa. *Elife* (2015) 4:e04851. doi: 10.7554/eLife.04851
187. Cejas PJ, Walsh MC, Pearce EL, Han D, Harms GM, Artis D, et al. TRAF6 inhibits Th17 differentiation and TGF-beta-mediated suppression of IL-2. *Blood* (2010) 115:4750–7. doi: 10.1182/blood-2009-09-242768
188. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol.* (2009) 27:693–733. doi: 10.1146/annurev.immunol.021908.132641
189. Sun SC. The non-canonical NF-kappaB pathway in immunity and inflammation. *Nat Rev Immunol.* (2017) 17:545–58. doi: 10.1038/nri.2017.52
190. Serre K, Mohr E, Benezech C, Bird R, Khan M, Caamano JH, et al. Selective effects of NF-kappaB1 deficiency in $CD4^+$ T cells on Th2 and TFh induction by alum-precipitated protein vaccines. *Eur J Immunol.* (2011) 41:1573–82. doi: 10.1002/eji.201041126
191. Kontgen F, Grumont RJ, Strasser A, Metcalf D, Li R, Tarlinton D, et al. Mice lacking the c-rel proto-oncogene exhibit defects in lymphocyte proliferation, humoral immunity, and interleukin-2 expression. *Genes Dev.* (1995) 9:1965–77. doi: 10.1101/gad.9.16.1965
192. Chen G, Hardy K, Bunting K, Daley S, Ma L, Shannon MF. Regulation of the IL-21 gene by the NF-kappaB transcription factor c-Rel. *J Immunol.* (2010) 185:2350–9. doi: 10.4049/jimmunol.1000317
193. Liu WH, Kang SG, Huang Z, Wu C-J, Jin HY, Maine CJ, et al. A miR-155-Peli1-c-Rel pathway controls the generation and function of T follicular helper cells. *J Exp Med.* (2016) 213:1901–19. doi: 10.1084/jem.20160204
194. Vasanthakumar A, Liao Y, Teh P, Pascutti MF, Oja AE, Garnham AL, et al. The TNF Receptor superfamily-NF-kappaB axis is critical to maintain

effector regulatory T cells in lymphoid and non-lymphoid tissues. *Cell Rep.* (2017) 20:2906–20. doi: 10.1016/j.celrep.2017.08.068
195. Sun L, Deng L, Ea CK, Xia ZP, Chen ZJ. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol Cell* (2004) 14:289–301. doi: 10.1016/S1097-2765(04)00236-9
196. Hu H, Wu X, Jin W, Chang M, Cheng X, Sun S-C. Noncanonical NF-κB regulates inducible costimulator (ICOS) ligand expression and T follicular helper cell development. *Proc Nat Acad Sci USA.* (2011) 108:12827–32. doi: 10.1073/pnas.1105774108
197. De Silva NS, Anderson MM, Carette A, Silva K, Heise N, Bhagat G, et al. Transcription factors of the alternative NF-kappaB pathway are required for germinal center B-cell development. *Proc Nat Acad Sci USA.* (2016) 113:9063–8. doi: 10.1073/pnas.1602728113
198. Gardam S, Sierro F, Basten A, Mackay F, Brink R. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. *Immunity* (2008) 28:391–401. doi: 10.1016/j.immuni.2008.01.009
199. Liao G, Zhang M, Harhaj EW, Sun SC. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. *J Biol Chem.* (2004) 279:26243–50. doi: 10.1074/jbc.M403286200
200. Vallabhapurapu S, Matsuzawa A, Zhang W, Tseng PH, Keats JJ, Wang H, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling. *Nat Immunol.* (2008) 9:1364–70. doi: 10.1038/ni.1678
201. Zarnegar BJ, Wang Y, Mahoney DJ, Dempsey PW, Cheung HH, He J, et al. Noncanonical NF-kappaB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nat Immunol.* (2008) 9:1371–8. doi: 10.1038/ni.1676
202. Xiao X, Balasubramanian S, Liu W, Chu X, Wang H, Taparowsky EJ, et al. OX40 signaling favors the induction of T(H)9 cells and airway inflammation. *Nat Immunol.* (2012) 13:981–90. doi: 10.1038/ni.2390
203. Bryce PJ, Oyoshi MK, Kawamoto S, Oettgen HC, Tsitsikov EN. TRAF1 regulates Th2 differentiation, allergic inflammation and nuclear localization of the Th2 transcription factor, NIP45. *Int Immunol.* (2006) 18:101–11. doi: 10.1093/intimm/dxh354
204. Pullen SS, Miller HG, Everdeen DS, Dang TTA, Crute JJ, Kehry MR. CD40-Tumor necrosis factor receptor-associated factor (TRAF) interactions: regulation of CD40 signaling through multiple TRAF binding sites and TRAF hetero-oligomerization. *Biochemistry* (1998) 37:11836–45. doi: 10.1021/bi981067q
205. Nguyen LT, Duncan GS, Mirtsos C, Ng M, Speiser DE, Shahinian A, et al. TRAF2 deficiency results in hyperactivity of certain TNFR1 signals and impairment of CD40-mediated responses. *Immunity* (1999) 11:379–89. doi: 10.1016/S1074-7613(00)80113-2
206. Jabara HH, Weng Y, Sannikova T, Geha RS. TRAF2 and TRAF3 independently mediate Ig class switching driven by CD40. *Int Immunol.* (2009) 21:477–88. doi: 10.1093/intimm/dxp013
207. Grech AP, Amesbury M, Chan T, Gardam S, Basten A, Brink R. TRAF2 differentially regulates the canonical and noncanonical pathways of NF-kappaB activation in mature B cells. *Immunity* (2004) 21:629–42. doi: 10.1016/j.immuni.2004.09.011
208. Lee SY, Reichlin A, Santana A, Sokol KA, Nussenzweig MC, Choi Y. TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. *Immunity* (1997) 7:703–13. doi: 10.1016/S1074-7613(00)80390-8
209. Cannons JL, Bertram EM, Watts TH. Cutting edge: profound defect in T cell responses in TNF receptor-associated factor 2 dominant negative mice. *J Immunol.* (2002) 169:2828–31. doi: 10.4049/jimmunol.169.6.2828
210. So T, Choi H, Croft M. OX40 complexes with phosphoinositide 3-kinase and protein kinase B (PKB) to augment TCR-dependent PKB signaling. *J Immunol.* (2011) 186:3547–55. doi: 10.4049/jimmunol.1003156
211. Xu Y, Cheng G, Baltimore D. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity* (1996) 5:407–15. doi: 10.1016/S1074-7613(00)80497-5
212. Xie P, Stunz LL, Larison KD, Yang B, Bishop GA. Tumor necrosis factor receptor-associated factor 3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. *Immunity* (2007) 27:253–67. doi: 10.1016/j.immuni.2007.07.012
213. Gardam S, Turner VM, Anderton H, Limaye S, Basten A, Koentgen F, et al. Deletion of cIAP1 and cIAP2 in murine B lymphocytes constitutively activates cell survival pathways and inactivates the germinal center response. *Blood* (2011) 117:4041–51. doi: 10.1182/blood-2010-10-312793
214. Zapata JM, Llobet D, Krajewska M, Lefebvre S, Kress CL, Reed JC. Lymphocyte-specific TRAF3 transgenic mice have enhanced humoral responses and develop plasmacytosis, autoimmunity, inflammation, and cancer. *Blood* (2009) 113:4595–603. doi: 10.1182/blood-2008-07-165456
215. Chang JH, Hu H, Jin J, Puebla-Osorio N, Xiao Y, Gilbert BE, et al. TRAF3 regulates the effector function of regulatory T cells and humoral immune responses. *J Exp Med.* (2014) 211:137–51. doi: 10.1084/jem.20131019
216. Michel M, Wilhelmi I, Schultz AS, Preussner M, Heyd F. Activation-induced tumor necrosis factor receptor-associated factor 3 (Traf3) alternative splicing controls the noncanonical nuclear factor kappaB pathway and chemokine expression in human T cells. *J Biol Chem.* (2014) 289:13651–60. doi: 10.1074/jbc.M113.526269
217. Takaori-Kondo A, Hori T, Fukunaga K, Morita R, Kawamata S, Uchiyama T. Both amino- and carboxyl-terminal domains of TRAF3 negatively regulate NF-kappaB activation induced by OX40 signaling. *Biochem Biophys Res Commun.* (2000) 272:856–63. doi: 10.1006/bbrc.2000.2860
218. Cherfils-Vicini J, Vingert B, Varin A, Tartour E, Fridman W-H, Sautès-Fridman C, et al. (2008). Characterization of immune functions in TRAF4-deficient mice. *Immunology* 124:562–74. doi: 10.1111/j.1365-2567.2008.02810.x
219. Nakano H, Sakon S, Koseki H, Takemori T, Tada K, Matsumoto M, et al. Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. *Proc Nat Acad Sci USA.* (1999) 96:9803–8. doi: 10.1073/pnas.96.17.9803
220. So T, Salek-Ardakani S, Nakano H, Ware CF, Croft M. TNF receptor-associated factor 5 limits the induction of Th2 immune responses. *J Immunol.* (2004) 172:4292–7. doi: 10.4049/jimmunol.172.7.4292
221. Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, et al. Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. *Genes Cells* (1999) 4:353–62. doi: 10.1046/j.1365-2443.1999.00265.x
222. Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, et al. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev.* (1999) 13:1015–24. doi: 10.1101/gad.13.8.1015
223. Jalukar SV, Hostager BS, Bishop GA. Characterization of the roles of TNF receptor-associated factor 6 in CD40-mediated B lymphocyte effector functions. *J Immunol.* (2000) 164:623–30. doi: 10.4049/jimmunol.164.2.623
224. Ahonen C, Manning E, Erickson LD, O'Connor B, Lind EF, Pullen SS, et al. The CD40-TRAF6 axis controls affinity maturation and the generation of long-lived plasma cells. *Nat Immunol.* (2002) 3:451–6. doi: 10.1038/ni792
225. Kobayashi T, Kim TS, Jacob A, Walsh MC, Kadono Y, Fuentes-Panana E, et al. TRAF6 is required for generation of the B-1a B cell compartment as well as T cell-dependent and -independent humoral immune responses. *PLoS ONE* (2009) 4:e4736. doi: 10.1371/journal.pone.0004736
226. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity* (2007) 26:371–81. doi: 10.1016/j.immuni.2007.02.009

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 15 November 2018
doi: 10.3389/fimmu.2018.02618

CD137 (4-1BB) Signalosome: Complexity Is a Matter of TRAFs

Juan M. Zapata[1,2]*, *Gema Perez-Chacon*[1,2], *Pablo Carr-Baena*[1], *Ivan Martinez-Forero*[3], *Arantza Azpilikueta*[3], *Itziar Otano*[3] *and Ignacio Melero*[3,4,5,6,7]*

[1] *Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), Madrid, Spain,* [2] *Instituto de Investigación Hospital Universitario La Paz, Madrid, Spain,* [3] *Departamento de Inmunologia and Inmunoterapia, Centro de Investigación Medica Aplicada, Universidad de Navarra, Pamplona, Spain,* [4] *MSD, London, United Kingdom,* [5] *Departamento de Inmunologia e Inmunoterapia, Clinica Universitaria, Universidad de Navarra, Pamplona, Spain,* [6] *Instituto de Investigacion Sanitaria de Navarra, Pamplona, Spain,* [7] *Centro de Investigación Biomédica en Red Cáncer (CIBERONC), Madrid, Spain*

CD137 (4-1BB, Tnsfr9) is a member of the TNF-receptor (TNFR) superfamily without known intrinsic enzymatic activity in its cytoplasmic domain. Hence, akin to other members of the TNFR family, it relies on the TNFR-Associated-Factor (TRAF) family of adaptor proteins to build the CD137 signalosome for transducing signals into the cell. Thus, upon CD137 activation by binding of CD137L trimers or by crosslinking with agonist monoclonal antibodies, TRAF1, TRAF2, and TRAF3 are readily recruited to the cytoplasmic domain of CD137, likely as homo- and/or heterotrimers with different configurations, initiating the construction of the CD137 signalosome. The formation of TRAF2-RING dimers between TRAF2 molecules from contiguous trimers would help to establish a multimeric structure of TRAF-trimers that is probably essential for CD137 signaling. In addition, available studies have identified a large number of proteins that are recruited to CD137:TRAF complexes including ubiquitin ligases and proteases, kinases, and modulatory proteins. Working in a coordinated fashion, these CD137-signalosomes will ultimately promote CD137-mediated T cell proliferation and survival and will endow T cells with stronger effector functions. Current evidence allows to envision the molecular events that might take place in the early stages of CD137-signalosome formation, underscoring the key roles of TRAFs and of K63 and K48-ubiquitination of target proteins in the signaling process. Understanding the composition and fine regulation of CD137-signalosomes assembly and disassembly will be key to improve the therapeutic activities of chimeric antigen receptors (CARs) encompassing the CD137 cytoplasmic domain and a new generation of CD137 agonists for the treatment of cancer.

Keywords: CD137, 4-1BB, TNFR, TRAF1, TRAF2, TRAF3, Immunotherapy, cytotoxic T lymphocytes (CTL)

OPEN ACCESS

Edited by:
Tania H. Watts,
University of Toronto, Canada

Reviewed by:
Shao-Cong Sun,
University of Texas MD Anderson Cancer Center, United States
Haopeng Wang,
ShanghaiTech University, China
Hyun Ho Park,
Chung-Ang University, South Korea

***Correspondence:**
Juan M. Zapata
jmzapata@iib.uam.es
Ignacio Melero
imelero@unav.es

Specialty section:
This article was submitted to T Cell Biology, a section of the journal Frontiers in Immunology

Received: *03 September 2018*
Accepted: *24 October 2018*
Published: *15 November 2018*

Citation:
Zapata JM, Perez-Chacon G, Carr-Baena P, Martinez-Forero I, Azpilikueta A, Otano I and Melero I (2018) CD137 (4-1BB) Signalosome: Complexity Is a Matter of TRAFs. Front. Immunol. 9:2618. doi: 10.3389/fimmu.2018.02618

BRIEF INTRODUCTION TO THE TRAF PROTEIN FAMILY

TNF Receptor Associated Factors (TRAFs) are a family of 6 proteins (TRAF1 to 6) characterized for having a protein region composed by a coiled coil followed by a seven-eight anti-parallel β-sheets at the C-terminus of the protein forming what has been coined as the TRAF domain (TD) (1, 2). This domain is also known as the Meprin and TRAF-C homology domains (MATH), since meprins, a family of extracellular proteases, also have a protein domain with high homology to the TD (3). In addition, there are also 3 proteins in humans encompassing internal *bona fide* TRAF

domains: tripartite motif (TRIM)-37, ubiquitin specific protease (USP)-7 and speckle-type POZ protein (SPOP) (4). Of note is that there is a protein known as TRAF7 that lacks a TD but has a RING and zinc finger domains similar to those of some members of the TRAF family proteins (5) and whose membership to the TRAF family is controversial.

TRAF1 to 6 were first identified as TNF-Receptor (TNFR) binding proteins, but it soon become evident that different members of the TRAF family were also involved in the regulation of pattern recognition receptors, including members of the Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and retinoic acid-inducible gene (RIG)-1-like Receptors (RLRs), thus demonstrating the key role of TRAF family proteins in the regulation of both innate and adaptive immunity [Reviewed by (6)]. Moreover, some members of the TRAF family also regulate cytokine receptors (6, 7). A role for TRAF family members in development has also been described (8–10).

TRAFs are the molecules that first engage the activated TNFR and act as scaffold proteins recruiting other proteins, including kinases, ubiquitin ligases and deubiquitinases among other regulatory proteins to conform the TNFR-signalosome. TRAF family members, with the exception of TRAF1, have a RING finger domain that endows some of them with the capacity to act as E3 ubiquitin ligases. Thus, TRAFs can ubiquitinate different components of the signalosome, including the TRAFs themselves, and modulate the activity of the complex (6).

There is a redundancy in the ability of different members of the TRAF family to interact with similar TRAF-binding peptidyl regions located in the cytosolic tails of the TNFRs [reviewed in (1, 2, 11)]. Moreover, besides this critical binding region, the surrounding amino acids to the peptide core motif in the cytosolic tail of TNFRs might also provide structural constrains that may have an effect on the binding affinity. In addition, the crystal structures of TDs bound to the cytosolic region of distinct TNFR family members have shown that particular structural features of the TD of each TRAF family member, in particular of those forming the TNFR-binding crevice, are critical in determining their specificities and binding affinities to the TNFRs [reviewed in (12)]. Altogether, these differences determine the binding specificity and affinity of the members of the TRAF family for the different TNFR family members (1, 11–14). Therefore, it is expected that a competition would be established between different TRAFs trimers to dock at the ligand-activated TNFR trimer, raising the possibility that neighboring TNFR trimers in the very same cell will hold TRAF trimers with different configurations. In addition, some TRAF family members can form heterotrimers (see below and **Figure 1**), adding further complexity to the system. Consequently, the composition of the signalosome mounted by each member of the TNFR family is likely to be highly influenced by the recruited TRAF family members. Besides, the signalosome composition would likely be cell type and activation state dependent, as it will be contingent on the expression levels and subcellular localization of the different proteins that could be part of this complex.

CD137 AS A MODEL OF HOW TRAFS CONFIGURE A TNFR-SIGNALOSOME

CD137 (4-1BB, TNFSFR9) is one of the TNFRs having a more restricted number of TRAF family members involved in its regulation, since only TRAF1, TRAF2, and TRAF3 interact with and control CD137 activity. CD137 is a member of the TNFR family whose expression is highly induced in CD8 T and NK lymphocytes upon activation, where it works as a critical costimulatory receptor (16–18). Moderate to low levels of CD137 expression could also be found in other activated immune populations, including CD4 T cells, B cells, monocytes, macrophages, granulocytes and dendritic cells and, in these cells, CD137 can also convey costimulatory signals (17, 19).

CD137 delivers potent costimulatory signals to the activated CTLs and memory T cells promoting cell proliferation and survival and also endowing CD8 T cells with CTL effector functions. As such, in the last 15 years, CD137 has become one of the most exciting targets to enhance anti-cancer immunity for its ability of boosting CTLs with anti-tumor effector functions (20–22).

CD137 binds to CD137-Ligand (CD137L, 4-1BBL, or tnfsf9), a member of the TNF superfamily (TNFSF). CD137L is mostly expressed on macrophages, activated B cells, and dendritic cells (23). In this regard, it is noteworthy that antigen presenting dendritic cells in tumors and tumor draining lymph nodes and tumor associated macrophages seem to be responsible for providing CD137L to cytotoxic T lymphocytes (CTLs) migrating to tumors (24). CD137L remains the sole intercellular ligand known for CD137, but binding of CD137 to extracellular matrix proteins, such as fibronectin, vitronectin, laminin and collagen VI (25) has been reported, albeit functional consequences of the binding to these additional putative ligands remain unknown. Interestingly, binding of CD137 to galectin-9, a member of the β-galactoside–binding family of lectins, has also been shown (26). Interestingly, galectin-9 binding to CD137 does not interfere with the binding of either CD137L or agonistic anti-CD137 mAbs to the receptor. Instead, it positively regulates CD137 function by keeping preassembled CD137 complexes together (26), which could be then further cross-linked by CD137L or by anti-CD137 mAbs.

The crystal structure of the CD137L trimer shows distinctive structural features that differ from those of other TNF family members. In this regard, CD137L trimer resembles a three-bladed propeller which is different from the cork-like shape of the trimers of other members of the family (27). This shape also confers some structural particularities to CD137/CD137L complex, which folds as a windmill-like shape structure. Despite these structural differences with other TNF and TNFR family members, these results are still fully consistent with a model for CD137/CD137L interaction similar to that of other members of the TNFR family, in which a trimeric ligand binding to three receptors conforms the basic unit of signaling (28, 29).

As for many other members of the TNFRSF, CD137 uses TRAFs as scaffold proteins to build its signalosome. CD137

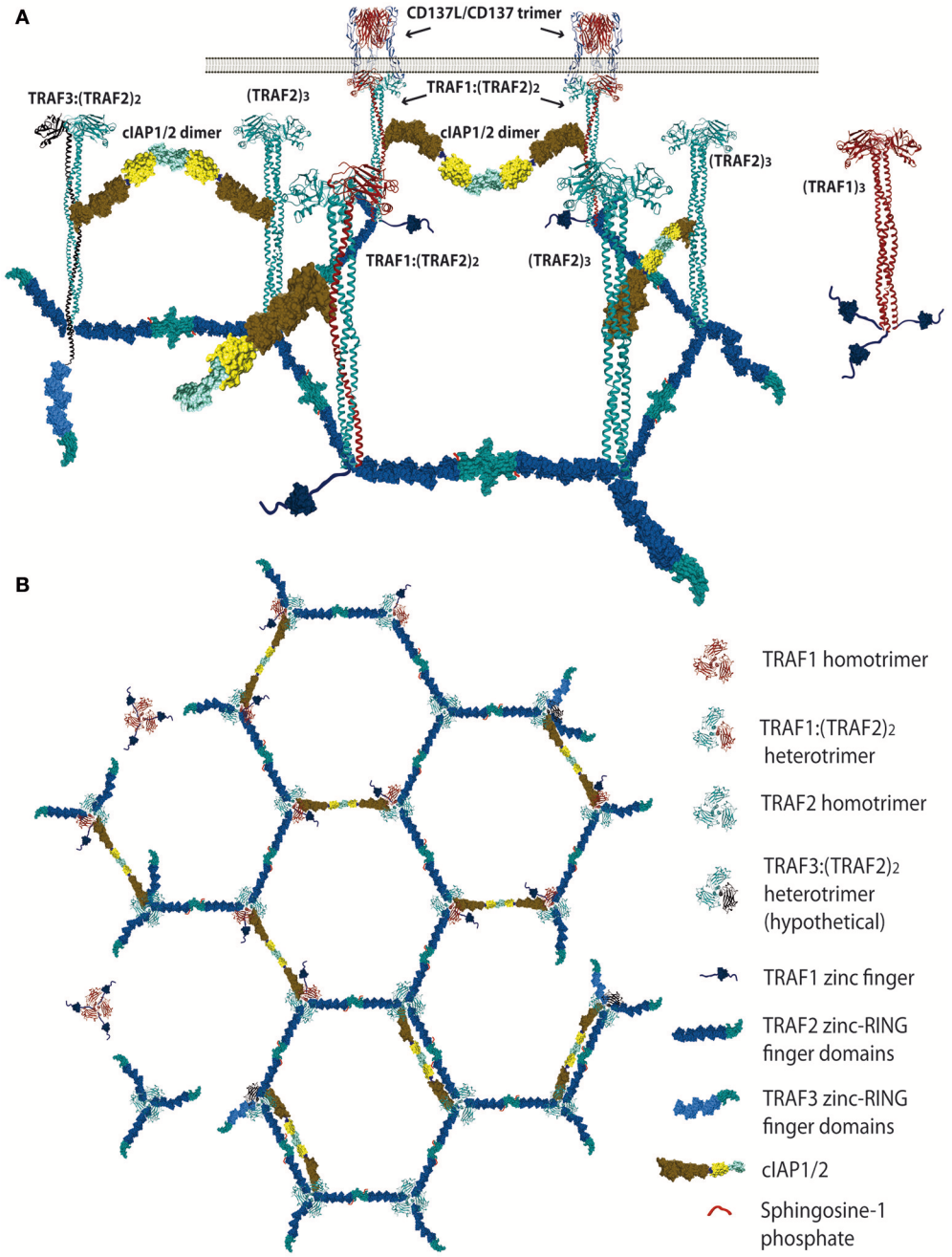


FIGURE 1 | Schematic representation of the proposed TRAF trimer configurations and interactions in the CD137L/CD137 hexagonal lattice. **(A)** Lateral view representing the various TRAF-trimer configurations that could be recruited to the activated CD137 trimers. The figure also shows the TRAF2-RING finger dimers that would likely be formed between the RING finger domains of two TRAF2 molecules from adjacent trimers, which is a requirement for E3 ubiquitin ligase activity. Similar interactions between the RING domains of cIAP1/2 from contiguous trimers are also expected. **(B)** It is show in top view how the CD137-recruited TRAF trimers would arrange forming a large hexagonal network that would be stabilized by the establishment of RING finger domains dimers between the TRAF2 molecules from adjacent trimers or between the RING finger domains of contiguous cIAP1/2 molecules. Further explanation in the text. Protein structure coordinates were obtained from the PDB database and molecular graphics were performed with UCSF Chimera (15).

has been found to bind to TRAF1, TRAF2, and TRAF3 (30–32) through two poly-acidic TRAF-binding consensus regions located in its cytosolic tail $_{234}$TTQEE$_{238}$ and $_{246}$PEEEE$_{250}$, which are similar to those found in other TNFR family members [reviewed in (1, 2)]. Point mutations studies showed that all three TRAFs seem to have binding preferences for the C-terminal $_{246}$PEEEE$_{250}$ TRAF-binding region, suggesting that they might compete with each other for interacting with the activated

receptor (31). Due to the proximity of the two TRAF binding sites, binding of one TRAF trimer to one of these regions, would render the other region unavailable by steric hindrance. However, this does not rule out the presence of different TRAFs associated to the same activated CD137 trimer, since TRAF1 and TRAF2 form heterotrimers that can associate to the activated TNFR (33).

Cross-linking of CD137 by either CD137L (30, 34) or bivalent agonistic antibodies (35) readily results in the recruitment of TRAF1 and TRAF2 to the receptor. The involvement of both TRAF family members in the regulation of CD137 signaling and function is further confirmed by several reports showing that CD137 activity is significantly affected in model systems lacking of either TRAF1 or TRAF2 (32, 36–38). However, the role of TRAF3 as a scaffold protein building the CD137 signalosome has not been confirmed and awaits further research, although the evidence indicating the induction of NF-kB2 activation by CD137 (38) implies that TRAF3 should be directly or indirectly recruited to the CD137 signalosome (see below). In addition, recent evidence shows that TRAF3, as well as TRAF1 and TRAF2, are essential for the activity of CD137-based chimeric antigen receptors (CARs) (39), further supporting TRAF3 role in CD137 function.

The absence of a RING finger domain in TRAF1 indicates that it lacks any E3 ubiquitin ligase activity and no other intrinsic enzymatic activity for TRAF1 has been identified so far (6, 40). However, TRAF1 interacts with and regulates the activity of a variety of ubiquitin ligases and proteases (33, 41, 42) and it plays critical roles in the regulation of several members of the TNFR family [Reviewed in (43)]. Initially, since TRAF1 expression is induced upon cell activation and it has similar TNFR-binding preferences than TRAF2, it was thought that TRAF1 would work toning down TNFR signaling in activated cells by outcompeting TRAF2 from binding to the TNFRs (43). Indeed, T cells from *Traf1*-deficient mice were hyper-responsive to TNF, supporting a role for TRAF1 as a negative TNFR2 regulator (44). However, it was soon recognized that TRAF1 was not just a TRAF2 competitor but, in some instances, rather the contrary. In this regard, TRAF1 positively modulates CD40 activity by cooperating with its activity and preventing TRAF2 degradation (45, 46). In addition, TRAF1 has been also implicated in CD137-mediated survival of activated CTL (47, 48) and of memory T cells (49).

The other TRAF family members that is critical for CD137 function is TRAF2. The RING domain that TRAF2 encompasses at its N-terminus endows it with an E3 ubiquitin ligase activity. Ubiquitin-conjugating protein (Ubc)-13 (Ube2N) is thought to be TRAF2 major E2 enzyme companion, providing TRAF2 with the capacity of mediating K63-ubiquitination and subsequent activation of itself and other target proteins (50–52). In addition, TRAF2 can also catalyze K48-ubiquitination of target proteins (53, 54). Interestingly, the crystal structure of the TRAF2 RING and the first zinc finger domains described by Wu et al. (55) revealed structural constrains that would preclude Ubc13 and other related E2 ubiquitin ligases from binding to the TRAF2 RING, raising questions on the actual ability of TRAF2 to act as an E3 ubiquitin ligase. However, these discrepancies were solved when sphingosine-1 phosphate (S1P) was identified as a cofactor required for TRAF2 E3 ubiquitin ligase activity (50). Indeed, S1P seems to act as a bridge between the RING finger domain of TRAF2 and the E2 proteins. Thus, in the presence of S1P, TRAF2 was able to ubiquitinate RIP1 and itself (and/or other TRAF2 molecules in the trimer) at K63 in the presence of Ubc13 or Ubc5 (Ube2D) (50).

CD137 SIGNALING: A RELATION OF KNOWN AND SUSPECTED EVENTS

While TRAF2 is expressed in resting and activated T lymphocytes, TRAF1 expression is induced upon activation (60, 61). Thus, as CD137 expression will also be induced in activated T cells (16, 17), both CD137 and TRAF1 will likely coexist in activated T cells where CD137 costimulatory activity is needed for CTL expansion and for boosting effector functions. Therefore, the composition of the CD137-TRAF signaling complexes would depends on the activation state of the cell and the relative expression levels of TRAF1 and TRAF2.

The kinetics of CD137 expression in activated CD8 T cells implies that at early activation stages low levels of CD137 will be found on the T cell surface (62, 63). However, even these low levels might be sufficient to trigger CD137 signaling upon interaction with the CD137L. In this regard, it has been proposed that the ligand-free form of TNFR family members exists on the cell surface as anti-parallel dimers arranged in a two-dimensional hexagonal lattice that brings three receptor monomers together at each lattice point [Reviewed in (28)]. This model would imply that even low level of ligand-free TNFRs might be already prearranged on the cell surface in high-density spots. In the case of CD137, galectin-9 might contribute to the maintenance of these bi-dimensional hexagonal structures (26). Assuming this model, when CD137L or other TNF family member and their corresponding TNFRs come together, the ligand trimer will shift the equilibrium from the CD137 dimeric interaction to the CD137 trimeric structure. The CD137/CD137L trimers will still occupy each lattice point preserving the hexagonal structure and maintaining neighboring activated CD137 trimers close, thus facilitating the establishment of molecular interactions between adjacent trimers. TRAF trimers will be readily recruited to the activated CD137 receptor binding to the poly-acidic TRAF-binding consensus regions located in CD137 cytosolic tail. As stated above, the composition of the TRAF trimers that would be recruited to the activated CD137 will likely depend on the expression levels of the TRAF family proteins that interact with CD137, which are TRAF1, TRAF2, and TRAF3. Since TRAF1 and TRAF2 have been shown to be critical for CD137 activity it is likely that these two TRAF family members will have a major role in building the CD137 signalosome. Wu and coworkers (33) have shown that TRAF1 and TRAF2 can associate in heterotrimers, but preferentially forming a trimer with a TRAF1:$(TRAF2)_2$ configuration. Therefore, a mix of TRAF1 and TRAF2 homotrimers and TRAF1:$(TRAF2)_2$ heterotrimers would be recruited to the activated CD137 in a way that would depends on their amounts and specific affinities to the TNFR.

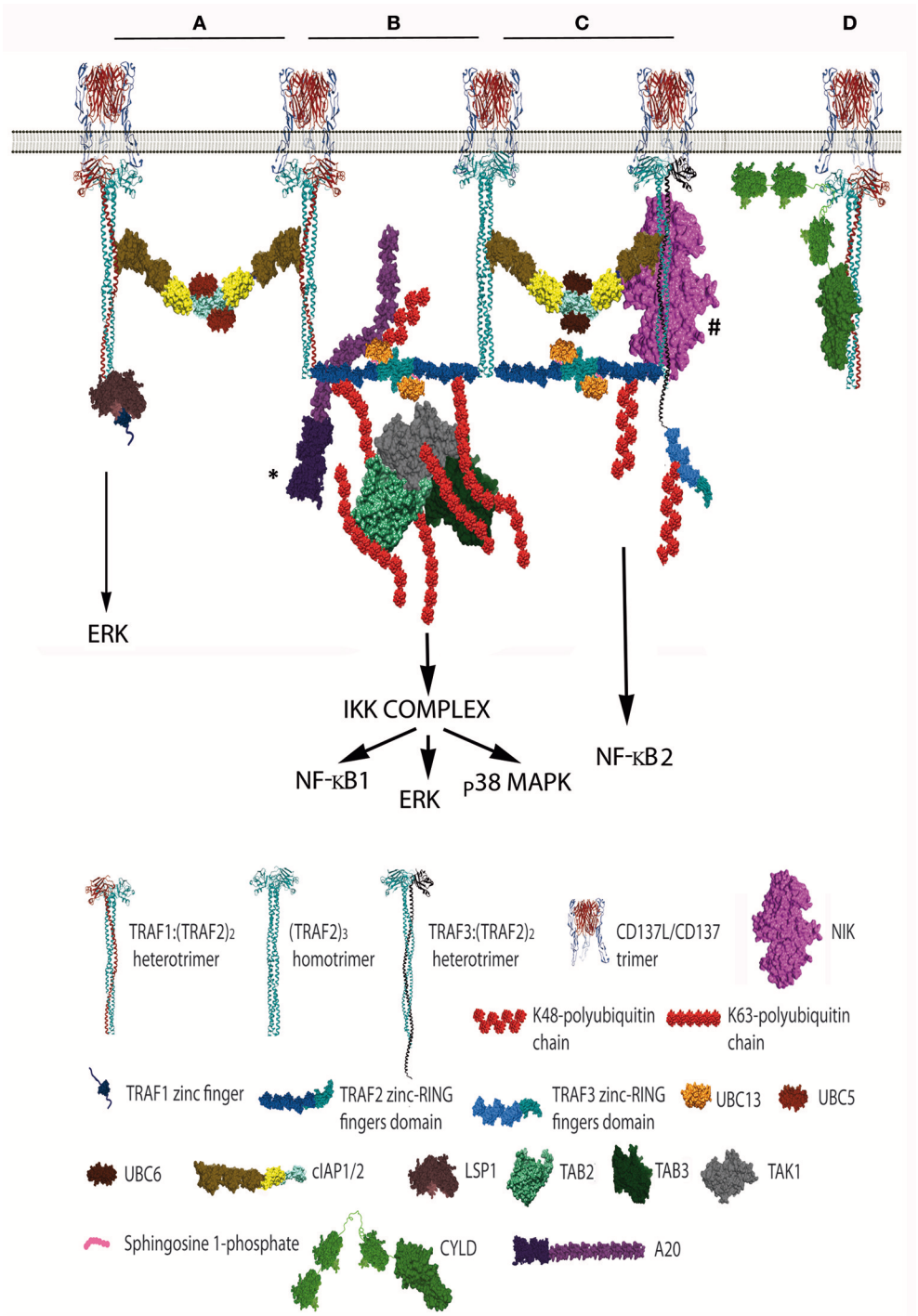


FIGURE 2 | Schematic representation of the distinct CD137 signalosomes that would be formed upon CD137 activation. This figure illustrates the distinct signalosomes that could be formed in response to CD137 activation depending on the TRAF trimer configurations that get associated to the activated CD137. **(A)** cIAP1/2 bridging between 2 TRAF1(TRAF2)$_2$ trimers. What other molecules, besides E2 proteins, would be specifically recruited to this configuration is yet unknown. The binding of Lymphocyte specific protein-1 to the N-terminal region of TRAF1 is shown. **(B)** The formation of a dimer between the RING finger domains of 2 TRAF2 molecules from adjacent trimers will trigger K63 ubiquitination of TRAF2 and the subsequent recruitment and activation of the TAK1/TAB1/TAB2/TAB3 complex (TAB1 is not shown). K63-TAK1-mediated IKKβ phosphorylation will activate the IKK complex activation initiating a signaling cascade that will result in NF-κB1 and ERK activation. A20 might inhibit this signaling cascade by K48-ubiquitinating Ubc13 thus inhibiting TRAF2 E3 ubiquitin ligase activity. * A20 can form dimers, but a sole A20 molecule is represented for clarity. **(C)** Hypothetical organization of a signalosome that includes a TRAF3:(TRAF2)$_2$ trimer. The cIAP1/2 molecules associated either to a TRAF2 homotrimer and the hypothetical TRAF3:(TRAF2)$_2$ trimer will form a dimer by the interaction of their RING fingers domains causing the activation of

(Continued)

FIGURE 2 | the E3 ubiquitin ligase activity. Thus, the cIAP1/2 dimer will K48-ubiquitinate TRAF3 and TRAF2 molecules targeting them for proteasome degradation and effectively releasing NIK from its interaction with TRAF3, resulting in the activation of NF-κB2 as has been observed following CD137 stimulation. # The TRAF region binding to NIK is still controversial, since reports indicating that is mediated by either the TRAF domain (56, 57) or the RING-zinc finger region (58, 59) are available. **(D)** CYLD interacts with the same crevice in the TRAF domain that binds to CD137 cytosolic tail. CYLD might works as a gate keeper preventing ligand-independent TRAF activation but it might also participate in the termination of CD137 signaling by outcompeting CD137 from binding to TRAF2 as shown in the figure. Further explanation in the text. Protein structure coordinates were obtained from the PDB database and molecular graphics were performed with UCSF Chimera (15). When this information was absent for a protein of interest, we modeled the proteins according to their domains using available structures of similar domains to provide an approximate representation of the protein structure and size.

Since the five zinc fingers and the RING finger domains of each TRAF2 molecule in the trimers will likely emanate from the intertwining coils in opposite directions (28, 64) and the active E3 ubiquitin ligase requires the formation of RING-finger dimers (65), TRAF2-RING finger dimers will likely be formed by the RING finger domains of two TRAF2 molecules from adjacent trimers, similar to what has been described for TRAF6 (66) (**Figure 1**). This inter-trimer bonding would help the clustering and stabilization of the two-dimensional hexagonal lattice (28, 64). In this case, since TRAF1 lacks of a RING finger domain, the presence of one TRAF1 molecule in a trimer would impede the formation of one intertrimer bonding but would not have any effect on the ability of the 2 TRAF2 molecules in the trimer to establish these TRAF2-RING dimers with neighboring TRAF2-containing trimers (**Figure 1**). The TRAF2-RINGs now in their active dimeric form will bind S1P and Ubc proteins (Ubc13 or UbcH5A) (**Figure 2**), getting ready to catalyze the K63-ubiquitination of TRAF2 itself and other target proteins (50). In addition TRAF2 trimers and TRAF1:$(TRAF2)_2$ heterotrimers, but not TRAF1 trimers, will recruit a single cIAP1/2 (33). Indeed, cIAP1/2 will interact through its BIR1 domain (67) with the TRAF trimers by asymmetrically engaging two cIAP-interacting motifs in the coiled coil of two TRAF molecules in the trimer (33, 42). Of note is that cIAP1/2 interaction with the TRAF1:$(TRAF2)_2$ heterotrimers is stronger than that with TRAF2 trimers and, therefore, cIAP1/2 would preferentially be bond to the TRAF1:$(TRAF2)_2$ heterotrimers. Interestingly, TRAF1 homotrimers have a cIAP2 dissociation constant two orders of magnitude weaker than that of TRAF2 homotrimers, effectively precluding the interaction of cIAP2 with TRAF1 homotrimers (33). The interaction of cIAP1/2 BIR1 domain with TRAFs would release the cIAP-RING from its inhibitory interaction with the cIAP-CARD domain (68), allowing the formation of cIAP1/2-RING dimers and the binding of the E2 ubiquitin ligases. Since only one cIAP1/2 molecule associates to a TRAF trimer, the cIAP1/2-RING dimer would have to be formed by two cIAP1/2 molecules each one associated to adjacent TRAF trimers in the hexagonal lattice (**Figures 1**, **2**), thus further bridging two neighbored activated TNFR complex. Altogether, these results indicate that albeit the basic signaling brick in CD137 (as well as of other TNFRs) would be a trimer, a trimer alone will not be able to signal as it seems absolutely necessary to establish inter-trimer bridging and multi-trimer clustering to build a functional signalosome (**Figures 1**, **2**).

In this regard, it is worth mentioning that since TRAF1 lacks RING finger domain and TRAF1 homotrimers cannot recruit cIAP1/2 to the CD137 signalosome, CD137-associated TRAF1 homotrimers would fail on bridging adjacent trimers through the formation of RING-dimers, which might result in the disruption of the hexagonal CD137 network and the inhibition of the signaling. While this scenario might provide a rationale for the TRAF1-mediated inhibitory effects on some members of the TNFR family (43), many evidence support a positive role for TRAF1 in CD137 signaling (37, 47–49, 69). Therefore, other proteins interacting with TRAF1 might not only provide new functionality to the signalosome but also might contribute to the clustering of the activated CD137 receptors. In this regard, it has been shown that recruitment to CD137 of leukocyte-specific protein-1, a protein involved in CD137-mediated ERK activation, is mediated by its interaction with TRAF1 (70) (**Figure 2A**). Furthermore, Watts and coworkers (71) have shown that the TRAF-domain of TRAF1 directly interacts with three components of the linear ubiquitination (LUBAC) complex, SHARPIN, HOIP, and HOIL-1. In addition, Greenfeld and coworkers (41) have shown that TRAF1 is a key component of the Epstein-Barr virus Late Membrane Protein (LMP)-1 signaling complex, a protein that mimics TNFRs and uses TRAF proteins as scaffold (72). In this model, LMP1 promoted the association between TRAF1 and LUBAC and stimulated the linear M1-linked poly-ubiquitination of TRAF1, thus allowing TRAF1-mediated recruitment of the M1-ubiquitin binding proteins IKKγ and deubiquitinase (DUB) A20 (41) (see below). TRAF2 was essential for both LUBAC interaction and M1-polyubiquitination of TRAF1 (41), strongly suggesting the participation of TRAF1:$(TRAF2)_2$ heterotrimers in this activity. In addition, binding of cIAP1 (73) and CYLD (74) to HOIP has also been reported. Although there is no evidence to date implicating LUBAC and M1-ubiquitination in the regulation of CD137 signalosome, research on this issue is warranted.

Soon after ligand activation, the growing CD137 signalosome gets decorated with K63-ubiquitinated proteins, mostly composed by K63-TRAF2 (36). Polyubiquitin chains linking the carboxyl terminus of ubiquitin molecules to the K63 of the next ubiquitin are well known as docking sites for downstream signaling components, and are required for building an effective signalosome (75–77). This is opposite to the polyubiquitination at K48, which in most cases targets proteins for proteosome-mediated degradation (77). TRAF2, associated to Ubc13 or UbcH5A, seems to be the main responsible of its own K63-ubiquitination (50) although cIAP1/2 associated to UbcH5A or Ubc13 also can catalyze K63-ubiquitination (78, 79). The next component of the CD137 signalosome getting recruited by K63-polyubiquitinated TRAF2 is a kinase complex composed by the transforming growth factor beta-activated

kinase (TAK)-1 and TAK binding proteins (TAB)-1, 2 and 3 and (**Figure 2B**). Once recruited, TAK1 will get K63-ubiquitinated by TRAF2 (80). Taking lessons from the mechanism recently described for TRAF6-mediated TAK1 activation (81), efficient TRAF6-mediated TAK1 activation requires the synthesis of long K63-polyubiquitin chains by TRAF6. These long K63-polyubiquitin chains would have to be recognized by TAB2 and 3 (82) irrespective of whether they remain conjugated to TRAF6 or been unanchored (81). Interestingly, A20 which is a component of the CD137 signalosome (see below), effectively removes long K63-linked polyubiquitin chains from TRAF6 without disassembling the chains themselves (83). Once activated, TAK1 will phosphorylate the inhibitor of nuclear factor κ-B kinase (IKK)-β leading to the activation of canonical NF-κB (75) and ERK1/2 (48). TAK1 will also induce the activation of mitogen activated kinases kinases (MKK) MAP kinases, leading to p38MAPK activation (84).

While ubiquitination is a chief mechanism controlling TRAF-mediated CD137 signaling, the regulation of TRAF activity by phosphorylation has also been described. In this regard, it has been shown that TRAF2 phosphorylation at T117 by PKC promotes both K63-ubiquitination of TRAF2 and the recruitment of the IKK complex to activated TNFRs (85). Moreover, TRAF1 phosphorylation at S139 by TANK-binding kinase inhibits NF-κB activation in response to CD137 engagement (86).

Besides the induction of the canonical NF-κB pathway by CD137, the activation of the alternative NF-κB pathway by this TNFR has also been reported (38). The molecular mechanism controlling NF-κB2 is different to that controlling the canonical NF-κB1 pathway. In non-activated cells, NF-κB2 activation is prevented by continuous NF-κB-inducing kinase (NIK) degradation by a complex formed by TRAF2/cIAP and TRAF3/NIK. In non-activated cells, this complex works promoting cIAP1/2 mediated K48-ubiquitination of NIK and its subsequent proteasome-mediated degradation. However, upon TNFR activation, binding of this complex to the receptor results in cIAP-dependent degradation of TRAF3 (and often also of TRAF2), releasing NIK and allowing p100 processing to the active p52 NF-κB subunit [reviewed in (87)]. Thus, the induction of NF-κB2 by CD137 (38) implies that TRAF3 and NIK would have to be recruited to the CD137 signalosome (**Figure 2C**). In support of this event, it has been shown that TRAF3 is degraded upon CD137 engagement (38). However, it is still unclear whether TRAF2 and TRAF3 would be recruited as homotrimers to adjacent CD137 trimers or as TRAF2/TRAF3 heterotrimers to one ligand-activated CD137 trimer. In this regard, there is evidence suggesting the existence of TRAF2/TRAF3 heterotrimers (88). In addition, it is noteworthy that TRAF1 has been shown to directly interact with NIK, suggesting that TRAF1:$(TRAF2)_2$-cIAP1/2 complexes can also be a component of the E3 ubiquitin ligase complex for NIK (33, 89). However, there are conflictive results on whether TRAF1 is an activator or an inhibitor of the NF-κB2 pathway. It has been proposed that the binding of TRAF1 to NIK causes the disruption of TRAF2:cIAP1/2 binding, resulting in NIK stabilization and NF-κB2 activation (89). However, studies on the role of TRAF1 in CD137-mediated NF-κB activation show that in the absence of TRAF1, NF-κB1 induction is restricted while NF-κB2 induction proceeds more efficiently (38). These results might indicate that the tighter association of cIAP1/2 to the TRAF1:$(TRAF2)_2$ heterotrimers compared to that of the TRAF2 homotrimers might restrict the ability of cIAP1/2 to shift their targets from NIK to TRAF3. Besides, it is also conceivable that an overabundance of TRAF1 might interact with all available TRAF2 molecules, thus precluding the formation of the TRAF2:TRAF3 heterotrimers. Interestingly, and as described above, TRAF1 protects TRAF2 from degradation (45, 46). Whether this protection could be caused by the inability of cIAP1/2 in the TRAF1:$(TRAF2)_2$ heterotrimers to K48-ubiquitinate TRAF2 while it could do it as part of the TRAF2 homotrimers deserves further investigation.

Adding just another level of complexity to an already crowded CD137 signalosome, we have recently observed the functional association of K63-DUBs A20 and CYLD to the CD137 signalosome. This interaction results in the downregulation of CD137-elicited K63-ubiquitination and signaling toward NF-κB activation in both primary T cells and transfected cell lines (90). A20 was first described as an ubiquitin-editing enzyme (91). It is composed of an N-terminal ovarian tumor (OTU) domain, which would catalyze the removal of K63-ubiquitin chains from target proteins, and a C-terminal zinc-finger domain region, which endows this protein with E3 ubiquitin ligase activity able to transfer K48-polyubiquitin chains to those target proteins, thereby promoting its proteasome-mediated degradation (92). However, recent evidence shows that A20 can efficiently remove K48-linkages but is almost inactive toward K63-linkages, raising questions on what is the actual mechanism by which A20 inhibits the NF-κB pathway [reviewed in (93)]. Interestingly, A20 is also able to inhibit K63-ubiquitination by promoting K48-ubiquitination and degradation of E2 ligases, such as Ubc13 and UbcH5C, thus effectively inhibiting the E3 ligase activity of TRAF2, cIAP1/2, and TRAF6 (94) (**Figure 2B**). In addition A20 can also inhibit NF-κB by interacting with and sequestering Nemo (IKKγ), thus impeding IKKβ activation without requiring the DUB and E3-ubiquitin ligase activities of A20 (95–97).

CYLD is another member of the DUB family. It contains three cytoskeletal-associated protein (CAP)-glycine conserved repeats at the N-terminus and a DUB domain at its C-terminus. CYLD has a TRAF-interacting motif and has been shown to interact with TRAF2 and to catalyze the removal of TRAF2-linked K63-ubiquitin chains, precluding IKK from being activated (98). Interestingly, phosphorylation of CYLD by IKK inhibits its DUB activity (99). This result opens the possibility that CYLD might works as a gate keeper preventing ligand-independent activation, and that once receptor signaling unlocks, CYLD would be kept inactive by the active IKK complex. Since CYLD has also been found associated to the CD137-signalosome (90), this suggests that CYLD might also participate in the termination of CD137 signaling by outcompeting CD137 from binding to TRAF2 (**Figure 2D**). Altogether, these results underscore the relevance of the ubiquitination and deubiquitination processes in the regulation of CD137 signaling, evidencing that the balance between K63- and K48-ubiquitination of key target proteins will

determine the outcome of the response. A summary of the role of TRAF1-3 in controlling CD137 signal transduction and function is provided in **Table 1**.

Finally, we have observed that upon ligation with anti-CD137 antibodies, CD137 signalosome becomes internalized and is transferred to an endosomal compartment in a K63-polyubiquitin-dependent manner (36). Nam and coworkers (34) showed that CD137 engagement caused its redistribution into lipid rafts, in a process that seems to be dependent on TRAF2 binding to Caveolin (100) and Filamin A (101), which are intrinsic components of the lipid rafts. Thus, the CD137 signalosome-containing endocyted vesicles might be caveolae that later fuse with early endosomes (102), but this awaits confirmation. Interestingly, So and Croft (103) have proposed that TRAF2-dependent recruitment of activated CD137 into lipid rafts might be behind the observed activation of PI3K-AKT signaling pathway by CD137. Lipid rafts are membrane microdomains that facilitate AKT recruitment and activation upon phosphatidylinositol-3,4,5-triphosphate accumulation in the plasma membrane (104). The mechanism involved in CD137-mediated PI3K/AKT activation is still unknown, although its relevance in promoting CD137-mediated T cell proliferation and apoptosis protection seems well sustained (105–107). As there is no evidence of a direct association of PI3K and/or AKT to the CD137 signalosome, PI3K-AKT ought to be activated by other signaling complexes, such as TCR/CD28, working together with CD137 (105, 107). Since activated TCR/CD28 reside in the lipid rafts, these lipid structures might work as multi-signaling hot-spots (103). Indeed, it would be plausible that the ligand-activated CD137 hexagonal lattice keeps trapped inside (in the center of the hexagons) TCR and CD28 complexes that would move together with the activated CD137 trimers to lipid-rafts, thus facilitating the response to antigen. However, since CD137-mediated AKT activation is delayed compared to that of ERK and NF-κB, taking hours instead of minutes (107), efficient CD137-mediated triggering of PI3K/AKT activity may require additional players (whose expression might even be induced by CD137 engagement) and/or further signaling-complexes compartmentalization to proceed.

Interestingly, we have observed that endocyted CD137 signalosome-containing vesicles remain decorated with K63-polyubiquitin chains, strongly suggesting that CD137 signaling is still active during this process (36). However, it is expected that the endosomes will later fuse with lysosomes to recycle its content. Interestingly, it has been shown that A20 can target TRAF2 to the lysosome for its degradation, which is dependent on the membrane tethering activity of A20, but not of its ubiquitin-modifying function (108). These CD137-mediated endocytosis experiments (36) were performed with agonist anti-CD137 mAbs and, therefore, it is yet to be determined whether CD137 engagement with CD137L would also cause the internalization of the complex, but it is likely that this will actually occurs for various reasons. First, CD137 internalization has been already observed in dendritic cells upon binding to CD137L fusion proteins used to target antigens for vaccination (109). Second, an accumulation of CD137 on the surface of *CD137L*-deficient T cells has been observed, probably as a result of the impossibility of CD137 to be internalized in the absence of CD137L (110). Third, because many key molecules in CD137 signaling, such as CD137, TRAF1, TRAF2 and cIAP1/2 are readily transcriptionally activated by NF-κB and AP1 transcription factors upon CD137 activation (111–114), restocking these molecules and ensuring CTL responsiveness to new CD137 costimulatory rounds. Finally, because it has been recently described that tonic chimeric antigen receptor (CAR)-derived CD137 signaling causes T cell toxicity by the continuous TRAF2-mediated NF-κB activation and increased Fas-dependent cell death (115). This result emphasizes the deleterious effects that unrestricted CD137-signaling would have in the cells and underscores the key role of the multiple mechanisms controlling CD137 signaling described above, including CD137 internalization.

UNDERSTANDING CD137 SIGNALING TO IMPROVE CD137-MEDIATED IMMUNOTHERAPY

CD137 has become one of the most relevant molecular targets in cancer immunotherapy for its ability to drive CTL and NK cells anti-tumor responses. Humanized anti-CD137 mAbs have entered the clinic (21). One of those (urelumab) showed promising anti-tumor effects as a monotherapy treatment in a phase I trial. Unfortunately, a follow-up Phase II trial revealed severe liver toxicity in a significant number of patients (10%) that resulted in two fatalities (116). Consequently, trials with urelumab as a monotherapy were terminated (117). A comprehensive safety analysis of patients treated with urelumab confirmed a strong association between hepatitis and the urelumab dose and resulted in dose reductions in subsequent clinical trials (118). In this regard, ongoing clinical trials with urelumab and other anti-human CD137 mAbs used in combinatory therapies are underway. Alternative approaches are needed to circumvent the off-target toxicity associated to these treatments while preserving their efficacy, for instance, by targeting these agonist antibodies or the natural ligand to surface molecules expressed on cells present in the tumor microenvironment (21). Another important strategy to improve anti-CD137 mAb anti-tumor activity, while limiting its side effects, would be boosting CD137-mediated signal transduction. Many aspects of CD137 signaling might be of interest for drug development, including interfering with negative regulators of CD137 signaling, promoting optimal complex/scaffold formation, and keeping signaling-CD137 endosomes from lysosome degradation, among others. These approaches have been neglected so far due to our limited understanding of the different mechanisms controlling CD137 signal transduction, a limitation that could also be extended to other members of the TNFR family.

These limitations would also apply to the usage of CD137 signaling for enhancing chimeric antigen receptor (CAR) T cell effectiveness. In this regard, transducing T cells with a CAR-construct containing the CD137 cytosolic tail together with the CAR-CD3ζ proved to be effective in increasing CTL cell survival,

TABLE 1 | Role of TRAF1,2 and 3 in CD137-mediated signaling.

	TRAF1	TRAF2	TRAF3
TRAF heterotrimers	TRAF2 (33)	TRAF1 (33) TRAF3 (suspected)	TRAF2 (suspected)
Ubiquitin ligase activity	no	K63 (49–52) K48 (53, 54)*	unknown
Substrates	no	TRAF2 (50) TAK1 (80)	unknown
Binding partners	cIAP1/2 (33) LSP-1 (70) NIK (33, 89) LUBAC (undetermined)	cIAP1/2 (33) ubcl3 (50) CYLD (98)	NIK (87)
Functional data	• CD137-mediated survival of memory T cells (49). • CD137-mediated antivirus responses (37, 49) • Required for activity of CD137-based chimeric antigen receptors (CARs) (39)	• CD137-mediated costimulatory signaling in T cells (32) • CD137-mediated tumor rejection in xenograft mice (36). • Required for activity of CD137-based chimeric antigen receptors (CARs) (39).	• Required for activity of CD137-based chimeric antigen receptors (CARs) (39)

The table summarizes the known function and binding partners of these TRAF proteins in the regulation of CD137 activity,as well as the available in vivo or ex vivo T cells functional data.
** not demonstrated in CD137 signaling.*

targeting of CTLs to the tumor and boosting anti-tumor activities (119). Remarkably, it has been recently demonstrated the clinical effectiveness of this therapy in the treatment of relapsed or refractory B-cell acute lymphoblastic leukemia (120, 121).

In the case of CD137 containing CARs, recent evidence shows that the activity of CD19-targeted CAR T cells with a CD137 endodomain is dependent on TRAF1, 2 and 3 and also on NF-κB activation (39). However, little is known on whether the molecular mechanisms controlling the extent of the response are similar to those of native CD137. In this regard, and as stated above, tonic chimeric antigen receptor (CAR)-derived CD137 signaling has been shown to cause T cell toxicity by the continuous TRAF2-mediated NF-κB activation and increased Fas-dependent cell death (115), thus highlighting the need of a better understanding of the molecular mechanisms controlling CD137 signaling. Moreover, the development of CAR T cells with CD137 intracellular tail acting in tandem with the cytoplasmic domain of CD3 ζ may promote a signaling crosstalk between these 2 pathways. Interestingly, and as discussed above, this crosstalking between CD137 and the TCR might be happening at certain extent in normal CD137-signaling (103). In any event, the CD137 component in the CAR T therapy is key to ensure the functional persistence and survival of the transduced T cells (119, 120) a feature ultimately needed for clinical efficacy, but also keeping in mind that unrestrained CD137 activity might also be deleterious for the cell (115). Translational research in the signal transduction pathways controling CD137-mediated responses should focus in the identification of druggable targets that would allow toning up or toning down CD137 activity as needed. In addition, developing tools for early and reliable detection of CD137-signaling events and/or their outcome would be paramount to define pharmacodynamic biomarkers and useful parameters to optimize new generations of CD137 agonists.

AUTHOR CONTRIBUTIONS

JZ, GP-C, PC-B, IM-F, AA, IO, and IM critically revised the manuscript for important intellectual content. JZ, GP-C, PC-B, and IM designed the figures. JZ and IM wrote the paper.

FUNDING

Funding was from Instituto de Salud Carlos III (ISCIII) (PI16/00895 to JZ), Ministerio de Economía y Competitividad (MINECO) RTC-16-5118-1 to JZ and IM, SAF 2014-52361-R and SAF 2017-83267-C2-1R to IM; and Cancer Research Institute (CRI) CLIP Grant 2017, Asociación Española Contra el Cancer, Fundación BBVA and I-ON Network-BMS to IM. The cost of this publication was paid in part by funds from the European Fund for Economic and Regional Development (FEDER).

REFERENCES

1. Chung JY, Park YC, Ye H, Wu H. All TRAFs are not created equal: common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J Cell Sci.* (2002) 115:679–88.
2. Zapata JM. TNF-receptor-associated factors as targets for drug development. *Expert Opin Ther Targets* (2003) 7:411–25. doi: 10.1517/14728222.7.3.411
3. Zapata JM, Martinez-Garcia V, Lefebvre S. Phylogeny of the TRAF/MATH domain. *Adv Exp Med Biol.* (2007) 597:1–24. doi: 10.1007/978-0-387-70630-6_1
4. Zapata JM, Pawlowski K, Haas E, Ware CF, Godzik A, Reed JC. A diverse family of proteins containing tumor necrosis factor receptor-associated factor domains. *J Biol Chem.* (2001) 276:24242–52. doi: 10.1074/jbc.M100354200
5. Xu LG, Li LY, Shu HB. TRAF7 potentiates MEKK3-induced AP1 and CHOP activation and induces apoptosis. *J Biol Chem.* (2004) 279:17278–82. doi: 10.1074/jbc.C400063200
6. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol Signal.* (2013) 8:7. doi: 10.1186/1750-2187-8-7

7. Wallis AM, Bishop GA. TRAF3 regulation of inhibitory signaling pathways in B and T lymphocytes by kinase and phosphatase localization. *J Leukoc Biol.* (2018) 103:1089–98. doi: 10.1002/JLB.2MIR0817-339RR
8. Regnier CH, Masson R, Kedinger V, Textoris J, Stoll I, Chenard MP, et al. Impaired neural tube closure, axial skeleton malformations, and tracheal ring disruption in TRAF4-deficient mice. *Proc Natl Acad Sci USA.* (2002) 99:5585–90. doi: 10.1073/pnas.052124799
9. Rousseau A, Rio MC, Alpy F. TRAF4, at the crossroad between morphogenesis and cancer. *Cancers* (2011) 3:2734–49. doi: 10.3390/cancers3022734
10. Lomaga M, Yeh W-C, Sarosi I, Duncan G, Furlonger C, Ho A, et al. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev.* (1999) 13:1015–24. doi: 10.1101/gad.13.8.1015
11. Li J, Yin Q, Wu H. Structural basis of signal transduction in the TNF receptor superfamily. *Adv Immunol.* (2013) 119:135–53. doi: 10.1016/B978-0-12-407707-2.00005-9
12. Park HH. Structure of TRAF family: current understanding of receptor recognition. *Front Immunol.* (2018) 9:1999. doi: 10.3389/fimmu.2018.01999
13. Foight GW, Keating AE. Comparison of the peptide binding preferences of three closely related TRAF paralogs: TRAF2, TRAF3, and TRAF5. *Protein Sci.* (2016) 25:1273–89. doi: 10.1002/pro.2881
14. Kim CM, Choi JY, Bhat EA, Jeong JH, Son YJ, Kim S, et al. Crystal structure of TRAF1 TRAF domain and its implications in the TRAF1-mediated intracellular signaling pathway. *Sci Rep.* (2016) 6:25526. doi: 10.1038/srep25526
15. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera–a visualization system for exploratory research and analysis. *J Comput Chem.* (2004) 25:1605–12. doi: 10.1002/jcc.20084
16. Shuford WW, Klussman K, Tritchler DD, Loo DT, Chalupny J, Siadak AW, et al. 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification *in vivo* of cytotoxic T cell responses. *J Exp Med.* (1997) 186:47–55. doi: 10.1084/jem.186.1.47
17. Cannons JL, Lau P, Ghumman B, DeBenedette MA, Yagita H, Okumura K, et al. 4-1BB ligand induces cell division, sustains survival, and enhances effector function of CD4 and CD8 T cells with similar efficacy. *J Immunol.* (2001) 167:1313–24. doi: 10.4049/jimmunol.167.3.1313
18. Lin W, Voskens CJ, Zhang X, Schindler DG, Wood A, Burch E, et al. Fc-dependent expression of CD137 on human NK cells: insights into "agonistic" effects of anti-CD137 monoclonal antibodies. *Blood* (2008) 112:699–707. doi: 10.1182/blood-2007-11-122465
19. Vinay DS, Kwon BS. 4-1BB signaling beyond T cells. *Cell Mol Immunol.* (2011) 8:281–4. doi: 10.1038/cmi.2010.82
20. Weigelin B, Bolanos E, Teijeira A, Martinez-Forero I, Labiano S, Azpilikueta A, et al. Focusing and sustaining the antitumor CTL effector killer response by agonist anti-CD137 mAb. *Proc Natl Acad Sci USA.* (2015) 112:7551–6. doi: 10.1073/pnas.1506357112
21. Chester C, Sanmamed MF, Wang J, Melero I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. *Blood* (2018) 131:49–57. doi: 10.1182/blood-2017-06-741041
22. Sanchez-Paulete AR, Labiano S, Rodriguez-Ruiz ME, Azpilikueta A, Etxeberria I, Bolanos E, et al. Deciphering CD137 (4-1BB) signaling in T-cell costimulation for translation into successful cancer immunotherapy. *Eur J Immunol.* (2016) 46:513–22. doi: 10.1002/eji.201445388
23. Pollok KE, Kim YJ, Hurtado J, Zhou Z, Kim KK, Kwon BS. 4-1BB T-cell antigen binds to mature B cells and macrophages, and costimulates anti-mu-primed splenic B cells. *Eur J Immunol.* (1994) 24:367–74. doi: 10.1002/eji.1830240215
24. Kang SW, Lee SC, Park SH, Kim J, Kim HH, Lee HW, et al. Anti-CD137 Suppresses tumor growth by blocking reverse signaling by CD137 ligand. *Cancer Res.* (2017) 77:5989–6000. doi: 10.1158/0008-5472.CAN-17-0610
25. Chalupny NJ, Peach R, Hollenbaugh D, Ledbetter JA, Farr AG, Aruffo A. T-cell activation molecule 4-1BB binds to extracellular matrix proteins. *Proc Natl Acad Sci USA.* (1992) 89:10360–4 doi: 10.1073/pnas.89.21.10360
26. Madireddi S, Eun SY, Lee SW, Nemcovicova I, Mehta AK, Zajonc DM, et al. Galectin-9 controls the therapeutic activity of 4-1BB-targeting antibodies. *J Exp Med.* (2014) 211:1433–48. doi: 10.1084/jem.20132687
27. Won EY, Cha K, Byun JS, Kim DU, Shin S, Ahn B, et al. The structure of the trimer of human 4-1BB ligand is unique among members of the tumor necrosis factor superfamily. *J Biol Chem.* (2010) 285:9202–10. doi: 10.1074/jbc.M109.084442
28. Vanamee ES, Faustman DL. Structural principles of tumor necrosis factor superfamily signaling. *Sci Signal.* (2018) 11:eaao4910. doi: 10.1126/scisignal.aao4910
29. Magis C, van der Sloot AM, Serrano L, Notredame C. An improved understanding of TNFL/TNFR interactions using structure-based classifications. *Trends Biochem Sci.* (2012) 37:353–63. doi: 10.1016/j.tibs.2012.06.002
30. Arch R, Thompson C. 4-1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclearfactor kB. *Mol Cell Biol.* (1998) 18:558–65. doi: 10.1128/MCB.18.1.558
31. Jang IK, Lee ZH, Kim YJ, Kim SH, Kwon BS. Human 4-1BB (CD137) signals are mediated by TRAF2 and activate nuclear factor-kappa B. *Biochem Biophys Res Commun.* (1998) 242:613–20. doi: 10.1006/bbrc.1997.8016
32. Saoulli K, Lee SY, Cannons JL, Yeh WC, Santana A, Goldstein MD, et al. CD28-independent, TRAF2-dependent costimulation of resting T cells by 4-1BB ligand. *J Exp Med.* (1998)187:1849–62.
33. Zheng C, Kabaleeswaran V, Wang Y, Cheng G, Wu H. Crystal structures of the TRAF2: cIAP2 and the TRAF1: TRAF2: cIAP2 complexes: affinity, specificity, and regulation. *Mol Cell* (2010) 38:101–13. doi: 10.1016/j.molcel.2010.03.009
34. Nam KO, Kang H, Shin SM, Cho KH, Kwon B, Kwon BS, et al. Cross-linking of 4-1BB activates TCR-signaling pathways in CD8+ T lymphocytes. *J Immunol.* (2005) 174:1898–905. doi: 10.4049/jimmunol.174.4.1898
35. Cannons JL, Hoeflich KP, Woodgett JR, Watts TH. Role of the stress kinase pathway in signaling via the T cell costimulatory receptor 4-1BB. *J Immunol.* (1999) 163:2990–8.
36. Martinez-Forero I, Azpilikueta A, Bolanos-Mateo E, Nistal-Villan E, Palazon A, Teijeira A, et al. T cell costimulation with anti-CD137 monoclonal antibodies is mediated by K63-polyubiquitin-dependent signals from endosomes. *J Immunol.* (2013) 190:6694–706. doi: 10.4049/jimmunol.1203010
37. Wang C, McPherson AJ, Jones RB, Kawamura KS, Lin GH, Lang PA, et al. Loss of the signaling adaptor TRAF1 causes CD8+ T cell dysregulation during human and murine chronic infection. *J Exp Med.* (2012) 209:77–91. doi: 10.1084/jem.20110675
38. McPherson AJ, Snell LM, Mak TW, Watts TH. Opposing roles for TRAF1 in the alternative versus classical NF-kappaB pathway in T cells. *J Biol Chem.* (2012) 287:23010–9. doi: 10.1074/jbc.M112.350538
39. Li G, Boucher JC, Kotani H, Park K, Zhang Y, Shrestha B, et al. 4-1BB enhancement of CAR T function requires NF-kappaB and TRAFs. *JCI Insight* (2018) 3:121322. doi: 10.1172/jci.insight.121322
40. Zapata JM, Reed JC. TRAF1: lord without a RING. *Science STKE* (2002) 2002:pe27. doi: 10.1126/stke.2002.133.pe27
41. Greenfeld H, Takasaki K, Walsh MJ, Ersing I, Bernhardt K, Ma Y, et al. TRAF1 coordinates polyubiquitin signaling to enhance epstein-barr virus lmp1-mediated growth and survival pathway activation. *PLoS Pathog.* (2015) 11:e1004890. doi: 10.1371/journal.ppat.1004890
42. Mace PD, Smits C, Vaux DL, Silke J, Day CL. Asymmetric recruitment of cIAPs by TRAF2. *J Mol Biol.* (2010) 400:8–15. doi: 10.1016/j.jmb.2010.04.055
43. Lee SY, Choi Y. TRAF1 and its biological functions. *Adv Exp Med Biol.* (2007) 597:25–31. doi: 10.1007/978-0-387-70630-6_2
44. Tsitsikov EN, Laouini D, Dunn IF, Sannikova TY, Davidson L, Alt FW, et al. TRAF1 is a negative regulator of TNF signaling: enhanced TNF signaling in TRAF1-deficient mice. *Immunity* (2001) 15:647–57. doi: 10.1016/S1074-7613(01)00207-2
45. Arron JR, Pewzner-Jung Y, Walsh MC, Kobayashi T, Choi Y. Regulation of the subcellular localization of tumor necrosis factor receptor-associated factor (TRAF)2 by TRAF1 reveals mechanisms of TRAF2 signaling. *J Exp Med.* (2002) 196:923–34 doi: 10.1084/jem.20020774
46. Xie P, Hostager BS, Munroe ME, Moore CR, Bishop GA. Cooperation between TNF receptor-associated factors 1 and 2 in CD40 signaling. *J Immunol.* (2006) 176:5388–400 doi: 10.4049/jimmunol.176.9.5388
47. Speiser DE, Lee SY, Wong B, Arron J, Santana A, Kong YY, et al. A regulatory role for TRAF1 in antigen-induced apoptosis of T cells. *J Exp Med.* (1997) 185:1777–83. doi: 10.1084/jem.185.10.1777

48. Sabbagh L, Pulle G, Liu Y, Tsitsikov EN, Watts TH. ERK-dependent Bim modulation downstream of the 4-1BB-TRAF1 signaling axis is a critical mediator of CD8 T cell survival *in vivo*. *J Immunol.* (2008) 180:8093–101 doi: 10.4049/jimmunol.180.12.8093
49. Watts TH, Lin GH, Wang C, McPherson AJ, Snell LM, Sabbagh L. Role of 4-1BBL and TRAF1 in the CD8 T cell response to influenza virus and HIV. *Adv Exp Med Biol.* (2011) 691:177–86. doi: 10.1007/978-1-4419-6612-4_19
50. Alvarez SE, Harikumar KB, Hait NC, Allegood J, Strub GM, Kim EY, et al. Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. *Nature* (2010) 465:1084–8. doi: 10.1038/nature09128
51. Shi CS, Kehrl JH. Tumor necrosis factor (TNF)-induced germinal center kinase-related (GCKR) and stress-activated protein kinase (SAPK) activation depends upon the E2/E3 complex Ubc13-Uev1A/TNF receptor-associated factor 2 (TRAF2). *J Biol Chem.* (2003) 278:15429–34. doi: 10.1074/jbc.M211796200
52. Habelhah H, Takahashi S, Cho SG, Kadoya T, Watanabe T, Ronai Z. Ubiquitination and translocation of TRAF2 is required for activation of JNK but not of p38 or NF-kappaB. *Embo J.* (2004) 23:322–32. doi: 10.1038/sj.emboj.7600044
53. Gonzalvez F, Lawrence D, Yang B, Yee S, Pitti R, Marsters S, et al. TRAF2 Sets a threshold for extrinsic apoptosis by tagging caspase-8 with a ubiquitin shutoff timer. *Mol Cell* (2012) 48:888–99. doi: 10.1016/j.molcel.2012.09.031
54. Robeson AC, Lindblom KR, Wojton J, Kornbluth S, Matsuura K. Dimer-specific immunoprecipitation of active caspase-2 identifies TRAF proteins as novel activators. *EMBO J.* (2018) 37:e97072. doi: 10.15252/embj.201797072
55. Yin Q, Lamothe B, Darnay BG, Wu H. Structural basis for the lack of E2 interaction in the RING domain of TRAF2. *Biochemistry* (2009) 48:10558–67. doi: 10.1021/bi901462e
56. Liao G, Zhang M, Harhaj EW, Sun SC. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. *J Biol Chem.* (2004) 279:26243–50. doi: 10.1074/jbc.M403286200
57. Sanjo H, Zajonc DM, Braden R, Norris PS, Ware CF. Allosteric regulation of the ubiquitin:NIK and ubiquitin:TRAF3 E3 ligases by the lymphotoxin-beta receptor. *J Biol Chem.* (2010) 285:17148–55. doi: 10.1074/jbc.M110.105874
58. He JQ, Saha SK, Kang JR, Zarnegar B, Cheng G. Specificity of TRAF3 in its negative regulation of the noncanonical NF-kappa B pathway. *J Biol Chem.* (2007) 282:3688–94. doi: 10.1074/jbc.M610271200
59. Lin WW, Hildebrand JM, Bishop GA. A complex relationship between TRAF3 and non-canonical NF-kappaB2 activation in B lymphocytes. *Front Immunol.* (2013) 4:477. doi: 10.3389/fimmu.2013.00477
60. Zapata JM, Krajewska M, Krajewski S, Kitada S, Welsh K, Monks A, et al. TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J Immunol.* (2000) 165:5084–96. doi: 10.4049/jimmunol.165.9.5084
61. Schwenzer R, Siemienski K, Liptay S, Schubert G, Peters N, Scheurich P, et al. The human tumor necrosis factor (TNF) receptor-associated factor 1 gene (TRAF1) is up-regulated by cytokines of the TNF ligand family and modulates TNF-induced activation of NF-kappaB and c-Jun N-terminal kinase. *J Biol Chem.* (1999) 274:19368–74. doi: 10.1074/jbc.274.27.19368
62. Lee SW, Park Y, Song A, Cheroutre H, Kwon BS, Croft M. Functional dichotomy between OX40 and 4-1BB in modulating effector CD8 T cell responses. *J Immunol.* (2006) 177:4464–72. doi: 10.4049/jimmunol.177.7.4464
63. Wen T, Bukczynski J, Watts TH. 4-1BB ligand-mediated costimulation of human T cells induces CD4 and CD8 T cell expansion, cytokine production, and the development of cytolytic effector function. *J Immunol.* (2002) 168:4897–906. doi: 10.4049/jimmunol.168.10.4897
64. Napetschnig J, Wu H. Molecular basis of NF-kappaB signaling. *Ann Rev Biophy.* (2013) 42:443–68. doi: 10.1146/annurev-biophys-083012-130338
65. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master manipulators of E2 ubiquitin-conjugating enzymes and ubiquitination. *Biochim Biophys Acta* (2014) 1843:47–60. doi: 10.1016/j.bbamcr.2013.05.026
66. Yin Q, Lin SC, Lamothe B, Lu M, Lo YC, Hura G, et al. E2 interaction and dimerization in the crystal structure of TRAF6. *Nat Struct Mol Biol.* (2009) 16:658–66. doi: 10.1038/nsmb.1605
67. Samuel T, Welsh K, Lober T, Togo SH, Zapata JM, Reed JC. Distinct BIR domains of cIAP1 mediate binding to and ubiquitination of tumor necrosis factor receptor-associated factor 2 and second mitochondrial activator of caspases. *J Biol Chem.* (2006) 281:1080–90. doi: 10.1074/jbc.M509381200
68. Lopez J, John SW, Tenev T, Rautureau GJ, Hinds MG, Francalanci F, et al. CARD-mediated autoinhibition of cIAP1's E3 ligase activity suppresses cell proliferation and migration. *Mol Cell* (2011) 42:569–83. doi: 10.1016/j.molcel.2011.04.008
69. Sabbagh L, Srokowski CC, Pulle G, Snell LM, Sedgmen BJ, Liu Y, et al. A critical role for TNF receptor-associated factor 1 and Bim down-regulation in CD8 memory T cell survival. *Proc Natl Acad Sci USA.* (2006) 103:18703–8. doi: 10.1073/pnas.0602919103
70. Sabbagh L, Andreeva D, Laramee GD, Oussa NA, Lew D, Bisson N, et al. Leukocyte-specific protein 1 links TNF receptor-associated factor 1 to survival signaling downstream of 4-1BB in T cells. *J Leukoc Biol.* (2013) 93:713–21. doi: 10.1189/jlb.1112579
71. Abdul-Sater AA, Edilova MI, Clouthier DL, Mbanwi A, Kremmer E, Watts TH. The signaling adaptor TRAF1 negatively regulates Toll-like receptor signaling and this underlies its role in rheumatic disease. *Nat Immunol.* (2017) 18:26–35. doi: 10.1038/ni.3618
72. Stunz LL, Bishop GA. Latent membrane protein 1 and the B lymphocyte-a complex relationship. *Crit Rev Immunol.* (2014) 34:177–98. doi: 10.1615/CritRevImmunol.2014010041
73. Borghi A, Haegman M, Fischer R, Carpentier I, Bertrand MJM, Libert C, et al. The E3 ubiquitin ligases HOIP and cIAP1 are recruited to the TNFR2 signaling complex and mediate TNFR2-induced canonical NF-kappaB signaling. *Biochem Pharmacol.* (2018) 153:292–8. doi: 10.1016/j.bcp.2018.01.039
74. Takiuchi T, Nakagawa T, Tamiya H, Fujita H, Sasaki Y, Saeki Y, et al. Suppression of LUBAC-mediated linear ubiquitination by a specific interaction between LUBAC and the deubiquitinases CYLD and OTULIN. *Genes Cells* (2014) 19:254–72. doi: 10.1111/gtc.12128
75. Chen ZJ. Ubiquitination in signaling to and activation of IKK. *Immunol Rev.* (2012) 246:95–106. doi: 10.1111/j.1600-065X.2012.01108.x
76. Chen J, Chen ZJ. Regulation of NF-kappaB by ubiquitination. *Curr Opin Immunol.* (2013) 25:4–12. doi: 10.1016/j.coi.2012.12.005
77. Komander D, Rape M. The ubiquitin code. *Annu Rev Biochem.* (2012) 81:203–29. doi: 10.1146/annurev-biochem-060310-170328
78. Mace PD, Linke K, Feltham R, Schumacher FR, Smith CA, Vaux DL, et al. Structures of the cIAP2 RING domain reveal conformational changes associated with ubiquitin-conjugating enzyme (E2) recruitment. *J Biol Chem.* (2008) 283:31633–40. doi: 10.1074/jbc.M804753200
79. Vandenabeele P, Bertrand MJ. The role of the IAP E3 ubiquitin ligases in regulating pattern-recognition receptor signalling. *Nat Rev Immunol.* (2012) 12:833–44. doi: 10.1038/nri3325
80. Fan Y, Yu Y, Shi Y, Sun W, Xie M, Ge N, et al. Lysine 63-linked polyubiquitination of TAK1 at lysine 158 is required for tumor necrosis factor alpha- and interleukin-1beta-induced IKK/NF-kappaB and JNK/AP-1 activation. *J Biol Chem.* (2010) 285:5347–60. doi: 10.1074/jbc.M109.076976
81. Hu L, Xu J, Xie X, Zhou Y, Tao P, Li H, et al. Oligomerization-primed coiled-coil domain interaction with Ubc13 confers processivity to TRAF6 ubiquitin ligase activity. *Nat Commun.* (2017) 8:814. doi: 10.1038/s41467-017-01290-0
82. Kanayama A, Seth RB, Sun L, Ea CK, Hong M, Shaito A, et al. TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Mol Cell* (2004) 15:535–48. doi: 10.1016/j.molcel.2004.08.008
83. Lin SC, Chung JY, Lamothe B, Rajashankar K, Lu M, Lo YC, et al. Molecular basis for the unique deubiquitinating activity of the NF-kappaB inhibitor A20. *J Mol Biol.* (2008) 376:526–40. doi: 10.1016/j.jmb.2007.11.092
84. Cannons JL, Choi Y, Watts TH. Role of TNF receptor-associated factor 2 and p38 mitogen-activated protein kinase activation during 4-1BB-dependent immune response. *J Immunol.* (2000) 165:6193–204. doi: 10.4049/jimmunol.165.11.6193
85. Li S, Wang L, Dorf ME. PKC phosphorylation of TRAF2 mediates IKKalpha/beta recruitment and K63-linked polyubiquitination. *Mol Cell* (2009) 33:30–42. doi: 10.1016/j.molcel.2008.11.023
86. Oussa NA, Soumounou Y, Sabbagh L. TRAF1 phosphorylation on Serine 139 modulates NF-kappaB activity downstream of 4-1BB in T cells. *Biochem Biophys Res Commun.* (2013) 432:129–34. doi: 10.1016/j.bbrc.2013.01.073

87. Sun SC. Non-canonical NF-kappaB signaling pathway. *Cell Res.* (2011) 21:71–85. doi: 10.1038/cr.2010.177
88. He L, Grammer AC, Wu X, Lipsky PE. TRAF3 forms heterotrimers with TRAF2 and modulates its ability to mediate NF-kB activation. *J Biol Chem.* (2004) 279:55855–65. doi: 10.1074/jbc.M407284200
89. Choudhary S, Kalita M, Fang L, Patel KV, Tian B, Zhao Y, et al. Inducible tumor necrosis factor (TNF) receptor-associated factor-1 expression couples the canonical to the non-canonical NF-kappaB pathway in TNF stimulation. *J Biol Chem.* (2013) 288:14612–23. doi: 10.1074/jbc.M113.464081
90. Azpilikueta A, Bolanos E, Lang V, Labiano S, Aznar MA, Etxeberria I, et al. Deubiquitinases A20 and CYLD modulate costimulatory signaling via CD137 (4-1BB). *Oncoimmunology* (2017) 7:e1368605. doi: 10.1080/2162402X.2017.1368605
91. Evans PC, Smith TS, Lai MJ, Williams MG, Burke DF, Heyninck K, et al. A novel type of deubiquitinating enzyme. *J Biol Chem.* (2003) 278:23180–6. doi: 10.1074/jbc.M301863200
92. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* (2004) 430:694–9. doi: 10.1038/nature02794
93. Kupka S, Reichert M, Draber P, Walczak H. Formation and removal of poly-ubiquitin chains in the regulation of tumor necrosis factor-induced gene activation and cell death. *FEBS J.* (2016) 283:2626–39. doi: 10.1111/febs.13644
94. Shembade N, Ma A, Harhaj EW. Inhibition of NF-kappaB signaling by A20 through disruption of ubiquitin enzyme complexes. *Science* (2010) 327:1135–9. doi: 10.1126/science.1182364
95. Skaug B, Chen J, Du F, He J, Ma A, Chen ZJ. Direct, noncatalytic mechanism of IKK inhibition by A20. *Mol Cell* (2011) 44:559–71. doi: 10.1016/j.molcel.2011.09.015
96. Tokunaga F, Nishimasu H, Ishitani R, Goto E, Noguchi T, Mio K, et al. Specific recognition of linear polyubiquitin by A20 zinc finger 7 is involved in NF-kappaB regulation. *EMBO J.* (2012) 31:3856–70. doi: 10.1038/emboj.2012.241
97. Verhelst K, Carpentier I, Kreike M, Meloni L, Verstrepen L, Kensche T, et al. A20 inhibits LUBAC-mediated NF-kappaB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J.* (2012) 31:3845–55. doi: 10.1038/emboj.2012.240
98. Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. *Nature* (2003) 424:801–5. doi: 10.1038/nature01802
99. Reiley W, Zhang M, Wu X, Granger E, Sun SC. Regulation of the deubiquitinating enzyme CYLD by IkappaB kinase gamma-dependent phosphorylation. *Mol Cell Biol.* (2005) 25:3886–95. doi: 10.1128/MCB.25.10.3886-3895.2005
100. Feng X, Gaeta ML, Madge LA, Yang JH, Bradley JR, Pober JS. Caveolin-1 associates with TRAF2 to form a complex that is recruited to tumor necrosis factor receptors. *J Biol Chem.* (2001) 276:8341–9. doi: 10.1074/jbc.M007116200
101. Leonardi A, Ellinger-Ziegelbauer H, Franzoso G, Brown K, Siebenlist U. Physical and functional interaction of filamin (actin-binding protein-280) and tumor necrosis factor receptor-associated factor 2. *J Biol Chem.* (2000) 275:271–8. doi: 10.1074/jbc.275.1.271
102. Parton RG, del Pozo MA. Caveolae as plasma membrane sensors, protectors and organizers. *Nat Rev Mol Cell Biol.* (2013) 14:98–112. doi: 10.1038/nrm3512
103. So T, Croft M. Regulation of PI-3-kinase and Akt signaling in T lymphocytes and other cells by TNFR family molecules. *Front Immunol.* (2013) 4:139. doi: 10.3389/fimmu.2013.00139
104. Lasserre R, Guo XJ, Conchonaud F, Hamon Y, Hawchar O, Bernard AM, et al. Raft nanodomains contribute to Akt/PKB plasma membrane recruitment and activation. *Nat Chem Biol.* (2008) 4:538–47. doi: 10.1038/nchembio.103
105. Starck L, Scholz C, Dorken B, Daniel PT. Costimulation by CD137/4-1BB inhibits T cell apoptosis and induces Bcl-xL and c-FLIP(short) via phosphatidylinositol 3-kinase and AKT/protein kinase B. *Eur J Immunol.* (2005) 35:1257–66. doi: 10.1002/eji.200425686
106. Lee HW, Nam KO, Park SJ, Kwon BS. 4-1BB enhances CD8+ T cell expansion by regulating cell cycle progression through changes in expression of cyclins D and E and cyclin-dependent kinase inhibitor p27kip1. *Eur J Immunol.* (2003) 33:2133–41. doi: 10.1002/eji.200323996
107. Lee DY, Choi BK, Lee DG, Kim YH, Kim CH, Lee SJ, et al. 4-1BB signaling activates the t cell factor 1 effector/beta-catenin pathway with delayed kinetics via ERK signaling and delayed PI3K/AKT activation to promote the proliferation of CD8+ T Cells. *PLoS ONE* (2013) 8:e69677. doi: 10.1371/journal.pone.0069677
108. Li L, Soetandyo N, Wang Q, Ye Y. The zinc finger protein A20 targets TRAF2 to the lysosomes for degradation. *Biochim Biophys Acta* (2009) 1793:346–53. doi: 10.1016/j.bbamcr.2008.09.013
109. Sharma RK, Schabowsky RH, Srivastava AK, Elpek KG, Madireddi S, Zhao H, et al. 4-1BB ligand as an effective multifunctional immunomodulator and antigen delivery vehicle for the development of therapeutic cancer vaccines. *Cancer Res.* (2010) 70:3945–54. doi: 10.1158/0008-5472.CAN-09-4480
110. Eun SY, Lee SW, Xu Y, Croft M. 4-1BB ligand signaling to T cells limits T cell activation. *J Immunol.* (2015) 194:134–41. doi: 10.4049/jimmunol.1401383
111. Kim JO, Kim HW, Baek KM, Kang CY. NF-kappaB and AP-1 regulate activation-dependent CD137 (4-1BB) expression in T cells. *FEBS Lett.* (2003) 541:163–70. doi: 10.1016/S0014-5793(03)00326-0
112. Dunn IF, Sannikova TY, Geha RS, Tsitsikov EN. Identification and characterization of two CD40-inducible enhancers in the mouse TRAF1 gene locus. *Mol Immunol.* (2000) 37:961–73. doi: 10.1016/S0161-5890(01)00015-3
113. Zhang B, Wang Z, Li T, Tsitsikov EN, Ding HF. NF-kappaB2 mutation targets TRAF1 to induce lymphomagenesis. *Blood* (2007) 110:743–51. doi: 10.1182/blood-2006-11-058446
114. Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AS Jr. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* (1998) 281:1680–3.
115. Gomes-Silva D, Mukherjee M, Srinivasan M, Krenciute G, Dakhova O, Zheng Y, et al. Tonic 4-1BB costimulation in chimeric antigen receptors impedes T cell survival and is vector-dependent. *Cell Rep.* (2017) 21:17–26. doi: 10.1016/j.celrep.2017.09.015
116. Melero I, Hirschhorn-Cymerman D, Morales-Kastresana A, Sanmamed MF, Wolchok JD. Agonist antibodies to TNFR molecules that costimulate T and NK cells. *Clin Cancer Res.* (2013) 19:1044–53. doi: 10.1158/1078-0432.CCR-12-2065
117. Bartkowiak T, Curran MA. 4-1BB Agonists: multi-potent potentiators of tumor immunity. *Front Oncol.* (2015) 5:117. doi: 10.3389/fonc.2015.00117
118. Segal NH, Logan TF, Hodi FS, McDermott D, Melero I, Hamid O, et al. Results from an integrated safety analysis of urelumab, an agonist Anti-CD137 monoclonal antibody. *Clin Cancer Res.* (2017) 23:1929–36. doi: 10.1158/1078-0432.CCR-16-1272
119. Song DG, Ye Q, Carpenito C, Poussin M, Wang LP, Ji C, et al. *In vivo* persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB). *Cancer Res.* (2011) 71:4617–27. doi: 10.1158/0008-5472.CAN-11-0422
120. Carpenito C, Milone MC, Hassan R, Simonet JC, Lakhal M, Suhoski MM, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc Natl Acad Sci USA.* (2009) 106:3360–5. doi: 10.1073/pnas.0813101106
121. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-Cell lymphoblastic leukemia. *N Engl J Med.* (2018) 378:439–48. doi: 10.1056/NEJMoa1709866

Conflict of Interest Statement: IM has served as a consultant for Bristol-Myers Squib, Roche-Genentech, Bayer, Alligator, Tusk, Bioncotech, Medimmune, Genmab, F-Star. IM receives commercial grants from Bristol Myers Squibb, Roche-Genentech and Alligator. IM-F is a full time employee for MSD.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 30 August 2018
doi: 10.3389/fimmu.2018.01986

Regulation of Interleukin-6 Receptor Signaling by TNF Receptor-Associated Factor 2 and 5 During Differentiation of Inflammatory CD4$^+$ T Cells

Hiroyuki Nagashima[1,2], *Naoto Ishii*[2] *and Takanori So*[2,3]*

[1] Lymphocyte Cell Biology Section, Molecular Immunology and Inflammation Branch, National Institute of Arthritis, Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD, United States, [2] Department of Microbiology and Immunology, Tohoku University Graduate School of Medicine, Sendai, Japan, [3] Laboratory of Molecular Cell Biology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan

There is growing evidence that tumor necrosis factor (TNF) receptor-associated factors (TRAFs) bind to unconventional membrane-bound receptors in many cell types and control their key signaling activity, in both positive and negative ways. TRAFs function in a variety of biological processes in health and disease, and dysregulation of TRAF expression or activity often leads to a patho-physiological outcome. We have identified a novel attribute of TRAF2 and TRAF5 in interleukin-6 (IL-6) receptor signaling in CD4$^+$ T cells. TRAF2 and TRAF5 are highly expressed by naïve CD4$^+$ T cells and constitutively bind to the signal-transducing receptor common chain gp130 via the C-terminal TRAF domain. The binding between TRAF and gp130 limits the early signaling activity of the IL-6 receptor complex by preventing proximal interaction of Janus kinases (JAKs) associated with gp130. In this reason, TRAF2 and TRAF5 in naïve CD4$^+$ T cells negatively regulate IL-6-mediated activation of signal transducer and activator of transcription 3 (STAT3) that is required for the development of IL-17-secreting CD4$^+$ T_H17 cells. Indeed, *Traf2*-knockdown in differentiating *Traf5*$^{-/-}$ CD4$^+$ T cells strongly promotes T_H17 development. *Traf5*$^{-/-}$ donor CD4$^+$ T cells exacerbate the development of neuroinflammation in experimental autoimmune encephalomyelitis (EAE) in wild-type recipient mice. In this review, we summarize the current understanding of the role for TRAF2 and TRAF5 in the regulation of IL-6-driven differentiation of pro-inflammatory CD4$^+$ T cells, especially focusing on the molecular mechanism by which TRAF2 and TRAF5 inhibit the JAK-STAT pathway that is initiated in the IL-6 receptor signaling complex.

Keywords: TRAF5, TRAF2, IL-6, T_H17, autoimmunity, inflammation

OPEN ACCESS

Edited by:
Tania H. Watts,
University of Toronto, Canada

Reviewed by:
Koji Yasutomo,
Tokushima University, Japan
Wai Wai Lin,
University of Iowa, United States

***Correspondence:**
Takanori So
tso@pha.u-toyama.ac.jp

Specialty section:
This article was submitted to T Cell Biology, a section of the journal Frontiers in Immunology

Received: *29 June 2018*
Accepted: *13 August 2018*
Published: *30 August 2018*

Citation:
Nagashima H, Ishii N and So T (2018) Regulation of Interleukin-6 Receptor Signaling by TNF Receptor-Associated Factor 2 and 5 During Differentiation of Inflammatory CD4$^+$ T Cells. Front. Immunol. 9:1986. doi: 10.3389/fimmu.2018.01986

INTRODUCTION

The tumor necrosis factor receptor-associated factor (TRAF) family molecules in mammals were initially discovered as cytoplasmic adaptor proteins interacting with one of the tumor necrosis factor receptor superfamily (TNFRSF) molecules, TNFR2 (1). TRAF molecules are also present in *Caenorhabditis elegans* and *Drosophila melanogaster* (2–4). There are six mammalian TRAF

molecules, TRAF1 to TRAF6, which share a conserved C-terminal TRAF-C domain that accommodates a short stretch of amino acids found in the cytoplasmic tail of receptors. Mammalian TRAFs critically participate in the signal transduction by receptors, such as TNFRSF molecules, Toll-like receptors (TLRs), nucleotide binding-oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), interleukin receptors, interferon receptors, transforming growth factor-β (TGF-β) receptor, the T-cell receptor (TCR) and platelet receptors. TRAFs link these receptors to various signaling cascades that are important in health and disease (3, 5–12).

One of the TRAF family molecules, TRAF5, is highly expressed in lung and moderately expressed in thymus, spleen, and kidney (13). In contrast to mice deficient in *Traf2*, *Traf3*, or *Traf6*, which become runted and die prematurely, *Traf5*$^{-/-}$ mice are born at the expected Mendelian ratios and exhibit no obvious abnormalities (14). One important question to be resolved is how TRAF5 specifically regulates cellular responses that are different and separate from those regulated by other TRAF family molecules.

Upon antigen exposure, naïve CD4$^+$ T cells differentiate into different effector CD4$^+$ helper T cell (T_H cell) subsets that control the functions of B cells, macrophages, and CD8$^+$ cytotoxic T cells through cell-to-cell contact and/or by secreting specific effector cytokines. There is growing evidence that TRAFs recruited to the TCR, costimulatory TNFRSF molecules, and cytokine receptors control key signaling events in CD4$^+$ T cells and are critical for the activation, differentiation, and survival of T_H cells in both positive and negative manners (11).

Although it has been well recognized that TRAF molecules play essential roles in T cell biology, the detailed functions and their molecular mechanisms of action are still enigmatic. In this review, we will highlight a novel function of TRAF2 and TRAF5 in the regulation of CD4$^+$ T_H17 cell differentiation that is controlled by pro-inflammatory cytokine IL-6 and its receptor signaling complex.

TRAF2 AND TRAF5 IN IL-6 RECEPTOR SIGNALING AND T_H17 DEVELOPMENT

The regulation of IL-6 receptor signaling by TRAF molecules was initially suggested by the observation that after culturing in IL-6-containing T_H17 skewing condition *in vitro*, differentiating CD4$^+$ T cells lacking *Traf5* produced a higher amount of IL-17 than did wild-type counterparts. However, *Traf5*-deficiency had no significant role for the development of T_H1, T_H2, T_H17, Treg cells in polarized *in vitro* cultures. Accordingly, *Traf5*$^{-/-}$ mice exhibited exacerbated T_H17 cell-dependent neuroinflammation in a model of experimental autoimmune encephalomyelitis (EAE). The enhanced EAE phenotype was recapitulated in irradiated wild-type mice that had been transferred with *Traf5*$^{-/-}$ CD4$^+$ T cells, demonstrating that TRAF5 expressed in CD4$^+$ T cells negatively regulates the generation of pathogenic T_H17 cells (15). These results strongly suggested that TRAF5 regulated IL-6 receptor signaling that is required for T_H17 differentiation.

Indeed, *Traf5*$^{-/-}$ naïve CD4$^+$ T cells stimulated with a complex of IL-6 and soluble IL-6R (IL-6–sIL-6R) without triggering of TCR and CD28 exhibited increased phosphorylation of JAK1 and STAT3. In addition, the retrovirally transduced *Traf5* gene in CD4$^+$ T cells suppressed the phosphorylation of STAT3 mediated by IL-6–sIL-6R (16, 17). The negative regulatory function of TRAF5 for STAT3 was also observed in primary CD8$^+$ T cells, but not in macrophages. One of the possible reasons would be that the expression of *Traf5* mRNA was almost five times lower in macrophages than in CD4$^+$ and CD8$^+$ T cells (15). These results strongly suggest that if a cell expresses substantial levels of endogenous TRAF5 and gp130, TRAF5 can repress IL-6 receptor signaling activity in this cell type. Importantly, TRAF5 exhibited no inhibitory role for the STAT3 phosphorylation mediated by signaling through IL-10 receptor or IL-21 receptor in CD4$^+$ T cells, demonstrating the specific action of TRAF5 for IL-6 receptor signaling (15).

By using a BAF/B03 cell line that stably expresses gp130 (BAF-gp130), we examined the role for TRAF family molecules in IL-6 receptor signaling and found that not only TRAF5 but also TRAF2 inhibited STAT3 phosphorylation and cell proliferation mediated by IL-6–sIL-6R, while TRAF1, TRAF3, TRAF4, and TRAF6 did not. In accordance with this, TRAF2 displayed a similar activity as TRAF5 in terms of the regulation of IL-6 receptor signaling and T_H17 development, which was confirmed by shRNA-mediated knockdown and overexpression of each *Traf* gene in differentiating wild-type CD4$^+$ T cells. TRAF2 did not inhibit the STAT3 phosphorylation downstream of IL-21 receptor in CD4$^+$ T cells (16), confirming the specificity of TRAF2 to the IL-6 receptor signaling. Thus, we concluded that both TRAF2 and TRAF5 work as negative regulators of the IL-6 receptor signaling pathway.

NF-κB-inducing kinase (NIK) is critical for T_H17 development, and both TRAF2 and TRAF3 limit NIK activity through ubiquitin-dependent degradation (18–21). In this reason, it was possible that TRAF2 and TRAF3 might inhibit T_H17 development via degradation of NIK. However, increasing or decreasing the expression of TRAF3 did not affect the sensitivity of the IL-6 receptor signaling and the development of T_H17 cells (16). In addition, it is unclear how TRAF2 regulates the differentiation of naïve CD4$^+$ T cells into T_H17 cells (20). Thus, we concluded that TRAF2 regulation of NIK expression levels is not the mechanism to limit the development of T_H17 cells.

Although naïve CD4$^+$ T cells from *Traf5*$^{-/-}$ and wild-type mice produced equivalent amounts of IL-6 in response to antigen stimulation (15), *Traf2*$^{-/-}$ macrophages and *Traf5*$^{-/-}$ B cells produced more IL-6 in response to TLR stimulation (22, 23). *Traf2*$^{-/-}$ *Tnfa*$^{-/-}$ mice displayed an inflammatory disorder and had elevated levels of IL-6 in serum (20). Thus, TRAF2 and TRAF5 might contribute to the development of T_H17 cells *in vivo* via negative regulation of IL-6 production.

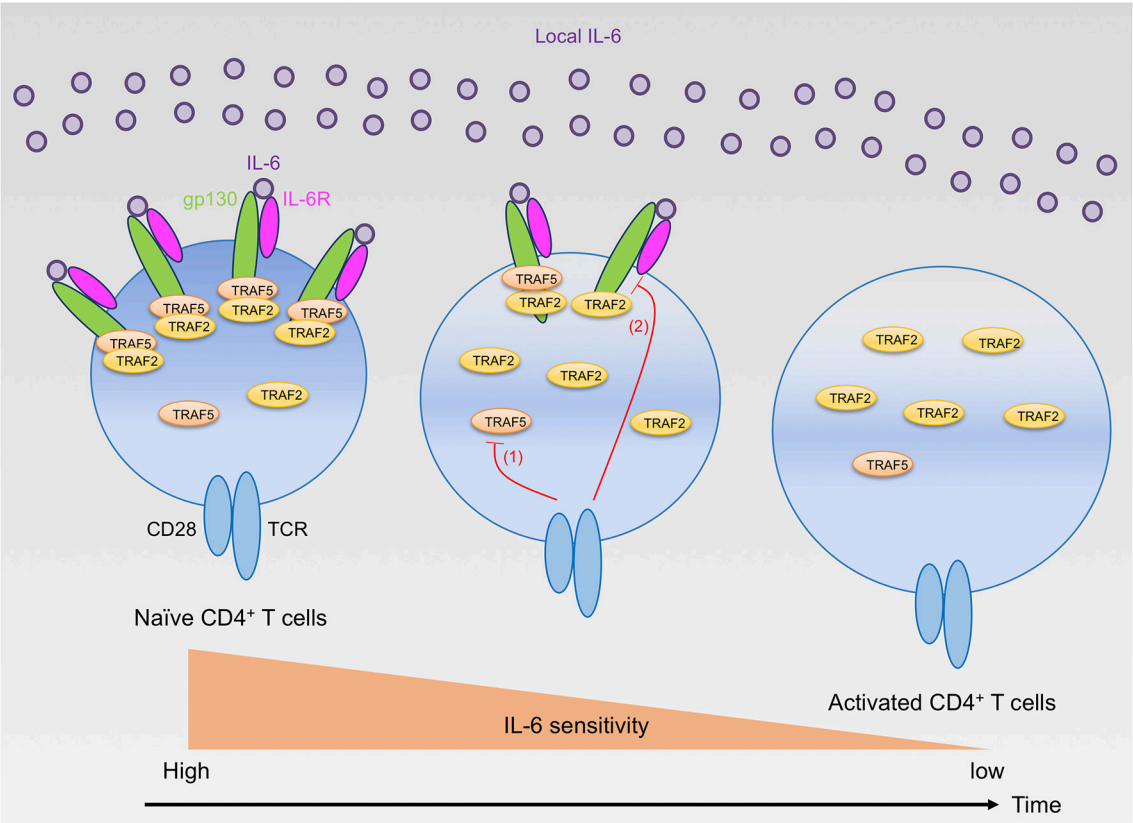


FIGURE 1 | Regulation of IL-6 receptor signaling sensitivity by TRAF2 and TRAF5 in $CD4^+$ T cells. Naïve $CD4^+$ T cells highly express gp130, IL-6R, TRAF2, and TRAF5. Naïve $CD4^+$ T cells can react to extracellular IL-6, but the signaling via the IL-6 receptor, IL-6R and gp130, is restrained by gp130-associated TRAF2 and TRAF5. After T cell activation by TCR and CD28, TRAF5 protein is rapidly downregulated (red line 1), while TRAF2 protein is maintained. Hence, TRAF5 limits the early IL-6 receptor signaling that is important for T_H17 development. In contrast, TRAF2 can inhibit the signaling activity of the IL-6 receptor complex even in the later phase of T_H17 differentiation. Moreover, the TCR and CD28 signaling also suppresses the expression of both gp130 and IL-6R (red line 2), and these receptor proteins are almost disappeared from the T cell surface within a few days after T cell activation. Therefore, activated $CD4^+$ T cells lose their responsiveness to IL-6 and cannot receive the instructive IL-6 receptor signals required for T_H17 development.

INHIBITORY ROLE FOR TRAF2 AND TRAF5 IN THE INITIAL STAGE OF T_H17 DEVELOPMENT

While TRAF2 and TRAF5 seemed to exhibit a similar role for the IL-6 receptor signaling pathway, detailed analyses revealed that the inhibition kinetic of TRAF2 for the IL-6 receptor signaling was different from that of TRAF5 due to different expression kinetics of respective TRAF proteins in developing $CD4^+$ T cells. TRAF5 was higly expressed by unactivated naive $CD4^+$ T cells, and *Traf5* mRNA and TRAF5 protein were rapidly disappeared within a few hours upon TCR triggering (16). This means that there is only a narrow window of time for the inhibition for IL-6 receptor signaling by TRAF5 in differentiating $CD4^+$ T cells. In contrast to this, *Traf2* mRNA and TRAF2 protein were stably detected during the course of $CD4^+$ T cell development, implying that TRAF2 can continuously suppress IL-6 receptor signaling as long as both gp130 and IL-6R are expressed in differentiating $CD4^+$ T cells. In comparison with the regulation of *Traf2* and *Traf5* mRNAs, the expression of *Traf1*, *Traf3*, *Traf4*, and *Traf6* mRNAs in $CD4^+$ T cells were oppositely regulated, and these mRNAs were rapidly upregulated after stimulation with TCR and CD28, demonstrating that the expression of respective TRAF molecules is differentially controlled in recently activated naïve $CD4^+$ T cells. In addition to this regulatory mechanism of TRAF molecules, after triggering of TCR and CD28, gp130 and IL-6R expressed by naïve $CD4^+$ T cells were downregulated in a time-dependent manner, and these molecules were hardly detected on the surface of activated $CD4^+$ T cells at 48 h after activation. In agreement with these results, addition of IL-6–sIL-6R at later time points of T_H differentiation could not effectively promote the development of T_H17 cells, and retrovirus-mediated transduction of short hairpin RNA (shRNA) that targets *Traf5* in differentiating wild-type $CD4^+$ T cells could not enhance the production of IL-17. On the other hand, shRNA-mediated knockdown of *Traf2* in differentiating $CD4^+$ T cells further promoted the development of T_H17 cells in both wild-type and $Traf5^{-/-}$ conditons (16). Thus, it is reasonable

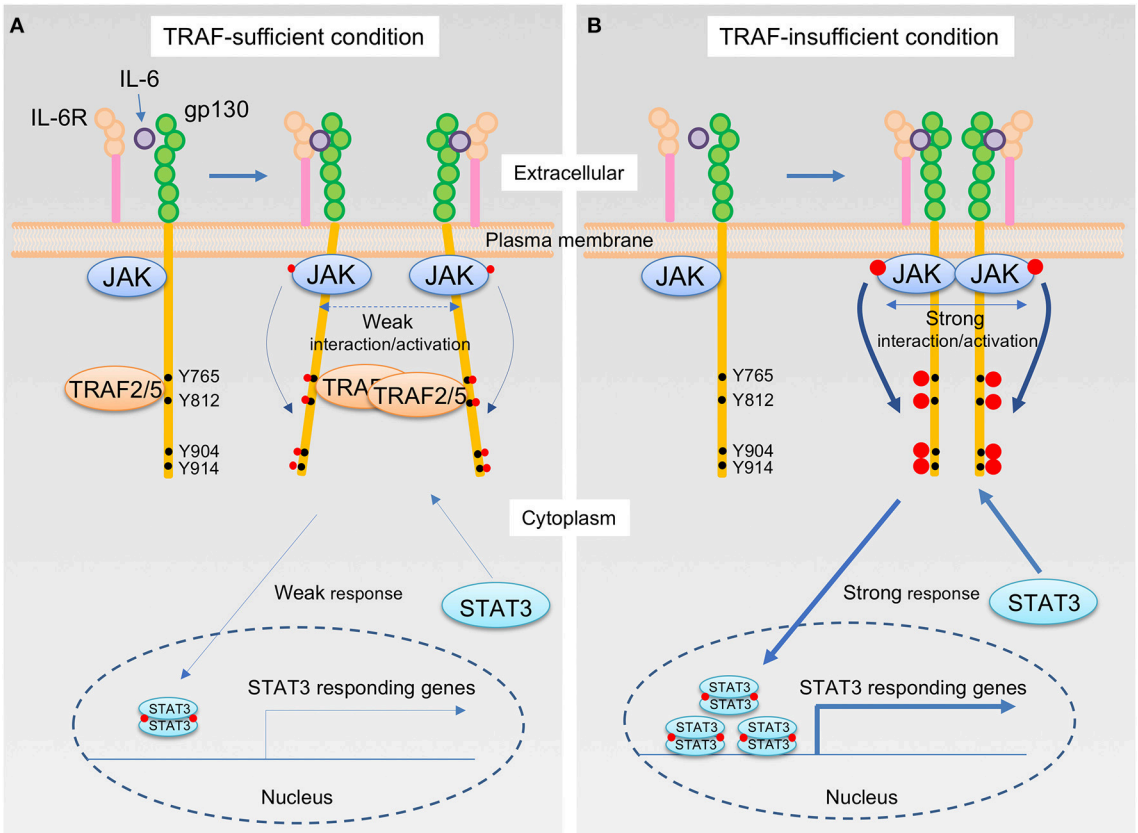


FIGURE 2 | The IL-6 receptor signaling pathway that is regulated by TRAF2 and TRAF5. **(A,B)** Upon interaction of IL-6 with the IL-6R, the complex of IL-6 and IL-6R next binds to the IL-6 receptor common chain gp130, which leads to dimerization of gp130. Janus kinase (JAK) is constitutively bound to the intracellular domains of gp130, and thus this event brings JAKs into close proximity, inducing transphosphorylation of each JAK on a tyrosine residue, indicated in red circle, that stimulates kinase activity of JAKs. The activated JAKs then phosphorylate the cytoplasmic tail of gp130 on specific tyrosine residues, generating binding sites for signal transducer and activator of transcription (STAT) including STAT3. Recruitment of a STAT3 to the phosphorylated gp130 brings the STAT3 close to the activated JAK, which then the activated JAK phosphorylates a tyrosine residue of the STAT3. Phosphorylated STAT3 molecules form a dimer, and STAT3 dimers translocate to the nucleus, then induce the gene transcription involved in T_H17 differentiation, including RAR-related orphan receptor-γt (RORγt) and IL-17. TRAF2 and TRAF5 constitutively bind to a cytoplasmic region of the gp130, which includes an amino acid sequence ^{774}VFSRSESTQPLLDSEERPEDLQLVD^{798} and locates between first two out of four distal phosphorylated tyrosine motifs in gp130, Y765, Y812, Y904, and Y914, that are recognized by STAT3. For this reason, JAK interactions are interrupted by the presence of TRAF2 and/or TRAF5, and this event causes a weaker interaction/activation of JAKs and subsequent attenuated responses in the IL-6 receptor signaling pathway **(A)**. On the other hand, in the absence of TRAF2 and/or TRAF5, a stronger association of JAKs facilitates an augmented JAK activation that leads to the enhanced STAT3 responses in the IL-6 receptor signaling pathway **(B)**.

to conclude that the instructive signals from IL-6 receptor for T_H17 development are restricted both by the negative action of TRAF2 and TRAF5 and by the expression levels of gp130, IL-6R and TRAF5 (**Figure 1**). The expression of TRAF5 protein can be regulated by the ubiquitin proteasome system (21, 24, 25), although it is not known whether this type of regulation of TRAF5 is ongoing in activated naïve $CD4^+$ T cells.

A MOLECULAR MECHANISM OF IL-6 RECEPTOR SIGNALING THAT IS REGULATED BY TRAF2 AND TRAF5

Naïve *Traf5*$^{-/-}$ $CD4^+$ T cells expressed the same level of IL-6R and gp130 as wild-type naïve $CD4^+$ T cells did, and the TRAF5 expression did not affect the STAT3 activation downstream of IL-10 receptor or IL-21 receptor in $CD4^+$ T cells (15). Thus, we thought that TRAF5 directly regulated a key signaling process in the IL-6 receptor complex. Indeed, endogenously expressed TRAF5 constitutively bound to a cytoplasmic region of gp130 in primary $CD4^+$ T cells. Co-immunoprecipitation assay using mutant proteins of TRAF5 and gp130 revealed that TRAF5 required its carboxy-terminal TRAF-C domain but not its amino-terminal RING/zinc-finger domains to interact with gp130 and that the TRAF5-C domain associated with a cytoplasmic region from residue 774 to residue 798 of gp130, gp130 (774-798), ^{774}Val-Phe-Ser-Arg-Ser-Glu-Ser-Thr-Gln-Pro-Leu-Leu-Asp-Ser-Glu-Glu-Arg-Pro-Glu-Asp-Leu-Gln-Leu-Val-Asp^{798}, which contains recognition elements for the TRAF-C domain (26–28). Similarly, TRAF2 bound to the same region in gp130 via the TRAF2-C domain. This cytoplasmic region of gp130 is highly conserved across various species including human (15, 16).

How do TRAF2 and TRAF5 negatively regulate IL-6 receptor signaling? Although the expression of TRAF5 did not inhibit the interaction between JAK1 and gp130, TRAF5 repressed the phosphorylation of JAK1, gp130, and STAT3 mediated by IL-6–sIL-6R. Therefore, it was hypothesized that TRAF5 limits the proximal interaction of JAK proteins and resulting their auto-phosphorylation by disturbing the optimal dimerization of gp130 upon interaction with IL-6 and IL-6R. By employing luciferase fragment complementation system using fusion proteins of JAK1 with either the N-terminal or the C terminal protein fragment of firefly luciferase, it was revealed that TRAF2 or TRAF5 suppressed JAK1-JAK1 interactions occurring after ligation of gp130 with IL-6–sIL-6R. Importantly, the JAK1-JAK1 interaction was intact in a mutant of gp130 (Δ774-798), which lacks the binding site for TRAF2 and TRAF5, even in the presence of TRAF2 or TRAF5. In addition, it was notable that RING and Zn finger domains of TRAF2 and TRAF5 are dispensable but TRAF-C domain is essential to suppress JAK1-JAK1 interactions to limit IL-6 receptor signaling (15, 17). This demonstrates that TRAF2 and TRAF5 require both TRAF-C domain and TRAF-binding region in gp130 to inhibit JAK1-JAK1 interactions and the following molecular events in the IL-6 receptor signaling pathway. Moreover, the expression of a peptide fragment of gp130 (769–800) fused with GFP in wild-type $CD4^+$ T cells promoted the T_H17 development (15), indicating that the peptide fragment of gp130 competitively inhibits the endogenous interaction between TRAFs and gp130. All of these data support the idea that TRAF2 and TRAF5 associated with gp130 via TRAF-C domains negatively regulate the JAK activation in the IL-6 receptor signaling complex that plays an essential role in initiating the JAK-STAT signaling pathway (**Figure 2**).

There has been some unsolved issues and controversy about the mechanisms regarding regulation of the IL-6 receptor signaling and the T_H17 development mediated by TRAF2 and TRAF5. Firstly, when $CD4^+$ T cells were stimulated with IL-6–sIL-6R instead of IL-6 alone, TRAF2 and TRAF5 could efficiently inhibit the IL-6 receptor signaling activity (15, 16). IL-6 *trans* signaling is activated via membrane-bound gp130 that interacts with a complex of sIL-6R and IL-6. IL-6 *trans* signaling or IL-6 cluster signaling plays a dominant role for priming pathogenic T_H17 cells (29, 30). This suggests that TRAF2 and TRAF5 may preferentially restrain IL-6 *trans* signaling activity. Secondly, the identified TRAF-binding region in gp130 is located between first two phosphorylated-tyrosine (p-Tyr) motifs in gp130, and thus it is possible that TRAF2 and TRAF5 may inhibit the binding of STAT3 to these p-Tyr motifs via making steric hindrance in gp130 (15, 16). Thirdly, it is not clear how gp130-associated TRAF proteins inhibit the proximal JAK interaction in the receptor complex. The binding between gp130 and JAK1 occurred independently of the interaction between gp130 and TRAF5 (17). TRAFs may restrain the formation of gp130 dimer and inhibit the reposition process of associated JAKs (**Figure 2**). Fourthly, TRAF2 and TRAF5 might recruit a protein tyrosine phosphatase to the IL-6 receptor signaling complex to inhibit the JAK-STAT signaling, although this mechanism is utilized by TRAF3 expressed in B cells (31). Fifthly, TRAF5 might work as a positive regulator for RAR-related orphan receptor-γt (RORγt) activity and augment RORγt-mediated T_H17 responses in a certain experimental setting (32), although this is inconsistent with the conclusion presented here.

CONCLUDING REMARKS

It is now clear that TRAF family molecules control a wide range of signaling mediated by membrane-bound receptors in many cell types including $CD4^+$ T cells. Findings highlighted here illustrate how TRAF2 and TRAF5 impact IL-6-mediated T_H17 generation and T_H17-driven immuno-pathology *in vivo* and *in vitro* and the molecular mechanisms by which TRAF2 and TRAF5 restrain IL-6 receptor signaling. The conclusion that TRAF2 and TRAF5 interacted with gp130 suppress proximal JAK-JAK interactions and resulting JAK phosphorylation in the receptor complex suggests that these TRAFs also regulate signals downstream of receptors for other IL-6 family cytokines that utilize gp130. Dysregulated TRAF5 expression might play an important role in autoimmune and inflammatory diseases in human (33). It will be absolutely important in the future to understand how TRAF2 and TRAF5 control signal transduction through unconventional cytokine receptors and to characterize its impact on immune responses and other relevant biological responses mediated by $CD4^+$ T cells and other cell types.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work supported by JSPS KAKENHI grant numbers JP15H04640 (TS) and JP18H02572 (TS), the Daiichi-Sankyo Foundation of Life Science (TS), and the Tamura Science and Technology Foundation (TS).

ACKNOWLEDGMENTS

We thank members of Department of Microbiology and Immunology, Tohoku University Graduate School of Medicine for their assistance and help. We thank the Biomedical Research Core and the Institute for Animal Experimentation (Tohoku University Graduate School of Medicine) for technical support.

REFERENCES

1. Rothe M, Wong SC, Henzel WJ, Goeddel DV. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* (1994) 78:681–92. doi: 10.1016/0092-8674(94)90532-0
2. Grech A, Quinn R, Srinivasan D, Badoux X, Brink R. Complete structural characterisation of the mammalian and Drosophila TRAF genes: implications

for TRAF evolution and the role of RING finger splice variants. *Mol Immunol.* (2000) 37:721–34. doi: 10.1016/S0161-5890(00)00098-5
3. Inoue J, Ishida T, Tsukamoto N, Kobayashi N, Naito A, Azuma S, et al. Tumor necrosis factor receptor-associated factor (TRAF) family: adapter proteins that mediate cytokine signaling. *Exp Cell Res.* (2000) 254:14–24. doi: 10.1006/excr.1999.4733
4. Wajant H, Henkler F, Scheurich P. The TNF-receptor-associated factor family: scaffold molecules for cytokine receptors, kinases and their regulators. *Cell Signal*. (2001) 13:389–400. doi: 10.1016/S0898-6568(01)00160-7
5. Chung JY, Park YC, Ye H, Wu H. (2002). All TRAFs are not created equal: common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J Cell Sci*. 115, 679–88.
6. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol.* (2003) 21:335–76. doi: 10.1146/annurev.immunol.21.120601.141126
7. Watts TH. TNF/TNFR family members in costimulation of T cell responses. *Annu Rev Immunol*. (2005) 23:23–68. doi: 10.1146/annurev.immunol.23.021704.115839
8. Croft M. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol*. (2009) 9:271–85. doi: 10.1038/nri2526
9. Ha H, Han D, Choi Y. TRAF-mediated TNFR-family signaling. *Curr Protoc Immunol.* (2009) Chapter 11:Unit11.9D. doi: 10.1002/0471142735.im1109ds87
10. Xie P. TRAF molecules in cell signaling and in human diseases. *J Mol signal.* (2013) 8:7. doi: 10.1186/1750-2187-8-7
11. So T, Nagashima H, Ishii N. TNF Receptor-Associated Factor (TRAF) Signaling Network in CD4(+) T-Lymphocytes. *Tohoku J Exp Med*. (2015) 236:139–54. doi: 10.1620/tjem.236.139
12. Bishop GA. TRAF3 as a powerful and multitalented regulator of lymphocyte functions. *J Leukoc Biol*. (2016) 100:919–26. doi: 10.1189/jlb.2MR0216-063R
13. Ishida TK, Tojo T, Aoki T, Kobayashi N, Ohishi T, Watanabe T, et al. TRAF5, a novel tumor necrosis factor receptor-associated factor family protein, mediates CD40 signaling. *Proc Nat Acad Sci USA*. (1996) 93:9437–42. doi: 10.1073/pnas.93.18.9437
14. Nakano H, Sakon S, Koseki H, Takemori T, Tada K, Matsumoto M, et al. Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. *Proc Nat Acad Sci USA*. (1999) 96:9803–8. doi: 10.1073/pnas.96.17.9803
15. Nagashima H, Okuyama Y, Asao A, Kawabe T, Yamaki S, Nakano H, et al. The adaptor TRAF5 limits the differentiation of inflammatory CD4(+) T cells by antagonizing signaling via the receptor for IL-6. *Nat Immunol*. (2014) 15:449–56. doi: 10.1038/ni.2863
16. Nagashima H, Okuyama Y, Hayashi T, Ishii N, So T. TNFR-associated factors 2 and 5 differentially regulate the instructive IL-6 receptor signaling required for Th17 development. *J Immunol*. (2016) 196:4082–9. doi: 10.4049/jimmunol.1501610
17. Kimura M, Nagashima H, Okuyama Y, Ishii N, So T. TRAF2 and TRAF5 associated with the signal transducing receptor gp130 limit IL-6-driven transphosphorylation of JAK1 through the inhibition of proximal JAK-JAK interaction. *Int Immunol*. (2018) 30:291–99. doi: 10.1093/intimm/dxy029
18. Gardam S, Sierro F, Basten A, Mackay F, Brink R. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. *Immunity* (2008) 28:391–401. doi: 10.1016/j.immuni.2008.01.009
19. Jin W, Zhou XF, Yu J, Cheng X, Sun SC. Regulation of Th17 cell differentiation and EAE induction by MAP3K NIK. *Blood* (2009) 113:6603–10. doi: 10.1182/blood-2008-12-192914
20. Lin WJ, Su YW, Lu YC, Hao Z, Chio II, Chen NJ, et al. Crucial role for TNF receptor-associated factor 2 (TRAF2) in regulating NFkappaB2 signaling that contributes to autoimmunity. *Proc Nat Acad Sci USA*. (2011) 108:18354–9. doi: 10.1073/pnas.1109427108
21. Yang XD, Sun SC. Targeting signaling factors for degradation, an emerging mechanism for TRAF functions. *Immunol Rev*. (2015) 266:56–71. doi: 10.1111/imr.12311
22. Buchta CM, Bishop GA. TRAF5 negatively regulates TLR signaling in B lymphocytes. *J Immunol*. (2014) 192:145–50. doi: 10.4049/jimmunol.1301901
23. Jin J, Xiao Y, Hu H, Zou Q, Li Y, Gao Y, et al. Proinflammatory TLR signalling is regulated by a TRAF2-dependent proteolysis mechanism in macrophages. *Nat Commun*. (2015) 6:5930. doi: 10.1038/ncomms6930
24. Bishop GA. The multifaceted roles of TRAFs in the regulation of B-cell function. *Nat Rev Immunol*. (2004) 4:775–86. doi: 10.1038/nri1462
25. Chen BB, Coon TA, Glasser JR, McVerry BJ, Zhao J, Zhao Y, et al. A combinatorial F box protein directed pathway controls TRAF adaptor stability to regulate inflammation. *Nat Immunol*. (2013) 14:470–9. doi: 10.1038/ni.2565
26. Arch RH, Thompson CB. 4-1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclear factor kappaB. *Mole Cell Biol.* (1998) 18:558–65. doi: 10.1128/MCB.18.1.558
27. Park YC, Burkitt V, Villa AR, Tong L, Wu H. Structural basis for self-association and receptor recognition of human TRAF2. *Nature* (1999) 398:533–8. doi: 10.1038/19110
28. Ye H, Park YC, Kreishman M, Kieff E, Wu H. The structural basis for the recognition of diverse receptor sequences by TRAF2. *Mole Cell*. (1999) 4:321–30. doi: 10.1016/S1097-2765(00)80334-2
29. Jones GW, McLoughlin RM, Hammond VJ, Parker CR, Williams JD, Malhotra R, et al. Loss of CD4+ T cell IL-6R expression during inflammation underlines a role for IL-6 trans signaling in the local maintenance of Th17 cells. *J Immunol*. (2010) 184:2130–9. doi: 10.4049/jimmunol.0901528
30. Heink S, Yogev N, Garbers C, Herwerth M, Aly L, Gasperi C, et al. Trans-presentation of IL-6 by dendritic cells is required for the priming of pathogenic TH17 cells. *Nat Immunol*. (2017) 18:74–85. doi: 10.1038/ni.3632
31. Lin WW, Yi Z, Stunz LL, Maine CJ, Sherman LA, Bishop GA. The adaptor protein TRAF3 inhibits interleukin-6 receptor signaling in B cells to limit plasma cell development. *Sci Signal*. (2015) 8:ra88. doi: 10.1126/scisignal.aaa5157
32. Wang X, Yang J, Han L, Zhao K, Wu Q, Bao L, et al. TRAF5-mediated Lys-63-linked polyubiquitination plays an essential role in positive regulation of RORgammat in promoting IL-17A expression. *J Biol Chem*. (2015) 290:29086–94. doi: 10.1074/jbc.M115.664573
33. Potter C, Eyre S, Cope A, Worthington J, Barton A. Investigation of association between the TRAF family genes and RA susceptibility. *Ann Rheum Dis.* (2007) 66:1322–6. doi: 10.1136/ard.2006.065706

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership