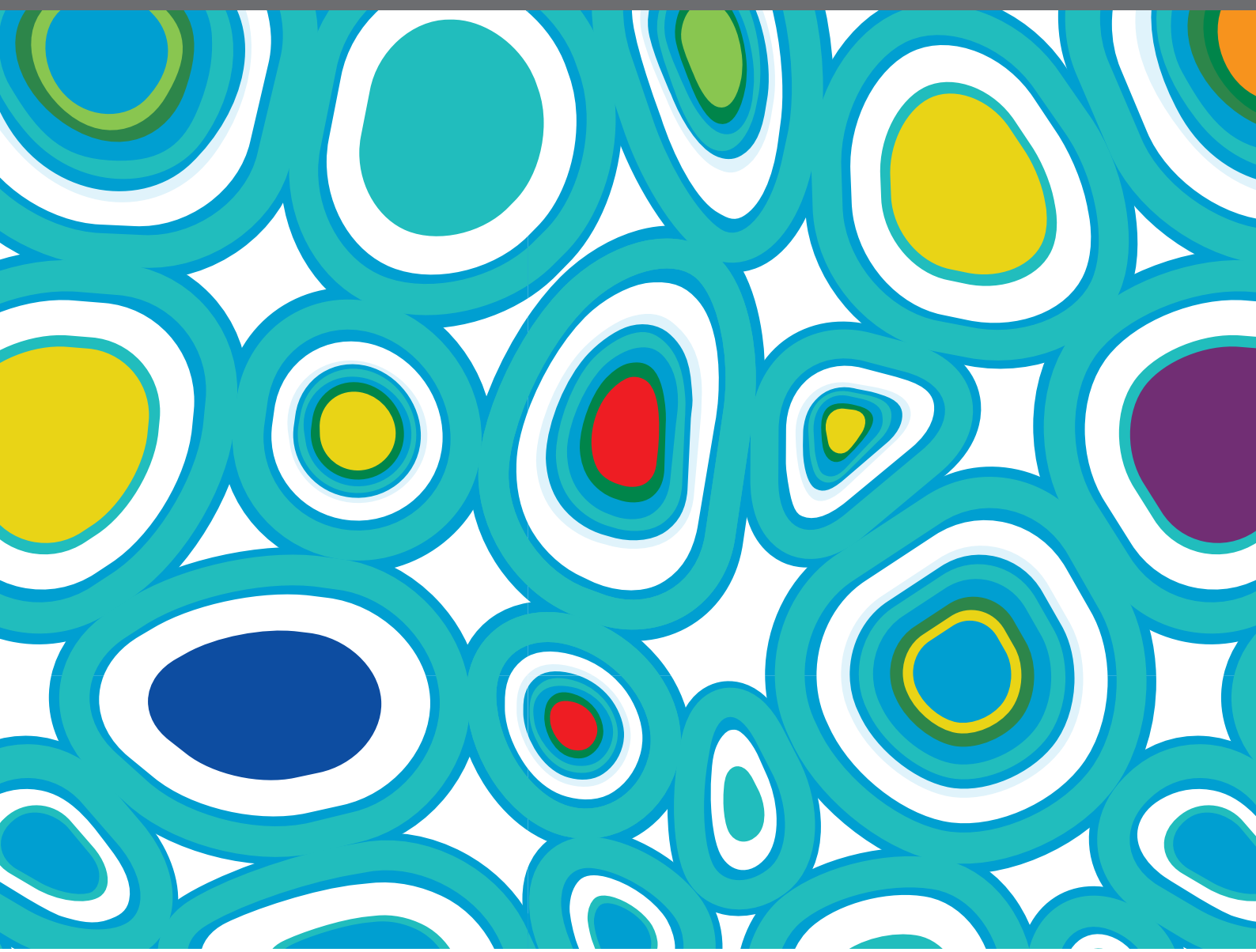
REGULATION OF UBIQUITINATION AND SUMOYLATION SIGNALING IN DISEASE

EDITED BY: Zhenghong Lin, Long Zhang, David W. Li, Xiaohua Yan and Chenghua Li

PUBLISHED IN: Frontiers in Cell and Developmental Biology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88976-731-1
DOI 10.3389/978-2-88976-731-1

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: frontiersin.org/about/contact

REGULATION OF UBIQUITINATION AND SUMOYLATION SIGNALING IN DISEASE

Topic Editors:
Zhenghong Lin, Chongqing University, China
Long Zhang, Zhejiang University, China
David W. Li, Sun Yat-sen University, China
Xiaohua Yan, Nanchang University, China
Chenghua Li, Sichuan University, China

Citation: Lin, Z., Zhang, L., Li, D. W., Yan, X., Li, C., eds. (2022). Regulation of Ubiquitination and Sumoylation Signaling in Disease.
Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-731-1

Table of Contents

frontiers | Frontiers in Cell and Developmental Biology

EDITORIAL
published: 12 July 2022
doi: 10.3389/fcell.2022.970683

Editorial: Regulation of Ubiquitination and Sumoylation Signaling in Disease

Zhenghong Lin[1]* *and Xiaohua Yan*[2]*

[1]School of Life Sciences, Chongqing University, Chongqing, China, [2]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Nanchang University, Nanchang, China

Keywords: ubiquitination, sumoylation, ISGylation, neddylation, PROTAC (proteolysis-targeting chimeric molecule)

Editorial on the Research Topic

Regulation of Ubiquitination and Sumoylation Signaling in Disease

Ubiquitination/Ubiquitin-like protein modifications play an important role in the regulation of diverse physiological processes, such as DNA damage repair, cell cycle progression, cell proliferation, apoptosis and differentiation, signal transduction and gene transcriptional regulation, vesicle transport, autophagy, immunity, etc. In addition, ubiquitination and ubiquitin-like protein modifications have also been widely involved in pathological processes including metabolic disorders, inflammation, tumorigenesis, amongst others. Therefore, in recent years, it has received increasing interest to identify novel molecular targets that could lead to the development of new drugs. A deeper understanding of the ubiquitination and ubiquitin-like modifications-mediated signaling pathways and their regulatory mechanisms is urgently needed in order to identify novel molecular players and therapeutic targets for treatment of cancer and other diseases. In this Research Topic, we collected nine articles to discuss the roles of the ubiquitination/ubiquitin-like modifications in regulating diverse signal transduction pathways, thereby providing new insights to understand why and how dysregulation of them may drive pathological progression or trigger disease and aimed to provide clues for the treatment of these diseases.

The ubiquitin-proteasome system (UPS) is the main machinery contributive to the control of protein degradation in eukaryotic cells. Ubiquitination can occur under the successive actions of E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, E3 ubiquitin-ligase enzymes, and/or E4. WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) is a member of the C2-WW-HECT E3 ubiquitin ligase protein family. The Kuang et al. summarized the recent advances of WWP1 E3 ubiquitin ligase in cancer progression. They discussed the factors that cause mRNA level increase of WWP1 gene, the regulation of enzymatic activity of WWP1 protein, and its autoinhibitory mechanism in steady state.

E4B belongs to the U-box E3 ubiquitin ligase family and functions as either an E3 or an E4 enzyme in protein ubiquitination. Lu et al. studied the differential degradation of TRA2A and PYCR2 by E3 ubiquitin ligase E4B. In their study, they validated the ubiquitination of TRA2A and PYCR2 by E4B *in vitro* and in mammalian cells. They found that E4B mediated the degradation by forming K11- and K48- linked polyubiquitin chains on TRA2A and PYCR2, respectively. Intriguingly, both E4B and its substrates TRA2A and PYCR2 are overexpressed in hepatocellular carcinoma (HCC) cells, and E4B-mediated ubiquitination does not lead to protein degradation of TRA2A or PYCR2. Therefore, they concluded that other factors may exist to control the degradation of TRA2A and PYCR2 in HCC.

Primary cilia are microtubule-based, non-motile sensory organelles present in most types of growth-arrested eukaryotic cells. They are regarded as the signal transduction hubs that receive and transmit external signals to the cells, thus controlling cell growth and differentiation. Mutation of ciliary structure-related genes has been reported to cause a wide array of developmental genetic

OPEN ACCESS

Edited and reviewed by:
Ana Cuenda,
Spanish National Research Council (CSIC), Spain

***Correspondence:**
Zhenghong Lin
zhenghonglin@cqu.edu.cn
Xiaohua Yan
yanxiaohua@ncu.edu.cn

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *16 June 2022*
Accepted: *20 June 2022*
Published: *12 July 2022*

Citation:
Lin Z and Yan X (2022) Editorial: Regulation of Ubiquitination and Sumoylation Signaling in Disease. Front. Cell Dev. Biol. 10:970683. doi: 10.3389/fcell.2022.970683

disorders. Senatore et al. summarized recent advances of primary cilia in controlling growth, differentiation and development. They discussed the interplay among UPS, autophagy and signaling pathways and concluded that they may act in synergy to control the ciliary homeostasis.

In contrast to polyubiquitination-mediated protein degradation, sumoylation, a ubiquitin-like post-translational modification, usually does not regulate protein stability but modulates signal transduction. Zhu et al. summarized the current understanding of the ubiquitination and sumoylation signaling in cellular metabolic regulation. They discussed how ubiquitination and sumoylation affect cancer metabolism by regulating the key enzymes involved in metabolic pathways to reshape metabolism and finally facilitate cancer progression.

Besides sumoylation, another ubiquitin-like post-translational modification is neddylation. It occurs *via* the activation of the neural precursor cell expressed, developmentally downregulated protein 8 (NEDD8) by three enzymes: an activating enzyme, a conjugating enzyme, and finally a ligase. Zhu et al. summarized the recent advances of the association between neddylation and immune response. They discussed the importance of NEDD8 in innate and adaptive immune cells and its regulatory role in the anti-viral pathways. Finally, they reminded us that the neddylation inhibitor MLN4924, as an anti-tumor medicine, has negative effects in immunity and need to take careful consideration when it is clinically used.

Apart from sumoylation and neddylation, ISGylation represents another ubiquitin-like post-translational modification. Zhang et al. summarized how ISGylation, especially ISG15, functions in innate antiviral immunity and pathogen defense responses. Through covalent binding with the host and viral target proteins, ISG15 inhibits the release of viral particles, hinder viral replication, and regulates the incubation period of viruses, thereby exerting strong antiviral effects.

Protein post-translational modification by ubiquitin is a reversible biochemical process. Deubiquitinating enzymes (DUBs) are responsible for removing ubiquitin or ubiquitin-like modifications from substrate proteins, thereby gaining increasing attention. Li et al. summarized the multiple functions of the deubiquitinase USP13 and its target inhibition. They discussed the structure and function of USP13 and its actions in various human diseases, in addition to the development of inhibitors. They hoped to provide some enlightenment for drug development and therapy of USP13-caused malignant diseases. Of relevance, Rossi and Rossi summarized the roles of USP19 in oncogenesis and cancer progression. They reviewed the current knowledge of USP19 as to the control of several cellular processes in different neoplasms, which highlights a complexity of USP19 function which possesses both positive and negative regulation activities in tumorigenesis and cancer progression. They suggested that USP19 might represent a novel putative pharmacologic target in oncology, underscoring the potential of identifying specific modulators to test in clinical settings.

Ubiquitination, as one of the most important post-translational events, is a dynamic process primarily responsible for protein degradation *via* proteasomes. Importantly, ubiquitination can be targeted for the treatment of human disease. Proteolysis-targeting chimera (PROTAC) is a recently emerged technique that has great potential to be clinically used in the treatment of cancer. Lospinoso Severini et al. summarized the recent advances of the PROTAC strategy as therapeutic option in glioblastoma. They discussed the advantages and limitations of PROTAC development and safety considerations for their application in clinical usage.

Together, understanding the biochemical nature and biological functions of protein post-translational modifications, especially ubiquitination and ubiquitination-like modifications, is of great significance in unravelling the molecular mechanisms underlying the development of human diseases, such as cancer. In addition, this is also a prerequisite for discovering new molecular targets and developing novel anti-cancer drugs.

AUTHOR CONTRIBUTIONS

ZL and XY wrote, reviewed and revised the manuscript.

FUNDING

This work was supported by Natural Science Foundation Project of CQ CSTC (Grant No. cstc2020jcyj-msxmX0154 to ZL), the National Natural Science Foundation of China (NSFC, grant Nos. 82172888 to ZL, 32060148 and 31871378 to XY), and by the Talent Plan of Jiangxi Province of China (Grant No. jxsq2018106037 to XY).

Conflict of Interest: 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cell and Developmental Biology

REVIEW
published: 12 October 2021
doi: 10.3389/fcell.2021.757493

WW Domain-Containing E3 Ubiquitin Protein Ligase 1: A Self-Disciplined Oncoprotein

Linghan Kuang[1,2], Yunhui Jiang[3], Chenghua Li[4] and Yongmei Jiang[1,2]**

[1] Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, Chengdu, China, [2] Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, Chengdu, China, [3] Pathology Department, The Second People's Hospital of Jingmen, Jingmen, China, [4] Center of Growth, Metabolism and Aging, Key Laboratory of Biological Resources and Ecological Environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu, China

WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) is a member of C2-WW-HECT E3 ligase family. Although it may execute carcinostatic actions in some scenarios, WWP1 functions as an oncoprotein under most circumstances. Here, we comprehensively review reports on regulation of WWP1 and its roles in tumorigenesis. We summarize the WWP1-mediated ubiquitinations of diverse proteins and the signaling pathways they involved, as well as the mechanisms how they affect cancer formation and progression. According to our analysis of database, in combination with previous reports, we come to a conclusion that WWP1 expression is augmented in various cancers. Gene amplification, as well as expression regulation mediated by molecules such as non-coding RNAs, may account for the increased mRNA level of WWP1. Regulation of enzymatic activity is another important facet to upregulate WWP1-mediated ubiquitinations. Based on the published data, we conclude that WWP1 employs interactions between multiple domains to autoinhibit its polyubiquitination activity in a steady state. Association of some substrates can partially release certain autoinhibition-related domains and make WWP1 have a moderate activity of polyubiquitination. Some cancer-related mutations can fully disrupt the inhibitory interactions and make WWP1 hyperactive. High expression level or hyperactivation of WWP1 may abnormally enhance polyubiquitinations of some oncoproteins or tumor suppressors, such as ΔNp63α, PTEN and p27, and ultimately promote cell proliferation, survival, migration and invasion in tumorigenesis. Given the dysregulation and oncogenic functions of WWP1 in some cancer types, it is promising to explore some therapeutic inhibitors to tune down its activity.

Keywords: WW domain-containing E3 ubiquitin protein ligase 1 (WWP1), tumorigenesis and progression, protein degradation, ubiquitination, C2-WW-HECT E3 ligase family, transforming growth factor-beta (TGFβ), epidermal growth factor receptor (EGFR), Wnt/b-catenin

OPEN ACCESS

Edited by:
Vanesa Gottifredi, IIBBA-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Leloir Institute Foundation, Argentina

Reviewed by:
Zaidoun Salah, Al-Quds University, Palestine
Shukuan Ling, China Astronaut Research and Training Center, China

****Correspondence:***
Chenghua Li
lichenghua@scu.edu.cn
Yongmei Jiang
jym85501201@163.com

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *12 August 2021*
Accepted: *21 September 2021*
Published: *12 October 2021*

Citation:
Kuang L, Jiang Y, Li C and Jiang Y (2021) WW Domain-Containing E3 Ubiquitin Protein Ligase 1: A Self-Disciplined Oncoprotein. Front. Cell Dev. Biol. 9:757493. doi: 10.3389/fcell.2021.757493

INTRODUCTION

WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) is also known as AIP5 (Atropin-1-interacting protein 5) or TIUL1 (TG-interacting ubiquitin ligase 1) (Wood et al., 1998; Seo et al., 2004; Zhi and Chen, 2012). It belongs to the C2-WW-HECT E3 ligase family, which also contains 8 extra members, i.e., WWP2 (also known as AIP2) (Zhang et al., 2019), NEDD4 (neural precursor

cell expressed developmentally downregulated protein 4, also known as NEDD4-1) (Huang et al., 2019), NEDD4L (NEDD4-like ubiquitin protein ligase, also known as NEDD4-2) (Goel et al., 2015), NEDL1 (NEDD4-like ubiquitin protein ligase-1) (Miyazaki et al., 2004), NEDL2 (Wei et al., 2015), Itch (named in reference to skin-scratching behavior in mice lacking this protein, also known as Itchy or AIP4) (Perry et al., 1998; Yin et al., 2020), SMURF1 (Smad ubiquitination regulatory factor 1), and SMURF2 (Zhi and Chen, 2012; Fu et al., 2020). Some of these members may be functionally redundant with WWP1, given that WWP1 knockout mice are viable and fertile with no obvious abnormalities (Chen and Matesic, 2007; Shu et al., 2013). *WWP1* is highly expressed in multiple tissues (Wood et al., 1998; Komuro et al., 2004), where it can ubiquitinate plenty of proteins and regulate diverse cellular processes including protein trafficking, degradation, and cell signal transduction. Thus, this E3 ligase should be finely regulated, because dysregulation of it is involved in a variety of diseases, such as malignancies, cardiovascular diseases, and immune disorders (Zhi and Chen, 2012). Vast evidence reveals that WWP1 is overexpressed in multiple cancer types, especially some breast and prostate cancers, while downregulated in several classes of carcinomas. In these tumor tissues, WWP1 either promotes or inhibits tumorigenesis via modulating the protein levels or functions of its substrates (Zhi and Chen, 2012).

THE *WW DOMAIN-CONTAINING E3 UBIQUITIN PROTEIN LIGASE 1* GENE AND ITS EXPRESSION

The *WWP1* gene is highly conserved from *C. elegance* to mammals (Huang et al., 2000). It locates on chromosome 8q21 and spans up to 142 kilobase pairs, containing 26 exons (Malbert-Colas et al., 2003). Numerous somatic mutations occur in the *WWP1* gene in different human cancers (Wang et al., 2019). According to our analysis using GEPIA database[1] (Tang et al., 2017), WWP1 mRNA is expressed in diverse tissues, such as brain, esophagus, breast, lung, liver, stomach, colon, prostate, and testis (**Figure 1A**). This is consistent with previous reports (Wood et al., 1998; Komuro et al., 2004). It has been reported that about 1 out of 2 or 3 prostate and breast cancers bear multiple copies of the *WWP1* gene due to gene amplification, resulting in an elevated expression level of this gene (Chen et al., 2007a,b; Nguyen Huu et al., 2008). Here, we comprehensively analyzed the expression profile of *WWP1* in different cancer types using GEPIA database (Tang et al., 2017). As shown in **Figure 1B**, besides breast invasive carcinoma (BRCA) and prostate adenocarcinoma (PRAD), WWP1 mRNA level is significantly upregulated in cholangio carcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), kidney chromophobe (KICH), acute myeloid leukemia (AML), liver hepatocellular carcinoma (LIHC), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), and thymoma (THYM). Gene amplification may be the main cause of the high expression level of WWP1 in these malignancies, where this E3 ligase probably executes oncogenic functions. On the other hand, WWP1 is downregulated in bladder urothelial carcinoma (BLCA), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), ovarian serous cystadenocarcinoma (OV), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS). The undermentioned regulation mechanisms may account for the reduced expression of WWP1, which is likely to function as a tumor-suppressive E3 ligase in these cancers.

Expression of WWP1 is regulated by multiple mechanisms. It was reported that transforming growth factor β (TGFβ) stimulates transcription of *WWP1* gene via an unknown mechanism (Chen and Matesic, 2007). This suggests that TGFβ and WWP1 form a feedback loop, since WWP1 can repress TGFβ signaling via downregulating several components of this cascade (Komuro et al., 2004; Seo et al., 2004). Tumor necrosis factor α (TNFα) can also promote expression of WWP1 at mRNA level (Zhao et al., 2011). Data from Ceshi Chen group suggest that p53 positively regulates expression of WWP1 (Li Y. et al., 2008). In combination with the evidence that WWP1 impairs transactivity of p53 (Laine and Ronai, 2007), this indicates another feedback loop. According to data from Ceshi Chen group, DNA damage drugs induce expression of WWP1 via enhanced p53 level (Li Y. et al., 2008). Data from our group suggest that DNA damage may stimulate transcription of WWP1 through a p53-dependent manner or a miR-452-involved mode (Chen et al., 2017). Pier Paolo Pandolfi group recently reported that MYC directly binds to the promoter of WWP1 gene and activates its transcription (Lee et al., 2019). Besides miR-452 (Goto et al., 2016; Chen et al., 2017), several non-coding RNAs have been found to regulate expression of WWP1: microRNAs, including miR-15b (Li et al., 2020), miR-21 (Tao et al., 2018), miR-30a-5p (Zhao et al., 2019), miR-129-5p and -3p (Ma et al., 2018), miR-142 (Tu et al., 2017; Wang et al., 2021), and miR-584-5p (Li et al., 2017), inhibit WWP1 expression likely via destabilizing the WWP1 mRNA; long non-coding RNA SNHG12 and circular RNA circWAC sponge miR-129-5p and miR-142, respectively, to derepress the expression of WWP1 (Li et al., 2019; Wang et al., 2021). All the known factors regulating WWP1 expression at the mRNA level are summarized in **Table 1**. These data indicate that multiple signals, transcription factors, and non-coding RNAs may affect tumorigenesis via modulating expression of WWP1.

PROTEIN STRUCTURE AND ACTIVITY REGULATION OF WW DOMAIN-CONTAINING E3 UBIQUITIN PROTEIN LIGASE 1

Due to alternative splicing post transcription, the *WWP1* gene generates at least 6 isotypes of protein in homo sapiens (Flasza et al., 2002; Zhi and Chen, 2012). The predominant isoform is the longest one, which encompasses 922 amino acid residues. Without special instructions, WWP1 refers to this isotype in

[1] http://gepia.cancer-pku.cn/

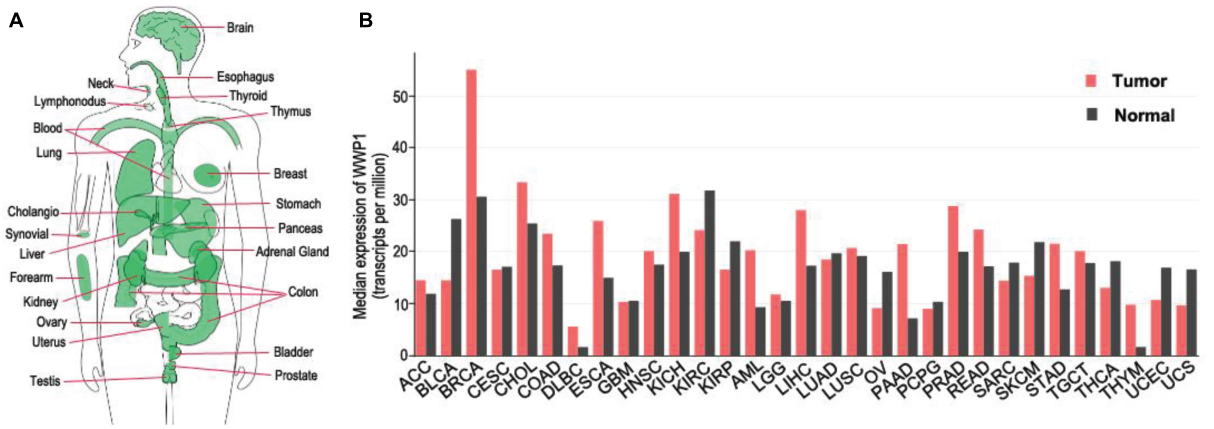


FIGURE 1 | Expression of WWP1 in human body and tumor tissues. **(A)** WWP1 expression of normal samples in bodymap. All organs expressing WWP1 mRNA are painted green. WWP1 expression is analyzed in website http://gepia.cancer-pku.cn/. **(B)** The gene expression profile across all tumor samples and paired normal tissues according to the analysis in website http://gepia.cancer-pku.cn/. TPM, transcripts per million; ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangio carcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; AML, Acute Myeloid Leukemia; LGG, Brain Lower Grade Glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine Carcinosarcoma.

general. It is comprised with an N-terminal Ca^{2+}-dependent lipid-binding (C2) domain, four WW domains (WW1∼4) in the middle, and a homologous to the E6-AP carboxyl terminus (HECT) domain (as depicted in **Figure 2A**). The structures and functions of other isoforms need further study. The C2 domain is responsible for protein-protein interaction and membrane targeting (Plant et al., 1997; Wiesner et al., 2007; Wang et al., 2010). Each WW domain contains 35∼40 residues in a triple strand β-sheet structure, which is characterized by two tryptophan (W) residues spaced 20∼22 residues apart. It is well known that the WW domains mediate the interaction with diverse substrates or adaptors, especially those containing PY motifs (Sudol et al., 1995; Mosser et al., 1998; Sudol and Hunter, 2000; Li Y. et al., 2008; Li et al., 2009). There is an autoinhibitory link, named 2,3-linker, between the second and the third WW domains (Wang et al., 2019). The HECT domain at the C-terminus endows WWP1 with E3 ligase activity. It can be divided into two lobes: the N-lobe can bind to an E2 enzyme such as UbcH5 and UbcH7, while the C-lobe is involved in the ubiquitination (Ub) process (Verdecia et al., 2003). The cystein 890 residue (C890) in the C-lobe is critical for ubiquitin transferring, because it can form a covalent bond with ubiquitin (Seo et al., 2004). There is a flexible hinge loop between both lobes, which can bend to execute the sequential addition of ubiquitin from E2 to the substrates (Verdecia et al., 2003; Lorenz et al., 2013).

WW domain-containing E3 ubiquitin protein ligase 1 can be post-translationally autoregulated to modulate its stability and E3 ligase activity. Ceshi Chen group reported that WWP1 protein undergoes autoubiquitination and proteasomal degradation (Chen and Matesic, 2007). Céline Prunier group found that an intra-molecular interaction between the C2 and/or WW and HECT domains of WWP1 makes WWP1 self-catalyze its monoubiquitination (mUb) at steady states, leading to stabilization of WWP1 and silence of its activity to polyubiquitinate (pUb) its substrates such as TGFβ type I receptor (TβR1). The intra-interaction is disrupted upon binding to the complex composed of TβR1 and Smad7, the latter of which is a bridging adaptor between TβR1 and WWP1. This switches the mUb activity of WWP1 toward a pUb activity, thereby driving the degradation of WWP1 itself as well as of TβR1. Removal of the WW domains can also convert auto-mUb of WWP1 to auto-pUb with both K48- and K63- linked polyubiquitin chains, which thereby facilitate proteasomal and lysosomal degradation of this E3 ligase (Courivaud et al., 2015). The replacement of a glutamate by a valine at position 798 (E798V) leads to constitutive pUb and degradation of WWP1 via disrupting this intra-interaction (Courivaud et al., 2015). This hyperactive mutant can cause prostate cancer in human (Chen et al., 2007a; Courivaud et al., 2015).

A multi-lock inhibitory mechanism for fine-tuning activity of WWP1 was recently proposed by Wenyu Wen group. Their data demonstrate that C2 domain cannot form a stable complex with HECT, and deletion of C2 domain alone or together with WW1 has little impact on the ligase activity. On the other hand, removing WW2/3/4 leads to a partial activation of WWP1, while deletion of the 2,3-linker strikingly increases WWP1 activity, indicating different importance of the WW and 2,3-linker domains in autoinhibition. Further, their data suggest that WWP1 employs the 2,3-linker, WW2∼4, and HECT domains to form a multilevel inhibitory machinery for tuning its enzymatic activity: WW2 and 2,3-linker interact with HECT;

TABLE 1 | Factors regulating WWP1 mRNA levels.

Factor	Molecule type	Effect on *WWP1* mRNA level	Mechanism	References
TGFβ	Extracellular signaling molecule	↑	unknown	Chen and Matesic, 2007
TNFα	Extracellular signaling molecule	↑	unknown	Zhao et al., 2011
p53	Transcription factor	↑	transactivation	Li Y. et al., 2008
MYC	Transcription factor	↑	transactivation	Lee et al., 2019
miR-452	microRNA	↓	Destabilizing *WWP1* mRNA	Goto et al., 2016
miR-15b	microRNA	↓	Destabilizing *WWP1* mRNA	Li et al., 2020
miR-21	microRNA	↓	Destabilizing *WWP1* mRNA	Tao et al., 2018
miR-30a-5p	microRNA	↓	Destabilizing *WWP1* mRNA	Zhao et al., 2019
miR-129-5p/3p	microRNA	↓	Destabilizing *WWP1* mRNA	Ma et al., 2018
miR-142	microRNA	↓	Destabilizing *WWP1* mRNA	Tu et al., 2017; Wang et al., 2021
miR-584-5p	microRNA	↓	Destabilizing *WWP1* mRNA	Li et al., 2017
SNHG12	Long non-coding RNA	↑	Sponging miR-129-5p and stabilizing *WWP1* mRNA	Li et al., 2019
circWAC	Circular RNA	↑	Sponging miR-142 and stabilizing *WWP1* mRNA	Wang et al., 2021

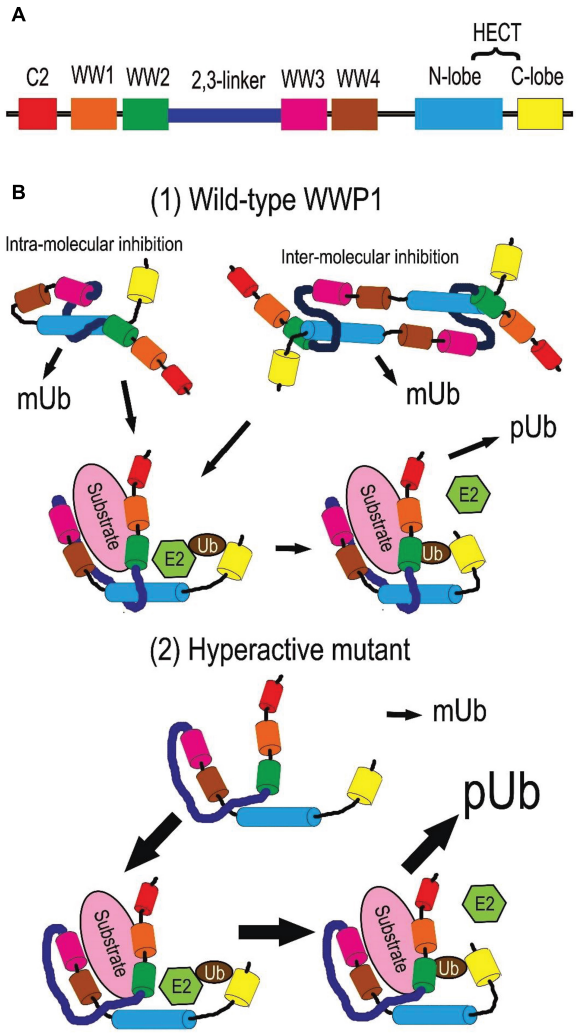


FIGURE 2 | Protein structure and activity regulation of WWP1. **(A)** Depiction of WWP1 protein structure. Each domain is depicted in a certain color (the same below). **(B)** Activity regulation of WWP1. (1) Wild-type WWP1 is autoinhibited via intra- or inter-molecular interactions in a steady state: the HECT domain is sequestered by the 2,3-linker and WW2∼4 domains. In this state, WWP1 has the monoubiquitination (mUb) activity to modify itself and some substrates. Binding of a substrate can partially disrupt the inhibitory interactions and release WW domains from N-lobe. This endows WWP1 with moderate polyubiquitination (pUb) activities: the N- and C-lobes collaborate to transfer ubiquitin chains (Ub) from E2 ligases sequentially. (2) Some cancer-related mutations severely break the autoinhibitory interactions and generate hyperactive WWP1 proteins, which induce elevated polyubiquitination of some substrates.

Tyr543 (Y543) in the HECT domain occupies the canonical PY motif-binding site of WW4; WW2 and WW3 stabilize both termini of 2,3-linker (Wang et al., 2019). Thus, WWP1 is locked in an inactive state by preventing ubiquitin transfer from an E2 ligase. When WW2 and WW4 are engaged by substrates or adaptors, especially those containing PY motifs, they can dissociate from HECT. This leads to a partial activation of WWP1 polyubiquitination activity. In some cases, the 2,3-linker is released from HECT for phosphorylation; dissociation of the WW domains and tyrosine/threonine phosphorylation on the 2,3-linker may cooperate to induce a fully active state of WWP1 (Riling et al., 2015; Grimsey et al., 2018; Jiang et al., 2019; Wang et al., 2019). Phosphorylation of Y543 in HECT may also elevate the ligase activity of WWP1. Due to the key roles of the abovementioned domains in autoinhibition, a significant number of cancer-related mutations of WWP1 are located in these domains, which may impair the autoinhibition and generate hyperactive variants of WWP1 (Wang et al., 2019).

Data from Yu-Ru Lee et al. indicate that the interaction between the 2,3-linker region and the HECT domain can be mediated by either homodimerization or intra-interaction, both of which lead to an autoinhibition of WWP1 E3 ligase activity. Two germline point mutations in the N-lobe, K740N and N745S, may hyperactivate the polyubiquitination activity of WWP1 (Lee et al., 2020).

Generally speaking, the manner of interaction between an E3 ligase and a substrate is a major determinant of the ubiquitination type. EGFR pathway substrate clone 15 (EPS15) does not have a PY motif. It employs its ubiquitin binding motif 2 (UIM2) to

recruit WWP1, which monoubiquitinates EPS15 (Woelk et al., 2006). UIMs usually bind ubiquitin with low affinities and fast dissociation kinetics, which make the substrate dissociate from the E3 ligase before a second round of ubiquitin addition occurs (Hicke et al., 2005; Hoeller et al., 2006; Ramanathan and Ye, 2012). Another monoubiquitination substrate of WWP1, p53, has no PY motif either (Laine and Ronai, 2007). Although RNF11 has a PY motif, it binds to the WW1 or WW3 domains of WWP1, instead of WW4 (Chen et al., 2008). All these associations may fail to disrupt the intra- or inter-molecular interaction between the WW4 domain and Y543 of the N-lobe. This may account for WWP1-mediated monoubiquitination of these proteins as well as of WWP1 itself. In the future, the exact binding sites of WWP1 with different substrates is worthwhile to map. On the other hand, how WWP1 mediates different types of polyubiquitination, i.e., K27-, K48-, or K63-linked ubiquitination, is poorly investigated.

Based on the reports mentioned above, we summarize the regulation of WWP1 activity as follows and depict it in **Figure 2B**. In a steady state, polyubiquitination (pUb) activity of WWP1 is autoinhibited through intra- or inter-molecular interactions: its WW2∼4 domains, especially WW4, sequester the N-lobe of the HECT domain, while the 2,3-linker also binds to the N-lobe. Association of a substrate protein or an adaptor with the WW domains disrupts the autoinhibitory interactions and releases the WW domains from HECT, inducing a partial activity of WWP1 to sequentially deliver ubiquitin chains (Ub) to the substrate. Some factors, e.g., some mutations in the autoinhibition-related domains, can severely break these interactions and release both the WW domains and the 2,3-linker, inducing a fully active WWP1, which aberrantly increases polyubiquitination of some substrates. On the other hand, some substrates recruit WWP1 with loose association manners, which may maintain the abovementioned interactions between domains of WWP1. These substrate proteins, as well as WWP1 itself, are consequently monoubiquitinated by WWP1, even in an autoinhibited state.

WW DOMAIN-CONTAINING E3 UBIQUITIN PROTEIN LIGASE 1-MEDIATED PROTEIN UBIQUITINATIONS AND THEIR ROLES IN TUMORIGENESIS

WW domain-containing E3 ubiquitin protein ligase 1 can directly add monoubiquitin or different polyubiquitin chains to a variety of substrate proteins and consequently modulate their stability, trafficking, or functions (Seo et al., 2004; Chen et al., 2005, 2008; Flasza et al., 2006; Zhi and Chen, 2012). These WWP1-mediated modifications affect tumorigenesis through kaleidoscopic pathways. In **Table 2**, we list the main substrates of WWP1 that we already know, and the signaling axes they involved, as well as the effects of these modifications on tumorigenesis. Though the ubiquitination (Ub) types of some substrates are yet to be identified, most degradation-related modifications are probably K48-linked polyubiquitination (K48 pUb), since K48-linked polyubiquitin chains are the canonical labels recognized by 26S proteasome for degradation (Grice and Nathan, 2016).

Like its homologues, Smad ubiquitination regulatory factors (SMURFs), WWP1 mediates polyubiquitination of several Smad proteins. Seo et al. (2004) found that WWP1 specifically interacts with Smad2, 3, 4, 6, and 7, which are key components of TGFβ signaling pathway (Colak and Ten Dijke, 2017). WWP1 associates with Smad7 to induce ubiquitination and subsequent proteasomal degradation of the TGFβ type 1 receptor (TβR1). On the other hand, WWP1 can mediate ubiquitin-dependent degradation of Smad2 in the presence of TG-interacting factor (TGIF), which is a TALE homeodomain protein and a transcriptional repressor (Seo et al., 2004). Furtherly, Run Shen et al. reported that Smad6 is involved in runt-related transcription factor 2 (Runx2) degradation mediated by WWP1 as well as by its homologues, SMURF1 and SMURF2 (Shen et al., 2006; Li X. et al., 2008). In parallel, Schnurr-3 (Shn3) can also recruit WWP1 to mediate Runx2 degradation (Jones et al., 2006). Moren et al. (2005) found that WW and HECT domain-containing ligases, including SMURF1, SMURF2, WWP1, and NEDD4-2, ubiquitinate and degrade Smad4 in the presence of Smad6 or Smad7. In these scenarios, Smad6, Smad7, Shn3, and TGIF function as adaptors for ubiquitination of other proteins (TβR1, Smad2, Smad4, and Runx2) mediated by WWP1 and its homologous E3 ligases. Via ubiquitinating the abovementioned proteins, WWP1 inhibits TGFβ-induced transcriptional responses and growth arrest in either normal kidney cells, including 293 and MDCK cells (Seo et al., 2004), or PC-3 prostate cancer cells (Chen et al., 2007a). In addition, WWP1 may be involved in downstream effects of TGFβ pathway. Kunhong Kim group reported that WWP1-mediated polyubiquitination and degradation of Casein kinase regulatory subunit, CK2β, is required for TGFβ-induced epithelial-mesenchymal transition (EMT) and metastasis of non-small cell lung cancer (NSCLC) cells via enhancing CK2 activity (Kim et al., 2018). Together, these data demonstrate that WWP1 can affect TGFβ pathway in multi-dimensions to promote neoplasia and progression of several cancer types, such as PCa and NSCLC.

WW domain-containing E3 ubiquitin protein ligase 1 can also regulate epidermal growth factor (EGF) signaling and its downstream pathways such as PI3K-AKT and Ras-ERK, which can promote cell proliferation and chemoresistance (Sharma et al., 2007). Overexpression or hyperactivation of epithelial growth factor receptor (EGFR) can drive formation and progression of multiple tumors, including lung cancer and breast cancer (Sharma et al., 2007). Previous studies suggest that WWP1 regulates EGFRs in different ways. According to data from Ceshi Chen and his colleagues, WWP1 monoubiquitinates RING finger protein 11 (RNF11), which is an E3 ligase mediating polyubiquitination and subsequent proteasomal degradation of EGFR. This RNF11-mediated regulation as well applies in HER2 (human epidermal growth factor receptor 2, also known as ErbB2). They further identified WW1/3 of WWP1 and the PY motif of RNF11 as the binding sites during the modification. This WWP1-mediated monoubiquitination (mUb) impairs RNF11-induced degradation of EGFR and HER2 (Chen et al., 2008).

TABLE 2 | WWP1-mediated protein ubiquitinations and their effects on tumorigenesis.

Substrate	Ub type	Effects on substrate	Effects of the ubiquitination on tumorigenesis	References
TβR1	pUb	Degradation	ImpairedTGFβ signaling and growth arrest of cancer cells, especially PCa cells	Komuro et al., 2004; Seo et al., 2004; Moren et al., 2005
Smad2	pUb	Degradation		
Smad4	pUb	Degradation		
CK2β	pUb	Degradation	Enhanced TGFβ-induced EMT and metastasis of NSCLC cells	Kim et al., 2018
RNF11	mUb	Activity repression	Increased survival of PCa and BrCa cells via upregulation of HER2 and EGFR	Chen et al., 2007a
EPS15	mUb	Decrease in association and co-localization with EGFR-containing endocytic vesicles	Unknown	Savio et al., 2016; Pascolutti et al., 2019
EGFR	K63 pUb	Enhanced recycling and protein stability	Increased stemness of NSCLC via activation of PI3K-AKT and Ras-ERK axes	Sharma et al., 2007; Yu et al., 2020
HER4	pUb	Degradation	Enhanced mammary epithelial cell proliferation and survival	Feng et al., 2009; Li et al., 2009
PTEN	K27 pUb	Inhibition of dimerization and membrane localization	Increased morbidity ofoligopolyposis, CRC, and PCa via derepression of PI3K-AKT-mTOR axis and	Lee et al., 2019, 2020
DVL2	K27 pUb	Stabilization and translocation to actin-rich projections	Enhanced invasion/metastasis of BrCa cells via upregulation of WNT-PCP pathway	Nielsen et al., 2019; Zhao et al., 2021
p53	mUb and pUb	Stabilization and translocation to cytoplasm	Inhibition of p53 tumor-suppressive activities	Laine and Ronai, 2007; Levine and Oren, 2009
TAp63α	pUb	Degradation	Survival and migration of cancer cells	Li Y. et al., 2008; Li et al., 2013;
ΔNp63α	K63 pUb	Degradation	*Inhibition of cell survival*but upregulation of cell migration	Chen et al., 2018
ΔNp73	pUb	Degradation	*Enhanced apoptosis in HeLa human cervix adenocarcinoma cells*	Chaudhary and Maddika, 2014
KLF2	pUb	Degradation	Unknown	Zhang et al., 2004; Chen et al., 2005; Dong and Chen, 2009
KLF5	K48 pUb	Degradation	*Inhibition of cell survival and metastasis in BrCa and PCa*	
p27	K48 pUb	Degradation	Cell proliferation and AML growth	Cao et al., 2011; Sanarico et al., 2018
LATS1	pUb	Degradation	BrCa cell proliferation	Yeung et al., 2013
JunB	pUb	Degradation	*Inhibition ofbone metastasis of BrCa and PCa tumors and cells*	Shen et al., 2006; Zhao et al., 2011; Jones et al., 2006; Shu et al., 2013
Runx2	pUb	Degradation		

Ub, ubiquitination; pUb, polyubiquitination; mUb, monoubiquitination; EMT, epithelial-mesenchymal transition; PCa, prostate cancer; BrCa, Breast cancer; NSCLC, non-small cell lung cancer; CRC, colorectal carcinoma; AML, acute myeloid leukemia. The tumor-suppressive effects of WWP1-mediated ubiquitination are in an italic font in the 4th column.

On the other hand, several groups reported that WWP1 monoubiquitinates endocytosis protein EPS15, which is involved in EGFR endocytosis and trafficking. This may subsequently modulate EPS15-mediated endocytosis and degradation of EGFR (Savio et al., 2016; Pascolutti et al., 2019). According to data from Zhuowei Hu group, WWP1 can directly ubiquitinate and upregulate EGFR: WWP1 directly binds to EGFR and induces K63-linked polyubiquitination in the EGFR juxtamembrane region, which enhances EGFR recycling and stability. This consequently upregulates EGFR and activates its downstream signaling pathways as well as stemness of non-small cell lung cancer (NSCLC) (Yu et al., 2020). Ceshi Chen group and Shelton Earp group found that WWP1 can directly ubiquitinate human epidermal growth factor receptor 4 (HER4, also known as ErbB4) and cause its degradation (Feng et al., 2009; Li et al., 2009). It has been reported that HER4 can activate the expression of the tumor suppressor BRCA1 (Muraoka-Cook et al., 2006), as well as the differentiation gene β-casein (Muraoka-Cook et al., 2008). There are also studies demonstrating that HER4 decreases mammary epithelial cell proliferation and survival (Muraoka-Cook et al., 2006; Naresh et al., 2006; Pitfield et al., 2006; Feng et al., 2007; Vidal et al., 2007). Therefore, the role as a negative regulator of

TABLE 3 | Validated hyperactive WWP1 mutants.

Mutation	Location in WWP1	Cancer type	Effects on tumorigenesis	References
R427W	2,3-linker	Breast cancer	Elevated pUb and degradation of ΔNp63α, inducing cell migration and invasion	Wang et al., 2019
S444L	2,3-linker			
H517Y	WW4			
P651A	N-lobe			
E697K	N-lobe			
K740N	N-lobe	Colon cancer	Aberrant pUb and inactivation of PTEN, enhancing cancer susceptibility	Lee et al., 2020
N745S	N-lobe			
E798V	N-lobe	Prostate cancer	Excessive TβR1 pUb and degradation, attenuating TGFβ cytostatic signaling	Chen et al., 2007a; Courivaud et al., 2015

HER4 may, to some extent, account for the positive regulation of WWP1 on proliferation and survival of mammary epithelial cells, as well as tumorigenesis of breast cancer (Chen et al., 2007b, 2009; Nguyen Huu et al., 2008). In brief, WWP1 regulates EGFR family proteins either directly or indirectly, leading to enhanced proliferation, survival, or stemness of PCa, BrCa, or NSCLC cells.

Phosphatase and tensin homolog (PTEN) is a classical tumor suppressor that antagonizes PI3K-AKT signaling (Rademacher and Eickholt, 2019). In its dimer configuration at the plasma membrane, PTEN is active to dephosphorylate the D3-phosphate of the second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3). This leads to a repression of the proto-oncogenic PI3K-AKT signaling pathway, and thus controls cell proliferation, growth, and metabolism (Lee et al., 2019). Data from Pier Paolo Pandolfi group demonstrate that WWP1 mediates K27 ubiquitination of PTEN and inhibits PTEN dimerization and membrane localization. This cytosol monomeric PTEN fails to dampen the growth-promoting signaling cascade consisting of PI3K, AKT, and mechanistic target of rapamycin (mTOR) (Lee et al., 2019). These effects increase the morbidity of oligopolyposis as well as colon and prostate cancers (Lee et al., 2019, 2020). All in all, WWP1-mediated K27 pUb of PTEN may promote tumorigenesis of colon and prostate cancers.

WNT signaling pathways, including the canonical WNT pathway and the WNT-planar cell polarity (WNT-PCP) relay, modulate actin cytoskeleton organization to promote cellular motility (VanderVorst et al., 2019). MacGurn group reported that WWP1 mediates ubiquitination of the WNT signal transducer, disheveled protein 2 (DVL2); this promotes redistribution of DVL2 to actin-rich projections (Nielsen et al., 2019). Another work collaborated by Yingxian Li and Shukuan Ling groups demonstrated that WWP1 mediates K27-linked polyubiquitination (K27 pUb) of DVL2 and subsequently stabilizes it (Zhao et al., 2021). WWP1-mediated ubiquitination of DVL2 initiates the WNT-PCP pathway, resulting in cell motility and breast cancer invasion/metastasis (Nielsen et al., 2019). These data reveal that WWP1-mediated K27 pUb of DVL2 promotes invasion and metastasis of breast cancer via activating WNT-PCP pathway.

p53 family transcription factors, including p53, p63, and p73 proteins, play crucial roles in kinds of cancers. Aaron Laine et al. reported that WWP1 directly binds to p53, though p53 does not have a PY motif. This physical interaction leads to modification of p53 with a monoubiquitin or an unidentified polyubiquitin chain, which intriguingly stabilizes p53 instead of targeting it for degradation. WWP1-mediated modifications also result in nuclear export of p53 and a concomitant decrease in its transcriptional activities (Laine and Ronai, 2007). This is likely to account for WWP1's oncogenic functions in some cancer types, given that p53 is a key tumor suppressor in most malignancies (Levine and Oren, 2009). Chaudhary and Maddika (2014) reported that WWP1 enhances apoptosis via degrading ΔNp73 in HeLa human cervix adenocarcinoma cells. According to data from our group and Ceshi Chen group, α isoforms of p63 (i.e., TAp63α and ΔNp63α) are polyubiquitinated by WWP1 and consequently targeted for degradation, which can be antagonized by isomerase Pin1 (Li Y. et al., 2008; Li et al., 2013). We speculate that WWP1/Pin1-involved protein stability may modulate p63α-mediated metastasis inhibition, especially in head and neck squamous cell carcinoma (HNSC), where ΔNp63α is the predominant p63 isoform, promoting cell proliferation and growth during the early stage and inhibiting metastasis during the late stage (Chen et al., 2018). Recently, Wang et al. (2019) demonstrated that constitutively active WWP1 promotes cell migration by enhancement of ΔNp63α proteolysis. On the other hand, TAp63α, which is also subject to WWP1-mediated degradation, is well known as a tumor suppressor to arrest cell cycle and inhibit cell migration (Su et al., 2010; Chen et al., 2018). These data indicate that WWP1-mediated ubiquitination of p53 family proteins may possess either oncogenic or carcinostatic functions in different scenarios.

WW domain-containing E3 ubiquitin protein ligase 1 can also polyubiquitinate and destabilize Krüppel-like factors 2 and 5 (KLF2 and KLF5) (Zhang et al., 2004; Chen et al., 2005). KLF5 is a key oncoprotein in breast and prostate cancers, where it promotes cell proliferation, survival, and angiogenesis (Dong and Chen, 2009). Downregulation of KLF5 desensitizes prostate cancer cells to chemotherapy (Jia et al., 2019). Deubiquitination of KLF5 boosts breast cancer cell proliferation and metastasis (Qin et al., 2015; Wu et al., 2019). Though it is well known that WWP1 acts as an oncoprotein in breast and prostate cancers,

WWP1-mediated ubiquitination and subsequent degradation of KLF5 indicate that WWP1 functions as a tumor suppressor via dampening KLF5's positive regulation on cell survival and metastasis of these tumors under some circumstances (Chen et al., 2005). YAP and TAZ, both of which are components Hippo pathway, can inhibit WWP1-mediated ubiquitination of KLF5 via competitively binding to the PY motif of KLF5. As a result, KLF5 is stabilized by YAP or TAZ, leading to enhanced proliferation and survival of breast cells or breast cancer cells (Zhao et al., 2012; Zhi et al., 2012). These data indicate that WWP1-mediated KLF5 degradation, which can be antagonized by YAP or TAZ, may inhibit tumorigenesis of breast and prostate cancers.

In addition, WWP1 is involved in tumorigenesis via ubiquitinating other proteins and targeting them for proteasomal degradation. The cyclin/CDK protein kinase inhibitor, p27, can be ubiquitinated by WWP1 (Cao et al., 2011), resulting in cell proliferation and growth of acute myeloid leukemia (AML) (Sanarico et al., 2018). The large tumor suppressor 1 (LATS1) is a key serine/threonine kinase in the Hippo signaling pathway. Xiaolong Yang group found that WWP1 promotes LATS1 degradation through polyubiquitination and the 26S proteasome pathway. This WWP1-mediated degradation of LATS1 increases cell proliferation in breast cancer cells (Yeung et al., 2013). Data from Lianping Xing group indicate that WWP1 may inhibit bone metastasis of prostate and breast cancer cells via destabilizing chemokine receptor CXCR4 as well as transcription factors Runx2 and JunB (Shu et al., 2013). Both transcription factors can be polyubiquitinated by WWP1 (Jones et al., 2006; Shen et al., 2006; Li X. et al., 2008; Zhao et al., 2011), while it is to be validated whether CXCR4 is a direct substrate of WWP1 (Subik et al., 2012).

MUTATIONS AND DYSREGULATION OF WW DOMAIN-CONTAINING E3 UBIQUITIN PROTEIN LIGASE 1 IN TUMORIGENESIS

As mentioned above, WWP1 is widely accepted as an oncoprotein and is upregulated in multiple cancer types due to gene amplification (Chen et al., 2007a,b; Nguyen Huu et al., 2008). Besides that, WWP1 can be upregulated at the transcriptional and post-transcriptional levels in cancers. Data from Lee et al. (2019) found that amplified and overexpressed MYC may augment transcription of WWP1 in human prostate cancers (PCa). Wang et al. (2021) reported that circular RNA circWAC acts as a miR-142 sponge to relieve the repressive effect of miR-142 on WWP1, resulting in an upregulation of WWP1 in triple-negative breast cancer (TNBC). Increased expression of WWP1 can lead to polyubiquitination and inactivation of PTEN. This can result in derepression of the PI3K-AKT pathway, which may account for neoplasia of PCa and chemotherapeutic resistance of TNBC (Lee et al., 2019; Wang et al., 2021). According to data from Francesca Bernassola group and their colleagues (Sanarico et al., 2018), in combination with our database analysis (**Figure 1B**), WWP1 expression is significantly elevated in acute myeloid leukemia (AML) patients and cell lines. Knockdown of WWP1 inhibits AML cell growth and delays leukemogenesis via the accumulation of p27, which is known to be polyubiquitinated and destabilized by WWP1 (Cao et al., 2011; Sanarico et al., 2018). This indicates that high level of WWP1 sustains the growth of AML, to some extent, via inducing p27 degradation. Dysregulation of WWP1 expression and its roles in other cancers needs to be further elucidated.

Numerous mutations have been reported in human cancers, which can cause dysregulation of WWP1 activity. Chen et al. (2007a) identified two sequence alterations, Glu798Val (E798V) and Thr241Ser (T241S), in prostate cancer xenografts. It remains unclear whether the change of T241S has any functional consequence. Glu798 resides on the N-lobe and is critical to the enzymatic activity of WWP1 (Verdecia et al., 2003). Data from Celine Prunier group demonstrate that E798V mutation dramatically boosts polyubiquitination activity of WWP1. This culminates in excessive TβR1 degradation and attenuated TGFβ cytostatic signaling, which may account for tumorigenesis of prostate cancers bearing this alteration (Chen et al., 2007a; Courivaud et al., 2015). Recently, Wenyu Wen group surveyed the COSMIC database and found 159 somatic WWP1 mutations in cancers. 85 of them fall into autoinhibition-related domains, i.e., WW2/3/4, 2,3-linker, and HECT. The authors assumed that these mutations promote oncogenesis via enhancing polyubiquitination activity of WWP1. To prove this hypothesis, they validated several mutations, including R427W, S444L, H517Y, P651A, and E697K, in their study. As expected, they found that all of them significantly increase WWP1 activity. Further, they found that WWP1 mutants facilitate cell migration via promoting ΔNp63α turnover (Wang et al., 2019). According to data from Yu-Ru Lee et al., germline mutations K740N and N745S, which are in the N-lobe of WWP1, can disrupt the 2,3-linker/HECT binding and consequently lead to hyperactivation of WWP1. The hyperactive WWP1 mutant proteins elevate polyubiquitination of PTEN, and result in PTEN inactivation, which in turn triggers hyperactivation of PI3K-AKT-mTOR signaling axis. As a consequence, WWP1 gain-of-function results in a genetic predisposition to oligopolyposis and early onset colon cancers in human individuals, as well as larger xenograft tumors of colorectal carcinoma (CRC) in mice (Lee et al., 2020). However, Philip Cole group recently performed an *in vitro* experiment and found that K740N and N745S mutations do not affect E3 ligase activity, and both mutants show similar dependencies to those of wild-type WWP1 in terms of allosteric activation (Jiang et al., 2021). This discrepancy may be due to the difference between modifications in mammalian cells and *in vitro* catalytic reactions with purified WWP1 from *E. Coli*. These main cancer-related WWP1 mutations are listed in **Table 3** in this review.

On the other hand, WWP1 is downregulated in several cancer types (**Figure 1B**) and may play as a tumor suppressor. It is poorly known how WWP1 is downregulated and what exact effects it has on tumorigenesis in these cancer cells. A recent investigation demonstrates that both mRNA and protein levels of WWP1

significantly decline in human glioma tissues and cell lines, compared with normal brain tissues and astrocytes, respectively. Upregulation of miR-30a-5p may lead to this downregulation of WWP1 at mRNA level, and consequently promotes glioma cell proliferation, migration, and invasion via an unknown mechanism (Zhao et al., 2019).

CONCLUSION REMARKS

To sum up, WWP1 is a cardinal E3 ligase, which mediates ubiquitination of a wide range of proteins. For the most part, WWP1-mediated modifications are K48 polyubiquitination, which promotes proteasomal degradation of substrate proteins. WWP1 also adds monoubiquitin or other types of polyubiquitin chains, which may affect trafficking, localization, lysosomal degradation, and enzymatic activity of the modified proteins. Though functioning as a tumor suppressor under some circumstances, WWP1 is generally accepted as an oncoprotein. Via a plethora of substrates, WWP1 regulates kaleidoscopic signaling pathways such as TGFβ, EGF, WNT, PI3K-AKT, and Hippo pathways, consequently promoting or inhibiting neoplasia and progression of diverse cancers (**Table 2**). The controversy about WWP1's effects on tumorigenesis may be due to opposite functions of different substrates, even related proteins (e.g., TAp63 and ΔNp63) or the same substrate (e.g., ΔNp63), as well as different ubiquitination types, in different scenarios.

Owing to gene amplification and regulation by transcription factors such as MYC and p53 as well as a batch of non-coding RNAs (**Table 1**), WWP1 is prone to overexpress in kinds of malignancies (**Figure 1B**), especially in breast and prostate cancers (Chen et al., 2007a,b; Nguyen Huu et al., 2008; Li et al., 2009, 2017, 2019, 2020; Tu et al., 2017; Ma et al., 2018; Tao et al., 2018; Lee et al., 2019; Zhao et al., 2019; Wang et al., 2021). No less than 6 WWP1 isoforms have been found in human cells (Flasza et al., 2002; Zhi and Chen, 2012). Different WWP1 isoforms may as well account for discrepant roles of this gene in different tissues or cancer types. Besides the elevated expression levels, enzymatic activation of this E3 ligase is also crucial to formation, growth, metastasis, or chemoresistance of some tumors (Chen et al., 2007a; Wang et al., 2019; Lee et al., 2020). Normally, polyubiquitination activity of WWP1 is tightly controlled via a multi-lock mechanism (**Figure 2B**). In a steady state, the interactions between autoinhibitory domains make WWP1 merely have the monoubiquitination activity. Binding of some substrates can sequentially disrupt these inhibitory interactions and partially activate WWP1. Some cancer-related mutations can result in abnormally hyperactive WWP1 proteins, which aberrantly increase polyubiquitinations of some oncoproteins or tumor suppressors and consequently promote tumorigenesis (Courivaud et al., 2015; Wang et al., 2019; Lee et al., 2020).

The hyperactive state and oncogenic functions of WWP1 in some cancer types make it possible as a therapeutic target. A recent investigation performed by Lee et al. (2019) found that a natural compound from cruciferous vegetables, indole-3-carbinol (I3C), can inhibit the enzymatic activity of WWP1 and the growth of prostate tumor induced by Hi-MYC in mice. This unravels a potential therapeutic strategy for prevention and treatment of some cancers through WWP1 suppression. In the future, more effective small molecules targeting WWP1 should be explored to make this hyperactive E3 ligase ease off in cancers. However, it should be cautious because WWP1 may as well have tumor-suppressive functions in some scenarios. For instance, though WWP1 overexpression in mammary epithelial cell lines MCF10A and 184B5 leads to increased proliferation (Chen et al., 2007b), knockdown of this E3 ligase promotes migration and bone metastasis of MDA-MB-231 breast cancer cells (Subik et al., 2012). On the other hand, in a same pathway WWP1 can be affected in different ways. For example, in the Hippo pathway, WWP1 executes its tumorigenic function by targeting LAST1 for degradation (Yeung et al., 2013), while two other components of this pathway, YAP and TAZ, antagonize WWP1-mediated degradation of KLF5 and consequently promote growth and metastasis of some breast and prostate cancers (Zhao et al., 2012; Zhi et al., 2012). This may increase the complexity of inhibiting WWP1 as a therapeutic strategy. In conclusion, several factors, including multiple WWP1 isoforms, diverse substrates, different ubiquitination types, and opposite functions of these modifications, may contribute to the complexity of WWP1's actions in cancers, which should be considered thoroughly to reduce side effects when choosing this E3 ligase as a therapeutic target.

AUTHOR CONTRIBUTIONS

LK and YuJ wrote the manuscript. CL and YoJ revised and proofread the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Science and Technology Department of Sichuan Province (20ZDYF3310 to YoJ), Science and Technology Bureau of Chengdu (2019-YF05-01178-SN to YoJ), National Natural Science Foundation of China (#32070747 to CL), and Technological Innovation Team Program of Colleges and Universities in Hubei Province (T201819 to YuJ).

REFERENCES

Cao, X., Xue, L., Han, L., Ma, L., Chen, T., and Tong, T. (2011). WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) delays cellular senescence by promoting p27(Kip1) degradation in human diploid fibroblasts. *J. Biol. Chem.* 286, 33447–33456. doi: 10.1074/jbc.M111.225565

Chaudhary, N., and Maddika, S. (2014). WWP2-WWP1 ubiquitin ligase complex coordinated by PPM1G maintains the balance between cellular p73 and DeltaNp73 levels. *Mol. Cell Biol.* 34, 3754–3764. doi: 10.1128/MCB.00101-14

Chen, C., and Matesic, L. E. (2007). The Nedd4-like family of E3 ubiquitin ligases and cancer. *Cancer Metastasis Rev.* 26, 587–604. doi: 10.1007/s10555-007-9091-x

Chen, C., Sun, X., Guo, P., Dong, X. Y., Sethi, P., Cheng, X., et al. (2005). Human Kruppel-like factor 5 is a target of the E3 ubiquitin ligase WWP1 for proteolysis in epithelial cells. *J. Biol. Chem.* 280, 41553–41561. doi: 10.1074/jbc.M506183200

Chen, C., Sun, X., Guo, P., Dong, X. Y., Sethi, P., Zhou, W., et al. (2007a). Ubiquitin E3 ligase WWP1 as an oncogenic factor in human prostate cancer. *Oncogene* 26, 2386–2394. doi: 10.1038/sj.onc.1210021

Chen, C., Zhou, Z., Ross, J. S., Zhou, W., and Dong, J. T. (2007b). The amplified WWP1 gene is a potential molecular target in breast cancer. *Int. J. Cancer* 121, 80–87. doi: 10.1002/ijc.22653

Chen, C., Zhou, Z., Liu, R., Li, Y., Azmi, P. B., and Seth, A. K. (2008). The WW domain containing E3 ubiquitin protein ligase 1 upregulates ErbB2 and EGFR through RING finger protein 11. *Oncogene* 27, 6845–6855. doi: 10.1038/onc.2008.288

Chen, C., Zhou, Z., Sheehan, C. E., Slodkowska, E., Sheehan, C. B., Boguniewicz, A., et al. (2009). Overexpression of WWP1 is associated with the estrogen receptor and insulin-like growth factor receptor 1 in breast carcinoma. *Int. J. Cancer* 124, 2829–2836. doi: 10.1002/ijc.24266

Chen, J., Shi, H., Chen, Y., Fan, S., Liu, D., and Li, C. (2017). DNA damage induces expression of WWP1 to target DeltaNp63alpha to degradation. *PLoS One* 12:e0176142. doi: 10.1371/journal.pone.0176142

Chen, Y., Peng, Y., Fan, S., Li, Y., Xiao, Z. X., and Li, C. (2018). A double dealing tale of p63: an oncogene or a tumor suppressor. *Cell Mol. Life Sci.* 75, 965–973. doi: 10.1007/s00018-017-2666-y

Colak, S., and Ten Dijke, P. (2017). Targeting TGF-beta signaling in cancer. *Trends Cancer* 3, 56–71. doi: 10.1016/j.trecan.2016.11.008

Courivaud, T., Ferrand, N., Elkhattouti, A., Kumar, S., Levy, L., Ferrigno, O., et al. (2015). Functional characterization of a WWP1/Tiul1 tumor-derived mutant reveals a paradigm of its constitutive activation in human cancer. *J. Biol. Chem.* 290, 21007–21018. doi: 10.1074/jbc.M115.642314

Dong, J. T., and Chen, C. (2009). Essential role of KLF5 transcription factor in cell proliferation and differentiation and its implications for human diseases. *Cell Mol. Life Sci.* 66, 2691–2706. doi: 10.1007/s00018-009-0045-z

Feng, S. M., Muraoka-Cook, R. S., Hunter, D., Sandahl, M. A., Caskey, L. S., Miyazawa, K., et al. (2009). The E3 ubiquitin ligase WWP1 selectively targets HER4 and its proteolytically derived signaling isoforms for degradation. *Mol. Cell Biol.* 29, 892–906. doi: 10.1128/MCB.00595-08

Feng, S. M., Sartor, C. I., Hunter, D., Zhou, H., Yang, X., Caskey, L. S., et al. (2007). The HER4 cytoplasmic domain, but not its C terminus, inhibits mammary cell proliferation. *Mol. Endocrinol.* 21, 1861–1876. doi: 10.1210/me.2006-0101

Flasza, M., Gorman, P., Roylance, R., Canfield, A. E., and Baron, M. (2002). Alternative splicing determines the domain structure of WWP1, a Nedd4 family protein. *Biochem. Biophys. Res. Commun.* 290, 431–437. doi: 10.1006/bbrc.2001.6206

Flasza, M., Nguyen Huu, N. S., Mazaleyrat, S., Clemence, S., Villemant, C., Clarke, R., et al. (2006). Regulation of the nuclear localization of the human Nedd4-related WWP1 protein by Notch. *Mol. Membr. Biol.* 23, 269–276. doi: 10.1080/09687860600665010

Fu, L., Cui, C. P., Zhang, X., and Zhang, L. (2020). The functions and regulation of Smurfs in cancers. *Semin. Cancer Biol.* 67(Pt 2), 102–116. doi: 10.1016/j.semcancer.2019.12.023

Goel, P., Manning, J. A., and Kumar, S. (2015). NEDD4-2 (NEDD4L): the ubiquitin ligase for multiple membrane proteins. *Gene* 557, 1–10. doi: 10.1016/j.gene.2014.11.051

Goto, Y., Kojima, S., Kurozumi, A., Kato, M., Okato, A., Matsushita, R., et al. (2016). Regulation of E3 ubiquitin ligase-1 (WWP1) by microRNA-452 inhibits cancer cell migration and invasion in prostate cancer. *Br. J. Cancer* 114, 1135–1144. doi: 10.1038/bjc.2016.95

Grice, G. L., and Nathan, J. A. (2016). The recognition of ubiquitinated proteins by the proteasome. *Cell Mol. Life Sci.* 73, 3497–3506. doi: 10.1007/s00018-016-2255-5

Grimsey, N. J., Narala, R., Rada, C. C., Mehta, S., Stephens, B. S., Kufareva, I., et al. (2018). A tyrosine switch on NEDD4-2 E3 ligase transmits GPCR inflammatory signaling. *Cell Rep.* 24, 3312–3323 e3315. doi: 10.1016/j.celrep.2018.08.061

Hicke, L., Schubert, H. L., and Hill, C. P. (2005). Ubiquitin-binding domains. *Nat. Rev. Mol. Cell Biol.* 6, 610–621. doi: 10.1038/nrm1701

Hoeller, D., Crosetto, N., Blagoev, B., Raiborg, C., Tikkanen, R., Wagner, S., et al. (2006). Regulation of ubiquitin-binding proteins by monoubiquitination. *Nat. Cell Biol.* 8, 163–169. doi: 10.1038/ncb1354

Huang, K., Johnson, K. D., Petcherski, A. G., Vandergon, T., Mosser, E. A., Copeland, N. G., et al. (2000). A HECT domain ubiquitin ligase closely related to the mammalian protein WWP1 is essential for *Caenorhabditis elegans* embryogenesis. *Gene* 252, 137–145. doi: 10.1016/s0378-1119(00)00216-x

Huang, X., Chen, J., Cao, W., Yang, L., Chen, Q., He, J., et al. (2019). The many substrates and functions of NEDD4-1. *Cell Death Dis.* 10:904. doi: 10.1038/s41419-019-2142-8

Jia, J., Zhang, H. B., Shi, Q., Yang, C., Ma, J. B., Jin, B., et al. (2019). KLF5 downregulation desensitizes castration-resistant prostate cancer cells to docetaxel by increasing BECN1 expression and inducing cell autophagy. *Theranostics* 9, 5464–5477. doi: 10.7150/thno.33282

Jiang, H., Dempsey, D. R., and Cole, P. A. (2021). Ubiquitin ligase activities of WWP1 germline variants K740N and N745S. *Biochemistry* 60, 357–364. doi: 10.1021/acs.biochem.0c00869

Jiang, H., Thomas, S. N., Chen, Z., Chiang, C. Y., and Cole, P. A. (2019). Comparative analysis of the catalytic regulation of NEDD4-1 and WWP2 ubiquitin ligases. *J. Biol. Chem.* 294, 17421–17436. doi: 10.1074/jbc.RA119.009211

Jones, D. C., Wein, M. N., Oukka, M., Hofstaetter, J. G., Glimcher, M. J., and Glimcher, L. H. (2006). Regulation of adult bone mass by the zinc finger adapter protein Schnurri-3. *Science* 312, 1223–1227. doi: 10.1126/science.1126313

Kim, S., Ham, S., Yang, K., and Kim, K. (2018). Protein kinase CK2 activation is required for transforming growth factor beta-induced epithelial-mesenchymal transition. *Mol. Oncol.* 12, 1811–1826. doi: 10.1002/1878-0261.12378

Komuro, A., Imamura, T., Saitoh, M., Yoshida, Y., Yamori, T., Miyazono, K., et al. (2004). Negative regulation of transforming growth factor-beta (TGF-beta) signaling by WW domain-containing protein 1 (WWP1). *Oncogene* 23, 6914–6923. doi: 10.1038/sj.onc.1207885

Laine, A., and Ronai, Z. (2007). Regulation of p53 localization and transcription by the HECT domain E3 ligase WWP1. *Oncogene* 26, 1477–1483. doi: 10.1038/sj.onc.1209924

Lee, Y. R., Chen, M., Lee, J. D., Zhang, J., Lin, S. Y., Fu, T. M., et al. (2019). Reactivation of PTEN tumor suppressor for cancer treatment through inhibition of a MYC-WWP1 inhibitory pathway. *Science* 364:eaau0159. doi: 10.1126/science.aau0159

Lee, Y. R., Yehia, L., Kishikawa, T., Ni, Y., Leach, B., Zhang, J., et al. (2020). WWP1 gain-of-function inactivation of PTEN in cancer predisposition. *N. Engl. J. Med.* 382, 2103–2116. doi: 10.1056/NEJMoa1914919

Levine, A. J., and Oren, M. (2009). The first 30 years of p53: growing ever more complex. *Nat. Rev. Cancer* 9, 749–758. doi: 10.1038/nrc2723

Li, C., Chang, D. L., Yang, Z., Qi, J., Liu, R., He, H., et al. (2013). Pin1 modulates p63alpha protein stability in regulation of cell survival, proliferation and tumor formation. *Cell Death Dis.* 4:e943. doi: 10.1038/cddis.2013.468

Li, J., Sun, S., Chen, W., and Yuan, K. (2019). Small nucleolar RNA host gene 12 (SNHG12) promotes proliferation and invasion of laryngeal cancer cells via sponging miR-129-5p and potentiating WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) expression. *Med. Sci. Monit.* 25, 5552–5560. doi: 10.12659/MSM.917088

Li, Q., Li, Z., Wei, S., Wang, W., Chen, Z., Zhang, L., et al. (2017). Overexpression of miR-584-5p inhibits proliferation and induces apoptosis by targeting WW domain-containing E3 ubiquitin protein ligase 1 in gastric cancer. *J. Exp. Clin. Cancer Res.* 36:59. doi: 10.1186/s13046-017-0532-2

Li, X., Huang, M., Zheng, H., Wang, Y., Ren, F., Shang, Y., et al. (2008). CHIP promotes Runx2 degradation and negatively regulates osteoblast differentiation. *J. Cell Biol.* 181, 959–972. doi: 10.1083/jcb.200711044

Li, Y., Wang, J., Ma, Y., Du, W., Feng, H., Feng, K., et al. (2020). MicroRNA-15b shuttled by bone marrow mesenchymal stem cell-derived extracellular vesicles binds to WWP1 and promotes osteogenic differentiation. *Arthritis Res. Ther.* 22:269. doi: 10.1186/s13075-020-02316-7

Li, Y., Zhou, Z., Alimandi, M., and Chen, C. (2009). WW domain containing E3 ubiquitin protein ligase 1 targets the full-length ErbB4 for ubiquitin-mediated degradation in breast cancer. *Oncogene* 28, 2948–2958. doi: 10.1038/onc.2009.162

Li, Y., Zhou, Z., and Chen, C. (2008). WW domain-containing E3 ubiquitin protein ligase 1 targets p63 transcription factor for ubiquitin-mediated proteasomal degradation and regulates apoptosis. *Cell Death Differ.* 15, 1941–1951. doi: 10.1038/cdd.2008.134

Lorenz, S., Cantor, A. J., Rape, M., and Kuriyan, J. (2013). Macromolecular juggling by ubiquitylation enzymes. *BMC Biol.* 11:65. doi: 10.1186/1741-7007-11-65

Ma, L., Chen, X., Li, C., Cheng, R., Gao, Z., Meng, X., et al. (2018). miR-129-5p and -3p co-target WWP1 to suppress gastric cancer proliferation and migration. *J. Cell Biochem.* doi: 10.1002/jcb.28027 [Epub ahead of print].

Malbert-Colas, L., Fay, M., Cluzeaud, F., Blot-Chabaud, M., Farman, N., Dhermy, D., et al. (2003). Differential expression and localisation of WWP1, a Nedd4-like protein, in epithelia. *Pflugers Arch.* 447, 35–43. doi: 10.1007/s00424-003-1152-6

Miyazaki, K., Fujita, T., Ozaki, T., Kato, C., Kurose, Y., Sakamoto, M., et al. (2004). NEDL1, a novel ubiquitin-protein isopeptide ligase for dishevelled-1, targets mutant superoxide dismutase-1. *J. Biol. Chem.* 279, 11327–11335. doi: 10.1074/jbc.M312389200

Moren, A., Imamura, T., Miyazono, K., Heldin, C. H., and Moustakas, A. (2005). Degradation of the tumor suppressor Smad4 by WW and HECT domain ubiquitin ligases. *J. Biol. Chem.* 280, 22115–22123. doi: 10.1074/jbc.M414027200

Mosser, E. A., Kasanov, J. D., Forsberg, E. C., Kay, B. K., Ney, P. A., and Bresnick, E. H. (1998). Physical and functional interactions between the transactivation domain of the hematopoietic transcription factor NF-E2 and WW domains. *Biochemistry* 37, 13686–13695. doi: 10.1021/bi981310l

Muraoka-Cook, R. S., Caskey, L. S., Sandahl, M. A., Hunter, D. M., Husted, C., Strunk, K. E., et al. (2006). Heregulin-dependent delay in mitotic progression requires HER4 and BRCA1. *Mol. Cell Biol.* 26, 6412–6424. doi: 10.1128/MCB.01950-05

Muraoka-Cook, R. S., Sandahl, M., Hunter, D., Miraglia, L., and Earp, H. S. III (2008). Prolactin and ErbB4/HER4 signaling interact via Janus kinase 2 to induce mammary epithelial cell gene expression differentiation. *Mol. Endocrinol.* 22, 2307–2321. doi: 10.1210/me.2008-0055

Naresh, A., Long, W., Vidal, G. A., Wimley, W. C., Marrero, L., Sartor, C. I., et al. (2006). The ERBB4/HER4 intracellular domain 4ICD is a BH3-only protein promoting apoptosis of breast cancer cells. *Cancer Res.* 66, 6412–6420. doi: 10.1158/0008-5472.CAN-05-2368

Nguyen Huu, N. S., Ryder, W. D., Zeps, N., Flasza, M., Chiu, M., Hanby, A. M., et al. (2008). Tumour-promoting activity of altered WWP1 expression in breast cancer and its utility as a prognostic indicator. *J. Pathol.* 216, 93–102. doi: 10.1002/path.2385

Nielsen, C. P., Jernigan, K. K., Diggins, N. L., Webb, D. J., and MacGurn, J. A. (2019). USP9X Deubiquitylates DVL2 to Regulate WNT Pathway Specification. *Cell Rep.* 28, 1074–1089 e1075. doi: 10.1016/j.celrep.2019.06.083

Pascolutti, R., Algisi, V., Conte, A., Raimondi, A., Pasham, M., Upadhyayula, S., et al. (2019). Molecularly distinct clathrin-coated pits differentially impact EGFR fate and signaling. *Cell Rep.* 27, 3049–3061 e3046. doi: 10.1016/j.celrep.2019.05.017

Perry, W. L., Hustad, C. M., Swing, D. A., O'Sullivan, T. N., Jenkins, N. A., and Copeland, N. G. (1998). The itchy locus encodes a novel ubiquitin protein ligase that is disrupted in a18H mice. *Nat. Genet.* 18, 143–146. doi: 10.1038/ng0298-143

Pitfield, S. E., Bryant, I., Penington, D. J., Park, G., and Riese, D. J. II (2006). Phosphorylation of ErbB4 on tyrosine 1056 is critical for ErbB4 coupling to inhibition of colony formation by human mammary cell lines. *Oncol. Res.* 16, 179–193. doi: 10.3727/000000006783981134

Plant, P. J., Yeger, H., Staub, O., Howard, P., and Rotin, D. (1997). The C2 domain of the ubiquitin protein ligase Nedd4 mediates Ca2+-dependent plasma membrane localization. *J. Biol. Chem.* 272, 32329–32336. doi: 10.1074/jbc.272.51.32329

Qin, J., Zhou, Z., Chen, W., Wang, C., Zhang, H., Ge, G., et al. (2015). BAP1 promotes breast cancer cell proliferation and metastasis by deubiquitinating KLF5. *Nat. Commun.* 6:8471. doi: 10.1038/ncomms9471

Rademacher, S., and Eickholt, B. J. (2019). PTEN in autism and neurodevelopmental disorders. *Cold Spring Harb. Perspect. Med.* 9:a036780. doi: 10.1101/cshperspect.a036780

Ramanathan, H. N., and Ye, Y. (2012). Cellular strategies for making monoubiquitin signals. *Crit. Rev. Biochem. Mol. Biol.* 47, 17–28. doi: 10.3109/10409238.2011.620943

Riling, C., Kamadurai, H., Kumar, S., O'Leary, C. E., Wu, K. P., Manion, E. E., et al. (2015). Itch WW domains inhibit Its E3 ubiquitin ligase activity by blocking E2-E3 ligase trans-thiolation. *J. Biol. Chem.* 290, 23875–23887. doi: 10.1074/jbc.M115.649269

Sanarico, A. G., Ronchini, C., Croce, A., Memmi, E. M., Cammarata, U. A., De Antoni, A., et al. (2018). The E3 ubiquitin ligase WWP1 sustains the growth of acute myeloid leukaemia. *Leukemia* 32, 911–919. doi: 10.1038/leu.2017.342

Savio, M. G., Wollscheid, N., Cavallaro, E., Algisi, V., Di Fiore, P. P., Sigismund, S., et al. (2016). USP9X controls EGFR fate by deubiquitinating the endocytic adaptor Eps15. *Curr. Biol.* 26, 173–183. doi: 10.1016/j.cub.2015.11.050

Seo, S. R., Lallemand, F., Ferrand, N., Pessah, M., L'Hoste, S., Camonis, J., et al. (2004). The novel E3 ubiquitin ligase Tiul1 associates with TGIF to target Smad2 for degradation. *EMBO J.* 23, 3780–3792. doi: 10.1038/sj.emboj.7600398

Sharma, S. V., Bell, D. W., Settleman, J., and Haber, D. A. (2007). Epidermal growth factor receptor mutations in lung cancer. *Nat. Rev. Cancer* 7, 169–181. doi: 10.1038/nrc2088

Shen, R., Chen, M., Wang, Y. J., Kaneki, H., Xing, L., O'Keefe, R. J., et al. (2006). Smad6 interacts with Runx2 and mediates Smad ubiquitin regulatory factor 1-induced Runx2 degradation. *J. Biol. Chem.* 281, 3569–3576. doi: 10.1074/jbc.M506761200

Shu, L., Zhang, H., Boyce, B. F., and Xing, L. (2013). Ubiquitin E3 ligase Wwp1 negatively regulates osteoblast function by inhibiting osteoblast differentiation and migration. *J. Bone Miner. Res.* 28, 1925–1935. doi: 10.1002/jbmr.1938

Su, X., Chakravarti, D., Cho, M. S., Liu, L., Gi, Y. J., Lin, Y. L., et al. (2010). TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. *Nature* 467, 986–990. doi: 10.1038/nature09459

Subik, K., Shu, L., Wu, C., Liang, Q., Hicks, D., Boyce, B., et al. (2012). The ubiquitin E3 ligase WWP1 decreases CXCL12-mediated MDA231 breast cancer cell migration and bone metastasis. *Bone* 50, 813–823. doi: 10.1016/j.bone.2011.12.022

Sudol, M., and Hunter, T. (2000). New wrinkles for an old domain. *Cell* 103, 1001–1004. doi: 10.1016/s0092-8674(00)00203-8

Sudol, M., Chen, H. I., Bougeret, C., Einbond, A., and Bork, P. (1995). Characterization of a novel protein-binding module–the WW domain. *FEBS Lett.* 369, 67–71. doi: 10.1016/0014-5793(95)00550-s

Tang, Z., Li, C., Kang, B., Gao, G., Li, C., and Zhang, Z. (2017). GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* 45, W98–W102. doi: 10.1093/nar/gkx247

Tao, H., Zhang, M., Yang, J. J., and Shi, K. H. (2018). MicroRNA-21 via dysregulation of WW domain-containing protein 1 regulate atrial fibrosis in atrial fibrillation. *Heart Lung Circ.* 27, 104–113. doi: 10.1016/j.hlc.2016.01.022

Tu, M., Tang, J., He, H., Cheng, P., and Chen, C. (2017). MiR-142-5p promotes bone repair by maintaining osteoblast activity. *J. Bone Miner. Metab.* 35, 255–264. doi: 10.1007/s00774-016-0757-8

VanderVorst, K., Dreyer, C. A., Konopelski, S. E., Lee, H., Ho, H. H., and Carraway, K. L. III (2019). Wnt/PCP signaling contribution to carcinoma collective cell migration and metastasis. *Cancer Res.* 79, 1719–1729. doi: 10.1158/0008-5472.CAN-18-2757

Verdecia, M. A., Joazeiro, C. A., Wells, N. J., Ferrer, J. L., Bowman, M. E., Hunter, T., et al. (2003). Conformational flexibility underlies ubiquitin ligation mediated by the WWP1 HECT domain E3 ligase. *Mol. Cell* 11, 249–259. doi: 10.1016/s1097-2765(02)00774-8

Vidal, G. A., Clark, D. E., Marrero, L., and Jones, F. E. (2007). A constitutively active ERBB4/HER4 allele with enhanced transcriptional coactivation and cell-killing activities. *Oncogene* 26, 462–466. doi: 10.1038/sj.onc.1209794

Wang, J., Peng, Q., Lin, Q., Childress, C., Carey, D., and Yang, W. (2010). Calcium activates Nedd4 E3 ubiquitin ligases by releasing the C2 domain-mediated auto-inhibition. *J. Biol. Chem.* 285, 12279–12288. doi: 10.1074/jbc.M109.086405

Wang, L., Zhou, Y., Jiang, L., Lu, L., Dai, T., Li, A., et al. (2021). CircWAC induces chemotherapeutic resistance in triple-negative breast cancer by targeting miR-142, upregulating WWP1 and activating the PI3K/AKT pathway. *Mol. Cancer* 20:43. doi: 10.1186/s12943-021-01332-8

Wang, Z., Liu, Z., Chen, X., Li, J., Yao, W., Huang, S., et al. (2019). A multi-lock inhibitory mechanism for fine-tuning enzyme activities of the HECT family E3 ligases. *Nat. Commun.* 10:3162. doi: 10.1038/s41467-019-11224-7

Wei, R., Qiu, X., Wang, S., Li, Y., Wang, Y., Lu, K., et al. (2015). NEDL2 is an essential regulator of enteric neural development and GDNF/Ret signaling. *Cell Signal* 27, 578–586. doi: 10.1016/j.cellsig.2014.12.013

Wiesner, S., Ogunjimi, A. A., Wang, H. R., Rotin, D., Sicheri, F., Wrana, J. L., et al. (2007). Autoinhibition of the HECT-type ubiquitin ligase Smurf2 through its C2 domain. *Cell* 130, 651–662. doi: 10.1016/j.cell.2007.06.050

Woelk, T., Oldrini, B., Maspero, E., Confalonieri, S., Cavallaro, E., Di Fiore, P. P., et al. (2006). Molecular mechanisms of coupled monoubiquitination. *Nat. Cell Biol.* 8, 1246–1254. doi: 10.1038/ncb1484

Wood, J. D., Yuan, J., Margolis, R. L., Colomer, V., Duan, K., Kushi, J., et al. (1998). Atrophin-1, the DRPLA gene product, interacts with two families of WW domain-containing proteins. *Mol. Cell Neurosci.* 11, 149–160. doi: 10.1006/mcne.1998.0677

Wu, Y., Qin, J., Li, F., Yang, C., Li, Z., Zhou, Z., et al. (2019). USP3 promotes breast cancer cell proliferation by deubiquitinating KLF5. *J. Biol. Chem.* 294, 17837–17847. doi: 10.1074/jbc.RA119.009102

Yeung, B., Ho, K. C., and Yang, X. (2013). WWP1 E3 ligase targets LATS1 for ubiquitin-mediated degradation in breast cancer cells. *PLoS One* 8:e61027. doi: 10.1371/journal.pone.0061027

Yin, Q., Wyatt, C. J., Han, T., Smalley, K. S. M., and Wan, L. (2020). ITCH as a potential therapeutic target in human cancers. *Semin. Cancer Biol.* 67(Pt 2), 117–130. doi: 10.1016/j.semcancer.2020.03.003

Yu, J. J., Zhou, D. D., Yang, X. X., Cui, B., Tan, F. W., Wang, J., et al. (2020). TRIB3-EGFR interaction promotes lung cancer progression and defines a therapeutic target. *Nat. Commun.* 11:3660. doi: 10.1038/s41467-020-17385-0

Zhang, R., Zhang, J., Luo, W., Luo, Z., and Shi, S. (2019). WWP2 Is one promising novel oncogene. *Pathol. Oncol. Res.* 25, 443–446. doi: 10.1007/s12253-018-0506-5

Zhang, X., Srinivasan, S. V., and Lingrel, J. B. (2004). WWP1-dependent ubiquitination and degradation of the lung Kruppel-like factor, KLF2. *Biochem. Biophys. Res. Commun.* 316, 139–148. doi: 10.1016/j.bbrc.2004.02.033

Zhao, D., Zhi, X., Zhou, Z., and Chen, C. (2012). TAZ antagonizes the WWP1-mediated KLF5 degradation and promotes breast cell proliferation and tumorigenesis. *Carcinogenesis* 33, 59–67. doi: 10.1093/carcin/bgr242

Zhao, D., Zhong, G., Li, J., Pan, J., Zhao, Y., Song, H., et al. (2021). Targeting E3 ubiquitin-ligase WWP1 prevents cardiac hypertrophy through destabilizing DVL2 via inhibition of K27-linked ubiquitination. *Circulation* 144, 694–711. doi: 10.1161/CIRCULATIONAHA.121.054827

Zhao, L., Huang, J., Zhang, H., Wang, Y., Matesic, L. E., Takahata, M., et al. (2011). Tumor necrosis factor inhibits mesenchymal stem cell differentiation into osteoblasts via the ubiquitin E3 ligase Wwp1. *Stem Cells* 29, 1601–1610. doi: 10.1002/stem.703

Zhao, P., Wang, M., An, J., Sun, H., Li, T., and Li, D. (2019). A positive feedback loop of miR-30a-5p-WWP1-NF-kappaB in the regulation of glioma development. *Int. J. Biochem. Cell Biol.* 112, 39–49. doi: 10.1016/j.biocel.2019.04.003

Zhi, X., and Chen, C. (2012). WWP1: a versatile ubiquitin E3 ligase in signaling and diseases. *Cell Mol. Life Sci.* 69, 1425–1434. doi: 10.1007/s00018-011-0871-7

Zhi, X., Zhao, D., Zhou, Z., Liu, R., and Chen, C. (2012). YAP promotes breast cell proliferation and survival partially through stabilizing the KLF5 transcription factor. *Am. J. Pathol.* 180, 2452–2461. doi: 10.1016/j.ajpath.2012.02.025

Conflict of Interest: 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cell and Developmental Biology

MINI REVIEW
published: 25 November 2021
doi: 10.3389/fcell.2021.788410

ISGylation in Innate Antiviral Immunity and Pathogen Defense Responses: A Review

Mengdi Zhang[1], Jingxian Li[2], Haiyan Yan[1], Jun Huang[2], Fangwei Wang[2], Ting Liu[2], Linghui Zeng[1] and Fangfang Zhou[3]**

[1]School of Medicine, Zhejiang University City College, Hangzhou, China, [2]MOE Laboratory of Biosystems Homeostasis and Protection and Innovation Center for Cell Signaling Network, Life Sciences Institute, Zhejiang University, Hangzhou, China, [3]Institute of Biology and Medical Sciences, Soochow University, Suzhou, China

The interferon-stimulating gene 15 (ISG15) protein is a ubiquitin-like protein induced by interferons or pathogens. ISG15 can exist in free form or covalently bind to the target protein through an enzymatic cascade reaction, which is called ISGylation. ISGylation has been found to play an important role in the innate immune responses induced by type I interferon, and is, thus, critical for the defense of host cells against RNA, DNA, and retroviruses. Through covalent binding with the host and viral target proteins, ISG15 inhibits the release of viral particles, hinder viral replication, and regulates the incubation period of viruses, thereby exerting strong antiviral effects. The SARS-CoV-2 papain-like protease, a virus-encoded deubiquitinating enzyme, has demonstrated activity on both ubiquitin and ISG15 chain conjugations, thus playing a suppressive role against the host antiviral innate immune response. Here we review the recent research progress in understanding ISG15-type ubiquitin-like modifications, with an emphasis on the underlying molecular mechanisms. We provide comprehensive references for further studies on the role of ISG15 in antiviral immunity, which may enable development of new antiviral drugs.

Keywords: ISG15, isgylation, immune response, innate antiviral immunity, SARS PLpro

OPEN ACCESS

Edited by:
Ana Cuenda,
Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
Zhenghong Lin,
Chongqing University, China
Zhongwei Li,
University of Southern California, Los Angeles, United States

****Correspondence:***
Fangfang Zhou
zhoufangfang@suda.edu.cn
Linghui Zeng
zenglh@zucc.edu.cn

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *02 October 2021*
Accepted: *22 October 2021*
Published: *25 November 2021*

Citation:
Zhang M, Li J, Yan H, Huang J, Wang F, Liu T, Zeng L and Zhou F (2021) ISGylation in Innate Antiviral Immunity and Pathogen Defense Responses: A Review. Front. Cell Dev. Biol. 9:788410. doi: 10.3389/fcell.2021.788410

INTRODUCTION

Interferon-stimulated gene 15 (ISG15) is a member of the family of interferon-stimulating genes (ISGs) (Takeuchi et al., 2019), which are fast and strong type I interferon (IFN)-stimulated reaction proteins that inhibit viral replication, whose function against virus invasion has been fully investigated (Loeb and Haas, 1992; Hermann and Bogunovic, 2017; Sooryanarain et al., 2017). Viral infection induces IFN synthesis, and the secreted IFN acts on nearby uninfected cells to resist the infection. After the virus enters the body, IFN binds to IFN receptors, which activate the Janus protein tyrosine kinase-signal transducer and activator of transcription pathway to form the interferon-stimulating factor 3 complex, which induces the expression of hundreds of ISGs, including ISG15, which can fight against the replication and invasion of the virus (Yuan and Krug, 2001).

Recently, the function of ISG15 as a ubiquitin-like protein has attracted much attention. ISG15 is the first identified ubiquitin-like protein, which contains two ubiquitin-like domains, and its amino acid sequence shows 50% homology with ubiquitin (Dos Santos and Mansur, 2017). Under physiological conditions, the ISG15 precursor protein can be cleaved into a mature 15-kDa form, exposing the carboxyl-terminated LRLRGG motif, which recognizes and binds to substrate

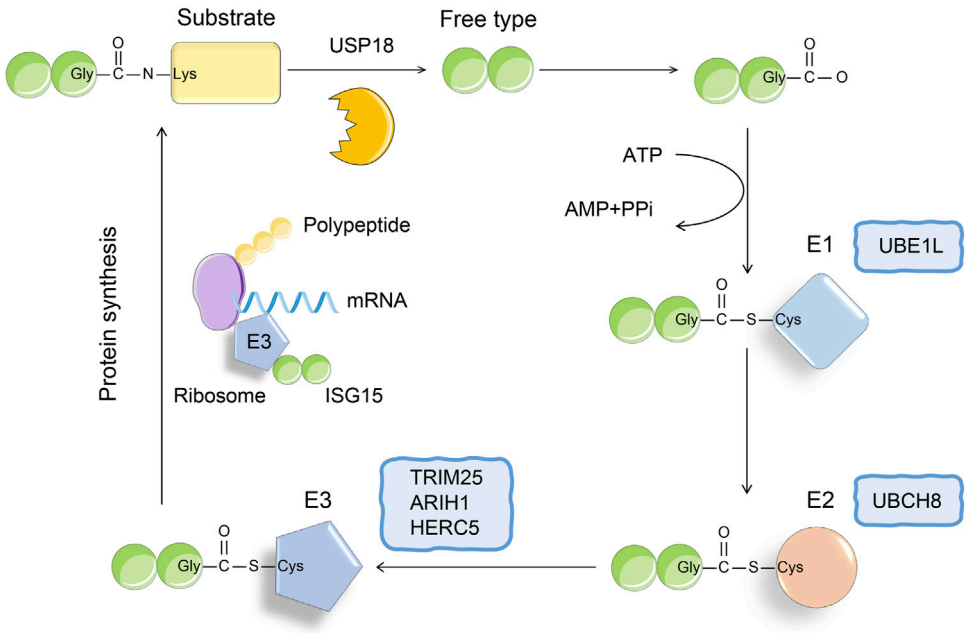


FIGURE 1 | The conjugation of ISG15. The binding process of ISG15 and substrate is similar to the three-step enzyme cascade reaction of ubiquitination. The formation of thioester bond between E1 activating enzyme (UBE1L) and ISG15 depends on ATP, thereby activating ISG15. Next, SIG15 is transferred to the cysteine active site of E2 ligase (UBCH8). Finally, E3 ligase binds to polysomes, thereby promoting the binding of ISG15 to the nascent target protein. The process of ISGylation is reversible, and USP18 as a deubiquitinating enzyme can specifically remove ISG15 from the binding protein.

lysine residues, resulting in its ISGylation (**Figure 1**) (Potter et al., 1999; Langevin et al., 2013; Zuo et al., 2016). Similar to ubiquitin modification, ISG modification of the substrate is also catalyzed by ubiquitin-activating enzyme E1, ubiquitin-binding enzyme E2, and ubiquitin ligase E3 (Mustachio et al., 2018). The removal of substrate ISGylation is catalyzed by deubiquitinases (DUB), and ubiquitin-specific peptidase 18 (USP18; also called UBP43) is a human-specific enzyme that removes ISG15 from conjugated proteins (Malakhov et al., 2002; Basters et al., 2017; Basters et al., 2018; Mustachio et al., 2018). Using ISG15 as bait, we obtained more than 300 candidate ISG15 substrates using immunoprecipitation-mass spectrometry. At present, more than 100 proteins have been established as substrates of ISG15, including p53, nuclear factor kappa B (NF-κB), KRAS, cyclin D, PTEN protein, STAT1, and retinoic acid-induced gene I (RIG-I) (Feng et al., 2008; Kim et al., 2008; Huang et al., 2014; Ganesan et al., 2016; Park et al., 2016; Mustachio et al., 2018). In this review, we discuss how ISG15 regulates viral replication, inflammation, cell proliferation and differentiation, and tumor genesis and development by modifying these proteins.

Currently, there are still many controversies regarding whether ISG15 exerts a tumor-suppressing effect or a cancer-promoting effect. Both unconjugated and conjugated ISG15 have demonstrated tumor-suppressing and cancer-promoting functions. Research results show that the tumor-suppressing function of unconjugated ISG15 is mainly related to its immune regulatory function (Desai, 2015). Yeung TL using laser microdissection and sequencing analysis that free ISG15 was highly expressed in serous ovarian cancer with high infiltration of $CD8^+$ T cells (Yeung et al., 2018). *In vitro* experiments indicated that free ISG15 can increase the ISG modification of extracellular signal-regulated protein kinase one and the viability of natural killer (NK) cells and $CD8^+$ T cells and enhance immune surveillance (Burks et al., 2015). Moreover, studies have shown that unconjugated ISG15 exerts a cancer-promoting function by enhancing the stem transformation and proliferation of tumor cells (Sainz et al., 2014; Chen et al., 2016). The same effect occurs in conjugated ISG15, which exerts a cancer-promoting effect by interacting with carcinogens (Burks et al., 2014), and a tumor-suppressing effect by regulating the function of p53 (Park et al., 2016; Jeon et al., 2017). Therefore, ISG15 can perform distinct functions depending on the cell type and physiological state, substrate, and subcellular location.

ISG15 AND INNATE IMMUNITY

Studies have shown that fibroblasts, monocytes, lymphocytes, neutrophils, plasma cells, and NK cells secrete small amounts of ISG15 under physiological conditions (Bogunovic et al., 2012). In addition, the expression of ISG15 can be affected by many factors. Viral and bacterial infections, LPS, DNA damage and other pathogenic stimuli can activate the expression of ISG15 (Malakhova et al., 2002; Pitha-Rowe et al., 2004). The free form of ISG15 binds to the LFA1 receptor on the surface of NK cells and T lymphocytes, increasing the release of type I and II IFNs and activating natural and acquired immunity (Swaim et al.,

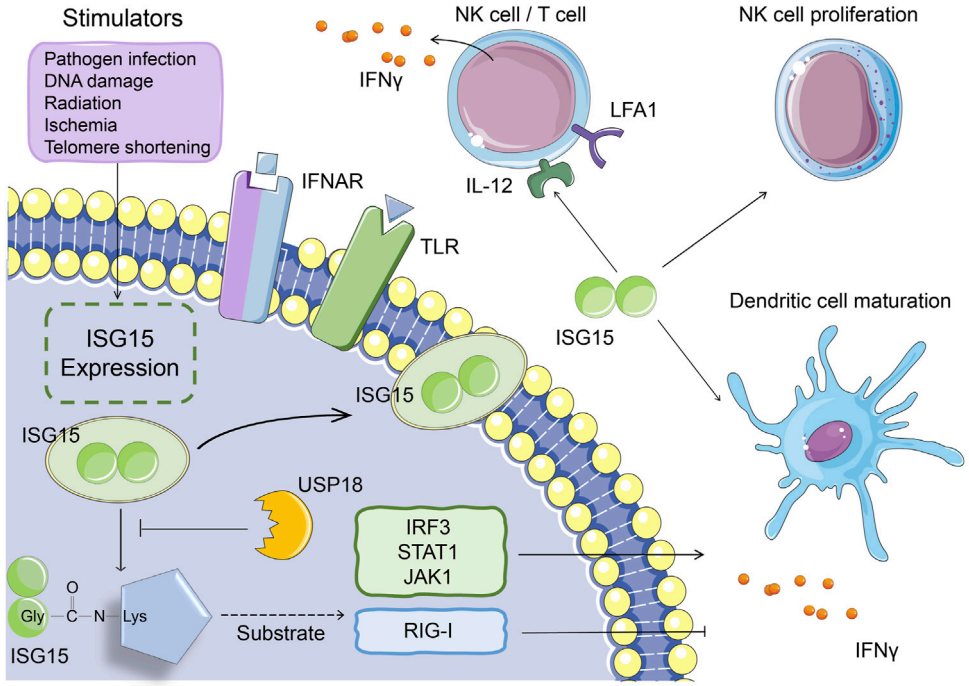


FIGURE 2 | The function of ISG15 in immune response. Under pathogenic stimuli such as viral and bacterial infections, LPS, and DNA damage, monocytes, lymphocytes, neutrophils, etc. can all secrete ISG15. Intracellular ISG15 can bind to proteins related to innate immune signaling pathways, activate IRF3, STAT1, JAK1 and other proteins, or inhibit protein activity (such as RIG-I), thereby promoting or inhibiting the secretion of IFNγ. The ISG15 secreted *in vitro* can bind to the LFA1 receptor on the cell surface, thereby promoting the secretion of IFNγ from NK cells and T cells. It can also induce the proliferation of NK cells and the maturation of dendritic cells.

2017). ISG15 can also induce the proliferation of NK cells, the IFNγ production of NK cells and T cells, the maturation of dendritic cells, and enhancement of antigen presentation and function as a chemokine that promotes the enrichment of neutrophils to inflammatory regions (**Figure 2**) (Morales et al., 2015; D'Cunha et al., 1996; Padovan et al., 2002; Owhashi et al., 2003; Recht et al., 1991).

Proteomic studies have identified that the immune-regulating factors interferon-regulated transcription factor 3 (IRF3), STAT1, and Janus kinase one function as substrates of ISG15 and that the ISGylation of these proteins increases the release of type I IFNs and ISGs, thereby extending the immune response signal cascade (Ganesan et al., 2016; Albert et al., 2018; Yoo et al., 2018; Malakhov et al., 2003). For example, when the host is infected, STAT1 ISGylation promotes the maintenance of phosphorylation and continuous activation of downstream signaling, which ultimately promotes a more powerful IFN response (Ganesan et al., 2016). In addition to positive regulation, ISG15 negatively regulates type I IFN signaling at multiple levels, such as ISGylation of the RIG-I protein, which inhibits IFN expression (**Figure 2**) (Zhao et al., 2005; Kim et al., 2008; Zhu et al., 2014; Du et al., 2018). On the one hand, because the process of covalent binding of ISG15 to the target protein is reversible, this binding can be dissociated by the ubiquitin-specific protease USP18, which indirectly regulates IFN expression. On the other hand, the deubiquitinating enzyme USP18 can also directly inhibit type I IFN receptor signaling, thereby suppressing the immune response (Arimoto et al., 2017). The non-covalent interactions of ISG15 and USP18 prevent the ubiquitination of USP18 by S-phase kinase-associated protein two and stabilize the downregulation of the IFN signaling pathway by USP18 (Tokarz et al., 2004; Zhang et al., 2015).

These results suggest that ISG15 can regulate immune function from multiple perspectives, such as stimulating immune cell maturation, regulating cytokine release, and affecting IFN signaling. In recent years, many studies have explored the role of ISG15 in antiviral innate immunity, especially in the process of viral infection, and the role of ISGylation of host and viral target proteins in immune defense. In this review, we explore this topic in detail.

ANTIVIRAL EFFECTS OF ISGYLATION ON HOST PROTEINS AND THEIR FUNCTIONS

Although the ISG15 protein was discovered in 1979, its nature and function were not elucidated for many years, until researchers discovered that IFN-induced ISG15 and its covalent form were implicated as a central player in the process of viral infection. Gene knockout, overexpression, genetic deletion of each component in the ISG15 cascade

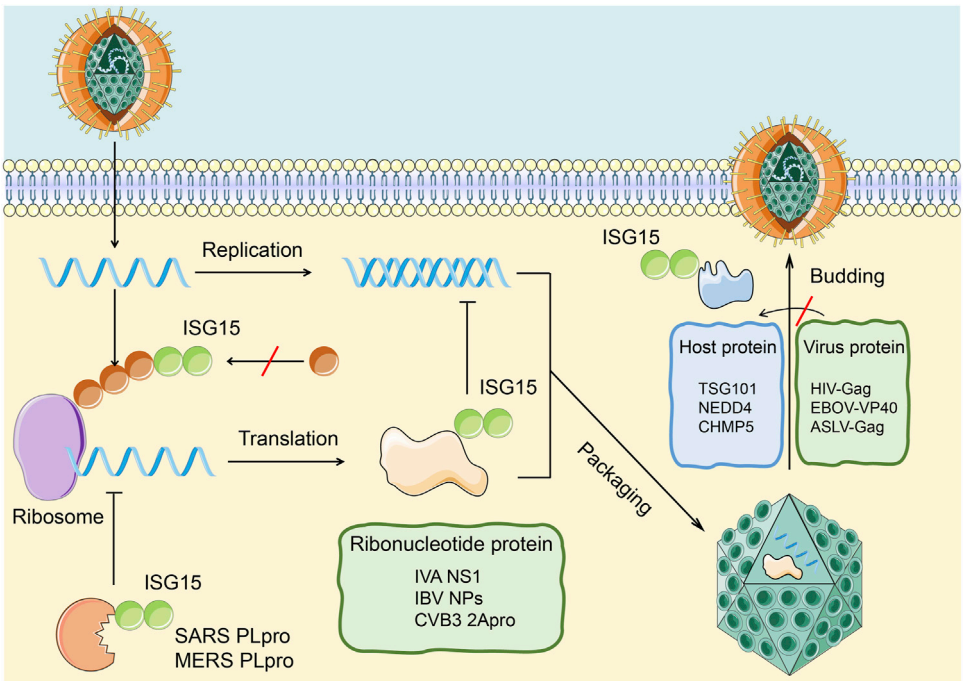


FIGURE 3 | Antiviral effects of ISGylation on host and viral proteins. ISG15 affects the infection of cells by the virus through covalently binding with viral proteins and host proteins. 1. The combination of ISG15 and the viral nucleoprotein (green) can destroy the protein oligomerization and the ability of the viral nucleoprotein to inhibit virus replication. The ubiquitin-like modification formed by this combination can be cleaved by PLpro to restore the replication ability of the virus.2. The combination of ISG15 and host protein (blue) can inhibit the interaction between host protein and virus protein, thereby inhibiting the release of virus particles in the cell.

reaction process, and various other methods have since been used to determine whether ISG15 is involved in the host antiviral immune response (Campbell and Lenschow, 2013).

ISG15 can affect the antiviral immune response by binding to the target proteins of the IFN, NF-κB, and c-Jun N-terminal kinase (JNK) pathways (Jeon et al., 2009). Among them, the key factor for type I IFN response, IRF3, is a target of ISG15. The combination of ISG15 and IRF3 inhibited the proteasomal degradation of IRF3 and enhanced the intracellular IFN response (Ganesan et al., 2016). Concurrently, the covalent binding of ISG to the antiviral effector molecules K193, K360, and K366 can weaken the interaction between IRF3 and peptidyl-prolyl-cis-trans isomerase one and hinder the ubiquitination of IRF3 (Shi et al., 2010). Therefore, IRF3 can maintain its own activity after the modification of ISG and improve the IRF3-mediated antiviral response by inhibiting its own degradation.

ISG15 can bind to protein kinase R (PKR), an IFN-inducible protein kinase activated by double-stranded RNA. Simultaneously ISG15 can also activate PKR in the absence of viral RNA. Activated PKR can inhibit protein translation by phosphorylation of eukaryotic initiation factor 2α, and PKR activated by ISG15 can further promote IFN production (Okumura et al., 2013). In addition, RIG-I is the target protein of ISG15, and RIG-I can activate the RNA sensors of IRF3 and NF-κB. The covalent combination of ISG15 and RIG-I can downregulate signal transduction mediated by RIG-I. Free ISG15 can regulate the level of RIG-I by promoting the interaction between RIG-I and the autophagy substrate protein p62 (Nakashima et al., 2015; Du et al., 2018). ISGylation of phosphorylated STAT1 can also maintain its activity by inhibiting its own polyubiquitination and proteasomal degradation (Ganesan et al., 2016). In another example, ISGylation of filamin B can negatively regulate IFN-α-mediated c-Jun N-terminal kinase signals and inhibit cell apoptosis (Jeon et al., 2009). ISG15 can also bind to ubiquitin-conjugating enzyme 13 to inhibit the ubiquitination of transforming growth factor kinase one and negatively regulates the NF-κB pathway (Takeuchi and Yokosawa, 2005).

On the one hand, ISG15 influences antiviral immunity by ISGylation of host cell proteins and the relevant immune signaling pathways. On the other hand, ISG15 can affect virus replication, release, and latency in the host body through the ubiquitin-like modification of the virus protein to achieve antiviral immunity (**Figure 3**). Relevant examples are described in detail below.

ISGYLATION OF VIRAL PROTEINS AND THEIR FUNCTIONS

Lenschow and Werneke's team demonstrated that ISG15-knockout mice were more susceptible to IAV and IBV, herpes simplex virus, norovirus, chikungunya virus, and other pathogens than wild-type mice (Lenschow et al., 2007; Werneke et al., 2011; Morales and Lenschow, 2013; Rodriguez et al., 2014). They demonstrated that both free and binding ISG15 expression is

upregulated after pathogen infection, and both forms of ISG15 exhibit antiviral activity. For example, after IAV infection, free ISG15 can bind to the NS1 protein with seven lysine residues, which are potential target sites for ISGylation, blocking the nuclear localization of the NS1 protein and inhibiting virus replication, RNA processing (Jumat et al., 2016). At the same time, the ISGylation of NS1 can inhibit the interaction with PKR, which relieve the inhibition of NS1 protein on innate immunity and restoring IFN-induced anti-IAV activity (Pincetic et al., 2010).

As for IBV, nucleoprotein and matrix protein M1 are also targets for covalent binding of ISG15. Nucleoprotein ISGylation hinders the oligomerization of a large number of other non-conjugated nucleoproteins, inhibits the formation of IBV ribonucleic acid protein, and reduces viral protein synthesis and viral replication (Durfee et al., 2010; Zhao et al., 2016). Rahnefeld et al. found that the coxsackie virus CVB3 2A protease ISGylation can inhibit the cleavage of eukaryotic translation initiation factor 4 G and reduce CVB3 replication (Rahnefeld et al., 2014).

Studies have shown that ISG15 can affect the release of HIV, Ebola virus, and avian sarcoma leukosis virus through different mechanisms. Pincetic and Okumura demonstrated that ISG15 inhibited the monoubiquitination of the HIV group-specific antigen protein, blocked its interaction with host tumor susceptibility gene 101, and inhibited the emergence and release of HIV (Okumura et al., 2006). When infected with Ebola, the ubiquitin ligase NEDD4 catalyzes the ubiquitination of the viral matrix protein VP40 and promotes the release of virus-like particles (Okumura et al., 2008). Lenschow and Malakhova demonstrated that ISG15 inhibits the transfer of ubiquitin-binding enzyme to NEDD4 and activity of NEDD4 ubiquitin-binding enzyme, thus inhibiting the budding and release of the Ebola virus (Malakhova and Zhang, 2008). ISGylation of charged multivesicular body protein 5 (CHMP5), a component of the endosome sorting complex, promotes its aggregation and the isolation of Vps4 coenzyme factor LIP5 and limits the membrane recruitment of Vps4 and its interaction with the avian sarcoma leukosis virus budding complex, thereby inhibiting the release of intracellular virus-like particles (Pincetic et al., 2010). In addition, researchers found that ISG15 can also affect the budding process of vesicular stomatitis virus by inhibiting the activity of NEDD4 and that ISG15 overexpression can significantly reduce the viral titer of its wild-type strains (Malakhova and Zhang, 2008).

Another study showed that ISG15 regulated the incubation period of the virus. Dai et al. used Illumina microarray technology to analyze the gene expression changes in primary human oral fibroblasts after infection with Kaposi's sarcoma-associated herpes virus and found that a series of IFN-stimulated genes were upregulated, especially ISG15 and ISG20, which maintain the virus incubation period by regulating Kaposi's sarcoma-associated herpes virus-specific microRNA (Dai et al., 2016). This reduces the expression of ISG15 during the incubation period of Kaposi's sarcoma-associated herpes virus infection and increases the expression of virus cleavage genes and the release of virus particles.

These results suggest that free or bound ISG15 produced by stress can regulate the function of viral proteins, inhibit viral replication, budding, and release. Thus, ISG15 may play a key role in inhibiting viral infection (**Table 1**).

ISG15 PARTICIPATES IN NON-VIRAL INNATE IMMUNE RESPONSES

Recent work has also highlighted the function of ISG15 in non-viral innate immune responses, such as pathogen defense responses, host damage and repair responses, and other host signaling pathways. $ISG15^{-/-}$ mice are more susceptible to *mycobacterium* than wild-type mice, verifying that the degree of *mycobacterium* drop is not a determinant of susceptibility enhancement. Significantly increased cytokine release was detected in $ISG15^{-/-}$ mice, and the cytokine storm induced by ISG15 knockout was blocked by tumor necrosis factor-α-specific antibodies (Bogunovic et al., 2012; Kimmey et al., 2017). During *Listeria monocytogenes* infection, the expression of ISG15 increases, which depends on the cytosolic DNA-sensing pathway, and enhanced secretion of IL-6 and IL-8 was detected in ISG15-overexpressing cells (Radoshevich et al., 2015). These studies demonstrate that ISG15 plays an antagonistic role in the host response to pathogens and regulates cytokine signal transduction. Exogenous stimuli, such as DNA damage, radiation, ischemia, and telomere shortening, can also induce immune cells to produce ISG15 (Liu et al., 2004).

SARS-COV-2 PAPAIN-LIKE PROTEASE: A DECONJUGATING PROTEASE

The SARS-CoV–coronavirus genome encodes two viral proteases: PLpro and 3C-like protease. The structure and function of PLpro has been a hot topic in the molecular biology of coronavirus recently. PLpro is involved in cutting the N-terminal part of the SARS-CoV replicase polymerin and is a regulatory protein molecule for the formation of the SARS-CoV replicase complex (Shin et al., 2020). Results showed that SARS-CoV PLpro protease is a virus-encoded DUB, which has an obvious deubiquitinating effect on cellular proteins (Klemm et al., 2020). PLpro is also active against ubiquitin and ISG15, which can negatively regulate the innate immune response to the virus (Shin et al., 2020). There are also OTU domain-containing proteases that can be encoded by Crimean-Congo hemorrhagic fever orthonairovirus, porcine reproductive and respiratory syndrome virus, and equine arteritis virus, which have properties similar to those of PLpro (Frias-Staheli et al., 2007). These proteins have been shown to reduce ubiquitin and ISG15 conjugates in cells. However, the researchers have compared SARS-CoV-2-PLpro with similar enzymes of other coronaviruses (SARS-CoV-1 and MERS). It was found that the SARS-CoV-2-PLpro enzyme processes ubiquitin and ISG15 in a different way with SARS-CoV-1-PLpro (Rut et al., 2020).

Recently, Huang and Zhang have made progress in elucidating the complex structure of SARS-CoV-2 PLpro and antiviral drug

TABLE1 | Interaction between ISG15 and viral proteins.

Viral proteins	Biological effects after ISGylation	Impact on viral infection	Reference
IVA NS1	ISG15 inhibits viral proteins nuclear translocation and restores host antiviral responses	Inhibits IBV replication	(Tang et al., 2010; Zhao et al., 2010)
IBV NPs	ISGylation of NPs inhibit the oligomerization of unmodified NPs, which impedes viral RNA synthesis	Inhibits IBV replication	Zhao et al. (2016)
CVB3 2Apro	ISG15 inhibits its protease activity to restore host protein translation	Inhibits CVB3 replication	Rahnefeld et al. (2014)
HIV Gag	ISG15 inhibits the monoubiquitination of Gag protein and block its interaction with TSG101	Inhibits the emergence and release of HIV.	Okumura et al. (2006)
EBOV VP40	ISGylation of NEDD4 ubiquitin-binding enzyme inhibits its interaction with VP40	Inhibits the budding and release of Ebola virus	(Yasuda et al., 2003; Okumura et al., 2008)
ASLV Gag	The ISGylation of CHMP5 limits the membrane recruitment of Vps4 and its interaction with the ASLV Gag	Inhibits the ASLV budding complex, then inhibits the release of intracellular virus-like particles	Pincetic et al. (2010)
SARS PLpro MERS PLpro	PLpro protease is a virus-encoded DUB, which active on ubiquitin like molecule ISG15	Negatively regulates the innate immune response to the virus	Rut et al. (2020)

discovery (Fu et al., 2021). They found that the small molecule inhibitor GRL0617 inhibited the activity of PLpro to shear the ubiquitin-like chain and the ubiquitin-like protein ISG15 chain *in vitro* and the ability to inhibit viral replication of SARS-CoV-2. The structure of the inhibitor and protein complex and two-dimensional NMR experiments revealed that GRL0617 interferes with protein–protein interaction between PLpro and ISG15, acting as an inhibitor for this interaction. They established that SARS-CoV-2 protease PLpro is a target for antiviral drug development at the cellular and atomic resolution crystal structure levels and identified the binding site of GRL0617 as a hot spot for antiviral drug development targeting PLpro using a variety of biophysical methods.

DISCUSSION

ISG15 is a ubiquitin-like protein, produced by IFN, viruses, lipopolysaccharides, and other stimuli. ISG15 exerts antiviral effects by covalently binding to target proteins, inhibiting the release and replication of viral particles, and regulating the incubation period of viruses. In addition to the ISG15 covalent conjugate, the ISG15 monomer can promote the proliferation of NK cells and dendritic cells and enhance the chemotactic activity of neutrophils. Moreover, ISG15 is implicated in host damage, DNA repair, autophagy, protein translation, and other processes. ISG15 is also associated with the occurrence of cancer. However, there are still many unsolved mysteries about the biological function of ISG15 and the molecular mechanism underlying the antiviral effects of the ubiquitin-like modification system.

The PLP2 domains of many human and animal coronaviruses, such as the SARS coronavirus, MHV-A59, NL-63, and 229E, have demonstrated DUB activity, and the catalytic sequence of the PLP domain of these coronaviruses is highly conserved. However, it is still unclear whether DUB activity and regulation of the host natural immune response are the common characteristics of all PLpros, and the functional relationship between DUB activity of PLpro and its IFN antagonism needs further study.

In general, the diversity and broad spectrum of substrates, complexity of the ISG enzyme system, and cross-linking with the ubiquitination pathway all determine the complexity of ISG15 function. Further understanding of the molecular trajectory of the ubiquitin-like protein ISG15 may lead to new therapeutic strategies for antiviral treatment, immune function regulation, and cancer treatment.

AUTHOR CONTRIBUTIONS

MZ and JL conceived and drafted the manuscript. FZ, FW, HY, JH, TL discussed the concepts of the manuscript. MZ drew the figures. FZ and LZ approved the version to be submitted. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by a special program from the Chinese National Natural Science Funds (31871405 and 82041009 to FZ), Jiangsu National Science Foundation (BK20180043 and19KJA550003 to FZ).

ACKNOWLEDGMENTS

We would like to apologize to those researchers whose related work we were not able to cite in this review.

REFERENCES

Albert, M., Bécares, M., Falqui, M., Fernández-Lozano, C., and Guerra, S. (2018). ISG15, a Small Molecule with Huge Implications: Regulation of Mitochondrial Homeostasis. *Viruses* 10. doi:10.3390/v10110629

Arimoto, K.-i., Löchte, S., Stoner, S. A., Burkart, C., Zhang, Y., Miyauchi, S., et al. (2017). STAT2 Is an Essential Adaptor in USP18-Mediated Suppression of Type I Interferon Signaling. *Nat. Struct. Mol. Biol.* 24, 279–289. doi:10.1038/nsmb.3378

Basters, A., Knobeloch, K. P., and Fritz, G. (2018). USP18 - a Multifunctional Component in the Interferon Response. *Biosci. Rep.* 38, BSR20180250. doi:10.1042/BSR20180250

Basters, A., Geurink, P. P., Röcker, A., Witting, K. F., Tadayon, R., Hess, S., et al. (2017). Structural Basis of the Specificity of USP18 toward ISG15. *Nat. Struct. Mol. Biol.* 24, 270–278. doi:10.1038/nsmb.3371

Bogunovic, D., Byun, M., Durfee, L. A., Abhyankar, A., Sanal, O., Mansouri, D., et al. (2012). Mycobacterial Disease and Impaired IFN-γ Immunity in Humans with Inherited ISG15 Deficiency. *Science* 337, 1684–1688. doi:10.1126/science.1224026

Burks, J., Reed, R. E., and Desai, S. D. (2015). Free ISG15 Triggers an Antitumor Immune Response against Breast Cancer: a New Perspective. *Oncotarget* 6, 7221–7231. doi:10.18632/oncotarget.3372

Burks, J., Reed, R. E., and Desai, S. D. (2014). ISGylation Governs the Oncogenic Function of Ki-Ras in Breast Cancer. *Oncogene* 33, 794–803. doi:10.1038/onc.2012.633

Campbell, J. A., and Lenschow, D. J. (2013). Emerging Roles for Immunomodulatory Functions of Free ISG15. *J. Interferon Cytokine Res.* 33, 728–738. doi:10.1089/jir.2013.0064

Chen, R.-H., Du, Y., Han, P., Wang, H.-B., Liang, F.-Y., Feng, G.-K., et al. (2016). ISG15 Predicts Poor Prognosis and Promotes Cancer Stem Cell Phenotype in Nasopharyngeal Carcinoma. *Oncotarget* 7, 16910–16922. doi:10.18632/oncotarget.7626

D'Cunha, J., Knight, E., Jr., Haas, A. L., Truitt, R. L., and Borden, E. C. (1996). Immunoregulatory Properties of ISG15, an Interferon-Induced Cytokine. *Proc. Natl. Acad. Sci.* 93, 211–215. doi:10.1073/pnas.93.1.211

Dai, L., Bai, L., Lin, Z., Qiao, J., Yang, L., Flemington, E. K., et al. (2016). Transcriptomic Analysis of KSHV-Infected Primary Oral Fibroblasts: The Role of Interferon-Induced Genes in the Latency of Oncogenic Virus. *Oncotarget* 7, 47052–47060. doi:10.18632/oncotarget.9720

Desai, S. D. (2015). ISG15: A Double Edged Sword in Cancer. *Oncoimmunology* 4, e1052935. doi:10.1080/2162402x.2015.1052935

Dos Santos, P. F., and Mansur, D. S. (2017). Beyond ISGlylation: Functions of Free Intracellular and Extracellular ISG15. *J. Interferon Cytokine Res.* 37, 246–253. doi:10.1089/jir.2016.0103

Du, Y., Duan, T., Feng, Y., Liu, Q., Lin, M., Cui, J., et al. (2018). LRRC25 Inhibits Type I IFN Signaling by Targeting ISG15-associated RIG-I for Autophagic Degradation. *EMBO J.* 37, 351–366. doi:10.15252/embj.201796781

Durfee, L. A., Lyon, N., Seo, K., and Huibregtse, J. M. (2010). The ISG15 Conjugation System Broadly Targets Newly Synthesized Proteins: Implications for the Antiviral Function of ISG15. *Mol. Cel* 38, 722–732. doi:10.1016/j.molcel.2010.05.002

Feng, Q., Sekula, D., Guo, Y., Liu, X., Black, C. C., Galimberti, F., et al. (2008). UBE1L Causes Lung Cancer Growth Suppression by Targeting Cyclin D1. *Mol. Cancer Ther.* 7, 3780–3788. doi:10.1158/1535-7163.mct-08-0753

Frias-Staheli, N., Giannakopoulos, N. V., Kikkert, M., Taylor, S. L., Bridgen, A., Paragas, J., et al. (2007). Ovarian Tumor Domain-Containing Viral Proteases Evade Ubiquitin- and ISG15-dependent Innate Immune Responses. *Cell Host & Microbe* 2, 404–416. doi:10.1016/j.chom.2007.09.014

Fu, Z., Huang, B., Tang, J., Liu, S., Liu, M., Ye, Y., et al. (2021). The Complex Structure of GRL0617 and SARS-CoV-2 PLpro Reveals a Hot Spot for Antiviral Drug Discovery. *Nat. Commun.* 12, 488. doi:10.1038/s41467-020-20718-8

Ganesan, M., Poluektova, L. Y., Tuma, D. J., Kharbanda, K. K., and Osna, N. A. (2016). Acetaldehyde Disrupts Interferon Alpha Signaling in Hepatitis C Virus-Infected Liver Cells by Up-Regulating USP18. *Alcohol. Clin. Exp. Res.* 40, 2329–2338. doi:10.1111/acer.13226

Hermann, M., and Bogunovic, D. (2017). ISG15: In Sickness and in Health. *Trends Immunol.* 38, 79–93. doi:10.1016/j.it.2016.11.001

Huang, Y. F., Wee, S., Gunaratne, J., Lane, D. P., and Bulavin, D. V. (2014). Isg15 Controls P53 Stability and Functions. *Cell Cycle* 13, 2200–2210. doi:10.4161/cc.29209

Jeon, Y. J., Choi, J. S., Lee, J. Y., Yu, K. R., Kim, S. M., Ka, S. H., et al. (2009). ISG15 Modification of Filamin B Negatively Regulates the Type I Interferon-induced JNK Signalling Pathway. *EMBO Rep.* 10, 374–380. doi:10.1038/embor.2009.23

Jeon, Y. J., Park, J. H., and Chung, C. H. (2017). Interferon-Stimulated Gene 15 in the Control of Cellular Responses to Genotoxic Stress. *Mol. Cell* 40, 83–89. doi:10.14348/molcells.2017.0027

Jumat, M. R., Wong, P., Lee, R. T. C., Maurer-Stroh, S., Tan, B. H., and Sugrue, R. J. (2016). Molecular and Biochemical Characterization of the NS1 Protein of Non-cultured Influenza B Virus Strains Circulating in Singapore. *Microb. Genom* 2, e000082. doi:10.1099/mgen.0.000082

Kim, M.-J., Hwang, S.-Y., Imaizumi, T., and Yoo, J.-Y. (2008). Negative Feedback Regulation of RIG-I-Mediated Antiviral Signaling by Interferon-Induced ISG15 Conjugation. *J. Virol.* 82, 1474–1483. doi:10.1128/jvi.01650-07

Kimmey, J. M., Campbell, J. A., Weiss, L. A., Monte, K. J., Lenschow, D. J., and Stallings, C. L. (2017). The Impact of ISGylation during *Mycobacterium tuberculosis* Infection in Mice. *Microbes Infect.* 19, 249–258. doi:10.1016/j.micinf.2016.12.006

Klemm, T., Ebert, G., Calleja, D. J., Allison, C. C., Richardson, L. W., Bernardini, J. P., et al. (2020). Mechanism and Inhibition of the Papain-like Protease, PLpro, of SARS-CoV-2. *EMBO J.* 39, e106275. doi:10.15252/embj.2020106275

Langevin, C., van der Aa, L. M., Houel, A., Torhy, C., Briolat, V., Lunazzi, A., et al. (2013). Zebrafish ISG15 Exerts a strong Antiviral Activity against RNA and DNA Viruses and Regulates the Interferon Response. *J. Virol.* 87, 10025–10036. doi:10.1128/jvi.01294-12

Lenschow, D. J., Lai, C., Frias-Staheli, N., Giannakopoulos, N. V., Lutz, A., Wolff, T., et al. (2007). From the Cover: IFN-Stimulated Gene 15 Functions as a Critical Antiviral Molecule against Influenza, Herpes, and Sindbis Viruses. *Proc. Natl. Acad. Sci.* 104, 1371–1376. doi:10.1073/pnas.0607038104

Liu, M., Hummer, B. T., Li, X., and Hassel, B. A. (2004). Camptothecin Induces the Ubiquitin-like Protein, ISG15, and Enhances ISG15 Conjugation in Response to Interferon. *J. Interferon Cytokine Res.* 24, 647–654. doi:10.1089/jir.2004.24.647

Loeb, K. R., and Haas, A. L. (1992). The Interferon-Inducible 15-kDa Ubiquitin Homolog Conjugates to Intracellular Proteins. *J. Biol. Chem.* 267, 7806–7813. doi:10.1016/s0021-9258(18)42585-9

Malakhov, M. P., Kim, K. I., Malakhova, O. A., Jacobs, B. S., Borden, E. C., and Zhang, D.-E. (2003). High-throughput Immunoblotting. *J. Biol. Chem.* 278, 16608–16613. doi:10.1074/jbc.m208435200

Malakhov, M. P., Malakhova, O. A., Kim, K. I., Ritchie, K. J., and Zhang, D.-E. (2002). UBP43 (USP18) Specifically Removes ISG15 from Conjugated Proteins. *J. Biol. Chem.* 277, 9976–9981. doi:10.1074/jbc.m109078200

Malakhova, O. A., and Zhang, D.-E. (2008). ISG15 Inhibits Nedd4 Ubiquitin E3 Activity and Enhances the Innate Antiviral Response*. *J. Biol. Chem.* 283, 8783–8787. doi:10.1074/jbc.c800030200

Malakhova, O., Malakhov, M., Hetherington, C., and Zhang, D.-E. (2002). Lipopolysaccharide Activates the Expression of ISG15-specific Protease UBP43 via Interferon Regulatory Factor 3. *J. Biol. Chem.* 277, 14703–14711. doi:10.1074/jbc.m111527200

Morales, D. J., and Lenschow, D. J. (2013). The Antiviral Activities of ISG15. *J. Mol. Biol.* 425, 4995–5008. doi:10.1016/j.jmb.2013.09.041

Morales, D. J., Monte, K., Sun, L., Struckhoff, J. J., Agapov, E., Holtzman, M. J., et al. (2015). Novel Mode of ISG15-Mediated protection against Influenza A Virus and Sendai Virus in Mice. *J. Virol.* 89, 337–349. doi:10.1128/jvi.02110-14

Mustachio, L. M., Lu, Y., Kawakami, M., Roszik, J., Freemantle, S. J., Liu, X., et al. (2018). Evidence for the ISG15-specific Deubiquitinase USP18 as an Antineoplastic Target. *Cancer Res.* 78, 587–592. doi:10.1158/0008-5472.can-17-1752

Nakashima, H., Nguyen, T., Goins, W. F., and Chiocca, E. A. (2015). Interferon-stimulated Gene 15 (ISG15) and ISG15-Linked Proteins Can Associate with Members of the Selective Autophagic Process, Histone Deacetylase 6 (HDAC6) and SQSTM1/p62. *J. Biol. Chem.* 290, 1485–1495. doi:10.1074/jbc.m114.593871

Okumura, A., Lu, G., Pitha-Rowe, I., and Pitha, P. M. (2006). Innate Antiviral Response Targets HIV-1 Release by the Induction of Ubiquitin-like Protein ISG15. *Proc. Natl. Acad. Sci.* 103, 1440–1445. doi:10.1073/pnas.0510518103

Okumura, A., Pitha, P. M., and Harty, R. N. (2008). ISG15 Inhibits Ebola VP40 VLP Budding in an L-domain-dependent Manner by Blocking Nedd4 Ligase Activity. *Proc. Natl. Acad. Sci.* 105, 3974–3979. doi:10.1073/pnas.0710629105

Okumura, F., Okumura, A. J., Uematsu, K., Hatakeyama, S., Zhang, D.-E., and Kamura, T. (2013). Activation of Double-Stranded RNA-Activated Protein Kinase (PKR) by Interferon-Stimulated Gene 15 (ISG15) Modification Down-Regulates Protein Translation. *J. Biol. Chem.* 288, 2839–2847. doi:10.1074/jbc.m112.401851

Owhashi, M., Taoka, Y., Ishii, K., Nakazawa, S., Uemura, H., and Kambara, H. (2003). Identification of a Ubiquitin Family Protein as a Novel Neutrophil Chemotactic Factor. *Biochem. Biophysical Res. Commun.* 309, 533–539. doi:10.1016/j.bbrc.2003.08.038

Padovan, E., Terracciano, L., Certa, U., Jacobs, B., Reschner, A., Bolli, M., et al. (2002). Interferon Stimulated Gene 15 Constitutively Produced by Melanoma Cells Induces E-Cadherin Expression on Human Dendritic Cells. *Cancer Res.* 62, 3453–3458. doi:10.1111/omi.12270/v1/review2

Park, J. H., Yang, S. W., Park, J. M., Ka, S. H., Kim, J.-H., Kong, Y.-Y., et al. (2016). Positive Feedback Regulation of P53 Transactivity by DNA Damage-Induced ISG15 Modification. *Nat. Commun.* 7, 12513. doi:10.1038/ncomms12513

Pincetic, A., Kuang, Z., Seo, E. J., and Leis, J. (2010). The Interferon-Induced Gene ISG15 Blocks Retrovirus Release from Cells Late in the Budding Process. *J. Virol.* 84, 4725–4736. doi:10.1128/jvi.02478-09

Pitha-Rowe, I., Hassel, B. A., and Dmitrovsky, E. (2004). Involvement of UBE1L in ISG15 Conjugation during Retinoid-Induced Differentiation of Acute Promyelocytic Leukemia. *J. Biol. Chem.* 279, 18178–18187. doi:10.1074/jbc.m309259200

Potter, J. L., Narasimhan, J., Mende-Mueller, L., and Haas, A. L. (1999). Precursor Processing of Pro-ISG15/UCRP, an Interferon-β-Induced Ubiquitin-like Protein. *J. Biol. Chem.* 274, 25061–25068. doi:10.1074/jbc.274.35.25061

Radoshevich, L., Impens, F., Ribet, D., Quereda, J. J., Nam Tham, T., Nahori, M. A., et al. (2015). ISG15 Counteracts Listeria Monocytogenes Infection. *Elife* 4, e06848. doi:10.7554/eLife.06848

Rahnefeld, A., Klingel, K., Schuermann, A., Diny, N. L., Althof, N., Lindner, A., et al. (2014). Ubiquitin-like Protein ISG15 (Interferon-stimulated Gene of 15 kDa) in Host Defense against Heart Failure in a Mouse Model of Virus-Induced Cardiomyopathy. *Circulation* 130, 1589–1600. doi:10.1161/circulationaha.114.009847

Recht, M., Borden, E. C., and Knight, E., Jr. (1991). A Human 15-kDa IFN-Induced Protein Induces the Secretion of IFN-Gamma. *J. Immunol.* 147, 2617–2623. doi:10.1111/j.1365-2249.1990.tb05403.x

Rodriguez, M. R., Monte, K., Thackray, L. B., and Lenschow, D. J. (2014). ISG15 Functions as an Interferon-Mediated Antiviral Effector Early in the Murine Norovirus Life Cycle. *J. Virol.* 88, 9277–9286. doi:10.1128/jvi.01422-14

Rut, W., Lv, Z., Zmudzinski, M., Patchett, S., Nayak, D., Snipas, S. J., et al. (2020). Olsen, Activity Profiling and Crystal Structures of Inhibitor-Bound SARS-CoV-2 Papain-Like Protease: A Framework for Anti-COVID-19 Drug Design. *Sci. Adv.* 6.

Sainz, B., Jr., Martín, B., Tatari, M., Heeschen, C., and Guerra, S. (2014). ISG15 Is a Critical Microenvironmental Factor for Pancreatic Cancer Stem Cells. *Cancer Res.* 74, 7309–7320. doi:10.1158/0008-5472.can-14-1354

Shi, H.-X., Yang, K., Liu, X., Liu, X.-Y., Wei, B., Shan, Y.-F., et al. (2010). Positive Regulation of Interferon Regulatory Factor 3 Activation by Herc5 via ISG15 Modification. *Mol. Cel Biol* 30, 2424–2436. doi:10.1128/mcb.01466-09

Shin, D., Mukherjee, R., Grewe, D., Bojkova, D., Baek, K., Bhattacharya, A., et al. (2020). Papain-like Protease Regulates SARS-CoV-2 Viral Spread and Innate Immunity. *Nature* 587, 657–662. doi:10.1038/s41586-020-2601-5

Sooryanarain, H., Rogers, A. J., Cao, D., Haac, M. E. R., Karpe, Y. A., and Meng, X. J. (2017). ISG15 Modulates Type I Interferon Signaling and the Antiviral Response during Hepatitis E Virus Replication. *J. Virol.* 91, e00621. doi:10.1128/JVI.00621-17

Swaim, C. D., Scott, A. F., Canadeo, L. A., and Huibregtse, J. M. (2017). Extracellular ISG15 Signals Cytokine Secretion through the LFA-1 Integrin Receptor. *Mol. Cel* 68, 581–590. e585. doi:10.1016/j.molcel.2017.10.003

Takeuchi, T., Koinuma, S., Yokosawa, H., and Arata, Y. (2019). An Approach for the Identification of Proteins Modified with ISG15. *Methods Mol. Biol.* 1934, 235–246. doi:10.1007/978-1-4939-9055-9_15

Takeuchi, T., and Yokosawa, H. (2005). ISG15 Modification of Ubc13 Suppresses its Ubiquitin-Conjugating Activity. *Biochem. Biophysical Res. Commun.* 336, 9–13. doi:10.1016/j.bbrc.2005.08.034

Tang, Y., Zhong, G., Zhu, L., Liu, X., Shan, Y., Feng, H., et al. (2010). Herc5 Attenuates Influenza A Virus by Catalyzing ISGylation of Viral NS1 Protein. *J. Immunol.* 184, 5777–5790. doi:10.4049/jimmunol.0903588

Tokarz, S., Berset, C., La Rue, J., Friedman, K., Nakayama, K.-I., Nakayama, K., et al. (2004). The ISG15 Isopeptidase UBP43 Is Regulated by Proteolysis via the SCFSkp2 Ubiquitin Ligase. *J. Biol. Chem.* 279, 46424–46430. doi:10.1074/jbc.m403189200

Werneke, S. W., Schilte, C., Rohatgi, A., Monte, K. J., Michault, A., Arenzana-Seisdedos, F., et al. (2011). ISG15 Is Critical in the Control of Chikungunya Virus Infection Independent of UbE1L Mediated Conjugation. *Plos Pathog.* 7, e1002322. doi:10.1371/journal.ppat.1002322

Yasuda, J., Nakao, M., Kawaoka, Y., and Shida, H. (2003). Nedd4 Regulates Egress of Ebola Virus-like Particles from Host Cells. *J. Virol.* 77, 9987–9992. doi:10.1128/jvi.77.18.9987-9992.2003

Yeung, T. L., Tsai, C. C., Leung, C. S., Au Yeung, C. L., Thompson, M. S., Lu, K. H., et al. (2018). ISG15 Promotes ERK1 ISGylation, CD8+ T Cell Activation and Suppresses Ovarian Cancer Progression. *Cancers (Basel)* 10, 464. doi:10.3390/cancers10120464

Yoo, L., Yoon, A.-R., Yun, C.-O., and Chung, K. C. (2018). Covalent ISG15 Conjugation to CHIP Promotes its Ubiquitin E3 Ligase Activity and Inhibits Lung Cancer Cell Growth in Response to Type I Interferon. *Cell Death Dis* 9, 97. doi:10.1038/s41419-017-0138-9

Yuan, W., and Krug, R. M. (2001). Influenza B Virus NS1 Protein Inhibits Conjugation of the Interferon (IFN)-induced Ubiquitin-like ISG15 Protein. *EMBO J.* 20, 362–371. doi:10.1093/emboj/20.3.362

Zhang, X., Bogunovic, D., Payelle-Brogard, B., Francois-Newton, V., Speer, S. D., Yuan, C., et al. (2015). Human Intracellular ISG15 Prevents Interferon-A/β Over-amplification and Auto-Inflammation. *Nature* 517, 89–93. doi:10.1038/nature13801

Zhao, C., Denison, C., Huibregtse, J. M., Gygi, S., and Krug, R. M. (2005). Human ISG15 Conjugation Targets Both IFN-Induced and Constitutively Expressed Proteins Functioning in Diverse Cellular Pathways. *Proc. Natl. Acad. Sci.* 102, 10200–10205. doi:10.1073/pnas.0504754102

Zhao, C., Hsiang, T.-Y., Kuo, R.-L., and Krug, R. M. (2010). ISG15 Conjugation System Targets the Viral NS1 Protein in Influenza A Virus-Infected Cells. *Proc. Natl. Acad. Sci. USA* 107, 2253–2258. doi:10.1073/pnas.0909144107

Zhao, C., Sridharan, H., Chen, R., Baker, D. P., Wang, S., and Krug, R. M. (2016). Influenza B Virus Non-structural Protein 1 Counteracts ISG15 Antiviral Activity by Sequestering ISGylated Viral Proteins. *Nat. Commun.* 7, 12754. doi:10.1038/ncomms12754

Zhu, Z., Yang, Y., Wei, J., Shao, D., Shi, Z., Li, B., et al. (2014). Type I Interferon-Mediated Immune Response against Influenza A Virus Is Attenuated in the Absence of P53. *Biochem. Biophysical Res. Commun.* 454, 189–195. doi:10.1016/j.bbrc.2014.10.067

Zuo, C., Sheng, X., Ma, M., Xia, M., and Ouyang, L. (2016). ISG15 in the Tumorigenesis and Treatment of Cancer: An Emerging Role in Malignancies of the Digestive System. *Oncotarget* 7, 74393–74409. doi:10.18632/oncotarget.11911

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

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cell and Developmental Biology

MINI REVIEW
published: 15 February 2022
doi: 10.3389/fcell.2022.854352

Proteolysis-Targeting Chimera (PROTAC): Is the Technology Looking at the Treatment of Brain Tumors?

Ludovica Lospinoso Severini[1], *Francesca Bufalieri*[1], *Paola Infante*[1]* and *Lucia Di Marcotullio*[1,2]*

[1]Department of Molecular Medicine, University of Rome La Sapienza, Rome, Italy, [2]Istituto Pasteur-Fondazione Cenci Bolognetti, University of Rome La Sapienza, Rome, Italy

Post-translational modifications, such as ubiquitylation, need to be tightly controlled to guarantee the accurate localization and activity of proteins. Ubiquitylation is a dynamic process primarily responsible for proteasome-mediated degradation of substrate proteins and crucial for both normal homeostasis and disease. Alterations in ubiquitylation lead to the upregulation of oncoproteins and/or downregulation of tumor suppressors, thus concurring in tumorigenesis. PROteolysis-TArgeting Chimera (PROTAC) is an innovative strategy that takes advantage by the cell's own Ubiquitin-Proteasome System (UPS). Each PROTAC molecule is composed by a ligand that recruits the target protein of interest (POI), a ligand specific for an E3 ubiquitin ligase enzyme, and a linker that connects these units. Upon binding to the POI, the PROTAC recruits the E3 inducing ubiquitylation-dependent proteasome degradation of the POI. To date, PROTAC technology has entered in clinical trials for several human cancers. Here, we will discuss the advantages and limitations of PROTACs development and safety considerations for their clinical application. Furthermore, we will review the potential of PROTAC strategy as therapeutic option in brain tumor, focusing on glioblastoma.

Keywords: protac (proteolysis targeting chimera), ubiquitylation (ubiquitination), cancer, glioblastoma, cancer therapy

OPEN ACCESS

Edited by:
Zhenghong Lin,
Chongqing University, China

Reviewed by:
Ribhav Mishra,
Northwestern University, United States
Jerry Vriend,
University of Manitoba, Canada

***Correspondence:**
Lucia Di Marcotullio
lucia.dimarcotullio@uniroma1.it
Paola Infante
paola.infante@uniroma1.it

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *13 January 2022*
Accepted: *31 January 2022*
Published: *15 February 2022*

Citation:
Lospinoso Severini L, Bufalieri F, Infante P and Di Marcotullio L (2022) Proteolysis-Targeting Chimera (PROTAC): Is the Technology Looking at the Treatment of Brain Tumors? Front. Cell Dev. Biol. 10:854352. doi: 10.3389/fcell.2022.854352

INTRODUCTION

The Ubiquitin-Proteasome System

The Ubiquitin-Proteasome System (UPS) is a cellular mechanism essential for maintaining the correct balance of protein turnover and cell homeostasis (Finley 2009; Hipp et al., 2019). UPS machinery includes chaperones and components of the proteolytic system (Kim et al., 2013): the first are required for an accurate protein folding; the latter converge on the 26S proteasome and guarantee the removal of unfolded and/or damaged proteins. To be targeted for proteasome-mediated degradation, proteins are covalently tagged with ubiquitin (Ub) moieties. This event requests the consequential activity of three enzymes: E1 Ub-activating enzyme (E1), E2 Ub-conjugating enzyme (E2), and E3 Ub-ligase (E3) (Kliza and Husnjak, 2020). First, an Ub molecule is activated by E1 in an ATP-dependent manner resulting in an E1-Ub conjugate. Then, a trans-thioesterification reaction allows the transfer of a molecule of Ub from E1 to E2. Lastly, an E3 binds at the same time the E2-Ub conjugate and the target protein favouring the transfer of Ub from the E2 to the substrate, directly or indirectly depending on the E3 family involved in the event (Infante et al., 2019; Sharma et al., 2021).

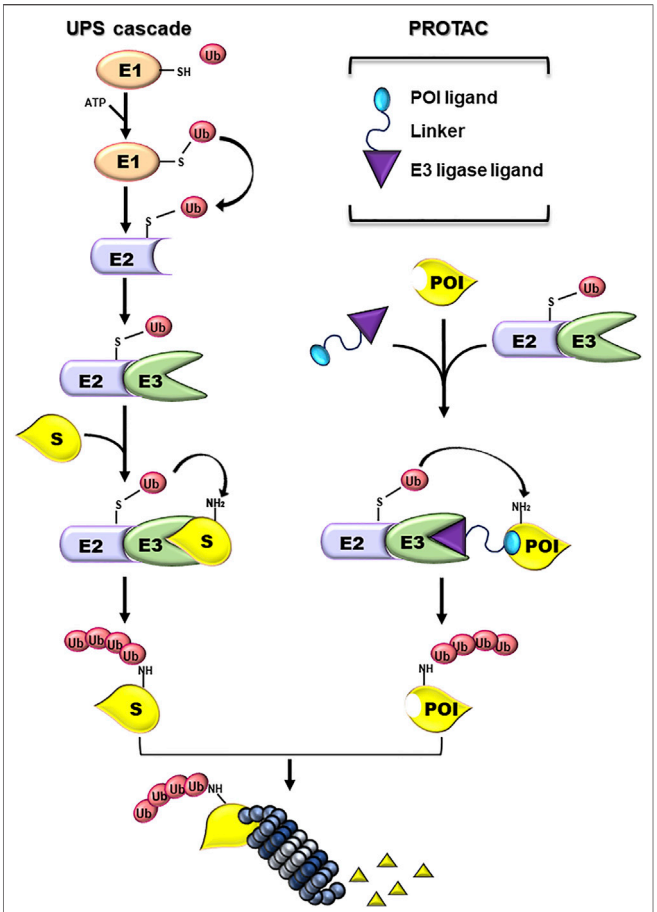


FIGURE 1 | Ubiquitin-proteasome and PROTAC systems. Schematic representation of the enzymatic cascade of the Ubiquin-Proteasome System (UPS cascade; left side). Ubiquitylation is triggered by the ATP-dependent activation of the ubiquitin by E1 activating enzyme. Next, the ubiquitin (Ub) is bound to the E2 Ub-conjugating enzyme and, subsequently, transferred to a Lys residue on a substrate protein (S) by an E3-Ub ligase (E3). The formation of a poly-Ub chain, formed by more than four Ub moieties, can lead to the degradation of the substrate by the proteasome. PROTAC components and their mechanism of action (PROTAC; right side). PROTACs are heterobifunctional small molecules consisting of a ligand specific for the protein of interest (POI) and another ligand for E3, connected by a linker. PROTACs work by recruiting an E3 ligase into proximity of a specific POI that can be tagged with Ub and degraded by the proteasome.

Both the number of Ub moieties and the lysine linkage of Ub-Ub conjugation determine the fate of the protein (Welchman et al., 2005). Ub-tagged substrates are mostly addressed to the proteasome for degradation (**Figure 1**).

The UPS is finely regulated by E3 ligases that confer specificity of ubiquitylation through the recognition of substrates, thus making these enzymes considerable druggable targets. So far, several small molecule inhibitors (SMIs) have been designed to hit E3s. For instance, Mouse double minute two homolog (Mdm2), the E3 responsible of the ubiquitylation and degradation of p53, is highly expressed in sarcomas and breast cancers (~20 and ~15%, respectively) (Karni-Schmidt et al., 2016; Oliner et al., 2016) and represents a significant drug target in these tumors. Nutlin-3a, a small inhibitor of Mdm2, binds the hydrophobic pocket at the N-terminal of Mdm2 necessary for its binding with p53, preventing Mdm2-p53 interaction and activating p53 oncosuppressor functions in malignant cells (Vassilev et al., 2004).

SMIs present some inevitable limitations, including the possibility to target only a moderate percentage (~20%) and an exiguous class, mainly enzymes, of human proteins (Schapira et al., 2019). Since most of disease-driven proteins are not enzymes, they are considered unconventional therapeutic targets. The urgent need to develop new strategies to target the undruggable proteome led to advances in antibody therapy (Jenkins et al., 2018; Dobosz and Dzieciątkowski 2019), although the difficulty to hit intracellular proteins still strongly limits the use of this option. The current emerging and successful strategy to target proteome is PROteolysis TArgeting Chimera (PROTAC) technology (Sakamoto et al., 2001; Schapira et al., 2019).

PROTACs take advantage of cell's own UPS machinery to specifically address a protein of interest (POI) towards a proteasome-mediated degradation (Sakamoto et al., 2001).

PROTAC Technology: The Two Side of the Coin

PROTACs are heterobifunctional molecules formed by two ligands connected by a linker. The first ligand (warhead) interacts with the POI, a different one binds with an E3, and the linker connects them (**Figure 1**) (An and Fu 2018). The proximity between the E3 and the POI mediated by PROTAC favors the ubiquitylation and catalyzes the degradation of the POI by the UPS.

PROTAC compounds have been developed more than 20 years ago (Sakamoto et al., 2001) and many efforts have been made in these 2 decades to improve their effectiveness. For example, peptide ligands in PROTAC structure have been modified in small molecules to ameliorate cell permeability (Schneekloth et al., 2008).

PROTACs show multiple advantages as compared to traditional SMIs, alongside several limitations. A PROTAC molecule can catalyse the degradation of multiple POI molecules, and its pharmacological effect is achieved at very low dosages compared to SMIs, thus reducing the toxicity. Of note, proteins considered as "undruggable" could be potentially targeted by PROTACs. This is relevant especially for transcription factors (TFs) involved in the progression of several malignancies (Bai et al., 2019; Zhou et al., 2019). For example, genomic alterations in c-MYC, FOXO1 or the androgen receptor (AR) have been described in neuroblastoma, breast, and prostate cancer, respectively (Bushweller 2019; Yu et al., 2019). Counteracting their expression through protein degradation represents a therapeutic strategy for these human malignancies. In this regard, two PROTACs targeting the AR and estrogenic receptor (ER) have reached the clinical practice in two phase I studies for the treatment of prostate and ER-positive breast cancer, respectively (Mullard 2019), sustaining the results obtained in this field.

Additionally, PROTACs can overcome SMIs resistance by targeting mutated POIs (Burslem et al., 2018; Zhao et al., 2019; Gonzalez et al., 2020), as well as the resistance resulting from POIs upregulation (Kregel et al., 2020).

However, some safety concerns associated with PROTACs need to be taken into consideration before supporting their entry in clinical practice. PROTACs limitations are mainly due to on-target and off-target toxicities. The on-target toxicities are related to the physiological functions of POI. Some proteins (i.e., kinases) hold enzymatic as well as scaffold functions, becoming essential for normal cellular functions. SMIs block only the enzymatic activity of POI, while the complete degradation induced by PROTACs interferes with both enzymatic and scaffolding function, eliciting undesirable consequences (Cromm et al., 2018; Nunes et al., 2019). Moreover, unlike SMI that can only partially inhibit the functions of their targets, a potent PROTAC can completely deplete its POIs. The partial inhibition consequent to SMIs treatment may be tolerable, while PROTAC-induced degradation could be harmful if POIs have essential functions for cell survival (Winter et al., 2015). The extent of cellular damage depends on the rate of the depleted protein resynthesis (Chan et al., 2018; Cromm et al., 2018; Olson et al., 2018; Testa et al., 2018; Smith et al., 2019). In addition, the inhibition of POIs mediated by SMIs is transient as opposed to the prolonged depletion PROTAC-mediated. In this case, the cellular/tissue context and the target features impact on the benefits or drawbacks of PROTACs. If a POI has redundant function in normal tissues, its prolonged degradation couldn't be devastating for cells (Mason et al., 2007; Eichhorn et al., 2014; Khan et al., 2019). On the contrary, targeting a POI indispensable for physiological cellular activities can cause on-target toxicities.

Off-target toxicities often arise from the "unintentional" degradation of proteins. This event may occur when the non-target protein is not directly bound to the PROTAC but is in complex with the POI or in its proximity (Hsu et al., 2020). Since PROTACs form a ternary complex between POI and E3, a phenomenon known as "Hook effect" can take place. In particular, the formation of the ternary complexes is inhibited with high PROTACs concentrations causing an excess of binary bindings PROTAC-POI or PROTAC-E3, thus invalidating target degradation (Pettersson and Crews 2019). Furthermore, the generation of PROTAC-E3 binary complexes can induce the degradation of lower-affinity non-targeted proteins (Moreau et al., 2020). This event may affect substrates essential for cellular homeostasis (Schmitt et al., 2002), or may cause the accumulation of off-target ubiquitylated proteins saturating the UPS and dysregulating the proteostasis.

PROTACs Optimization Strategies

PROTAC is a relatively new research field with rapid developments that, however, still needs laborious optimization. Biological and physical-chemical properties of this technology can be fine-tuned. The linker length is a crucial structural element that can be improved. Too short linkers may cause a steric clash that disrupts ternary complex, thus impairing PROTAC activity. Conversely, too long linkers can give two heads of a PROTAC more motility, thus changing molecule stability. Moreover, an excessive linker length increases the molecular weight and reduces cell permeability of a PROTAC.

The first linker used in PROTAC design has been a flexible one, such as polyethylene glycol (PEG), which improves water solubility (Bai et al., 2019; Khan et al., 2019) or polymethylene chains. Recently, "click chemistry" based on coppercatalyzed azide-alkyne cycloaddition (CuAAC) and the Diels–Alder (DA) reaction has been applied in PROTAC preparation (Wang et al., 2020). The resulting PROTACs can be faster validated for their degradation capability and can self-assembly as active molecules in live cells (Lebraud et al., 2016).

The rigidity of the linker represents another important aspect that impacts on pharmacokinetic properties and oral bioavailability of PROTACs (Farnaby et al., 2019; Testa et al., 2020). Nevertheless, the design of an optimal rigid linker could be difficult if the cocrystal structure of the ternary complex is unknown.

The human genome encodes for more than 600 E3s, but only 1% of them have been explored for substrate degradation (Khan et al., 2020). Since E3s define target specificity, this feature could be useful to increase efficacy and decrease toxicity of the PROTACs. For example, one PROTAC optimization strategy is based on E3 specific expression in tissues (i.e. the F-box and leucine-rich repeat protein 16, FBXL16, is specifically expressed in cerebral cortex (Clifford et al., 1999)) and/or cellular compartments (i.e. the DDB1- and CUL4-associated factor 16, DCAF16, localizes only in the nucleus (Robb et al., 2017).

PROTACs in Human Cancers

Cancer is a multistep process characterized by abnormal cellular proliferation and dissemination due to genomic and epigenomic alterations (Hanahan and Weinberg 2011). The identification of molecular alterations involved in the oncogenic features has become attractive for the development of novel therapeutics (Ocaña et al., 2018). The clinical use of proteasome inhibitors in oncology demonstrates how the disbalance in protein homeostasis reflects an oncogenic vulnerability in some malignancies (Inobe and Matouschek 2014; Schapira et al., 2019). Indeed, an accurate proteostasis is crucial in cells characterized by a high rate of protein turnover, such as tumor cells, that consequently need a very efficient and quick protein synthesis and degradation (Bard et al., 2019; Pohl and Dikic 2019).

Several PROTACs have been developed in the last 20 years, but unfortunately only few of them are selective for tumor cells. Many PROTACs recruit E3 ligases that are ubiquitously expressed in both normal and tumor tissues, thus leading to on-target toxicities. Multiple strategies can be followed to achieve the selective degradation of tumor-specific POIs mediated by PROTACs.

If the POI is tumor specific, it is possible to target it with any available E3s expressed in the tumor tissues (Burslem et al., 2019). Alternatively, if the POI is characteristic of a tumor-derived tissue, it is possible to optimize PROTACs taking advantages of any available tissue-specific E3 (Schapira et al., 2019; Sun et al., 2019). Further, a tumor-associated POI could be expressed in normal tissues and involved in physiological cell functions but showing an upregulated expression in cancer tissues. The use of tumor specific E3s highly expressed in tumor cells, but lowly or absent expression in normal tissues, could offer an increased advantage to selectively kill cancer cell, thus minimizing toxicity to normal tissues. The development of a B-cell lymphoma-extra-large (BCL-XL) PROTAC is a recent example (Chung et al., 2020; Kolb et al., 2021).

The availability of public -omics data has incentivized the identification of tissue-selective E3s (Consortium 2015; Melé et al., 2015) opening the route to achieve the selective and tumor specific degradation of a target protein by PROTACs.

Several research groups have recently investigated the activity of the light-controllable photo-PROTACs, which can be controlled under visible or UVA light to drive tumor specific degradation of POIs (Pfaff et al., 2019; Xue et al., 2019; Liu et al., 2020; Reynders et al., 2020). This strategy can only be accomplished in a clinical setting using photodynamic therapy for limited types of cancer.

PROTACs efficacy has been demonstrated in several preclinical studies (Bai et al., 2019; Khan et al., 2019; Li et al., 2019). Of note, PROTAC technology has also been shown to stimulate an anticancer immune response by inducing the presentation of peptides derived from the degradation of POI to antigen-presenting cells (Moser et al., 2017; Jensen et al., 2018). Moreover, PROTAC could be used to generate new MHC-I peptides on the cell surface favouring the formation of new immunopeptidome "targetable" by T-cell based therapeutics (Lai et al., 2018). Mass spectrometry analysis can help to understand and explore the impact of PROTAC treatment on peptide repertoire of MHC-I presentation and potential perturbation of biological pathways.

PROTAC strategy can be used to exploit E3s having tumor suppressor natural substrates (Hines et al., 2019), as well as PROTAC-incorporation into nanoparticles which can be incapsulated with antibodies, can help to specifically reach the tumoral environment and malignant cells (Beck et al., 2017; Niza et al., 2019; Pillow et al., 2020).

Recently, strategies similar to PROTACs have been developed to induce the degradation of RNAs (i.e., oncogenic micro-RNAs) through the recruitment of nucleases. These molecules, known as ribonuclease Targeting Chimeras (RIBOTACs) stands as innovative future anticancer therapeutics (Costales et al., 2020a; Costales et al., 2020b). Overall, PROTACs and similar technologies stand as promising class of biological drugs useful in cancer therapy.

PROTACs as Therapeutic Option for Glioblastoma

Central nervous system (CNS) cancers are a group of heterogeneous tumor entities with wide differences regarding the site of onset, molecular biology, clinical behaviour, and etiology (Kristensen et al., 2019; Lospinoso Severini et al., 2020). Among them, glioblastoma (GB) is the most malignant and lethal in adults (Louis et al., 2016). Classified as grade IV diffuse glioma by the World Health Organization (WHO), GB encompasses more than 54% of gliomas with an median survival of about 15 months (Ostrom et al., 2014; Louis et al., 2016). Current standard therapy for newly diagnosed GB is based on maximal surgical resection, followed by radiation and chemotherapy, based on the administration of temozolomide (TMZ), an oral alkylating agent (Stupp et al., 2005; Stupp et al., 2009). Despite the aggressiveness of this therapeutic strategy, it has limited effectiveness making GB an incurable tumor that often returns as relapse (Lieberman 2017). The main hallmarks of this malignancy that hinder its treatments are rapid progression, invasiveness of cancer cells in the surrounding region of the brain, inter- and intra-tumoral genetic and molecular heterogeneity and the presence of drug-resistance GB stem-like cells (GSCs), which favour tumor relapse (Brennan et al., 2012; Meyer et al., 2015; Gangoso et al., 2021).

Transcriptomic and genomic profiling have allowed the identification of genetic alterations patterns affecting molecular drivers involved in GB tumorigenesis, including epidermal growth factor receptor (*EGFR),* phosphatase and tensin homolog (*PTEN*), cyclin dependent kinase 4/6 (*CDK4/6*) and cyclin dependent kinase inhibitor 2A/B (*CDKN2A/B*), neurofibromatosis type 1 (*NF1*), platelet-derived growth factor receptor alpha (*PDGFRα),* and isocitrate dehydrogenase (*IDH*) genes (Verhaak et al., 2010; Dunn et al., 2012; Stoyanov and Dzhenkov 2018).

The delineation of the aberrant molecular networks that cause the malignant phenotype of GB have highlighted key processes, which can be therapeutically exploited. So far, several targeted therapies for GB have been tested, most of which aim to block growth factor receptors (i.e., EGFR) and downstream pathways frequently altered in GB (i.e., PI3K/AKT/mTOR and MAPK/ERK) (Le Rhun et al., 2019). However, none of these approaches have been formally validated as effective in clinical trials, likely due to molecular compensatory mechanism, insufficient target coverage or toxicity (Touat et al., 2017; Le Rhun et al., 2019). Different immunotherapeutic approaches have also been investigated for the treatment of GB, but the presence of the tumor immunosuppressive microenvironment limits their benefits (Bufalieri et al., 2020a; Weenink et al., 2020; Bufalieri et al., 2021; Medikonda et al., 2021).

Recently, the UPS is emerging as a promising source for the development of new therapeutic options for GB, and in particular PROTACs represent an interesting targeted therapy for the treatment of this devastating tumor (Bufalieri et al., 2020b; Scholz et al., 2020; Maksoud 2021; Farrell and Jarome 2021).

Two different PROTAC strategies able to induce the degradation of CDK4 and/or CDK6 have been tested in GB cells. CDK4 and CDK6 are crucial for cell cycle regulation and are attractive targets for the treatments of various types of cancers, including GB, frequently

characterized by a CDK4/6 pathway dysregulation (Network 2008; Brennan et al., 2013; Bronner et al., 2019). In 2019, Zhao and Burgess tested the activity of PROTACs based on two selective CDK4/6 inhibitors, palbociclib (Ibrance®, Pfizer, New York, USA) and ribociclib (Kisqali®, Novartis, Basel, Switzerland) in breast cancer and GB cell lines (Zhao and Burgess 2019). These drugs have been approved by US Food Drug Administration (FDA) as combination therapy for ER-positive, HER2-negative advanced breast cancer and are currently used in ongoing clinical trials, including some for the treatment of GB (NCT03158389; NCT02345824; NCT02933736; NCT03834740; NCT03355794; NCT03355794). PROTACs of palbociclib and ribociclib (called pal-pom and rib-pom, respectively) consist in the conjugation of these two drugs to pomalidomide (pom), a cereblon (CRBN) E3 ligand, by cycloadding a known azide derived from pomalidomide to N-propargyl derivatives of palbociclib or ribociclib. U87 GB cells treated with pal-pom and rib-pom at 20–200 nM have a significant depletion of CDK4 protein levels, showing the effectiveness of these PROTACs to counteract the aberrant overexpression of this kinase in GB (Zhao and Burgess 2019). In addition, Su and others designed and synthesized a PROTAC by linking the CDK6 inhibitor palbociclib and E3 CRBN recruiter pom, testing its effect in GB cells (Su et al., 2019). In this study Nutlin-3b, VH032, and bestatin were also used as recruiting moiety for the E3 ligases Mdm2 and VHL, and inhibitor of apoptosis (cIAP), respectively. Interestingly, the authors found that in U251 GB cells CDK4 and CDK6 were degraded only with PROTAC recruiting CRBN, but not the other E3s, and that CDK4 degradation was less significant compared to those of CDK6. Furthermore, CDK6 degraders with shorter linker possessed higher degradation capacity, favouring the recruitment of CRBN towards CDK6 (Su et al., 2019). Although in-depth studies on the biological effect and anti-tumor potential of these PROTACs are still needed, these data suggest the potential application of PROTAC technology for the specific CDK4/6 degradation for the treatment of GB.

The first *in vivo* evidence of the potential of PROTACs as anticancer agents for GB was provided by a recent work in which the authors exploited the ability of a high-selective histone deacetylase 6 (HDAC6) inhibitor, J22352, to impair GB tumor growth (Liu et al., 2019). Indeed, the overexpression of HDAC6 in GB is associated with proliferation and resistance to TMZ, thus targeting this enzyme stands as a promising strategy for GB therapeutic interventions (Wang et al., 2016). J22352 shows PROTAC-like property, leading to the ubiquitylation and subsequent proteasome degradation of HDAC6. As consequence, the decrease of HDAC6 expression level significantly inhibits GB tumor growth in U87MG cells, both *in vitro* and *in vivo,* by increasing autophagic cancer cell death and eliciting the anti-tumor immune response (Liu et al., 2019).

These pioneering studies on the effects of PROTACs in GB cells and the evidence that PROTACs are already developed against oncoproteins relevant for the progression of this tumor, including EGFR (Zhang et al., 2020; Zhao et al., 2020), mitogen-activated MAP-kinases (MAPs) (Pandey et al., 2016; Trauner and Shemet 2019) and bromodomain and extraterminal (BET) protein BRD4 (Xu et al., 2018; Yang et al., 2019; Hu and Crews 2021; Yang et al., 2021), suggest the great potential for the use of this technology for the treatment of GB.

DISCUSSION

In the last two decades, targeting UPS has emerged as an extraordinary clinical opportunity, leading to the development of new and effective therapeutic options in human diseases, especially in cancer.

In this field, PROTAC has been one of the first strategies developed, aimed to degrade rather than inhibit protein targets. Thanks to their mechanism of action, PROTACs have shown the peculiarity to improve current cancer therapies based on the use of SMIs. Indeed, while SMIs act by occupying pockets on target proteins in a stoichiometric manner, a single PROTAC molecule can induce the degradation of its target through many rounds, even after dissociation of the PROTAC from POI (Lai and Crews 2017). This mechanism of action provides several advantages (**Figure 2**). Foremost PROTACs can be administered at lower dosages compared to SMIs achieving comparable effects, thus reducing toxicity. Moreover, PROTACs are less sensitive to drug resistance compared to traditional drugs. Indeed, PROTACs are potentially able to degrade multiple subunits of a protein complex, thus reducing the possibility to develop resistance-mutations in the protein of interest (Hu and Crews 2021). However, genomic alterations in the components of the E3s complex can cause resistance to PROTACs, underling the urgent need to find novel ligands for other druggable E3 ligases (Ottis et al., 2019; Zhang et al., 2019).

Given that many PROTACs targets are proteins involved in oncogenic proliferation and metastasis, PROTAC technology rapidly moved from laboratory to clinics especially for the treatment of human cancers (Zeng et al., 2021). At present, two Phase II clinical trials for the PROTACs ARV-471 and ARV-110 are ongoing, for the treatment of breast and prostate cancer, respectively. ARV-471 is an orally available PROTAC developed by Arvinas for the targeting of ER and its mutated forms, ER^{Y537S} ER^{D538G}, resistant to endocrine therapy in ER-positive breast cancer (Martin et al., 2017). ARV-110, another orally available PROTAC, selectively degrades AR and inhibits pancreatic tumor growth, both in mice models and patient-derived organoids, better than enzalutamide, a known AR inhibitor (Neklesa et al., 2019). ARV-110 have been tested in Phase I clinical trial for castration-resistant prostate cancer (CRPC) and a Phase II clinical trial is ongoing to evaluate its pharmacokinetics and pharmacodynamics as well as its safety and tolerability, in CRPC patients (Petrylak et al., 2020).

Despite the rapid preclinical development of PROTACs as novel cancer therapeutics, many aspects need to be addressed. One of the biggest challenges is that PROTACs have high molecular weights, often larger than 1,000 Da, which could limit their cell permeability, pharmacokinetic abilities, oral bioavailability, and their capability to bypass the blood-brain

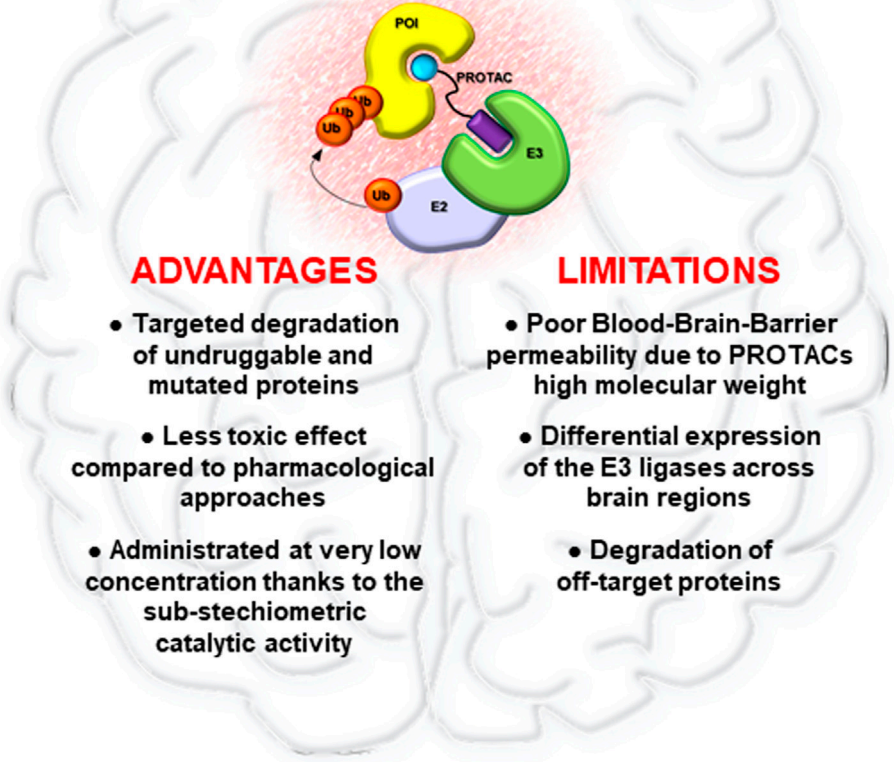


FIGURE 2 | Advantages and limitations of PROTAC technology in brain tumors. Pro and cons of PROTACs application for the treatment of brain malignancies.

barrier (**Figure 2**). In particular, this last aspect could represent a relevant limit for the clinical application of PROTACs in brain tumors, for which it will be essential to improve drug delivery systems for PROTACs, such as nano-vehicles, active transporter or alternative administration regimens (Banks 2016; Dong 2018).

One of the biggest weaknesess in the development of new PROTACs is the lack of knowledge for many E3s, especially regarding their tissue-specific expression and correlation to human diseases. So far, only a few E3s and ubiquitin ligase binders have been explored for the design of PROTACs. This aspect raises the need to study the biological functions and expression of E3 ligases as well as to solve their structures to accelerate the synthesis of new PROTACs. Moving forward, chemo-proteomic platforms, DNA-encoded library screening, and fragment-based ligand discovery will be useful both for the identification of E3s tissue, tumor, or compartment specific, and of ligands for incurable disease-related targets (Jacquemard and Kellenberger 2019). Despite the use of small molecule binders of only a few E3s, a fast progress has been made in this field, set the ground for a bright future of PROTACs in drug discovery and precision medicine. Overall, PROTAC technology shows unique advantages and great therapeutic potentials, thus possibly revolutionizing drug development and providing clinical benefits.

AUTHOR CONTRIBUTIONS

LLS and FB performed the literature research and drafted a first version of the manuscript. LDM and PI supervised and coordinated the work as well as wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This paper was funded by the Fondazione AIRC (Associazione Italiana per la Ricerca sul Cancro) Grant IG20801 to LDM, MFAG 2021-ID. 26536 to PI, Progetti di Ricerca di Università Sapienza di Roma, Italian Ministry of Health Grant PRIN 2017BF3PXZ to LDM, Dipartimenti di Eccellenza-L. 232/2016, Institut Pasteur/Cenci Bolognetti Foundation to LDM. LLS was supported by a FIRC-AIRC fellowship for Italy.

REFERENCES

An, S., and Fu, L. (2018). Small-molecule PROTACs: An Emerging and Promising Approach for the Development of Targeted Therapy Drugs. *EBioMedicine* 36, 553–562. doi:10.1016/j.ebiom.2018.09.005

Bai, L., Zhou, H., Xu, R., Zhao, Y., Chinnaswamy, K., McEachern, D., et al. (2019). A Potent and Selective Small-Molecule Degrader of STAT3 Achieves Complete Tumor Regression *In Vivo*. *Cancer Cell* 11 1136, 498–511. e417. doi:10.1016/j.ccell.2019.10.002

Banks, W. A. (2016). From Blood-Brain Barrier to Blood-Brain Interface: New Opportunities for CNS Drug Delivery. *Nat. Rev. Drug Discov.* 15, 275–292. doi:10.1038/nrd.2015.21

Bard, J. A. M., Bashore, C., Dong, K. C., and Martin, A. (2019). The 26S Proteasome Utilizes a Kinetic Gateway to Prioritize Substrate Degradation. *Cell* 177, 286–298. e215. doi:10.1016/j.cell.2019.02.031

Beck, A., Goetsch, L., Dumontet, C., and Corvaïa, N. (2017). Strategies and Challenges for the Next Generation of Antibody-Drug Conjugates. *Nat. Rev. Drug Discov.* 16, 315–337. doi:10.1038/nrd.2016.268

Brennan, C. W., Verhaak, R. G., McKenna, A., Campos, B., Noushmehr, H., Salama, S. R., et al. (2013). The Somatic Genomic Landscape of Glioblastoma. *Cell* 155, 462–477. doi:10.1016/j.cell.2013.09.034

Brennan, D., Chen, X., Cheng, L., Mahoney, M., and Riobo, N. A. (2012). Noncanonical Hedgehog Signaling. *Vitam Horm.* 88, 55–72. doi:10.1016/b978-0-12-394622-5.00003-1

Bronner, S. M., Merrick, K. A., Murray, J., Salphati, L., Moffat, J. G., Pang, J., et al. (2019). Design of a Brain-Penetrant CDK4/6 Inhibitor for Glioblastoma. *Bioorg. Med. Chem. Lett.* 29 (29), 2294–2301. doi:10.1016/j.bmcl.2019.06.021

Bufalieri, F., Caimano, M., Lospinoso Severini, L., Basili, I., Paglia, F., Sampirisi, L., et al. (2020a). The RNA-Binding Ubiquitin Ligase MEX3A Affects Glioblastoma Tumorigenesis by Inducing Ubiquitylation and Degradation of RIG-I. *Cancers (Basel)* 12, 12. doi:10.3390/cancers12020321

Bufalieri, F., Lospinoso Severini, L., Caimano, M., Infante, P., and Di Marcotullio, L. (2020b). DUBs Activating the Hedgehog Signaling Pathway: A Promising Therapeutic Target in Cancer. *Cancers (Basel)* 12, 1518. doi:10.3390/cancers12061518

Bufalieri, F., Basili, I., Di Marcotullio, L., and Infante, P. (2021). Harnessing the Activation of RIG-I like Receptors to Inhibit Glioblastoma Tumorigenesis. *Front. Mol. Neurosci.* 14, 710171. doi:10.3389/fnmol.2021.710171

Burslem, G. M., Schultz, A. R., Bondeson, D. P., Eide, C. A., Savage Stevens, S. L., Druker, B. J., et al. (2019). Targeting BCR-ABL1 in Chronic Myeloid Leukemia by PROTAC-Mediated Targeted Protein Degradation. *Cancer Res.* 79 (79), 4744–4753. doi:10.1158/0008-5472.CAN-19-1236

Burslem, G. M., Smith, B. E., Lai, A. C., Jaime-Figueroa, S., McQuaid, D. C., Bondeson, D. P., et al. (2018). The Advantages of Targeted Protein Degradation over Inhibition: An RTK Case Study. *Cell Chem Biol* 25 (25), 67–e3. doi:10.1016/j.chembiol.2017.09.009

Bushweller, J. H. (2019). Targeting Transcription Factors in Cancer - from Undruggable to Reality. *Nat. Rev. Cancer* 19 (19), 611–624. doi:10.1038/s41568-019-0196-7

Chan, K. H., Zengerle, M., Testa, A., and Ciulli, A. (2018). Impact of Target Warhead and Linkage Vector on Inducing Protein Degradation: Comparison of Bromodomain and Extra-terminal (BET) Degraders Derived from Triazolodiazepine (JQ1) and Tetrahydroquinoline (I-Bet726) BET Inhibitor Scaffolds. *J. Med. Chem.* 61 (61), 504–513. doi:10.1021/acs.jmedchem.6b01912

Chung, C. W., Dai, H., Fernandez, E., Tinworth, C. P., Churcher, I., Cryan, J., et al. (2020). Structural Insights into PROTAC-Mediated Degradation of Bcl-xL. *ACS Chem. Biol.* 15 (15), 2316–2323. doi:10.1021/acschembio.0c00266

Clifford, S. C., Walsh, S., Hewson, K., Green, E. K., Brinke, A., Green, P. M., et al. (1999). Genomic organization and chromosomal localization of the humanCUL2 gene and the role of von Hippel-Lindau tumor suppressor-binding protein (CUL2 and VBP1) mutation and loss in renal-cell carcinoma development. *Genes Chromosom. Cancer* 26, 20–28. doi:10.1002/(sici)1098-2264(199909)26:1<20:aid-gcc3>3.0.co;2-6

Consortium, G. (2015). Human Genomics. The Genotype-Tissue Expression (GTEx) Pilot Analysis: Multitissue Gene Regulation in Humans. *Science* 348, 648–660. doi:10.1126/science.1262110

Costales, M. G., Aikawa, H., Li, Y., Childs-Disney, J. L., Abegg, D., Hoch, D. G., et al. (2020a). Small-molecule Targeted Recruitment of a Nuclease to Cleave an Oncogenic RNA in a Mouse Model of Metastatic Cancer. *Proc. Natl. Acad. Sci. U S A.* 117 (117), 2406–2411. doi:10.1073/pnas.1914286117

Costales, M. G., Childs-Disney, J. L., Haniff, H. S., and Disney, M. D. (2020b). How We Think about Targeting RNA with Small Molecules. *J. Med. Chem.* 63 (63), 8880–8900. doi:10.1021/acs.jmedchem.9b01927

Cromm, P. M., Samarasinghe, K. T. G., Hines, J., and Crews, C. M. (2018). Addressing Kinase-independent Functions of Fak via PROTAC-Mediated Degradation. *J. Am. Chem. Soc.* 140 (140), 17019–17026. doi:10.1021/jacs.8b08008

Dobosz, P., and Dzieciątkowski, T. (2019). The Intriguing History of Cancer Immunotherapy. *Front. Immunol.* 10, 2965. doi:10.3389/fimmu.2019.02965

Dong, X. (2018). Current Strategies for Brain Drug Delivery. *Theranostics* 8, 1481–1493. doi:10.7150/thno.21254

Dunn, G. P., Rinne, M. L., Wykosky, J., Genovese, G., Quayle, S. N., Dunn, I. F., et al. (2012). Emerging Insights into the Molecular and Cellular Basis of Glioblastoma. *Genes Dev.* 26 (26), 756–784. doi:10.1101/gad.187922.112

Eichhorn, J. M., Alford, S. E., Sakurikar, N., and Chambers, T. C. (2014). Molecular Analysis of Functional Redundancy Among Anti-apoptotic Bcl-2 Proteins and its Role in Cancer Cell Survival. *Exp. Cel Res.* 322, 415–424. doi:10.1016/j.yexcr.2014.02.010

Farnaby, W., Koegl, M., Roy, M. J., Whitworth, C., Diers, E., Trainor, N., et al. (2019). BAF Complex Vulnerabilities in Cancer Demonstrated via Structure-Based PROTAC Design. *Nat. Chem. Biol.* 15 (15), 672–680. doi:10.1038/s41589-019-0294-6

Farrell, K., and Jarome, T. J. (2021). Is PROTAC Technology Really a Game Changer for central Nervous System Drug Discovery. *Expert Opin. Drug Discov.* 16, 833–840. doi:10.1080/17460441.2021.1915979

Finley, D. (2009). Recognition and Processing of Ubiquitin-Protein Conjugates by the Proteasome. *Annu. Rev. Biochem.* 78, 477–513. doi:10.1146/annurev.biochem.78.081507.101607

Gangoso, E., Southgate, B., Bradley, L., Rus, S., Galvez-Cancino, F., McGivern, N., et al. (2021). Glioblastomas Acquire Myeloid-Affiliated Transcriptional Programs via Epigenetic Immunoediting to Elicit Immune Evasion. *Cell.* 29 (184), 2454–2470. doi:10.1016/j.cell.2021.03.023

Gonzalez, T. L., Hancock, M., Sun, S., Gersch, C. L., Larios, J. M., David, W., et al. (2020). Targeted Degradation of Activating Estrogen Receptor α Ligand-Binding Domain Mutations in Human Breast Cancer. *Breast Cancer Res. Treat.* 180, 611–622. doi:10.1007/s10549-020-05564-y

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of Cancer: the Next Generation. *Cell* 144, 646–674. doi:10.1016/j.cell.2011.02.013

Hines, J., Lartigue, S., Dong, H., Qian, Y., and Crews, C. M. (2019). MDM2-Recruiting PROTAC Offers Superior, Synergistic Antiproliferative Activity via Simultaneous Degradation of BRD4 and Stabilization of P53. *Cancer Res.* 79, 251–262. doi:10.1158/0008-5472.can-18-2918

Hipp, M. S., Kasturi, P., and Hartl, F. U. (2019). The Proteostasis Network and its Decline in Ageing. *Nat. Rev. Mol. Cel Biol* 20, 421–435. doi:10.1038/s41580-019-0101-y

Hsu, J. H., Rasmusson, T., Robinson, J., Pachl, F., Read, J., Kawatkar, S., et al. (2020). EED-targeted PROTACs Degrade EED, EZH2, and SUZ12 in the PRC2 Complex. *Cel Chem Biol* 27 (27), 41–e17. doi:10.1016/j.chembiol.2019.11.004

Hu, Z., and Crews, C. M. (2021). Recent Developments in PROTAC-Mediated Protein Degradation: From Bench to Clinic. *Chembiochem* 23, e202100270. doi:10.1002/cbic.202100270

Infante, P., Lospinoso Severini, L., Bernardi, F., Bufalieri, F., and Di Marcotullio, L. (2019). Targeting Hedgehog Signalling through the Ubiquitylation Process: The Multiple Roles of the HECT-E3 Ligase Itch. *Cells* 8, 98. doi:10.3390/cells8020098

Inobe, T., and Matouschek, A. (2014). Paradigms of Protein Degradation by the Proteasome. *Curr. Opin. Struct. Biol.* 24, 156–164. doi:10.1016/j.sbi.2014.02.002

Jacquemard, C., and Kellenberger, E. (2019). A Bright Future for Fragment-Based Drug Discovery: what Does it Hold. *Expert Opin. Drug Discov.* 14, 413–416. doi:10.1080/17460441.2019.1583643

Jenkins, R. W., Barbie, D. A., and Flaherty, K. T. (2018). Mechanisms of Resistance to Immune Checkpoint Inhibitors. *Br. J. Cancer* 118, 9–16. doi:10.1038/bjc.2017.434

Jensen, S. M., Potts, G. K., Ready, D. B., and Patterson, M. J. (2018). Specific MHC-I Peptides Are Induced Using PROTACs. *Front. Immunol.* 9, 2697. doi:10.3389/fimmu.2018.02697

Karni-Schmidt, O., Lokshin, M., and Prives, C. (2016). The Roles of MDM2 and MDMX in Cancer. *Annu. Rev. Pathol.* 11 (11), 617–644. doi:10.1146/annurev-pathol-012414-040349

Khan, S., Zhang, X., Lv, D., Zhang, Q., He, Y., Zhang, P., et al. (2019). A Selective BCL-XL PROTAC Degrader Achieves Safe and Potent Antitumor Activity. *Nat. Med.* 25 (25), 1938–1947. doi:10.1038/s41591-019-0668-z

Khan, S., He, Y., Zhang, X., Yuan, Y., Pu, S., Kong, Q., et al. (2020). PROteolysis TArgeting Chimeras (PROTACs) as Emerging Anticancer Therapeutics. *Oncogene* 0639, 4909–4924. doi:10.1038/s41388-020-1336-y

Kim, Y. E., Hipp, M. S., Bracher, A., Hayer-Hartl, M., and Ulrich Hartl, F. (2013). Molecular Chaperone Functions in Protein Folding and Proteostasis. *Annu. Rev. Biochem.* 82, 323–355. doi:10.1146/annurev-biochem-060208-092442

Kliza, K., and Husnjak, K. (2020). Resolving the Complexity of Ubiquitin Networks. *Front. Mol. Biosci.* 7, 21. doi:10.3389/fmolb.2020.00021

Kolb, R., De, U., Khan, S., Luo, Y., Kim, M. C., Yu, H., et al. (2021). Proteolysis-targeting Chimera against BCL-X. *Nat. Commun.* 24 (12), 1281. doi:10.1038/s41467-021-21573-x

Kregel, S., Wang, C., Han, X., Xiao, L., Fernandez-Salas, E., Bawa, P., et al. (2020). Androgen Receptor Degraders Overcome Common Resistance Mechanisms Developed during Prostate Cancer Treatment. *Neoplasia* 22 (22), 111–119. doi:10.1016/j.neo.2019.12.003

Kristensen, B. W., Priesterbach-Ackley, L. P., Petersen, J. K., and Wesseling, P. (2019). Molecular Pathology of Tumors of the central Nervous System. *Ann. Oncol.* 30, 1265–1278. doi:10.1093/annonc/mdz164

Lai, A. C., and Crews, C. M. (2017). Induced Protein Degradation: an Emerging Drug Discovery Paradigm. *Nat. Rev. Drug Discov.* 16, 101–114. doi:10.1038/nrd.2016.211

Lai, J., Wang, Y., Wu, S.-S., Ding, D., Sun, Z.-Y., Zhang, Y., et al. (2018). Elimination of Melanoma by Sortase A-Generated TCR-like Antibody-Drug Conjugates (TL-ADCs) Targeting Intracellular Melanoma Antigen MART-1. *Biomaterials* 178, 158–169. doi:10.1016/j.biomaterials.2018.06.017

Le Rhun, E., Preusser, M., Roth, P., Reardon, D. A., van den Bent, M., Wen, P., et al. (2019). Molecular Targeted Therapy of Glioblastoma. *Cancer Treat. Rev.* 80, 101896. doi:10.1016/j.ctrv.2019.101896

Lebraud, H., Wright, D. J., Johnson, C. N., and Heightman, T. D. (2016). Protein Degradation by In-Cell Self-Assembly of Proteolysis Targeting Chimeras. *ACS Cent. Sci.* 2 (2), 927–934. doi:10.1021/acscentsci.6b00280

Li, Y., Yang, J., Aguilar, A., McEachern, D., Przybranowski, S., Liu, L., et al. (2019). Discovery of MD-224 as a First-In-Class, Highly Potent, and Efficacious Proteolysis Targeting Chimera Murine Double Minute 2 Degrader Capable of Achieving Complete and Durable Tumor Regression. *J. Med. Chem.* 62 (62), 448–466. doi:10.1021/acs.jmedchem.8b00909

Lieberman, F. (2017). Glioblastoma Update: Molecular Biology, Diagnosis, Treatment, Response Assessment, and Translational Clinical Trials. *F1000Res* 6, 1892. doi:10.12688/f1000research.11493.1

Liu, J., Chen, H., Ma, L., He, Z., Wang, D., Liu, Y., et al. (2020). Light-induced Control of Protein Destruction by Opto-PROTAC. *Sci. Adv.* 2, eaay5154. doi:10.1126/sciadv.aay5154

Liu, J.-R., Yu, C.-W., Hung, P.-Y., Hsin, L.-W., and Chern, J.-W. (2019). High-selective HDAC6 Inhibitor Promotes HDAC6 Degradation Following Autophagy Modulation and Enhanced Antitumor Immunity in Glioblastoma. *Biochem. Pharmacol.* 163, 458–471. doi:10.1016/j.bcp.2019.03.023

Lospinoso Severini, L., Ghirga, F., Bufalieri, F., Quaglio, D., Infante, P., and Di Marcotullio, L. (2020). The SHH/GLI Signaling Pathway: a Therapeutic Target for Medulloblastoma. *Expert Opin. Ther. Targets* 24, 1159–1181. doi:10.1080/14728222.2020.1823967

Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., et al. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a Summary. *Acta Neuropathol.* 06131, 803–820. doi:10.1007/s00401-016-1545-1

Maksoud, S. (2021). The Role of the Ubiquitin Proteasome System in Glioma: Analysis Emphasizing the Main Molecular Players and Therapeutic Strategies Identified in Glioblastoma Multiforme. *Mol. Neurobiol. Jul* 58, 3252–3269. doi:10.1007/s12035-021-02339-4

Martin, L. A., Ribas, R., Simigdala, N., Schuster, E., Pancholi, S., Tenev, T., et al. (2017). Discovery of Naturally Occurring ESR1 Mutations in Breast Cancer Cell Lines Modelling Endocrine Resistance. *Nat. Commun.* 8 (8), 1865. doi:10.1038/s41467-017-01864-y

Mason, K. D., Carpinelli, M. R., Fletcher, J. I., Collinge, J. E., Hilton, A. A., Ellis, S., et al. (2007). Programmed Anuclear Cell Death Delimits Platelet Life Span. *Cell* 128 (128), 1173–1186. doi:10.1016/j.cell.2007.01.037

Medikonda, R., Dunn, G., Rahman, M., Fecci, P., and Lim, M. (2021). A Review of Glioblastoma Immunotherapy. *J. Neurooncol.* 151, 41–53. doi:10.1007/s11060-020-03448-1

Melé, M., Ferreira, P. G., Reverter, F., DeLuca, D. S., Monlong, J., Sammeth, M., et al. (2015). The Human Transcriptome across Tissues and Individuals. *Science* 348, 660–665. doi:10.1126/science.aaa0355

Meyer, M., Reimand, J., Lan, X., Head, R., Zhu, X., Kushida, M., et al. (2015). Single Cell-Derived Clonal Analysis of Human Glioblastoma Links Functional and Genomic Heterogeneity. *Proc. Natl. Acad. Sci. USA* 112, 851–856. doi:10.1073/pnas.1320611111

Moreau, K., Coen, M., Zhang, A. X., Pachl, F., Castaldi, M. P., Dahl, G., et al. (2020). Proteolysis-targeting Chimeras in Drug Development: A Safety Perspective. *Br. J. Pharmacol.* 04177, 1709–1718. doi:10.1111/bph.15014

Moser, S. C., Voerman, J. S. A., Buckley, D. L., Winter, G. E., and Schliehe, C. (2017). Acute Pharmacologic Degradation of a Stable Antigen Enhances its Direct Presentation on MHC Class I Molecules. *Front. Immunol.* 8, 1920. doi:10.3389/fimmu.2017.01920

Mullard, A. (2019). Arvinas's PROTACs Pass First Safety and PK Analysis. *Nat. Rev. Drug Discov.* 1118, 895. doi:10.1038/d41573-019-00188-4

Neklesa, T., Snyder, L. B., Willard, R. R., Vitale, N., Pizzano, J., Gordon, D. A., et al. (2019). ARV-110: An Oral Androgen Receptor PROTAC Degrader for Prostate Cancer. *J. Clin. Oncol.* 37, 1200. doi:10.1200/jco.2019.37.7_suppl.259

Network, C. G. A. R. (2008). Comprehensive Genomic Characterization Defines Human Glioblastoma Genes and Core Pathways. *Nature* 455 (455), 1061–1068. doi:10.1038/nature07385

Niza, E., Noblejas-López, M. D. M., Bravo, I., Nieto-Jiménez, C., Castro-Osma, J. A., Canales-Vázquez, J., et al. (2019). Trastuzumab-Targeted Biodegradable Nanoparticles for Enhanced Delivery of Dasatinib in HER2+ Metastasic Breast Cancer. *Nanomaterials (Basel)* 9, 9. doi:10.3390/nano9121793

Nunes, J., McGonagle, G. A., Eden, J., Kiritharan, G., Touzet, M., Lewell, X., et al. (2019). Targeting IRAK4 for Degradation with PROTACs. *ACS Med. Chem. Lett.* 10 (10), 1081–1085. doi:10.1021/acsmedchemlett.9b00219

Ocaña, A., García-Alonso, S., Amir, E., and Pandiella, A. (2018). Refining Early Antitumoral Drug Development. *Trends Pharmacol. Sci.* 39 (39), 922–925. doi:10.1016/j.tips.2018.09.003

Oliner, J. D., Saiki, A. Y., and Caenepeel, S. (2016). The Role of MDM2 Amplification and Overexpression in Tumorigenesis. *Cold Spring Harb Perspect. Med.* 6, 6. doi:10.1101/cshperspect.a026336

Olson, C. M., Jiang, B., Erb, M. A., Liang, Y., Doctor, Z. M., Zhang, Z., et al. (2018). Pharmacological Perturbation of CDK9 Using Selective CDK9 Inhibition or Degradation. *Nat. Chem. Biol.* 14, 163–170. doi:10.1038/nchembio.2538

Ostrom, Q. T., Gittleman, H., Liao, P., Rouse, C., Chen, Y., Dowling, J., et al. (2014). CBTRUS Statistical Report: Primary Brain and central Nervous System Tumors Diagnosed in the United States in 2007-2011. *Neuro Oncol.* 16 Suppl 4 (Suppl. 4), iv1–63. doi:10.1093/neuonc/nou223

Ottis, P., Palladino, C., Thienger, P., Britschgi, A., Heichinger, C., Berrera, M., et al. (2019). Cellular Resistance Mechanisms to Targeted Protein Degradation Converge toward Impairment of the Engaged Ubiquitin Transfer Pathway. *ACS Chem. Biol.* 14 (14), 2215–2223. doi:10.1021/acschembio.9b00525

Pandey, V., Bhaskara, V. K., and Babu, P. P. (2016). Implications of Mitogen-Activated Protein Kinase Signaling in Glioma. *J. Neurosci. Res.* 94, 114–127. doi:10.1002/jnr.23687

Petrylak, D. P., Gao, X., Vogelzang, N. J., Garfield, M. H., Taylor, I., Dougan Moore, M., et al. (2020). First-in-human Phase I Study of ARV-110, an Androgen Receptor (AR) PROTAC Degrader in Patients (Pts) with Metastatic Castrate-Resistant Prostate Cancer (mCRPC) Following Enzalutamide (ENZ) And/or Abiraterone (ABI). *Am. Soc. Clin. Oncol.* 39 (45). 6935–6949. doi:10.1200/jco.2020.38.15_suppl.3500

Pettersson, M., and Crews, C. M. (2019). PROteolysis TArgeting Chimeras (PROTACs) - Past, Present and Future. *Drug Discov. Today Tech.* 31, 15–27. doi:10.1016/j.ddtec.2019.01.002

Pfaff, P., Samarasinghe, K. T. G., Crews, C. M., and Carreira, E. M. (2019). Reversible Spatiotemporal Control of Induced Protein Degradation by Bistable PhotoPROTACs. *ACS Cent. Sci.* 5 (5), 1682–1690. doi:10.1021/acscentsci.9b00713

Pillow, T. H., Adhikari, P., Blake, R. A., Chen, J., Del Rosario, G., Deshmukh, G., et al. (2020). Antibody Conjugation of a Chimeric BET Degrader Enables *In Vivo* Activity. *ChemMedChem* 15, 17–25. doi:10.1002/cmdc.201900497

Pohl, C., and Dikic, I. (2019). Cellular Quality Control by the Ubiquitin-Proteasome System and Autophagy. *Science* 366 (15366), 818–822. doi:10.1126/science.aax3769

Reynders, M., Matsuura, B. S., Bérouti, M., Simoneschi, D., Marzio, A., Pagano, M., et al. (2020). PHOTACs Enable Optical Control of Protein Degradation. *Sci. Adv.* 6 (6), eaay5064. doi:10.1126/sciadv.aay5064

Robb, C. M., Contreras, J. I., Kour, S., Taylor, M. A., Abid, M., Sonawane, Y. A., et al. (2017). Chemically Induced Degradation of CDK9 by a Proteolysis Targeting Chimera (PROTAC). *Chem. Commun.* 53, 7577–7580. doi:10.1039/c7cc03879h

Sakamoto, K. M., Kim, K. B., Kumagai, A., Mercurio, F., Crews, C. M., and Deshaies, R. J. (2001). Protacs: Chimeric Molecules that Target Proteins to the Skp1-Cullin-F Box Complex for Ubiquitination and Degradation. *Proc. Natl. Acad. Sci. U S A.* 98 (98), 8554–8559. doi:10.1073/pnas.141230798

Schapira, M., Calabrese, M. F., Bullock, A. N., and Crews, C. M. (2019). Targeted Protein Degradation: Expanding the Toolbox. *Nat. Rev. Drug Discov.* 18 (18), 949–963. doi:10.1038/s41573-019-0047-y

Schmitt, C., Tonnelle, C., Dalloul, A., Chabannon, C., Debré, P., and Rebollo, A. (2002). Aiolos and Ikaros: Regulators of Lymphocyte Development, Homeostasis and Lymphoproliferation. *Apoptosis* 7, 277–284. doi:10.1023/a:1015372322419

Schneekloth, A. R., Pucheault, M., Tae, H. S., and Crews, C. M. (2008). Targeted Intracellular Protein Degradation Induced by a Small Molecule: En Route to Chemical Proteomics. *Bioorg. Med. Chem. Lett.* 18 (18), 5904–5908. doi:10.1016/j.bmcl.2008.07.114

Scholz, N., Kurian, K. M., Siebzehnrubl, F. A., and Licchesi, J. D. F. (2020). Targeting the Ubiquitin System in Glioblastoma. *Front. Oncol.* 10, 574011. doi:10.3389/fonc.2020.574011

Sharma, A., Khan, H., Singh, T. G., Grewal, A. K., Najda, A., Kawecka-Radomska, M., et al. (2021). Pharmacological Modulation of Ubiquitin-Proteasome Pathways in Oncogenic Signaling. *Int. J. Mol. Sci. Nov* 04, 22. doi:10.3390/ijms222111971

Smith, B. E., Wang, S. L., Jaime-Figueroa, S., Harbin, A., Wang, J., Hamman, B. D., et al. (2019). Differential PROTAC Substrate Specificity Dictated by Orientation of Recruited E3 Ligase. *Nat. Commun.* 10 (10), 131. doi:10.1038/s41467-018-08027-7

Stoyanov, G. S., and Dzhenkov, D. L. (2018). On the Concepts and History of Glioblastoma Multiforme - Morphology, Genetics and Epigenetics. *Folia Med. (Plovdiv).* 60, 48–66. doi:10.1515/folmed-2017-0069

Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J., et al. (2005). Radiotherapy Plus Concomitant and Adjuvant Temozolomide for Glioblastoma. *N. Engl. J. Med.* 352 (352), 987–996. doi:10.1056/NEJMoa043330

Stupp, R., Hegi, M. E., Mason, W. P., van den Bent, M. J., Taphoorn, M. J., Janzer, R. C., et al. (2009). Effects of Radiotherapy with Concomitant and Adjuvant Temozolomide versus Radiotherapy Alone on Survival in Glioblastoma in a Randomised Phase III Study: 5-year Analysis of the EORTC-NCIC Trial. *Lancet Oncol.* 10, 459–466. doi:10.1016/s1470-2045(09)70025-7

Su, S., Yang, Z., Gao, H., Yang, H., Zhu, S., An, Z., et al. (2019). Potent and Preferential Degradation of CDK6 via Proteolysis Targeting Chimera Degraders. *J. Med. Chem.* 62 (62), 7575–7582. doi:10.1021/acs.jmedchem.9b00871

Sun, Y., Ding, N., Song, Y., Yang, Z., Liu, W., Zhu, J., et al. (2019). Degradation of Bruton's Tyrosine Kinase Mutants by PROTACs for Potential Treatment of Ibrutinib-Resistant Non-hodgkin Lymphomas. *Leukemia* 0833, 2105–2110. doi:10.1038/s41375-019-0440-x

Testa, A., Hughes, S. J., Lucas, X., Wright, J. E., and Ciulli, A. (2020). Structure-Based Design of a Macrocyclic PROTAC. *Angew. Chem. Int. Ed. Engl.* 59 (59), 1727–1734. doi:10.1002/anie.201914396

Testa, A., Lucas, X., Castro, G. V., Chan, K. H., Wright, J. E., Runcie, A. C., et al. (2018. 3-Fluoro-4-hydroxyprolines: Synthesis, Conformational Analysis, and Stereoselective Recognition by the VHL E3 Ubiquitin Ligase for Targeted Protein Degradation. *J. Am. Chem. Soc.* 140 (140), 9299–9313. doi:10.1021/jacs.8b05807

Touat, M., Idbaih, A., Sanson, M., and Ligon, K. L. (2017). Glioblastoma Targeted Therapy: Updated Approaches from Recent Biological Insights. *Ann. Oncol.* 28, 1457–1472. doi:10.1093/annonc/mdx106

Trauner, D., and Shemet, A. (2019). Selective Targeting of P38 MAPK Isoforms Using PROTACs. *Synfacts* 15, 0438. doi:10.1038/s41467-018-08027-7

Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., et al. (2004). *In Vivo* activation of the P53 Pathway by Small-Molecule Antagonists of MDM2. *Science* 303, 844–848. doi:10.1126/science.1092472

Verhaak, R. G. W., Hoadley, K. A., Purdom, E., Wang, V., Qi, Y., Wilkerson, M. D., et al. (2010). Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 17, 98–110. doi:10.1016/j.ccr.2009.12.020

Wang, Y., Jiang, X., Feng, F., Liu, W., and Sun, H. (2020). Degradation of Proteins by PROTACs and Other Strategies. *Acta Pharmaceutica Sinica B* 10, 207–238. doi:10.1016/j.apsb.2019.08.001

Wang, Z., Hu, P., Tang, F., Lian, H., Chen, X., Zhang, Y., et al. (2016). HDAC6 Promotes Cell Proliferation and Confers Resistance to Temozolomide in Glioblastoma. *Cancer Lett.* 379 (379), 134–142. doi:10.1016/j.canlet.2016.06.001

Weenink, B., French, P. J., Sillevis Smitt, P. A. E., Debets, R., and Geurts, M. (2020). Immunotherapy in Glioblastoma: Current Shortcomings and Future Perspectives. *Cancers (Basel)* 12, 12. doi:10.3390/cancers12030751

Welchman, R. L., Gordon, C., and Mayer, R. J. (2005). Ubiquitin and Ubiquitin-like Proteins as Multifunctional Signals. *Nat. Rev. Mol. Cel Biol* 6, 599–609. doi:10.1038/nrm1700

Winter, G. E., Buckley, D. L., Paulk, J., Roberts, J. M., Souza, A., Dhe-Paganon, S., et al. (2015). Phthalimide Conjugation as a Strategy for *In Vivo* Target Protein Degradation. *Science* 348, 1376–1381. doi:10.1126/science.aab1433

Xu, L., Chen, Y., Mayakonda, A., Koh, L., Chong, Y. K., Buckley, D. L., et al. (2018). Targetable BET Proteins- and E2F1-dependent Transcriptional Program Maintains the Malignancy of Glioblastoma. *Proc. Natl. Acad. Sci. U S A.* 115 (115), E5086–E5095. doi:10.1073/pnas.1712363115

Xue, G., Wang, K., Zhou, D., Zhong, H., and Pan, Z. (2019). Light-Induced Protein Degradation with Photocaged PROTACs. *J. Am. Chem. Soc.* 141, 14118370–14118374. doi:10.1021/jacs.9b06422

Yang, C.-Y., Qin, C., Bai, L., and Wang, S. (2019). Small-molecule PROTAC Degraders of the Bromodomain and Extra Terminal (BET) Proteins - A Review. *Drug Discov. Today Tech.* 31, 43–51. doi:10.1016/j.ddtec.2019.04.001

Yang, H., Wei, L., Xun, Y., Yang, A., and You, H. (2021). BRD4: An Emerging Prospective Therapeutic Target in Glioma. *Mol. Ther. - Oncolytics* 21 (21), 1–14. doi:10.1016/j.omto.2021.03.005

Yu, J. M., Sun, W., Wang, Z. H., Liang, X., Hua, F., Li, K., et al. (2019). TRIB3 Supports Breast Cancer Stemness by Suppressing FOXO1 Degradation and Enhancing SOX2 Transcription. *Nat. Commun.* 10 (10), 5720. doi:10.1038/s41467-019-13700-6

Zeng, S., Huang, W., Zheng, X., Cheng, L., Zhang, Z., Wang, J., et al. (2021). Proteolysis Targeting Chimera (PROTAC) in Drug Discovery Paradigm: Recent Progress and Future Challenges. *Eur. J. Med. Chem. Jan* 15, 112981. doi:10.1016/j.ejmech.2020.112981

Zhang, H., Zhao, H.-Y., Xi, X.-X., Liu, Y.-J., Xin, M., Mao, S., et al. (2020). Discovery of Potent Epidermal Growth Factor Receptor (EGFR) Degraders by Proteolysis Targeting Chimera (PROTAC). *Eur. J. Med. Chem.* 189, 112061. doi:10.1016/j.ejmech.2020.112061

Zhang, L., Riley-Gillis, B., Vijay, P., and Shen, Y. (2019). Acquired Resistance to BET-PROTACs (Proteolysis-Targeting Chimeras) Caused by Genomic Alterations in Core Components of E3 Ligase Complexes. *Mol. Cancer Ther.* 18, 1302–1311. doi:10.1158/1535-7163.mct-18-1129

Zhao, B., and Burgess, K. (2019). PROTACs Suppression of CDK4/6, Crucial Kinases for Cell Cycle Regulation in Cancer. *Chem. Commun. (Camb)* 55 (55), 2704–2707. doi:10.1039/c9cc00163h

Zhao, H. Y., Yang, X. Y., Lei, H., Xi, X. X., Lu, S. M., Zhang, J. J., et al. (2020). Discovery of Potent Small Molecule PROTACs Targeting

Mutant EGFR. *Eur. J. Med. Chem.* 208, 112781. doi:10.1016/j.ejmech.2020.112781

Zhao, Q., Ren, C., Liu, L., Chen, J., Shao, Y., Sun, N., et al. (2019). Discovery of SIAIS178 as an Effective BCR-ABL Degrader by Recruiting Von Hippel-Lindau (VHL) E3 Ubiquitin Ligase. *J. Med. Chem.* 62 (62), 9281–9298. doi:10.1021/acs.jmedchem.9b01264

Zhou, H., Bai, L., Xu, R., Zhao, Y., Chen, J., McEachern, D., et al. (2019). Structure-Based Discovery of SD-36 as a Potent, Selective, and Efficacious PROTAC Degrader of STAT3 Protein. *J. Med. Chem.* 62 (62), 11280–11300. doi:10.1021/acs.jmedchem.9b01530

Conflict of Interest: 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Cell and Developmental Biology

REVIEW
published: 21 March 2022
doi: 10.3389/fcell.2022.849625

Regulation of Glucose, Fatty Acid and Amino Acid Metabolism by Ubiquitination and SUMOylation for Cancer Progression

Shunqin Zhu[1,2†], *Hongyu Gu*[1,2†], *Cheng Peng*[1,2], *Fanwei Xia*[1], *Huan Cao*[1] *and Hongjuan Cui*[1,2*]

[1]State Key Laboratory of Silkworm Genome Biology, School of Life Sciences, Southwest University, Chongqing, China, [2]Cancer Center, Reproductive Medicine Center, Medical Research Institute, Southwest University, Chongqing, China

Ubiquitination and SUMOylation, which are posttranslational modifications, play prominent roles in regulating both protein expression and function in cells, as well as various cellular signal transduction pathways. Metabolic reprogramming often occurs in various diseases, especially cancer, which has become a new entry point for understanding cancer mechanisms and developing treatment methods. Ubiquitination or SUMOylation of protein substrates determines the fate of modified proteins. Through accurate and timely degradation and stabilization of the substrate, ubiquitination and SUMOylation widely control various crucial pathways and different proteins involved in cancer metabolic reprogramming. An understanding of the regulatory mechanisms of ubiquitination and SUMOylation of cell proteins may help us elucidate the molecular mechanism underlying cancer development and provide an important theory for new treatments. In this review, we summarize the processes of ubiquitination and SUMOylation and discuss how ubiquitination and SUMOylation affect cancer metabolism by regulating the key enzymes in the metabolic pathway, including glucose, lipid and amino acid metabolism, to finally reshape cancer metabolism.

Keywords: ubiquitination, SUMOylation, cancer, metabolic reprogramming, glucose metabolism, lipid metabolism

OPEN ACCESS

Edited by:
Zhenghong Lin,
Chongqing University, China

Reviewed by:
Hai-Long Piao,
Dalian Institute of Chemical Physics (CAS), China
Rong Cai,
Shanghai Jiao Tong University, China

***Correspondence:**
Hongjuan Cui
hongjuan.cui@gmail.com

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *06 January 2022*
Accepted: *01 March 2022*
Published: *21 March 2022*

Citation:
Zhu S, Gu H, Peng C, Xia F, Cao H and Cui H (2022) Regulation of Glucose, Fatty Acid and Amino Acid Metabolism by Ubiquitination and SUMOylation for Cancer Progression. Front. Cell Dev. Biol. 10:849625. doi: 10.3389/fcell.2022.849625

INTRODUCTION

Metabolic reprogramming occurs in many diseases, including diabetes, obesity, inflammation, cancer, and neurodegenerative diseases. Compared with normal cells, tumor cells grow malignantly and require a continuous supply of energy and nutrients, namely, biological macromolecules. The classic Warburg effect in tumor cells is defined as tumor cells undergoing glycolytic metabolism to rapidly meet energy requirements, even under aerobic conditions (Hanahan and Weinberg, 2011). This condition is considered the basic feature of tumor metabolism, which is universal in different tumor cells and is also used to diagnose and treat cancer (Moreau et al., 2017). In the pathogenesis of cancer and diabetes, the dysregulation of amino acid transporters (AATs) changes intracellular amino acid levels, leading to metabolic reprogramming and finally the occurrence of diseases (Kandasamy et al., 2018). The intricate relationship between metabolism and disease prompts us to understand and discuss the underlying mechanisms.

Ubiquitination and SUMOylation are important posttranslational modifications that control cell metabolism, signaling and differentiation. However, any abnormality in their

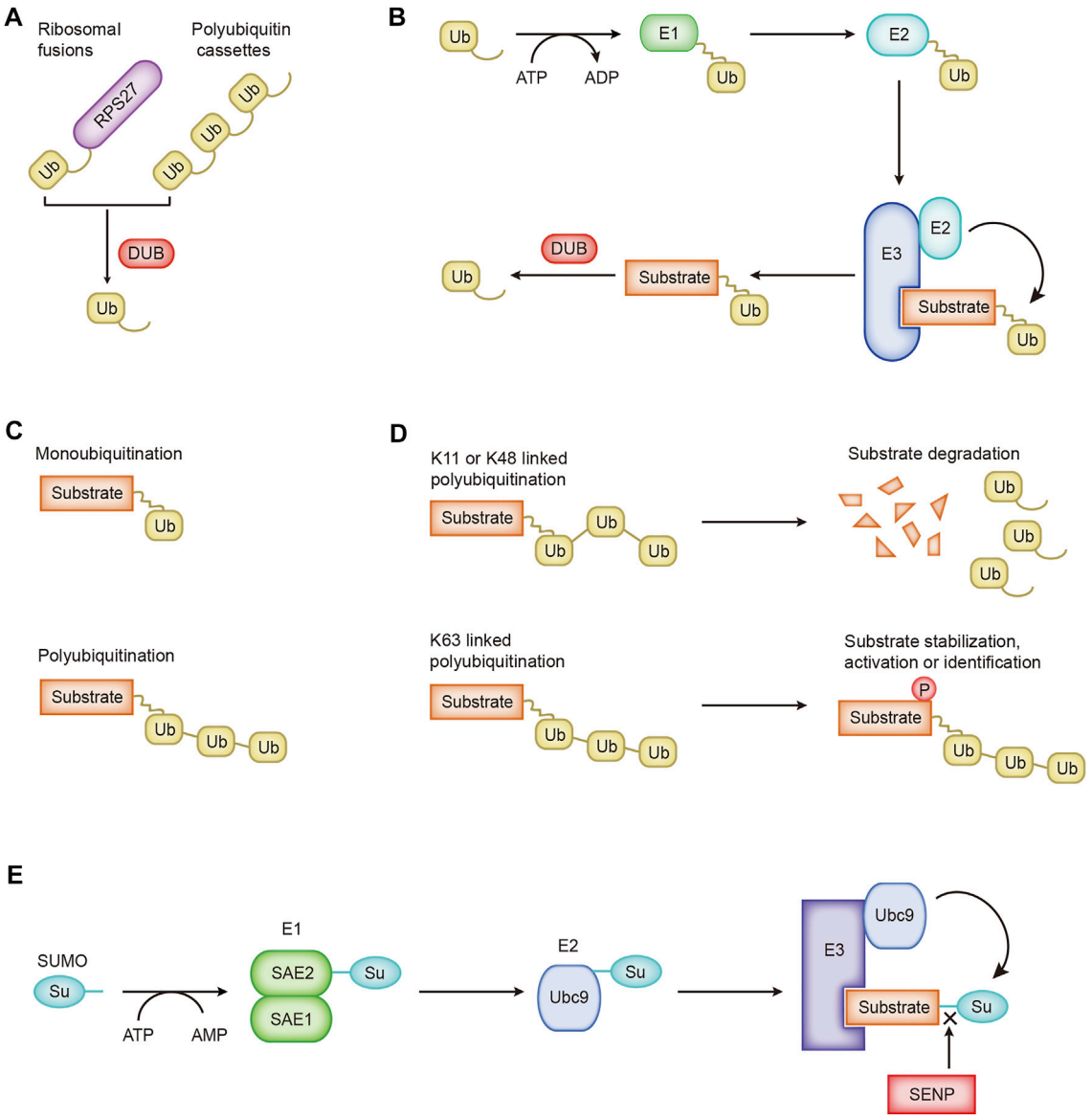


FIGURE 1 | The processes of ubiquitination and SUMOylation. **(A)** Cells obtain ubiquitin from ribosomal fusions and polyubiquitin cassettes. **(B)** The cascade of ubiquitin transfer. **(C)** Different forms of ubiquitin are linked to the substrate. **(D)** Regulation of K11-, K48-, and K63-linked ubiquitination. **(E)** The SUMO cascade.

functions leads to disease occurrence and progression (Popovic et al., 2014; Zhao et al., 2020). Genetic and epigenetic aberrations are the main cause of the dysregulation of ubiquitination and SUMOylation (Flotho and Melchior, 2013; Popovic et al., 2014). Recent studies have shown how ubiquitination and SUMOylation affect metabolic diseases. Cell metabolism is a complex and efficient process regulated by numerous proteins. Ubiquitination and SUMOylation perfectly control cell metabolism through the precise and timely regulation of substrate proteins. Here, we focus on metabolic disorders in cancer and how ubiquitination and SUMOylation affect metabolic reprogramming. Finally, we predict potential research directions and clinical applications in the future.

The Processes of Ubiquitination and SUMOylation

The precise communication of signals between and within cells supports the ability of cells to perform different functions and move toward different fates. Ubiquitination is the basic mechanism for establishing these signal exchanges. Ubiquitin is widely present in eukaryotic cells. It is a highly conserved protein consisting of 76 amino acids. Cells obtain ubiquitin from two sources, ribosomal fusions and polyubiquitin cassettes, which are cleaved by deubiquitinase to obtain monomeric ubiquitin molecules (**Figure 1A**). UBA52 and RPS27A encode ribosomal fusions, and polyubiquitin cassettes are encoded by UBB and UBC (Rape, 2018). Three enzymes participate in the ubiquitin

cascade: the E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin-protein ligase (Yau and Rape, 2016). The ubiquitination of the substrate usually includes three continuous processes. 1) ATP provides energy, and the ubiquitin-activating enzyme E1 activates ubiquitin to provide it a high-energy thioester. 2) Ubiquitin-activating enzyme E1 transfers the activated ubiquitin to ubiquitin-conjugating enzyme E2. 3) Ubiquitin ligase E3 transfers the ubiquitin from E2 to the substrate protein (**Figure 1B**). Special cases where the ubiquitin-conjugating enzyme E2 directly transfers ubiquitin to the substrate protein have also been identified. For example, the UBE2E family of E2 enzymes directly ubiquitinate the substrate SETDB1 in a manner independent of ubiquitin ligase E3, and conjugated ubiquitin is protected from active deubiquitination (Sun and Fang, 2016). Ubiquitin molecules may be cleaved from ubiquitinated proteins by deubiquitinating enzymes (DUBs) (**Figure 1B**). As the opposite process of ubiquitination, the process of deubiquitination plays the opposite role. Approximately 40 E2 ubiquitin-conjugating enzymes, ~600 E3 ubiquitin ligases, and ~100 DUBs have been identified and constitute the extremely complex and sophisticated ubiquitination/deubiquitination machinery in cells.

Ubiquitin molecules are conjugated to substrate proteins and perform different functions. Ubiquitination is divided into monoubiquitination and polyubiquitination according to the number of ubiquitin molecules attached to one lysine residue on the substrate (**Figure 1C**). Monoubiquitination is the simplest process. A single ubiquitin molecule is attached to one lysine of a substrate protein, which usually affects the interaction between proteins (Hicke and Dunn, 2003). Ubiquitin molecules are conjugated one after the other to form a ubiquitin chain, which eventually binds to one lysine of the substrate protein, which is polyubiquitination. Ubiquitin molecules contain seven lysines, and ubiquitin molecules are covalently connected to form different types of ubiquitin chains. Different types of ubiquitin chains lead to different fates for substrate proteins. Ubiquitin may be attached to the substrate through seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) or the first methionine (M1) (Komander and Rape, 2012). K11-, K48-, and K63-linked polyubiquitination have been extensively studied. K11-linked polyubiquitination is related to the degradation of proteins during mitosis (MatSUMOto et al., 2010). K48-linked polyubiquitination-labeled proteins undergo 26S proteasome-mediated recognition and degradation, a crucial regulatory process in cells that promotes the progression of diseases such as cancer (Chen et al., 2018; Zhang et al., 2019). The ubiquitin–proteasome system participates in the degradation of more than 80% of the proteins in the cells (Glickman and Ciechanover, 2002). K63-linked polyubiquitination mediates protein stabilization, activation, and identification, which are essential for intracellular signal transduction (**Figure 1D**; Song et al., 2016).

A new ubiquitin-like protein called Sentrin was first reported in 1996 that binds to the death domain of FAS and regulates cell death signaling (Okura et al., 1996). A subsequent critical report named this ubiquitin-like protein SUMO (small ubiquitin-like modifier), and it covalently modifies the Ran GTPase activator protein RanGAP1 (Mahajan et al., 1997). Later, researchers adopted the name SUMO for this ubiquitin-like protein. Similar to the process of ubiquitination, SUMOylation is catalyzed by specific enzymes: SUMO-specific activating (E1), conjugating (E2), and ligating (E3) enzymes (**Figure 1E**). The first step is to cut the COOH termini of the SUMO protein to reveal the diglycine residues required for conjugation. A SUMO-activating enzyme contains two subunits, SAE1/Aos1 and SAE2/Uba2, conjugated with SUMO protein. ATP is hydrolyzed to generate energy for E1 and SUMO to form high-energy thioester bonds for conjugation. Next, SUMO is transferred to the only SUMO-conjugating enzyme UBC9. Finally, through catalysis by SUMO E3 ligase, SUMO is attached to the lysine residue of the substrate. Substrate proteins modified by SUMO may be unmodified by family SENPs (Sentrin/SUMO-specific proteases) (**Figure 1E**; Yeh, 2009). SUMO-1, SUMO-2, and SUMO-3 are the main SUMO proteins. SUMO-1 usually modifies the substrate as a monomer, while SUMO-2 and SUMO-3 form poly-SUMO chains. Monomeric SUMO or poly-SUMO chains interact with other proteins through the SUMO-interactive motif. SUMOylation changes the protein structure, leading to changes in the protein location, activity, and stability.

According to previous research, SUMOylation is a process independent of ubiquitination. However, an increasing number of studies have shown the inseparable relationship between SUMOylation and ubiquitination. These processes are jointly involved in protein degradation. The number of enzymes involved in SUMOylation is relatively small, but SUMOylation modifies various substrates. The SUMOylation pathway is related to many human diseases. An understanding of the ingenious mechanism of SUMOylation and its role in diseases may help us develop new therapeutic strategies for disease (Chang and Yeh, 2020).

UBIQUITINATION, SUMOYLATION AND GLUCOSE METABOLISM IN CANCER

Metabolic Reprogramming of Glycolysis

Glycolysis, one of the most important catabolic pathways in organisms, oxidizes glucose to generate pyruvate without the participation of oxygen and produces NADH and a small amount of ATP (**Figure 2**).

Tumor cells grow extremely fast, and their oxygen demand exceeds the oxygen supply capacity of blood vessels, resulting in a weaker ability to obtain oxygen and a subsequent state of hypoxia. The process of glycolytic reprogramming in tumor cells is described below. Hypoxia inducible transcription factor 1α (HIF-1α) is activated under hypoxic conditions, and HIF-1α enters the nucleus and interacts with hypoxia response elements to induce the expression of glycolytic enzymes and glucose transport proteins, finally accelerating glycolysis (Semenza, 2003; Weidemann and Johnson, 2008).

VHL (von Hippel-Lindau protein) is an E3 ubiquitin ligase that interacts with HIF-1α and mediates the ubiquitination of HIF-1α, leading to its degradation (Ivan et al., 2001; Jaakkola

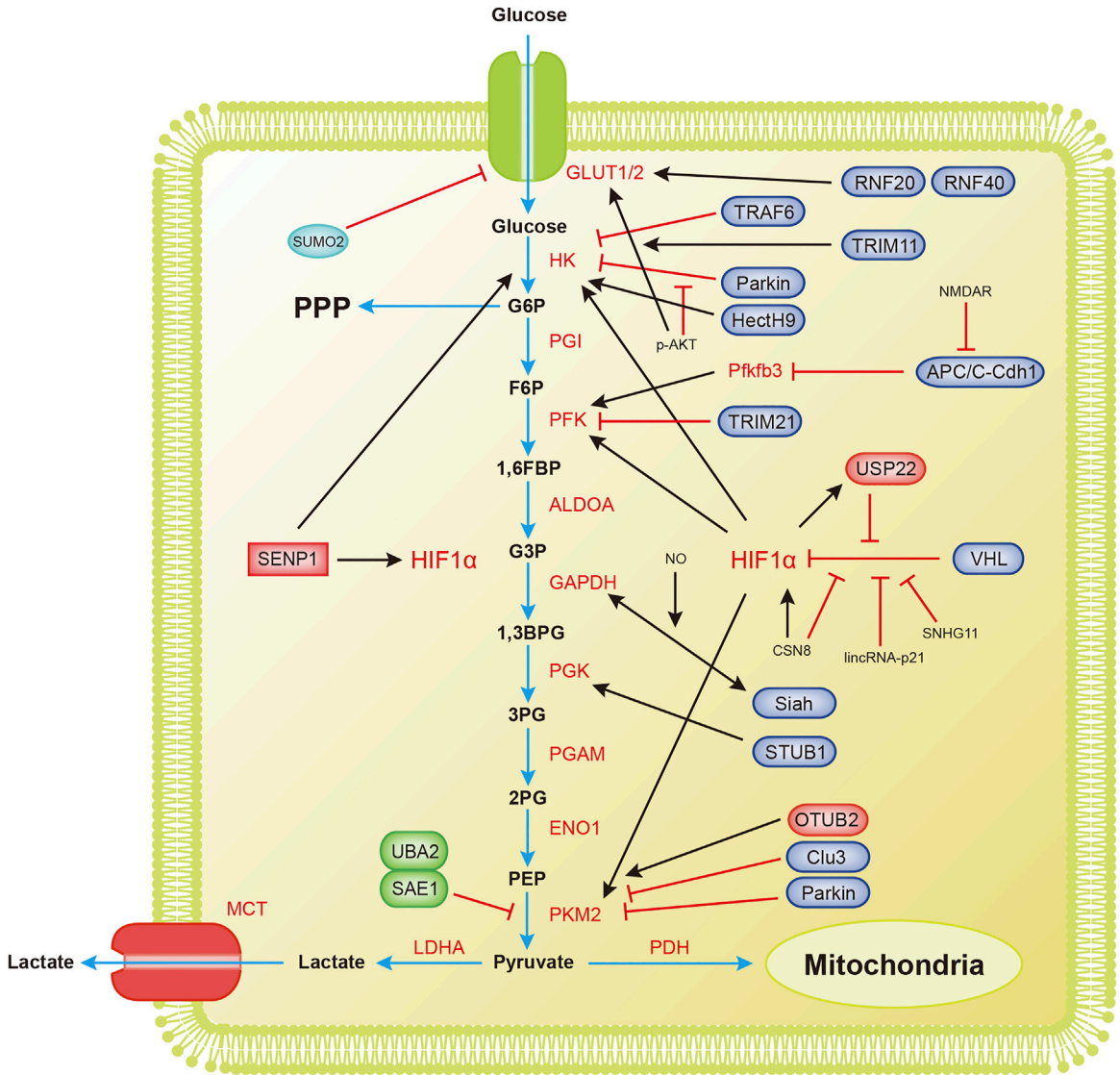


FIGURE 2 | Overview of the mechanisms by which ubiquitination and SUMOylation regulate glycolysis. The blue arrow represents the flow of glycolytic substances. The black and red arrows represent activation and inhibition, respectively. 1,3BPG, 1,3-bisphosphoglycerate; 1,6FBP, fructose 1,6-biophosphate; 2PG, 3-phosphoglycerate; 3PG, 3-phosphoglycerate; ALDOA, aldose; APC/C-Cdh1, anaphase-promoting complex/cyclosome (APC/C)-Cdh1; ENO1, enolase; F6P, fructose 6-phosphate; G3P, glyceraldehyde 3-phosphate; G6P, glucose 6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT1/2, glucose transporter 1/2; HIF1α, hypoxia-inducible factor 1α; HK, hexokinase; LDHA, lactate dehydrogenase A; MCT, monocarboxylic acid transporter; OTUB2, OTU deubiquitinating enzyme; PDH, pyruvate dehydrogenase; PFK, phosphofructokinase; Pfkfb3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase3; PGAM, phosphoglucomutase; PGI, glucose-6-phosphate isomerase; PGK, phosphoglycerate kinase; PKM2, pyruvate kinase M2-type; PPP, pentose phosphate pathway; RNF20/40, ring finger protein 20/40; SAE1, SUMO-activating enzyme 1; SENP1, sentrin/SUMO-specific proteases 1; STUB1, U-box containing protein 1; TPI, triode phosphate isomerase; TRAF6, TNF receptor-associated factor 6; TRIM11, tripartite motif (TRIM)-containing protein 11; TRIM21, tripartite motif (TRIM)-containing protein 21; UBA2, SUMO-activating enzyme 2; USP22, ubiquitin-specific protease 22; VHL, von Hippel-Lindau protein.

et al., 2001). The degradation of HIF-1α inhibits the metabolic reprogramming of tumors and tumor growth. Hypoxia-induced lincRNA-p21 is a hypoxia-responsive lncRNA that binds to HIF-1α and VHL, interrupting the interaction between HIF-1α and VHL and thereby reducing the degradation of HIF-1α (Wei and Lin, 2012; Yang et al., 2014). The lncRNA SNHG11 binds to the VHL recognition site on HIF-1α, thereby blocking the interaction of VHL and HIF-1α, preventing its ubiquitination and degradation, and promoting tumor metastasis (Xu et al., 2020). Under hypoxic conditions, HIF-1α transcription upregulates USP22 and TP53. USP22 promotes the stemness and glycolysis of hepatocellular carcinoma cells induced by hypoxia by deubiquitinating and stabilizing HIF-1α. Wild-type TP53, but not mutant TP53, inhibits the upregulation of USP22 induced by HIF1α. Patients with a loss-of-function mutation of TP53 have a worse prognosis (Ling et al., 2020). CSN8 increases

HIF-1α mRNA expression, stabilizes the HIF-1α protein by reducing its ubiquitination, induces the epidermal-mesenchymal transition (EMT) of primary colorectal cancer cells, and increases migration and invasion (Ju et al., 2020). The SUMOylation of HIF-1α reduces its stability. SENP1 is critical for increasing the stability of HIF-1α induced by hypoxia (Cheng et al., 2007). Hypoxia enhances the stemness of hepatocellular carcinoma cells and hepatocarcinogenesis by enhancing the deSUMOylation of HIF-1α by SENP1 and increasing the stability and transcriptional activity of HIF-1α (Cui et al., 2017).

Hexokinase (HK) is the first rate-limiting enzyme in glucose metabolism. Parkin ubiquitin ligase ubiquitinates HK1 (Okatsu et al., 2012). Parkin is a critical protein in the tumor suppressor pathway and a stress-activated effector. It inhibits the proliferation and metastasis of malignant cells by interfering with metabolic reprogramming. This reprogramming includes the ubiquitination of HK1, which affects the glycolysis process (Agarwal et al., 2021). Activated AKT inhibits HK1 ubiquitination, thereby promoting tumorigenesis. The long noncoding RNA LINC00470 positively regulates the activation of AKT in glioblastoma, inhibits HK1 ubiquitination, and leads to glycolytic reprogramming (Liu et al., 2018). HK2 is an essential regulator of glycolysis that couples metabolism and proliferation activities in cancer cells. The E3 ubiquitin ligase TRAF6 modifies HK2 by K63 ubiquitination, a process that is important for the recognition of HK2 by the autophagy receptor protein SQSTM1/p62 and subsequent selective autophagic degradation. This result reveals the relationship between autophagy and glycolysis in liver cancer (Jiao et al., 2018). The nonproteolytic ubiquitination of HK2 by HectH9 regulates the mitochondrial localization and function of HK2. Loss of HectH9 inhibits HK2, thereby hindering tumor glucose metabolism and growth (Lee et al., 2019). HK2 is SUMOylated at K315 and K492 and deSUMOylated by SENP1. HK2 lacking SUMO prefers to bind to mitochondria, increasing glucose consumption and lactate production and reducing mitochondrial respiration. This metabolic reprogramming strengthens prostate cancer cell proliferation and chemotherapy resistance (Shangguan et al., 2021).

PFK1 is the second rate-limiting enzyme in glycolysis. With the participation of ATP, it catalyzes the production of fructose 1,6-bisphosphate from 6-phosphofructose. E3 ubiquitin ligase tripartite motif (TRIM)-containing protein 21 (TRIM21) targets PFK1 for ubiquitination and degradation. Cells transfer from stiff to a soft substrate, and the disassembly of stress fibers releases TRIM21, which degrades PFK1 and subsequently reduces the rate of glycolysis. However, transformed non-small cell lung cancer cells maintain high levels of glycolysis by downregulating TRIM21 and isolating residual TRIM21 in the substrate-insensitive stress-fiber subset, regardless of the changing environmental mechanics (Park et al., 2020).

Pyruvate kinase M2 (PKM2) is the catalytic enzyme required for the last step of glycolysis and the third rate-limiting enzyme. Parkin mediates the ubiquitination of PKM2 and reduces its enzymatic activity (Liu et al., 2016). Overexpression of cannabinoid receptor-interacting protein 1 (CNRIP1) activates Parkin, leading to PKM2 degradation and thereby promoting cell growth and metastasis in intrahepatic cholangiocarcinoma (ICC) (Chen D. et al., 2021). OTUB2, an OTU deubiquitinating enzyme, directly interacts with PKM2. It promotes glycolysis by preventing Parkin from ubiquitinating PKM2 and enhancing the activity of PKM2 (Yu et al., 2021). Studies have found that PKM2 regulates apoptosis and promotes tumor proliferation. PKM2 translocates to mitochondria in response to oxidative stress, and it phosphorylates Bcl2 at threonine (T) 69. Phosphorylated Bcl2 prevents its ubiquitination and degradation by Cul3-based E3 ligase (Liang et al., 2017). SUMO-activating enzyme 1 (SAE1) and SUMO-activating enzyme 2 (UBA2) mediate the SUMOylation of PKM2, which then promotes its phosphorylation and nuclear localization, reduces its pyruvate kinase (PK) activity and promotes glycolysis (Wang et al., 2020b). PKM2, which undergoes SUMOylation at lysine 270 (K270), triggers a conformational change from a tetramer to a dimer. This conformational change reduces the PK activity of PKM2 and leads to the nuclear translocation of PKM2. SUMO-modified PKM2 recruits RUNX1 and promotes its degradation through the SUMO-interacting motif, which inhibits the myeloid differentiation of NB4 and U937 leukemia cells (Xia et al., 2021).

Other enzymes in the glycolysis process are also ubiquitinated and/or SUMOylated, profoundly affecting glycolysis. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (Pfkfb3) generates fructose-2,6-bisphosphate, which strongly activates PFK1 and promotes glycolysis. Pfkfb3 in neurons is ubiquitinated by the E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C)-Cdh1, causing Pfkfb3 to be degraded by proteases (Almeida et al., 2010; Zhu et al., 2019). Astrocytes have low APC/C-Cdh1 activity and therefore express Pfkfb3 at higher levels than neurons (Herrero-Mendez et al., 2009). A report has shown that glutamate receptors (NMDARs) stabilize Pfkfb3 by inhibiting APC/C-Cdh1, thereby changing neuronal metabolism and leading to oxidative damage and neurodegeneration (Rodriguez-Rodriguez et al., 2012). Interestingly, lysine demethylase KDM2A was recently reported to target Pfkfb3 for ubiquitination and degradation as a process to inhibit the proliferation of multiple myeloma (Liu et al., 2021). GAPDH is an abundant protein involved in glycolysis and catalyzes the conversion of glyceraldehyde 3-phosphate to glycerate 1,3-diphosphate. Activated nitric oxide (NO) nitrosylates GAPDH. This nitrosylation inhibits the catalytic activity of GAPDH and allows it to bind Siah, an E3 ubiquitin ligase. The GAPDH/Siah protein complex enters the nucleus and degrades the Siah target protein to cause cell death (Hara et al., 2006). Phosphoglycerate kinase 1 (PGK1) is related to the progression of many cancers and is an important enzyme in glycolysis. The E3 ubiquitin ligase STUB1 targets PGK1 for ubiquitination and subsequent degradation. The lncRNA LINC00926 promotes STUB1-mediated ubiquitination of PGK1 to downregulate PGK1 expression, thereby suppressing the growth of breast cancer (Chu et al., 2021). Interestingly, another lncRNA, GBCDRlnc1, inhibits the ubiquitination of PGK1 and endows gallbladder cancer cells with chemotherapy resistance by activating autophagy (Cai et al., 2019).

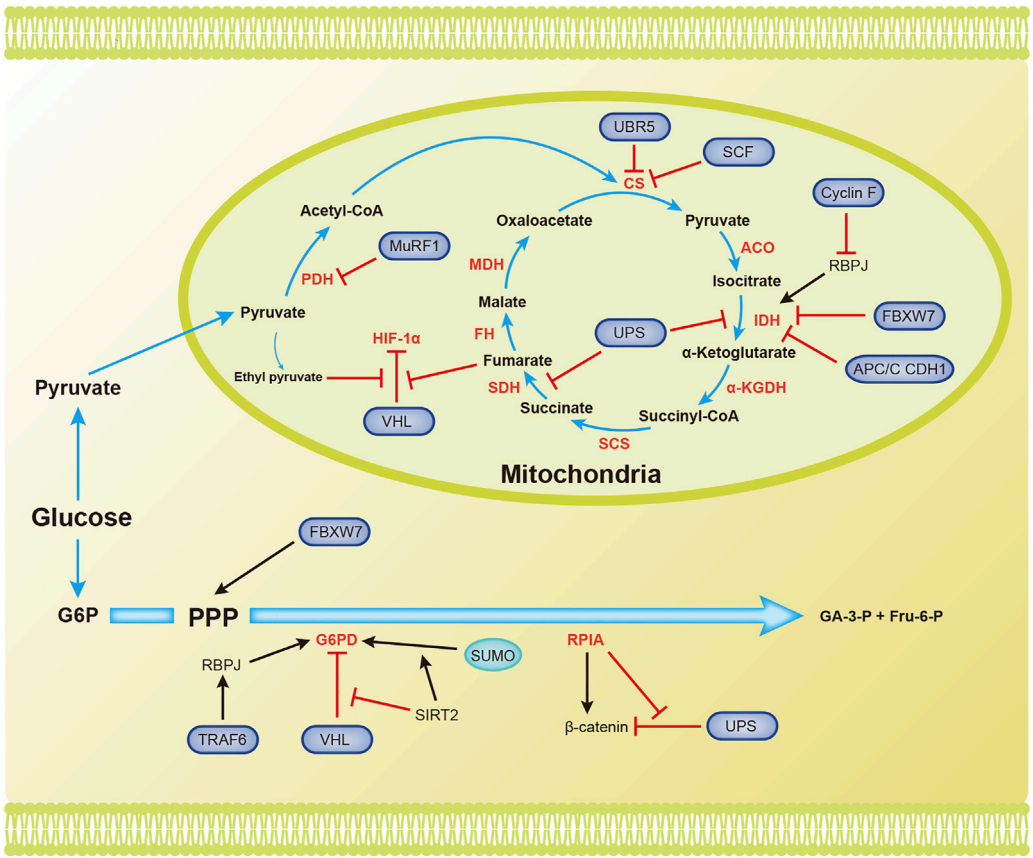


FIGURE 3 | Overview of the mechanisms by which ubiquitination and SUMOylation regulate the TCA cycle and PPP. The blue arrow represents the flow of metabolites. The black and red arrows represent activation and inhibition, respectively. ACO, aconitase; CS, citrate synthase; FBW7, F-box and WD repeat domain-containing protein 7; FH, fumarate hydratase; Fru-6-P, fructose 6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GA-3-P, glyceraldehyde 3-phosphate; IDH, isocitrate dehydrogenase; MDH, malate dehydrogenase; PDH, pyruvate dehydrogenase; RPIA, ribose-5-phosphate isomerase A; SCF, Skp1-Cul1-F-box protein; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase (Complex II); SIRT2, silent information regulator 2; SM22α, smooth muscle (SM) 22α protein; UPS, ubiquitin proteasome system; α-KGDH, α-ketoglutarate dehydrogenase.

Glucose transport is also important for glycolysis. Glucose transporters GLUT1 and GLUT2 mediate glucose transport, and glycolytic reprogramming will also alter the expression of GLUT1 and GLUT2. In response to EGF, the E3 ligase Skp2 mediates the nonproteolytic ubiquitination modification of AKT, thereby activating the AKT pathway and promoting GLUT1 expression and glycolysis (Chan et al., 2012). According to a recent study, the circular RNA circRNF13 binds to the 3′-UTR of the SUMO2 gene and prolongs the half-life of the SUMO2 mRNA. Upregulated SUMO2 promotes GLUT1 degradation through its SUMOylation and ubiquitination, which inhibits glycolysis and ultimately inhibits the proliferation and metastasis of nasopharyngeal carcinoma (Mo et al., 2021). Hypoxia-induced SUMOylation of HIF1α also promotes the expression of GLUT1 (Cheng et al., 2007). The E3 ubiquitin ligase TRIM11 promotes glycolysis in breast cancer by participating in the AKT/GLUT1 signaling pathway (Song et al., 2019). E3 ubiquitin ligases ring finger protein 20 (RNF20) and RNF40 target and regulate GLUT2 levels to affect islet β-cell function (Wade et al., 2019).

Metabolic Reprogramming of the Tricarboxylic Acid (TCA) Cycle

Pyruvate is converted to acetyl-CoA, which then enters the TCA cycle. The TCA cycle requires the presence of oxygen. Acetyl-CoA is completely oxidized in the TCA cycle to generate NADH, FADH2, CO_2, and GTP (**Figure 3**).

Several enzymes in the TCA cycle function as tumor suppressors and inhibit the progression of tumor cells. Their ubiquitination and/or SUMOylation affect their functions, thereby reducing their suppressive effects on tumors. The ubiquitin ligase MuRF1, muscle-specific RING-finger protein 1, interacts with PDH to regulate its stability (Hirner et al., 2008). Citrate synthase (CS) is posttranscriptionally regulated by UBR5-mediated ubiquitination (Peng et al., 2019). SCF (Ucc1) ubiquitin ligase also mediates the ubiquitination and degradation of CS by proteases (Nakatsukasa et al., 2015). Isocitrate dehydrogenase 1 and 2 (IDH1/2) catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG), and recurrent mutations in these genes have been confirmed in

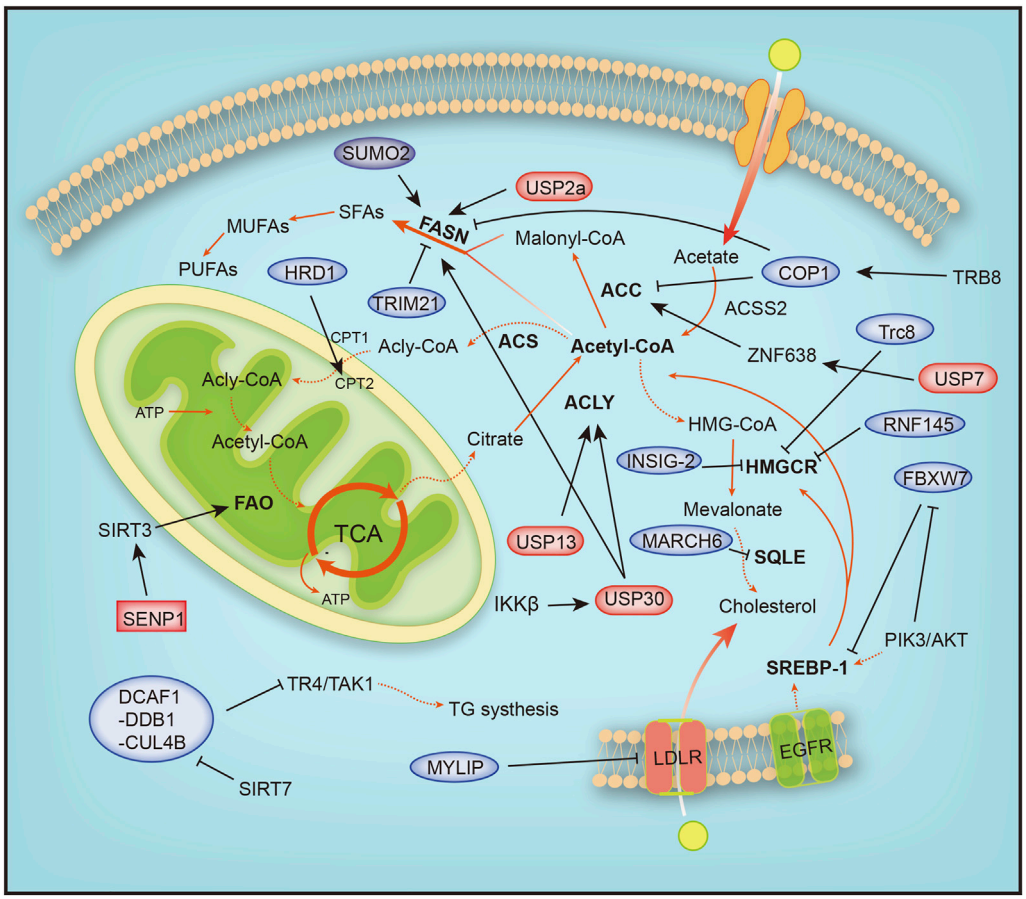


FIGURE 4 | Overview of the mechanisms by which ubiquitination and SUMOylation regulate lipometabolism. ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; ACS, acyl-CoA synthetase; ACSS2, acyl-CoA synthetase short-chain family member 2; ATP, adenosine triphosphate; COP1, constitutive photomorphogenic protein 1; CPT1, carnitine palmitoyl-transferase1; CPT2, carnitine palmitoyl-transferase 2; DCAF1-DDB1-CUL4B, DDB1-CUL4-associated factor 1 (DCAF1)/damage-specific DNA binding protein 1 (DDB1)/cullin 4B; EGFR, epidermal growth factor receptor; FAO, fatty acid oxidation; FASN, fatty acid synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HRD1, HMG-CoA reductase degradation protein 1; INSIG-2, insulin-induced gene 2; LDLR, low-density lipoprotein receptor; MARCH6, membrane-associated RING-CH 6; MUFA, monounsaturated FA; MYLIP, myosin regulatory light chain-interacting protein; PIK3, phosphoinositide-3-kinase; PUFA, polyunsaturated FA; RNF145, ring finger protein 145; SENP1, SUMO-specific protease 1; SFA, saturated FA; SIRT1, silent information regulator 1; SIRT3, silent information regulator 3; SQLE, squalene epoxidase; SREBP1/2, sterol regulatory element-binding protein 1/2; SUMO2, small ubiquitin-like modifier 2; TCA, tricarboxylic acid cycle; TR4/TAK1, testicular nuclear receptor 4; Trc8, translocation in renal carcinoma chromosome 8 gene; Trim 21, tripartite motif 21; USP13, ubiquitin specific protease 13; USP2a, ubiquitin specific protease 2a; USP30, ubiquitin specific protease 30, USP7, ubiquitin specific protease 7; ZNF638, zinc finger protein 638.

glioblastoma and acute myeloid leukemia (Dang et al., 2010). Cyclin F, the substrate recognition subunit of the Skp1-Cul1-F-box protein (SCF) E3 ubiquitin ligase complex, mediates the polyubiquitination of RBPJ at Lys315 and subsequent degradation under metabolic stress. RBPJ regulates IDH expression, regardless of IDH mutation. Therefore, Cyclin F attenuates the carcinogenic ability of IDH by reducing the expression of RBPJ (Deshmukh et al., 2018). Succinate dehydrogenase (SDH) catalyzes the production of fumaric acid from succinate. Decreased SDH activity will cause succinate to accumulate in cells, and succinate inhibits HIF-α hydroxylases. This inhibition will cause the E3 ligase pVHL to dissociate from HIF-1α and ultimately maintain the stability and activation of HIF-1α (Selak et al., 2005). F-box and WD repeat domain containing 7 (FBXW7), another SCF E3 ubiquitin ligase substrate recognition element, is inversely related to the expression of IDH1 in gliomas. Deletion of FBXW7 significantly increases IDH1 expression by inhibiting the degradation of sterol regulatory element binding protein 1 (SREBP1). This process weakens the cellular buffering capacity against radiation-induced oxidative stress and enhances radiation sensitivity (Yang et al., 2021). IDH2 is an enzyme that produces NADPH, and NADPH blocks ROS in cells. APC/C CDH1 mediates the ubiquitination of IDH2 and contributes to the increase in ROS levels during mitosis (Lambhate et al., 2021). Succinate dehydrogenase subunit A (SDHA) is specifically ubiquitinated in organelles by the ubiquitin proteasome system (UPS). Inhibition of UPS-mediated SDHA ubiquitination and

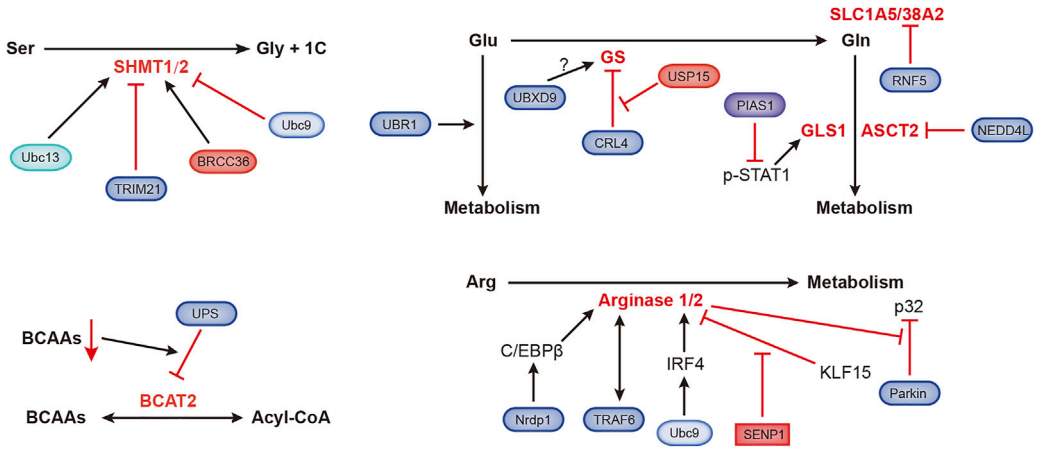


FIGURE 5 | Ubiquitination- and SUMOylation-mediated regulation of amino acid metabolism. The black and red arrows represent activation and inhibition, respectively. ASCT2, alanine, serine, cysteine-preferring transporter 2; BCAAs, branched-chain amino acids; BCAT2, branched-chain amino acid transaminase 2; BRCC36, Zn^{2+}-dependent deubiquitinating enzyme; CRL4, Cullin-RING ubiquitin ligase 4; GLS1, glutaminase 1; GS, glutamine synthetase; IRF4, interferon regulatory factor 4; KLF15, Kruppel-like factor 15; NEDD4L, NEDD4-like E3 ubiquitin protein ligase; Nrdp1, ring finger protein 41; PIAS1, protein inhibitor of activated STAT1; RNF5, ring finger protein 5; SHMT1/2, serine hydroxymethyltransferase 1/2; SLC1A5/38A2, L-glutamine carrier proteins; Ubc9, ubiquitin-conjugating enzyme; Ubc13, SUMO E2-conjugating enzyme; UBR1, ubiquitin protein ligase E3 component n-recognin 1; UBXD9, UBX domain containing 9; USP15, ubiquitin-specific protease 15.

degradation promotes the production of ATP, malate, and citrate (Lavie et al., 2018). SDH5 depletion inhibits p53 degradation through the ubiquitin/proteasome pathway, thereby promoting apoptosis and increasing the radiosensitivity of NSCLC cells (Zong et al., 2019). Ethyl pyruvate, a derivative of pyruvate, also inhibits pVHL-mediated degradation of HIF-1α (Kim et al., 2010). Fumarase is an enzyme in the TCA cycle that participates in DNA repair in cells. The ubiquitination of lysine 79 in fumarase inhibits its function in the TCA cycle and DNA damage repair. This report reveals how posttranslational modifications affect fumarase function (Wang S. et al., 2020). Tumor cells use Gln in the TCA cycle to maintain biosynthesis and support rapid growth and proliferation. The ubiquitin ligase RNF5 interacts with the L-glutamine carrier proteins SLC1A5 and SLC38A2 (SLC1A5/38A2) to mediate their ubiquitination and degradation. This degradation reduces the uptake of Gln and components of the TCA cycle, leading to autophagy and cell death (Jeon et al., 2015). To the best of our knowledge, few studies have assessed the involvement of SUMOylation in regulating the TCA cycle.

Metabolic Reprogramming of the Pentose Phosphate Pathway

In addition to the final production of NADPH, glyceraldehyde 3-phosphate (GA-3-P), and fructose 6-phosphate (Fru-6-P), the pentose phosphate pathway also generates many intermediates that provide raw materials for cellular biosynthesis (**Figure 3**).

Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the PPP and is important for maintaining NADPH levels. High glucose concentrations promote the degradation of G6PD through pVHL-mediated ubiquitination of G6PD, which leads to ROS accumulation and podocyte injury (Wang M. et al., 2019). TRAF6 mediates K63 ubiquitination of the smooth muscle (SM) 22α protein, promoting the interaction between SM22α and G6PD. Ubiquitinated SM22α enhances the activity of G6PD by mediating the membrane translocation of G6PD (Dong et al., 2015). According to a recent study, silent information regulator 2 (SIRT2) interacts with G6PD to increase its activity through deacetylation, leading to a decrease in ubiquitination and an increase in SUMOylation to increase G6PD stability (Ni et al., 2021). The lack of the E3 ubiquitin ligase FBW7 reduces substrate flux through the PPP and accelerates the production of reactive oxygen species (ROS) in macrophages (Wang et al., 2020a). The dysregulation of ribose-5-phosphate isomerase A (RPIA) in the PPP promotes liver, lung, and breast tumorigenesis. Unlike its function in the PPP, RPIA enters the nucleus to form a complex with β-catenin, and this interaction prevents the ubiquitination and subsequent degradation of β-catenin in colorectal cancer. This mechanism explains the role of RPIA in promoting tumorigenesis (Chou et al., 2018).

UBIQUITINATION AND SUMOYLATION IN LIPID METABOLISM IN CANCER

Eukaryotic cells degrade proteins through two pathways, autophagy and the ubiquitin–proteasome system (UPS), which are responsible for 10–20% and 80–90% of intracellular protein hydrolysis, respectively (Ciechanover and Kwon, 2015). The UPS is responsible for the selective hydrolysis of proteins by adding ubiquitin molecules to the substrate and degrading it in the proteasome. Ubiquitination is crucial for lipid metabolism. In recent studies, a correlation between the role of ubiquitin in the signaling pathways of lipid metabolism and the effect of the ubiquitin system on the development of human cancer has been

identified. Lipometabolism includes fatty acid oxidation (FAO), fatty acid synthesis (FAS), cholesterol metabolism, ketone body metabolism and acetate metabolism. Acetyl-CoA is the substrate for *de novo* synthesis of fatty acids, and intracellular ubiquitination includes proteolytic and nonproteolytic pathways. In addition to controlling protein stability, it is crucial for regulating lipid metabolism homeostasis, inflammation, autophagy, and DNA damage repair (Popovic et al., 2014). The uncontrollable ubiquitin system abnormally activates or inhibits certain cellular metabolic pathways, thus affecting cancer development. Next, we focused on how ubiquitination and SUMOylation affect cancer by affecting the intracellular lipid metabolism (**Figure 4**).

Fatty Acid Metabolic Reprogramming

The proliferation of cancer cells requires a large amount of material and energy, during which the metabolism of cells is reprogrammed. The TCA cycle is characterized by condensing acetyl coenzyme A and oxaloacetic acid to generate citric acid, anaplerosis of oxaloacetic acid, the production of intermediate products, and the energy release process (**Figure 4**). Glutamine is a precursor of mitochondrial oxaloacetic, supplying oxaloacetic acid required for the TCA cycle. Highly invasive ovarian cancer (OVCA) cells depend on glutamine, and glutamine is transformed into α-ketoglutarate (α-KG or 2-oxoglutarate). α-KG is reduced to isocitrate by isocitrate dehydrogenase and regenerated into citric acid. Citric acid produced from glutamine is transported into the cytoplasm to produce acetyl-CoA and participate in fatty acid synthesis (Metallo et al., 2011; Mullen et al., 2011). ACLY is expressed at the highest levels in the liver and white adipose tissue, and ACLY is upregulated and activated in a variety of tumor tissues (Chypre et al., 2012). Overexpression of USP13 is associated with malignant development and a poor prognosis of OVCA, and USP13 deubiquitinates and stabilizes ACLY. The decomposition of glutamine, ATP production, and fatty acid synthesis are accelerated. USP13 inhibition significantly blocks acetyl-CoA production and OVCA proliferation (Han et al., 2016). Strategies targeting USP13 have been proposed as a potential treatment for OVCA.

Fatty acid oxidation is a complex process characterized by a multistep reaction, and fatty acids are acylated by acyl-CoA synthase to form acyl-CoA on the endoplasmic reticulum and mitochondrial outer membrane. The oncoprotein Src is activated via autophosphorylation by two carnitine fatty acid transferases (CPT1 and CPT2) that enter the mitochondrial matrix and undergo fatty acid β oxidation. HMG-CoA reductase degradation protein 1 (HRD1), an E3 ubiquitin ligase, controls cholesterol production and regulates FAO metabolism by regulating the key rate-limiting enzyme HMG-COA reductase (HMGCR). In glutamine-deficient TNBC, HRD1 expression is downregulated, and CPT2 is stabilized by K48-linked ubiquitination, leading to increased FAO metabolism. Inhibition of CPT2 expression significantly reduces the proliferation of TNBC cells (Hampton and Rine, 1994; Hampton et al., 1996). In addition, CB839, a glutaminase (GLS) inhibitor, effectively treated TNBC cells with high HRD1 expression (Guo et al., 2021). Tumor cell metabolism is accompanied by reprogramming, as evidenced by increased glutamine and glucose uptake. Triple-negative breast cancer (TNBC) is a heterogeneous subtype of breast cancer with a high local recurrence rate and high risk of metastasis. Dysregulation of fatty acid oxidation is often associated with the progression of TNBC (Sun et al., 2020). TNBC has the metabolic characteristics of dependence on glutamine and accelerated fatty acid oxidation (FAO). When cells grow rapidly, glutamine consumption increases, resulting in a local glutamine deficiency. While fatty acids may be used as alternative energy sources for cell growth, studies have found that inhibiting MYC-dependent FAO in TNBC is an important target for the treatment of TNBC (Camarda et al., 2016).

Lipid metabolic reprogramming also frequently occurs in liver cancer, and lipid synthesis and metabolism are increased during liver cancer. The mitochondrial deubiquitination enzyme USP30 plays an important role in hepatocellular carcinoma (HCC) driven by high fat diets (HFDs). USP30 is phosphorylated and stabilized by IκB kinase β (IKKβ), which promotes USP30 deubiquitination and stabilizes ACLY and fatty acid synthase (FASN) to induce lipid generation and tumorigenesis. USP30 inhibitors significantly inhibit lipid synthesis and tumorigenesis (Gu et al., 2021). USP7 plays an important role in HCC caused by aberrant *de novo* lipogenesis (DNL). The expression of ubiquitin-specific peptidase 7 (USP7), a deubiquitinating enzyme, is positively correlated with malignant cancer by deubiquitinating MDM2, which inhibits the activation of P53 (Kon et al., 2010). USP7 deubiquitinates and stabilizes zinc finger protein 638 (ZNF638), a zinc finger protein, and promotes the transcription of ZNF638 by stabilizing cAMP-responsive element-binding protein (CREB). The USP7/ZNF638 axis activates AKT/mTORC1/S6K signaling, promotes the accumulation of cleaved-SREBP1C, and deubiquitinates nuclear cleaved-SREBP1C. Next, the USP7-ZNF638-cleaved-SREBP1C complex upregulates the expression of acetyl-CoA carboxylase (ACACA), FASN, and stearoyl-CoA desaturase (SCD) to enhance DNL and tumorigenesis (Ni et al., 2020).

Abnormal fat formation is associated with a number of malignant features, including clinically increased tumor invasion, activation of the AKT signaling pathway, and inhibition of adenosine monophosphate-activated protein kinase. The AKT-mTORC1-RPS6 signaling pathway enhances lipogenesis to promote the HCC process. In cancer, unconstrained lipogenesis is necessary to maintain a steady supply of lipids and lipid precursors that facilitate membrane production and postlipid transformation. AKT overactivation leads to increased lipid biosynthesis and higher levels of lipid-producing proteins. The AKT/mTORC1 pathway induces adipogenesis through transcriptional and posttranscriptional mechanisms. AKT promotes *de novo* adipogenesis by blocking the proteasomal degradation of SREBP1, SREBP2, and FASN. SREBP1 is an important transcription factor involved in fatty acid metabolism that upregulates fatty acid synthesis and extracellular lipid uptake. AKT inhibits SREBP1 and SREBP2 ubiquitination by inhibiting the E3 ligase FBXW7 after phosphorylation by GSK-3β to activate the expression of lipid metabolism genes (Sundqvist

et al., 2005; Tu et al., 2012; Zhao et al., 2012). In addition, SIRT1-mediated deubiquitination of SREBP promotes its ubiquitination and degradation (Walker et al., 2010). Moreover, AKT also upregulates USP2a, a deubiquitination enzyme, and blocks the degradation of FASN. In summary, ubiquitination and SUMOylation are important effector mechanisms of the AKT/mTORC1 axis in human HCC (Calvisi et al., 2011). PTEN is a tumor suppressor that inhibits the PI3K/AKT signaling pathway and is modified by ubiquitylation, SUMOylation and phosphorylation in the process of PTEN function and nuclear transport. PTEN neddylation is promoted by XIAP ligase and removed by NEDP1 deneddylase. Lys197 and Lys402 are the main neddylation sites of PTEN. In contrast, neddylated PTEN promotes tumor proliferation and metabolism by dephosphorylating the FASN protein, inhibiting trim21-mediated ubiquitylation and degradation of FASN, and promoting *de novo* fatty acid synthesis. This phenomenon suggests that neddylation transforms PTEN from a cancer suppressor to an oncogene (Lee et al., 2018; Xie et al., 2021).

During fatty acid synthesis, acetyl-CoA is utilized only after activated acetyl-CoA carboxylase (ACC), a rate-limiting enzyme in fatty acid synthesis, is degraded by phosphorylation and ubiquitination by increasing catecholamine levels during fasting. The phosphorylated form is inhibited, and the dephosphorylated form is activated. Tribbles 3 (TRB3) induces ACC degradation to inhibit fatty acid synthesis and promote oxidative lipid decomposition, and TRB3 interacts with E3 ubiquitin ligase constitutive photomorphogenic protein 1 (COP1) to promote ACC degradation (Qi et al., 2006).

FASN is a key enzyme involved in the synthesis of fatty acids. FASN is expressed at relatively low levels in normal tissues, but it is abnormally upregulated in tumors. The synthesis of fatty acids affects the metabolism of sugar, fat and cholesterol by activating PPARα (Chakravarthy et al., 2005). SHP2, a tyrosine phosphatase with an SH2 domain, interacts with the E3 ubiquitin ligase COP1 to regulate the ubiquitination of FASN. Moreover, SHP2 also regulates the expression of SREBP1c, which directly activates FASN gene expression via the PI3K-AKT signaling pathway (Yu et al., 2013). In addition, overactivation of the PI3K-Akt signaling pathway is accompanied by overactivation of ErbB1 (epidermal growth factor receptor) or ErbB2 (HER2/NEU) and abnormal expression of FASN. FASN inhibitors significantly increase the sensitivity of cells to anti-ErbB drugs by promoting the ubiquitination and degradation of PI3K effector proteins (Tomek et al., 2011). On the one hand, these treatments inhibit lipid synthesis in tumor cells, and on the other hand, they block PI3K signaling in ovarian cancer. Therefore, tumor therapy targeting FASN is a promising. SIRT7 binds to the DDB1-CUL4-associated factor 1 (DCAF1)/damage-specific DNA binding protein 1 (DDB1)/cullin 4B (CUL4B) E3 ubiquitin ligase complex to block the degradation of TR4/TAK1, resulting in increased synthesis and storage of fatty acids (Yoshizawa et al., 2014; Zhu et al., 2019). In breast cancer cell lines, SUMOylation prevents FASN degradation by the proteasome, and the reduction in SUMOylation caused by SUMO2 silencing reduces the stability of FASN and inhibits the development of tumor cells (Floris et al., 2020).

In addition, ubiquitination plays a crucial role in other diseases of the human immune system. Fatty acid metabolism includes cytoplasmic fatty acid synthesis (FAS) and mitochondrial fatty acid oxidation (FAO). Inducible regulatory T (iTreg) cells inhibit excessive or abnormal immune responses and are essential for maintaining immune homeostasis. Functional defects in Tregs may lead to autoimmune diseases, such as inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE). Differentiation of activated T (Th0) cells into inducible regulatory T (iTreg) cells requires cellular metabolic reprogramming from fatty acid synthesis to fatty acid oxidation. In the differentiation of inducible regulatory T cells stimulated by TGF-β signaling, ATP-citrate lyase (ACLY) is ubiquitinated by Cul3-KLHL25. ACLY degradation promotes iTreg cell differentiation by promoting cellular fatty acid oxidation and maintaining the stability of the immune system to prevent the occurrence of autoimmune diseases. In addition, ACLY transforms citrate into acetyl-CoA and oxaloacetate, providing acetyl-CoA substrates for *de novo* fatty acid synthesis. Ubiquitination of ACLY inhibits *de novo* fatty acid synthesis, resulting in impaired cell proliferation (Tian et al., 2021). Sirt3 regulates the mitochondrial adaptation to metabolic stress, and SUMOylation inhibits Sirt3 in mitochondria. When cells starve, Sirt3 is deSUMOylated by SENP1, a SUMO-specific protease that activates Sirt3 and increases fatty acid metabolism (Wang T. et al., 2019).

Cholesterol Metabolic Reprogramming

Cholesterol plays a vital role in maintaining cell homeostasis. It is most abundant in the eukaryotic plasma membrane, regulates cell membrane fluidity and material transport and is a precursor of intermediate metabolites such as bile acids and steroids (Haines, 2001; Hannich et al., 2011). Cholesterol metabolism is strictly regulated in the human body. A single disorder blocks arteries and causes heart attacks and strokes. Our body controls the amount of cholesterol through an enzyme called 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). In the next sections, we will focus on the functions of ubiquitination and SUMOylation in cholesterol metabolism (**Figure 4**).

HMGCR is the rate-limiting enzyme in cholesterol and nonsteroidal isoprene biosynthesis and the therapeutic target of statins (Sun et al., 2020). When cholesterol levels in cells are reduced, sterol response element binding proteins (SREBPs) bind to SREs in the promoter region of HMGCR to promote its transcription (Osborne, 1991), and the HMGCR mRNA and protein half-lives are extended, ensuring the supply of mevalonate (Goldstein and Brown, 1990). RNF145, a sterol-responsive-resident E3 ligase, gradually accumulates with depletion of sterols and sensitively responds to the cholesterol level. Reduced cellular cholesterol levels, the formation of RNF145, increased cellular cholesterol levels, and RNF145 mediate HMGCR ubiquitination and proteasomal degradation. Alternatively, in the presence of an RNF145 and GP78 deficiency, uBE2G2 ligase 1 partially regulates HMGCR activity (Loregger et al., 2017; Jiang et al., 2018; Menzies et al., 2018). Hypoxia-

induced expression of insulin-induced gene 2, insig-2, inhibits cholesterol synthesis by mediating sterol-induced ubiquitination and HMGCR degradation (Hwang et al., 2017). The low expression of the ubiquitin E3 ligase Trc8 in resistant cancer cells (MDR) that overexpress P-glycoprotein (Pgp) and multidrug resistance-related protein 1 (MRP1) leads to dysregulated cholesterol metabolism, decreased ubiquitination of HMGCR, increased cholesterol synthesis and storage, and the progression of colorectal cancer cells (Gelsomino et al., 2013).

Squalene epoxidase (SQLE) is a rate-limiting enzyme involved in cholesterol synthesis. Overexpression of SQLE impairs angiogenesis; however, angiogenesis is also attenuated when SQLE is silenced. The ERAD-associated ubiquitin ligase MARCH6 regulates endothelial cholesterol homeostasis by promoting SQLE degradation (Tan et al., 2020). Moreover, the E3 ligase MYLIP regulates cholesterol uptake by ubiquitinating LDL receptors, promoting LDLR degradation and blocking cholesterol uptake (Zelcer et al., 2009).

The effects of ubiquitination and deubiquitination on tumor metabolism are complex and require further study. The E3 ubiquitin ligase/DUB substrate network regulates tumor processes and is influenced by the cell type and environment for executive function. Understanding ubiquitination and SUMOylation in cancer metabolism is crucial to identifying new targets for cancer therapy. For example, the use of novel ubiquitin proteasome inhibitors to treat multiple myeloma is a therapeutic approach that links the ubiquitin system to the treatment of tumorigenesis and metabolic diseases. With the continuous development of proteomics technology, researchers have been able to accurately target and track ubiquitin-dependent tumorigenesis and diseases and develop more therapeutic targets.

UBIQUITINATION AND SUMOYLATION IN AMINO ACID METABOLISM IN CANCER

Amino acids are materials required for protein synthesis in organisms. Tumor cells grow rapidly and require a large amino acid supply, which leads to the reprogramming of amino acid metabolism in tumor cells (**Figure 5**).

Organic groups containing only one carbon atom, called one-carbon units, produced during amino acid catabolism, are involved in important nucleotide synthesis and methylation in cells. Serine hydroxymethyltransferase (SHMT) catalyzes the metabolism of serine to produce one-carbon units and glycine, supporting rapidly proliferation of tumor cells. SHMT1 can be ubiquitinated and SUMOylated at the same lysine residue, Ubc13-mediated ubiquitination is required for SHMT1 nuclear export and stabilization, and Ubc9-mediated SUMOylation promotes SHMT1 nuclear degradation (Anderson et al., 2012). Serine hydroxymethyltransferase 2 (SHMT2) catalyzes the conversion of serine and glycine to support the proliferation of cancer cells. TRIM21 promotes the ubiquitination and degradation of SHMT2 in a glucose-dependent manner (Wei et al., 2018). BRCC36 is a deubiquitinating subunit that forms the BRISC complex. ABRO1, another subunit of the BRISC complex, binds to SHMT2α to prevent deubiquitination of the latter. The BRISC-bound SHMT2α is catalytically inactive, which inhibits SHMT2α function (Rabl et al., 2019).

The E3 ubiquitin ligase UBR1 regulates glutamate metabolism, which is essential for neuronal development and signal transduction (Chitturi et al., 2018). Glutamine (Gln) dependence is a characteristic of tumor metabolism, and the ubiquitin ligase RNF5 regulates the expression of the glutamine carrier proteins SLC1A5 and SLC38A2. Paclitaxel promotes the ubiquitination and subsequent degradation of SLC1A5/38A2 by RNF5 (Jeon et al., 2015). The E3 ligase NEDD4L inhibits mitochondrial metabolism by reducing the level of the glutamine transporter ASCT2 (Lee et al., 2020). Transcription of glutaminase (GLS1) is coactivated by p-STAT1 and p300. PIAS1 is an E3 SUMO ligase that inhibits activated STAT1 (p-STAT1). Kr-POK interacts with PIAS1, disrupting the interaction between PIAS1 and p-STAT1 and subsequently increasing the activity of p-STAT1 to promote GLS1 transcription. This process promotes cell growth, migration and motility (Hur et al., 2017; Li et al., 2020). Glutamine synthetase (GS) is the endogenous substrate of Cullin-RING ubiquitin ligase 4 (CRL4). When the extracellular glutamine concentration is high, acetylated GS is ubiquitinated by CRL4 and then degraded by the proteasome (Nguyen et al., 2016). Valosin-containing protein (VCP)/p97 promotes the degradation of ubiquitylated GS (Nguyen et al., 2017). p97 also binds to UBXD9, a member of the UBXD protein family. GS type III has been identified as a new interacting partner of UBXD9, but the specific regulatory mechanism is still unknown (Riehl et al., 2021). A recent study showed that USP15 antagonizes CRL4-mediated ubiquitination of GS and prevents GS degradation. USP15 is expressed at high levels in immunomodulatory drug (IMiD)-resistant cells, and the loss of USP15 renders these resistant cells sensitive to IMiD (Nguyen, 2021).

Arginase is very important in arginine metabolism, catalyzing the conversion of arginine into urea. Nrdp1 mediates the ubiquitination of the transcription factor C/EBPβ at the K63 site, which promotes the activation of C/EBPβ and subsequently upregulates arginase 1 (Arg1) expression (Ye et al., 2012). TRAF6 and arginase 1 are related, and they are expressed at high levels in myeloid-derived suppressor cells (MDSCs) (Song et al., 2021). Ubc9-mediated SUMOylation of interferon regulatory factor 4 (IRF4) increases its nuclear localization and stability. IRF4 induces the transcription of Arg1 and promotes the progression of the macrophage M2 program (Wang F. et al., 2019). KLF15 (Kruppel-like factor 15) binds to the Arg2 (arginase 2) promoter, which hinders Arg2 transcription. Under hypoxic conditions, SENP1-mediated deSUMOylation of KLF15 leads to the translocation of KLF15 from the nucleus to the cytoplasm, which induces the expression of Arg2 (Pandey et al., 2018). Arg2 was recently shown to regulate Parkin-dependent p32 degradation, promoting Ca^{2+}-dependent eNOS activation (Koo et al., 2021).

The level of branched-chain amino acids (BCAAs) in plasma is related to the risk of pancreatic cancer. Branched-chain amino acid transaminase 2 (BCAT2) reversibly catalyzes BCAA degradation to branched-chain acyl-CoA. BCAA deprivation stimulates the degradation of acetylated BCAT2 through by

the ubiquitin–proteasomeubiquitin-proteasome pathway (Lei et al., 2020).

CONCLUSION

Similar to the human resources (HR) department in a company, which hires, trains, assigns work or fires personnel to make this huge machine (company) run normally, the cell is a more complex machine, and its signal transduction is more ingenious and delicate. Ubiquitination and SUMOylation are the HR of cells. They determine the fates of proteins and cellular signal transduction and determine the fate of the entire cell. Metabolic reprogramming occurs in various diseases, and an increasing number of studies are explaining the relationship between metabolism and diseases, especially cancer.

The regulation of ubiquitination and SUMOylation are similar in many respects. They both modify the substrate protein, and the modification binds covalently to the lysine residues of these proteins to determine the fate of the proteins. The motifs they recognize are different. Few proteins are involved in regulating ubiquitination and SUMOylation, but these processes both regulate a large number of proteins. Ubiquitin and SUMO proteins only show 18% sequence similarity, but their structures are similar. The main difference is that SUMO proteins have a long and flexible N-terminal extension. Compared with ubiquitination, SUMOylation is a relatively simple process. As described in the present review, SUMOylation also regulates fewer metabolic processes.

Here, we summarize the research on how ubiquitination and SUMOylation affect metabolic reprogramming. Based on the results from these recent studies, we realize that ubiquitination, SUMOylation and metabolic reprogramming are closely related. Neither is an independent process, and their effects are mutual. Ubiquitination and SUMOylation reshape metabolism by affecting important regulatory factors in the metabolic process (Lavie et al., 2018). The altered metabolic substrate level in metabolic reprogramming also affects the processes of ubiquitination and SUMOylation (Selak et al., 2005). Although many studies have been conducted in this field, research on SUMOylation in metabolic reprogramming is lacking. As stated in a recent review, SUMO: from bench to bedside (Chang and Yeh, 2020), research on SUMOylation in metabolic reprogramming must be a focus of future studies. We noticed that epigenetic regulation is also involved, increasing the complexity (Wang T. et al., 2019). Ubiquitination and SUMOylation are also involved in other cellular signal transduction pathways, including autophagy and ferroptosis (Varshavsky, 2017; Chen X. et al., 2021). These regulatory mechanisms are still unknown. Research designed to understand the complex regulatory mechanism must continue.

Ubiquitination and SUMOylation may be the direction of targeted therapy. An understanding of their relationship with cancer metabolism supports the development of new treatment strategies. Future research should also focus on treatment strategies that correct abnormal ubiquitination and SUMOylation. The development of new chemotherapeutic drugs targeting ubiquitination and/or SUMOylation combined with immunotherapy may overcome the limitations of chemotherapy and immunotherapy. This approach shows great promise and will provide benefits to human health.

AUTHOR CONTRIBUTIONS

SZ and HG wrote the manuscript. CP, FX, and HUC were Illustrators. HOC conducted and revised manuscript. All the authors have read and approved the final version of the manuscript.

FUNDING

This work was supported by Chongqing Research Program of Basic Research and Frontier Technology (No. cstc2019jcyj-msxmX0429 to SZ), the National Natural Science Foundation of China (No. 81672502 to HCui), Venture & Innovation Support Program for Chongqing Overseas Returnees (No. cx2019131 to SZ). National Undergraduate Training Programs for Innovation and Entrepreneurship of China (No. 202010635062 to SZ).

REFERENCES

Agarwal, E., Goldman, A. R., Tang, H. Y., Kossenkov, A. V., Ghosh, J. C., Languino, L. R., et al. (2021). A Cancer Ubiquitome Landscape Identifies Metabolic Reprogramming as Target of Parkin Tumor Suppression. *Sci. Adv.* 7, eabg7287. doi:10.1126/sciadv.abg7287

Almeida, A., Bolanos, J. P., and Moncada, S. (2010). E3 Ubiquitin Ligase APC/C-Cdh1 Accounts for the Warburg Effect by Linking Glycolysis to Cell Proliferation. *Proc. Natl. Acad. Sci.* 107, 738–741. doi:10.1073/pnas.0913668107

Anderson, D. D., Eom, J. Y., and Stover, P. J. (2012). Competition between SUMOylation and Ubiquitination of Serine Hydroxymethyltransferase 1 Determines its Nuclear Localization and its Accumulation in the Nucleus. *J. Biol. Chem.* 287, 4790–4799. doi:10.1074/jbc.m111.302174

Cai, Q., Wang, S., Jin, L., Weng, M., Zhou, D., Wang, J., et al. (2019). Long Non-coding RNA GBCDRlnc1 Induces Chemoresistance of Gallbladder Cancer Cells by Activating Autophagy. *Mol. Cancer* 18, 82. doi:10.1186/s12943-019-1016-0

Calvisi, D. F., Wang, C., Ho, C., Ladu, S., Lee, S. A., Mattu, S., et al. (2011). Increased Lipogenesis, Induced by AKT-mTORC1-RPS6 Signaling, Promotes Development of Human Hepatocellular Carcinoma. *Gastroenterology* 140, 1071–1083. doi:10.1053/j.gastro.2010.12.006

Camarda, R., Zhou, A. Y., Kohnz, R. A., Balakrishnan, S., Mahieu, C., Anderton, B., et al. (2016). Inhibition of Fatty Acid Oxidation as a Therapy for MYC-Overexpressing Triple-Negative Breast Cancer. *Nat. Med.* 22, 427–432. doi:10.1038/nm.4055

Chakravarthy, M. V., Pan, Z., Zhu, Y., Tordjman, K., Schneider, J. G., Coleman, T., et al. (2005). "New" Hepatic Fat Activates PPARα to Maintain Glucose, Lipid, and Cholesterol Homeostasis. *Cel Metab.* 1, 309–322. doi:10.1016/j.cmet.2005.04.002

Chan, C.-H., Li, C.-F., Yang, W.-L., Gao, Y., Lee, S.-W., Feng, Z., et al. (2012). The Skp2-SCF E3 Ligase Regulates Akt Ubiquitination, Glycolysis, Herceptin Sensitivity, and Tumorigenesis. *Cell* 149, 1098–1111. doi:10.1016/j.cell.2012.02.065

Chang, H.-M., and Yeh, E. T. H. (2020). SUMO: From Bench to Bedside. *Physiol. Rev.* 100, 1599–1619. doi:10.1152/physrev.00025.2019

Chen, D., Wu, H., Feng, X., Chen, Y., Lv, Z., Kota, V. G., et al. (2021a). DNA Methylation of Cannabinoid Receptor Interacting Protein 1 Promotes Pathogenesis of Intrahepatic Cholangiocarcinoma through Suppressing Parkin-Dependent Pyruvate Kinase M2 Ubiquitination. *Hepatology* 73, 1816–1835. doi:10.1002/hep.31561

Chen, J., Ou, Y., Yang, Y., Li, W., Xu, Y., Xie, Y., et al. (2018). KLHL22 Activates Amino-acid-dependent mTORC1 Signalling to Promote Tumorigenesis and Ageing. *Nature* 557, 585–589. doi:10.1038/s41586-018-0128-9

Chen, X., Yu, C., Kang, R., Kroemer, G., and Tang, D. (2021b). Cellular Degradation Systems in Ferroptosis. *Cell Death Differ* 28, 1135–1148. doi:10.1038/s41418-020-00728-1

Cheng, J., Kang, X., Zhang, S., and Yeh, E. T. H. (2007). SUMO-specific Protease 1 Is Essential for Stabilization of HIF1α during Hypoxia. *Cell* 131, 584–595. doi:10.1016/j.cell.2007.08.045

Chitturi, J., Hung, W., Rahman, A. M. A., Wu, M., Lim, M. A., Calarco, J., et al. (2018). The UBR-1 Ubiquitin Ligase Regulates Glutamate Metabolism to Generate Coordinated Motor Pattern in *Caenorhabditis elegans*. *Plos Genet.* 14, e1007303. doi:10.1371/journal.pgen.1007303

Chou, Y.-T., Jiang, J.-K., Yang, M.-H., Lu, J.-W., Lin, H.-K., Wang, H.-D., et al. (2018). Identification of a Noncanonical Function for Ribose-5-Phosphate Isomerase A Promotes Colorectal Cancer Formation by Stabilizing and Activating β-catenin via a Novel C-Terminal Domain. *Plos Biol.* 16, e2003714. doi:10.1371/journal.pbio.2003714

Chu, Z., Huo, N., Zhu, X., Liu, H., Cong, R., Ma, L., et al. (2021). FOXO3A-induced LINC00926 Suppresses Breast Tumor Growth and Metastasis through Inhibition of PGK1-Mediated Warburg Effect. *Mol. Ther.* 29, 2737–2753. doi:10.1016/j.ymthe.2021.04.036

Chypre, M., Zaidai, N., and Smans, K. (2012). ATP-Citrate Lyase: A Mini-Review. *Biochem. Biophys. Res. Commun.* 422 (1), 1–4. doi:10.1016/j.bbrc.2012.04.144

Ciechanover, A., and Kwon, Y. T. (2015). Degradation of Misfolded Proteins in Neurodegenerative Diseases: Therapeutic Targets and Strategies. *Exp. Mol. Med.* 47, e147. doi:10.1038/emm.2014.117

Cui, C.-P., Wong, C. C.-L., Kai, A. K.-L., Ho, D. W.-H., Lau, E. Y.-T., Tsui, Y.-M., et al. (2017). SENP1 Promotes Hypoxia-Induced Cancer Stemness by HIF-1α deSUMOylation and SENP1/HIF-1α Positive Feedback Loop. *Gut* 66, 2149–2159. doi:10.1136/gutjnl-2016-313264

Dang, L., Jin, S., and Su, S. M. (2010). IDH Mutations in Glioma and Acute Myeloid Leukemia. *Trends Mol. Med.* 16, 387–397. doi:10.1016/j.molmed.2010.07.002

Deshmukh, R. S., Sharma, S., and Das, S. (2018). Cyclin F-dependent Degradation of RBPJ Inhibits IDH1R132H-Mediated Tumorigenesis. *Cancer Res.* 78, 6386–6398. doi:10.1158/0008-5472.can-18-1772

Dong, L.-H., Li, L., Song, Y., Duan, Z.-L., Sun, S.-G., Lin, Y.-L., et al. (2015). TRAF6-Mediated SM22α K21 Ubiquitination Promotes G6PD Activation and NADPH Production, Contributing to GSH Homeostasis and VSMC Survival *In Vitro* and *In Vivo*. *Circ. Res.* 117, 684–694. doi:10.1161/circresaha.115.306233

Floris, A., Mazarei, M., Yang, X., Robinson, A. E., Zhou, J., Barberis, A., et al. (2020). SUMOylation Protects FASN against Proteasomal Degradation in Breast Cancer Cells Treated with Grape Leaf Extract. *Biomolecules* 10, 529. doi:10.3390/biom10040529

Flotho, A., and Melchior, F. (2013). SUMOylation: a Regulatory Protein Modification in Health and Disease. *Annu. Rev. Biochem.* 82, 357–385. doi:10.1146/annurev-biochem-061909-093311

Gameiro, C. M., Bell, P. A., Mattaini, E. L., Yang, K. R., Hiller, K., Jewell, C. M., et al. (2011). Reductive Glutamine Metabolism by IDH1 Mediates Lipogenesis under Hypoxia. *Nature* 481, 380–384. doi:10.1038/nature10602

Gelsomino, G., Corsetto, P. A., Campia, I., Montorfano, G., Kopecka, J., Castella, B., et al. (2013). Omega 3 Fatty Acids Chemosensitize Multidrug Resistant colon Cancer Cells by Down-Regulating Cholesterol Synthesis and Altering Detergent Resistant Membranes Composition. *Mol. Cancer* 12, 137. doi:10.1186/1476-4598-12-137

Glickman, M. H., and Ciechanover, A. (2002). The Ubiquitin-Proteasome Proteolytic Pathway: Destruction for the Sake of Construction. *Physiol. Rev.* 82, 373–428. doi:10.1152/physrev.00027.2001

Goldstein, J. L., and Brown, M. S. (1990). Regulation of the Mevalonate Pathway. *Nature* 343, 425–430. doi:10.1038/343425a0

Gu, L., Zhu, Y., Lin, X., Lu, B., Zhou, X., Zhou, F., et al. (2021). The IKKβ-USP30-ACLY Axis Controls Lipogenesis and Tumorigenesis. *Hepatology* 73, 160–174. doi:10.1002/hep.31249

Guo, X., Wang, A., Wang, W., Wang, Y., Chen, H., Liu, X., et al. (2021). HRD1 Inhibits Fatty Acid Oxidation and Tumorigenesis by Ubiquitinating CPT2 in Triple-negative Breast Cancer. *Mol. Oncol.* 15, 642–656. doi:10.1002/1878-0261.12856

Haines, T. H. (2001). Do sterols Reduce Proton and Sodium Leaks through Lipid Bilayers? *Prog. lipid Res.* 40, 299–324. doi:10.1016/s0163-7827(01)00009-1

Hampton, R. Y., Gardner, R. G., and Rine, J. (1996). Role of 26S Proteasome and HRD Genes in the Degradation of 3-Hydroxy-3-Methylglutaryl-CoA Reductase, an Integral Endoplasmic Reticulum Membrane Protein. *MBoC* 7, 2029–2044. doi:10.1091/mbc.7.12.2029

Hampton, R. Y., and Rine, J. (1994). Regulated Degradation of HMG-CoA Reductase, an Integral Membrane Protein of the Endoplasmic Reticulum, in Yeast. *J. Cel. Biol.* 125, 299–312. doi:10.1083/jcb.125.2.299

Han, C. L., Yang, H. H., Choi, J., Baddour, A., Achreja, Y., Liu, Y., et al. (2016). Amplification of USP13 Drives Ovarian Cancer Metabolism. *Nat. Commun.* 7, 13525. doi:10.1038/ncomms13525

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of Cancer: the Next Generation. *Cell* 144, 646–674. doi:10.1016/j.cell.2011.02.013

Hannich, J. T., Umebayashi, K., and Riezman, H. (2011). Distribution and Functions of Sterols and Sphingolipids. *Cold Spring Harb Perspect. Biol.* 3, a004762. doi:10.1101/cshperspect.a004762

Hara, M. R., Thomas, B., Cascio, M. B., Bae, B.-I., Hester, L. D., Dawson, V. L., et al. (2006). Neuroprotection by Pharmacologic Blockade of the GAPDH Death cascade. *Proc. Natl. Acad. Sci.* 103, 3887–3889. doi:10.1073/pnas.0511321103

Herrero-Mendez, A., Almeida, A., Fernández, E., Maestre, C., Moncada, S., and Bolaños, J. P. (2009). The Bioenergetic and Antioxidant Status of Neurons Is Controlled by Continuous Degradation of a Key Glycolytic Enzyme by APC/C-Cdh1. *Nat. Cel Biol* 11, 747–752. doi:10.1038/ncb1881

Hicke, L., and Dunn, R. (2003). Regulation of Membrane Protein Transport by Ubiquitin and Ubiquitin-Binding Proteins. *Annu. Rev. Cel Dev. Biol.* 19, 141–172. doi:10.1146/annurev.cellbio.19.110701.154617

Hirner, S., Krohne, C., Schuster, A., Hoffmann, S., Witt, S., Erber, R., et al. (2008). MuRF1-dependent Regulation of Systemic Carbohydrate Metabolism as Revealed from Transgenic Mouse Studies. *J. Mol. Biol.* 379, 666–677. doi:10.1016/j.jmb.2008.03.049

Hur, M.-W., Yoon, J.-H., Kim, M.-Y., Ko, H., and Jeon, B.-N. (2017). Kr-POK (ZBTB7c) Regulates Cancer Cell Proliferation through Glutamine Metabolism. *Biochim. Biophys. Acta (Bba) - Gene Regul. Mech.* 1860, 829–838. doi:10.1016/j.bbagrm.2017.05.005

Hwang, S., Nguyen, A. D., Jo, Y., Engelking, L. J., Brugarolas, J., and DeBose-Boyd, R. A. (2017). Hypoxia-inducible Factor 1α Activates Insulin-Induced Gene 2 (Insig-2) Transcription for Degradation of 3-Hydroxy-3-Methylglutaryl (HMG)-CoA Reductase in the Liver. *J. Biol. Chem.* 292, 9382–9393. doi:10.1074/jbc.m117.788562

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., et al. (2001). HIFα Targeted for VHL-Mediated Destruction by Proline Hydroxylation: Implications for O 2 Sensing. *Science* 292, 464–468. doi:10.1126/science.1059817

Jaakkola, P., Mole, D. R., Tian, Y.-M., Wilson, M. I., Gielbert, J., Gaskell, S. J., et al. (2001). Targeting of HIF-α to the von Hippel-Lindau Ubiquitylation Complex by O 2 -Regulated Prolyl Hydroxylation. *Science* 292, 468–472. doi:10.1126/science.1059796

Jeon, Y. J., Khelifa, S., Ratnikov, B., Scott, D. A., Feng, Y., Parisi, F., et al. (2015). Regulation of Glutamine Carrier Proteins by RNF5 Determines Breast Cancer Response to ER Stress-Inducing Chemotherapies. *Cancer Cell* 27, 354–369. doi:10.1016/j.ccell.2015.02.006

Jiang, L.-Y., Jiang, W., Tian, N., Xiong, Y.-N., Liu, J., Wei, J., et al. (2018). Ring finger Protein 145 (RNF145) Is a Ubiquitin Ligase for Sterol-Induced Degradation of HMG-CoA Reductase. *J. Biol. Chem.* 293, 4047–4055. doi:10.1074/jbc.ra117.001260

Jiao, L., Zhang, H.-L., Li, D.-D., Yang, K.-L., Tang, J., Li, X., et al. (2018). Regulation of Glycolytic Metabolism by Autophagy in Liver Cancer Involves Selective Autophagic Degradation of HK2 (Hexokinase 2). *Autophagy* 14, 671–684. doi:10.1080/15548627.2017.1381804

Ju, S., Wang, F., Wang, Y., and Ju, S. (2020). CSN8 Is a Key Regulator in Hypoxia-Induced Epithelial-Mesenchymal Transition and Dormancy of Colorectal Cancer Cells. *Mol. Cancer* 19, 168. doi:10.1186/s12943-020-01285-4

Kandasamy, P., Gyimesi, G., Kanai, Y., and Hediger, M. A. (2018). Amino Acid Transporters Revisited: New Views in Health and Disease. *Trends Biochem. Sci.* 43, 752–789. doi:10.1016/j.tibs.2018.05.003

Kim, S.-Y., Choi, J.-S., Park, C., and Jeong, J.-W. (2010). Ethyl Pyruvate Stabilizes Hypoxia-Inducible Factor 1 Alpha via Stimulation of the TCA Cycle. *Cancer Lett.* 295, 236–241. doi:10.1016/j.canlet.2010.03.006

Komander, D., and Rape, M. (2012). The Ubiquitin Code. *Annu. Rev. Biochem.* 81, 203–229. doi:10.1146/annurev-biochem-060310-170328

Kon, N., Kobayashi, Y., Li, M., Brooks, C. L., Ludwig, T., and Gu, W. (2010). Inactivation of HAUSP *In Vivo* Modulates P53 Function. *Oncogene* 29, 1270–1279. doi:10.1038/onc.2009.427

Koo, B. H., Won, M. H., Kim, Y. M., and Ryoo, S. (2021). Arginase II Protein Regulates Parkin-dependent P32 Degradation that Contributes to Ca2+-dependent eNOS Activation in Endothelial Cells. *Cardiovasc. Res.* 1, cvab163. doi:10.1093/cvr/cvab163

Lambhate, S., Bhattacharjee, D., and Jain, N. (2021). APC/C CDH1 ubiquitinates IDH2 contributing to ROS increase in mitosis. *Cellular Signalling* 86, 110087. doi:10.1016/j.cellsig.2021.110087

Lavie, J., De Belvalet, H., Sonon, S., Ion, A. M., Dumon, E., Melser, S., Lacombe, D., Dupuy, J.-W., Lalou, C., and Bénard, G. (2018). Ubiquitin-Dependent Degradation of Mitochondrial Proteins Regulates Energy Metabolism. *Cel Reports* 23, 2852–2863. doi:10.1016/j.celrep.2018.05.013

Lee, D.-E., Yoo, J. E., Kim, J., Kim, S., Kim, S., Lee, H., and Cheong, H. (2020). NEDD4L downregulates autophagy and cell growth by modulating ULK1 and a glutamine transporter. *Cell Death Dis* 11, 38. doi:10.1038/s41419-020-2242-5

Lee, H.-J., Li, C.-F., Ruan, D., He, J., Montal, E. D., Lorenz, S., Girnun, G. D., and Chan, C.-H. (2019). Non-proteolytic ubiquitination of Hexokinase 2 by HectH9 controls tumor metabolism and cancer stem cell expansion. *Nat Commun* 10, 2625. doi:10.1038/s41467-019-10374-y

Lee, Y.-R., Chen, M., and Pandolfi, P. P. (2018). The functions and regulation of the PTEN tumour suppressor: new modes and prospects. *Nat Rev Mol Cel Biol* 19, 547–562. doi:10.1038/s41580-018-0015-0

Lei, M.-Z., Li, X.-X., Zhang, Y., Li, J.-T., Zhang, F., Wang, Y.-P., Yin, M., Qu, J., and Lei, Q.-Y. (2020). Acetylation promotes BCAT2 degradation to suppress BCAA catabolism and pancreatic cancer growth. *Sig Transduct Target. Ther* 5, 70. doi:10.1038/s41392-020-0168-0

Li, C., McManus, F. P., Plutoni, C., Pascariu, C. M., Nelson, T., Alberici Delsin, L. E., Emery, G., and Thibault, P. (2020). Quantitative SUMO proteomics identifies PIAS1 substrates involved in cell migration and motility. *Nat Commun* 11, 834. doi:10.1038/s41467-020-14581-w

Liang, J., Cao, R., Wang, X., Zhang, Y., Wang, P., Gao, H., Li, C., Yang, F., Zeng, R., and Wei, P., (2017). Mitochondrial PKM2 Regulates Oxidative Stress-Induced Apoptosis by Stabilizing Bcl2. *Cell Res* 27, 329–351. doi:10.1038/cr.2016.159

Ling, S., Shan, Q., Zhan, Q., Ye, Q., Liu, P., Xu, S., et al. (2020). USP22 Promotes Hypoxia-Induced Hepatocellular Carcinoma Stemness by a HIF1α/USP22 Positive Feedback Loop upon TP53 Inactivation. *Gut* 69, 1322–1334. doi:10.1136/gutjnl-2019-319616

Liu, C., Zhang, Y., She, X., Fan, L., Li, P., Feng, J., et al. (2018). A Cytoplasmic Long Noncoding RNA LINC00470 as a New AKT Activator to Mediate Glioblastoma Cell Autophagy. *J. Hematol. Oncol.* 11, 77. doi:10.1186/s13045-018-0619-z

Liu, K., Li, F., Han, H., Chen, Y., Mao, Z., Luo, J., et al. (2016). Parkin Regulates the Activity of Pyruvate Kinase M2. *J. Biol. Chem.* 291, 10307–10317. doi:10.1074/jbc.m115.703066

Liu, X., Li, J., Wang, Z., Meng, J., Wang, A., Zhao, X., et al. (2021). KDM2A Targets PFKFB3 for Ubiquitylation to Inhibit the Proliferation and Angiogenesis of Multiple Myeloma Cells. *Front. Oncol.* 11, 653788. doi:10.3389/fonc.2021.653788

Loregger, A., Raaben, M., Tan, J., Scheij, S., Moeton, M., van den Berg, M., et al. (2017). Haploid Mammalian Genetic Screen Identifies UBXD8 as a Key Determinant of HMGCR Degradation and Cholesterol Biosynthesis. *Atvb* 37, 2064–2074. doi:10.1161/atvbaha.117.310002

Mahajan, R., Delphin, C., Guan, T., Gerace, L., and Melchior, F. (1997). A Small Ubiquitin-Related Polypeptide Involved in Targeting RanGAP1 to Nuclear Pore Complex Protein RanBP2. *Cell* 88, 97–107. doi:10.1016/s0092-8674(00)81862-0

MatSUMOto, M. L., Wickliffe, K. E., Dong, K. C., Yu, C., Bosanac, I., Bustos, D., et al. (2010). K11-linked Polyubiquitination in Cell Cycle Control Revealed by a K11 Linkage-specific Antibody. *Mol. Cel.* 39, 477–484. doi:10.1016/j.molcel.2010.07.001

Menzies, S. A., Volkmar, N., van den Boomen, D. J., Timms, R. T., Dickson, A. S., Nathan, J. A., et al. (2018). The Sterol-Responsive RNF145 E3 Ubiquitin Ligase Mediates the Degradation of HMG-CoA Reductase Together with Gp78 and Hrd1. *eLife* 7, e40009. doi:10.7554/eLife.40009

Mo, Y., Wang, Y., Zhang, S., Xiong, F., Yan, Q., Jiang, X., et al. (2021). Circular RNA circRNF13 Inhibits Proliferation and Metastasis of Nasopharyngeal Carcinoma via SUMO2. *Mol. Cancer* 20, 112. doi:10.1186/s12943-021-01409-4

Moreau, P., Attal, M., Caillot, D., Macro, M., Karlin, L., Garderet, L., et al. (2017). Prospective Evaluation of Magnetic Resonance Imaging and [18F] Fluorodeoxyglucose Positron Emission Tomography-Computed Tomography at Diagnosis and before Maintenance Therapy in Symptomatic Patients with Multiple Myeloma Included in the IFM/DFCI 2009 Trial: Results of the IMAJEM Study. *Jco* 35, 2911–2918. doi:10.1200/jco.2017.72.2975

Mullen, A. R., Wheaton, W. W., Jin, E. S., Chen, P.-H., Sullivan, L. B., Cheng, T., et al. (2011). Reductive Carboxylation Supports Growth in Tumour Cells with Defective Mitochondria. *Nature* 481, 385–388. doi:10.1038/nature10642

Nakatsukasa, K., Nishimura, T., Byrne, S. D., Okamoto, M., Takahashi-Nakaguchi, A., Chibana, H., et al. (2015). The Ubiquitin Ligase SCFUcc1 Acts as a Metabolic Switch for the Glyoxylate Cycle. *Mol. Cel.* 59, 22–34. doi:10.1016/j.molcel.2015.04.013

Nguyen, T. V., Li, J., Lu, C.-C., Mamrosh, J. L., Lu, G., Cathers, B. E., et al. (2017). p97/VCP Promotes Degradation of CRBN Substrate Glutamine Synthetase and Neosubstrates. *Proc. Natl. Acad. Sci. USA* 114, 3565–3571. doi:10.1073/pnas.1700949114

Nguyen, T. V. (2021). USP15 Antagonizes CRL4(CRBN)-mediated Ubiquitylation of Glutamine Synthetase and Neosubstrates. *Proc. Natl. Acad. Sci. U S A.* 118, e2111391118. doi:10.1073/pnas.2111391118

Ni, W., Lin, S., Bian, S., Zheng, W., Qu, L., Fan, Y., et al. (2020). USP7 Mediates Pathological Hepatic De Novo Lipogenesis through Promoting Stabilization and Transcription of ZNF638. *Cel Death Dis* 11, 843. doi:10.1038/s41419-020-03075-8

Ni, Y., Yang, Z., Agbana, Y. L., Bai, H., Wang, L., Yang, L., et al. (2021). Silent Information Regulator 2 Promotes clear Cell Renal Cell Carcinoma Progression through Deacetylation and Small Ubiquitin-related Modifier 1 Modification of Glucose 6-phosphate Dehydrogenase. *Cancer Sci.* 112, 4075–4086. doi:10.1111/cas.15085

Okatsu, K., Iemura, S.-i., Koyano, F., Go, E., Kimura, M., Natsume, T., et al. (2012). Mitochondrial Hexokinase HKI Is a Novel Substrate of the Parkin Ubiquitin Ligase. *Biochem. Biophysical Res. Commun.* 428, 197–202. doi:10.1016/j.bbrc.2012.10.041

Okura, T., Gong, L., Kamitani, T., Wada, T., Okura, I., Wei, C. F., et al. (1996). Protection against Fas/APO-1- and Tumor Necrosis Factor-Mediated Cell Death by a Novel Protein, Sentrin. *J. Immunol.* 157, 4277–4281.

Osborne, T. F. (1991). Single Nucleotide Resolution of Sterol Regulatory Region in Promoter for 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase. *J. Biol. Chem.* 266, 13947–13951. doi:10.1016/s0021-9258(18)92794-8

Pandey, D., Nomura, Y., Rossberg, M. C., Hori, D., Bhatta, A., Keceli, G., et al. (2018). Hypoxia Triggers SENP1 (Sentrin-specific Protease 1) Modulation of KLF15 (Kruppel-like Factor 15) and Transcriptional Regulation of Arg2 (Arginase 2) in Pulmonary Endothelium. *Atvb* 38, 913–926. doi:10.1161/atvbaha.117.310660

Park, J. S., Burckhardt, C. J., Lazcano, R., Solis, L. M., Isogai, T., Li, L., et al. (2020). Mechanical Regulation of Glycolysis via Cytoskeleton Architecture. *Nature* 578, 621–626. doi:10.1038/s41586-020-1998-1

Peng, M., Yang, D., Hou, Y., Liu, S., Zhao, M., Qin, Y., et al. (2019). Intracellular Citrate Accumulation by Oxidized ATM-Mediated Metabolism Reprogramming via PFKP and CS Enhances Hypoxic Breast Cancer Cell Invasion and Metastasis. *Cel Death Dis* 10, 228. doi:10.1038/s41419-019-1475-7

Popovic, D., Vucic, D., and Dikic, I. (2014). Ubiquitination in Disease Pathogenesis and Treatment. *Nat. Med.* 20, 1242–1253. doi:10.1038/nm.3739

Qi, L., Heredia, J. E., Altarejos, J. Y., Screaton, R., Goebel, N., Niessen, S., et al. (2006). TRB3 Links the E3 Ubiquitin Ligase COP1 to Lipid Metabolism. *Science* 312, 1763–1766. doi:10.1126/science.1123374

Rabl, J., Bunker, R. D., Schenk, A. D., Cavadini, S., Gill, M. E., Abdulrahman, W., et al. (2019). Structural Basis of BRCC36 Function in DNA Repair and

Immune Regulation. *Mol. Cel.* 75, 483–497. e489. doi:10.1016/j.molcel.2019.06.002

Rape, M. (2018). Ubiquitylation at the Crossroads of Development and Disease. *Nat. Rev. Mol. Cel Biol* 19, 59–70. doi:10.1038/nrm.2017.83

Riehl, J., Rijal, R., Nitz, L., Clemen, C. S., Hofmann, A., and Eichinger, L. (2021). Domain Organization of the UBX Domain Containing Protein 9 and Analysis of its Interactions with the Homohexameric AAA + ATPase P97 (Valosin-Containing Protein). *Front. Cel Dev. Biol.* 9, 748860. doi:10.3389/fcell.2021.748860

Rodriguez-Rodriguez, P., Fernandez, E., Almeida, A., and Bolaños, J. P. (2012). Excitotoxic Stimulus Stabilizes PFKFB3 Causing Pentose-Phosphate Pathway to Glycolysis Switch and Neurodegeneration. *Cel Death Differ* 19, 1582–1589. doi:10.1038/cdd.2012.33

Selak, M. A., Armour, S. M., MacKenzie, E. D., Boulahbel, H., Watson, D. G., Mansfield, K. D., et al. (2005). Succinate Links TCA Cycle Dysfunction to Oncogenesis by Inhibiting HIF-α Prolyl Hydroxylase. *Cancer Cell* 7, 77–85. doi:10.1016/j.ccr.2004.11.022

Semenza, G. L. (2003). Targeting HIF-1 for Cancer Therapy. *Nat. Rev. Cancer* 3, 721–732. doi:10.1038/nrc1187

Shangguan, X., He, J., Ma, Z., Zhang, W., Ji, Y., Shen, K., et al. (2021). SUMOylation Controls the Binding of Hexokinase 2 to Mitochondria and Protects against Prostate Cancer Tumorigenesis. *Nat. Commun.* 12, 1812. doi:10.1038/s41467-021-22163-7

Song, G., Liu, B., Li, Z., Wu, H., Wang, P., Zhao, K., et al. (2016). E3 Ubiquitin Ligase RNF128 Promotes Innate Antiviral Immunity through K63-Linked Ubiquitination of TBK1. *Nat. Immunol.* 17, 1342–1351. doi:10.1038/ni.3588

Song, W., Wang, Z., Gu, X., Wang, A., Chen, X., Miao, H., et al. (2019). TRIM11 Promotes Proliferation and Glycolysis of Breast Cancer Cells via Targeting AKT/GLUT1 Pathway. *Ott* 12, 4975–4984. doi:10.2147/ott.s207723

Song, G., Zhang, Y., Tian, J., Ma, J., Yin, K., Xu, H., et al. (2021). TRAF6 Regulates the Immunosuppressive Effects of Myeloid-Derived Suppressor Cells in Tumor-Bearing Host. *Front. Immunol.* 12, 649020. doi:10.3389/fimmu.2021.649020

Sun, L., and Fang, J. (2016). E3-Independent Constitutive Monoubiquitination Complements Histone Methyltransferase Activity of SETDB1. *Mol. Cel.* 62, 958–966. doi:10.1016/j.molcel.2016.04.022

Sun, X., Wang, M., Wang, M., Yu, X., Guo, J., Sun, T., et al. (2020). Metabolic Reprogramming in Triple-Negative Breast Cancer. *Front. Oncol.* 10, 428. doi:10.3389/fonc.2020.00428

Sundqvist, A., Bengoechea-Alonso, M. T., Ye, X., Lukiyanchuk, V., Jin, J., Harper, J. W., et al. (2005). Control of Lipid Metabolism by Phosphorylation-dependent Degradation of the SREBP Family of Transcription Factors by SCFFbw7. *Cel Metab.* 1, 379–391. doi:10.1016/j.cmet.2005.04.010

Tan, J. M. E., van der Stoel, M. M., van den Berg, M., van Loon, N. M., Moeton, M., Scholl, E., et al. (2020). The MARCH6-SQLE Axis Controls Endothelial Cholesterol Homeostasis and Angiogenic Sprouting. *Cel Rep.* 32, 107944. doi:10.1016/j.celrep.2020.107944

Tian, M., Hao, F., Jin, X., Sun, X., Jiang, Y., Wang, Y., et al. (2021). ACLY Ubiquitination by CUL3-KLHL25 Induces the Reprogramming of Fatty Acid Metabolism to Facilitate iTreg Differentiation. *eLife* 10, e62394. doi:10.7554/eLife.62394

Tomek, K., Wagner, R., Varga, F., Singer, C. F., Karlic, H., and Grunt, T. W. (2011). Blockade of Fatty Acid Synthase Induces Ubiquitination and Degradation of Phosphoinositide-3-Kinase Signaling Proteins in Ovarian Cancer. *Mol. Cancer Res.* 9, 1767–1779. doi:10.1158/1541-7786.mcr-10-0467

Tu, K., Zheng, X., Yin, G., Zan, X., Yao, Y., and Liu, Q. (2012). Evaluation of Fbxw7 Expression and its Correlation with Expression of SREBP-1 in a Mouse Model of NAFLD. *Mol. Med. Rep.* 6, 525–530. doi:10.3892/mmr.2012.953

Van Nguyen, T., Lee, J. E., Sweredoski, M. J., Yang, S.-J., Jeon, S.-J., Harrison, J. S., et al. (2016). Glutamine Triggers Acetylation-dependent Degradation of Glutamine Synthetase via the Thalidomide Receptor Cereblon. *Mol. Cel.* 61, 809–820. doi:10.1016/j.molcel.2016.02.032

Varshavsky, A. (2017). The Ubiquitin System, Autophagy, and Regulated Protein Degradation. *Annu. Rev. Biochem.* 86, 123–128. doi:10.1146/annurev-biochem-061516-044859

Wade, A. K., Liu, Y., Bethea, M. M., Toren, E., Tse, H. M., and Hunter, C. S. (2019). LIM-domain Transcription Complexes Interact with Ring-finger Ubiquitin Ligases and Thereby Impact Islet β-cell Function. *J. Biol. Chem.* 294, 11728–11740. doi:10.1074/jbc.ra118.006985

Walker, A. K., Yang, F., Jiang, K., Ji, J.-Y., Watts, J. L., Purushotham, A., et al. (2010). Conserved Role of SIRT1 Orthologs in Fasting-dependent Inhibition of the Lipid/cholesterol Regulator SREBP. *Genes Dev.* 24, 1403–1417. doi:10.1101/gad.1901210

Wang, C., Xiao, Y., Lao, M., Wang, J., Xu, S., Li, R., et al. (2020b). Increased SUMO-Activating Enzyme SAE1/UBA2 Promotes Glycolysis and Pathogenic Behavior of Rheumatoid Fibroblast-like Synoviocytes. *JCI Insight* 5, 1. doi:10.1172/jci.insight.135935

Wang, C., Chao, Y., Xu, W., Liu, Z., Wang, H., and Huang, K. (2020a). Myeloid FBW7 Deficiency Disrupts Redox Homeostasis and Aggravates Dietary-Induced Insulin Resistance. *Redox Biol.* 37, 101688. doi:10.1016/j.redox.2020.101688

Wang, F., Sun, F., Luo, J., Yue, T., Chen, L., Zhou, H., et al. (2019a). Loss of Ubiquitin-Conjugating Enzyme E2 (Ubc9) in Macrophages Exacerbates Multiple Low-Dose Streptozotocin-Induced Diabetes by Attenuating M2 Macrophage Polarization. *Cel Death Dis* 10, 892. doi:10.1038/s41419-019-2130-z

Wang, M., Hu, J., Yan, L., Yang, Y., He, M., Wu, M., et al. (2019b). High Glucose-Induced Ubiquitination of G6PD Leads to the Injury of Podocytes. *FASEB j.* 33, 6296–6310. doi:10.1096/fj.201801921r

Wang, S., Ramamurthy, D., Tan, J., Liu, J., Yip, J., Chua, A., et al. (2020c). Post-translational Modifications of Fumarase Regulate its Enzyme Activity and Function in Respiration and the DNA Damage Response. *J. Mol. Biol.* 432, 6108–6126. doi:10.1016/j.jmb.2020.09.021

Wang, T., Cao, Y., Zheng, Q., Tu, J., Zhou, W., He, J., et al. (2019c). SENP1-Sirt3 Signaling Controls Mitochondrial Protein Acetylation and Metabolism. *Mol. Cel.* 75, 823–834. e825. doi:10.1016/j.molcel.2019.06.008

Wei, W., and Lin, H.-K. (2012). The Key Role of Ubiquitination and SUMOylation in Signaling and Cancer: a Research Topic. *Front. Oncol.* 2, 187. doi:10.3389/fonc.2012.00187

Wei, Z., Song, J., Wang, G., Cui, X., Zheng, J., Tang, Y., et al. (2018). Deacetylation of Serine Hydroxymethyl-Transferase 2 by SIRT3 Promotes Colorectal Carcinogenesis. *Nat. Commun.* 9, 4468. doi:10.1038/s41467-018-06812-y

Weidemann, A., and Johnson, R. S. (2008). Biology of HIF-1α. *Cel Death Differ* 15, 621–627. doi:10.1038/cdd.2008.12

Xia, L., Jiang, Y., Zhang, X.-H., Wang, X.-R., Wei, R., Qin, K., et al. (2021). SUMOylation Disassembles the Tetrameric Pyruvate Kinase M2 to Block Myeloid Differentiation of Leukemia Cells. *Cel Death Dis* 12, 101. doi:10.1038/s41419-021-03400-9

Xie, P., Peng, Z., Chen, Y., Li, H., Du, M., Tan, Y., et al. (2021). Neddylation of PTEN Regulates its Nuclear Import and Promotes Tumor Development. *Cel Res* 31, 291–311. doi:10.1038/s41422-020-00443-z

Xu, L., Huan, L., Guo, T., Wu, Y., Liu, Y., Wang, Q., et al. (2020). LncRNA SNHG11 Facilitates Tumor Metastasis by Interacting with and Stabilizing HIF-1α. *Oncogene* 39, 7005–7018. doi:10.1038/s41388-020-01512-8

Yang, F., Zhang, H., Mei, Y., and Wu, M. (2014). Reciprocal Regulation of HIF-1α and LincRNA-P21 Modulates the Warburg Effect. *Mol. Cel.* 53, 88–100. doi:10.1016/j.molcel.2013.11.004

Yang, Z., Hu, N., Wang, W., Hu, W., Zhou, S., Shi, J., et al. (2021). Loss of FBXW7 Correlates with Increased IDH1 Expression in Glioma and Enhances IDH1-Mutant Cancer Cell Sensitivity to Radiation. *Cancer Res.* 82 (3), 497–509. doi:10.1158/0008-5472.can-21-0384

Yau, R., and Rape, M. (2016). The Increasing Complexity of the Ubiquitin Code. *Nat. Cel Biol* 18, 579–586. doi:10.1038/ncb3358

Ye, S., Xu, H., Jin, J., Yang, M., Wang, C., Yu, Y., et al. (2012). The E3 Ubiquitin Ligase Neuregulin Receptor Degradation Protein 1 (Nrdp1) Promotes M2 Macrophage Polarization by Ubiquitinating and Activating Transcription Factor CCAAT/Enhancer-Binding Protein β (C/EBPβ). *J. Biol. Chem.* 287 (32), 26740–26748. doi:10.1074/jbc.M112.383265

Yeh, E. T. H. (2009). SUMOylation and De-SUMOylation: Wrestling with Life's Processes. *J. Biol. Chem.* 284, 8223–8227. doi:10.1074/jbc.r800050200

Yoshizawa, T., Karim, M. F., Sato, Y., Senokuchi, T., Miyata, K., Fukuda, T., et al. (2014). SIRT7 Controls Hepatic Lipid Metabolism by Regulating the Ubiquitin-Proteasome Pathway. *Cel Metab.* 19, 712–721. doi:10.1016/j.cmet.2014.03.006

Yu, J., Deng, R., Zhu, H. H., Zhang, S. S., Zhu, C., Montminy, M., et al. (2013). Modulation of Fatty Acid Synthase Degradation by Concerted Action of P38 MAP Kinase, E3 Ligase COP1, and SH2-Tyrosine Phosphatase Shp2. *J. Biol. Chem.* 288, 3823–3830. doi:10.1074/jbc.m112.397885

Yu, S., Zang, W., Qiu, Y., Liao, L., and Zheng, X. (2021). Deubiquitinase OTUB2 Exacerbates the Progression of Colorectal Cancer by Promoting PKM2 Activity and Glycolysis. *Oncogene* 41 (1), 46–56. doi:10.1038/s41388-021-02071-2

Zelcer, N., Hong, C., Boyadjian, R., and Tontonoz, P. (2009). LXR Regulates Cholesterol Uptake through Idol-dependent Ubiquitination of the LDL Receptor. *Science* 325, 100–104. doi:10.1126/science.1168974

Zhang, G., Zhu, Q., Fu, G., Hou, J., Hu, X., Cao, J., et al. (2019). TRIP13 Promotes the Cell Proliferation, Migration and Invasion of Glioblastoma through the FBXW7/c-MYC axis. *Br. J. Cancer* 121, 1069–1078. doi:10.1038/s41416-019-0633-0

Zhao, X., Feng, D., Wang, Q., Abdulla, A., Xie, X.-J., Zhou, J., et al. (2012). Regulation of Lipogenesis by Cyclin-dependent Kinase 8-mediated Control of SREBP-1. *J. Clin. Invest.* 122, 2417–2427. doi:10.1172/jci61462

Zhao, Y., He, J., Li, Y., Lv, S., and Cui, H. (2020). NUSAP1 Potentiates Chemoresistance in Glioblastoma through its SAP Domain to Stabilize ATR. *Sig Transduct Target. Ther.* 5, 44. doi:10.1038/s41392-020-0137-7

Zhu, S., Dong, Z., Ke, X., Hou, J., Zhao, E., Zhang, K., et al. (2019). The Roles of Sirtuins Family in Cell Metabolism during Tumor Development. *Semin. Cancer Biol.* 57, 59–71. doi:10.1016/j.semcancer.2018.11.003

Zong, Y., Li, Q., Zhang, F., Xian, X., Wang, S., Xia, J., et al. (2019). SDH5 Depletion Enhances Radiosensitivity by Regulating P53: A New Method for Noninvasive Prediction of Radiotherapy Response. *Theranostics* 9, 6380–6395. doi:10.7150/thno.34443

Conflict of Interest: 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Cell and Developmental Biology

REVIEW
published: 04 April 2022
doi: 10.3389/fcell.2022.875124

USP13: Multiple Functions and Target Inhibition

*Xiaolong Li[1], Ge Yang[1], Wenyao Zhang[1], Biying Qin[1], Zifan Ye[1], Huijing Shi[1], Xinmeng Zhao[1], Yihang Chen[1], Bowei Song[1], Ziqing Mei[2], Qi Zhao[3] and Feng Wang[1]**

[1]Key Laboratory of Molecular Medicine and Biotherapy, Department of Biology, School of Life Science, Beijing Institute of Technology, Beijing, China, [2]School of Chemistry and Biological Engineering, University of Science and Technology Beijing, Beijing, China, [3]University of Macau, China

As a deubiquitination (DUB) enzyme, ubiquitin-specific protease 13 (USP13) is involved in a myriad of cellular processes, such as mitochondrial energy metabolism, autophagy, DNA damage response, and endoplasmic reticulum-associated degradation (ERAD), by regulating the deubiquitination of diverse key substrate proteins. Thus, dysregulation of USP13 can give rise to the occurrence and development of plenty of diseases, in particular malignant tumors. Given its implications in the stabilization of disease-related proteins and oncology targets, considerable efforts have been committed to the discovery of inhibitors targeting USP13. Here, we summarize an overview of the recent advances of the structure, function of USP13, and its relations to diseases, as well as discovery and development of inhibitors, aiming to provide the theoretical basis for investigation of the molecular mechanism of USP13 action and further development of more potent druggable inhibitors.

Keywords: deubiquitination, ubiquitin-specific protease 13, structure, disease, inhibitor

OPEN ACCESS

Edited by:
Xiaohua Yan,
Nanchang University, China

Reviewed by:
Tao Wan,
University of Nebraska System, United States
Shangyu Dang,
Hong Kong University of Science and Technology, Hong Kong SAR, China

****Correspondence:***
Feng Wang
wfeng@bit.edu.cn

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *13 February 2022*
Accepted: *08 March 2022*
Published: *04 April 2022*

Citation:
Li X, Yang G, Zhang W, Qin B, Ye Z, Shi H, Zhao X, Chen Y, Song B, Mei Z, Zhao Q and Wang F (2022) USP13: Multiple Functions and Target Inhibition. Front. Cell Dev. Biol. 10:875124. doi: 10.3389/fcell.2022.875124

INTRODUCTION

Ubiquitination, as a crucial post-translational modification in eukaryotic cells, is involved in various cellular activities, including DNA damage repair (DDR), cell signal transduction, cell cycle regulation, and innate immune signaling pathways (Harrigan et al., 2018; Ciechanover, 2003; Ravid and Hochstrasser, 2008; Luan et al., 2016). In the process of ubiquitination, the ubiquitin (Ub) molecule is covalently attached to substrate proteins (or ubiquitin itself) through isopeptide bonds or peptide bonds by the E1-E2-E3 ligase cascade (or LUBAC complex) (Ciechanover, 2003; Dittmar and Winklhofer, 2019). Like other post-translational modifications, ubiquitination is reversible, and its reverse process, deubiquitination, is catalyzed by DUBs(Dandrea and Pellman, 1998) (**Figure 1**). DUBs can remove ubiquitins from substrate proteins (or poly-ubiquitin chains), edit ubiquitin chains and process ubiquitin precursors (Komander et al., 2009). These two processes coordinate to accurately maintain the proteostasis and ubiquitin balance in quantity.

To date, seven structurally distinct DUB families have been described, including ubiquitin-specific proteases (USPs), ovarian tumor proteases (OTUs), ubiquitin C-terminal hydrolase (UCHs), Machado–Josephin domain-containing proteases (MJDs), motifs interacting with the ubiquitin-containing novel DUB family (MINDYs), JAB1, MPN, MOV34 family (JAMMs), and zinc finger containing Ub peptidase 1 (ZUP1) (Kwasna et al., 2018; Cho et al., 2020; Wang and Wang, 2021). JAMMs are zinc metallopeptidases, while the other six DUB families are cysteine peptidases. The USPs family has the largest number of members with diverse functions, providing the potential for developing drugs with more specific effects (Sippl et al., 2011; Yuan et al., 2018; Cruz et al., 2021).

USP13, belonging to the USPs family, is known to be extensively engaged in diverse cellular processes, such as mitochondrial energy metabolism, autophagy, DNA damage response, ERAD and

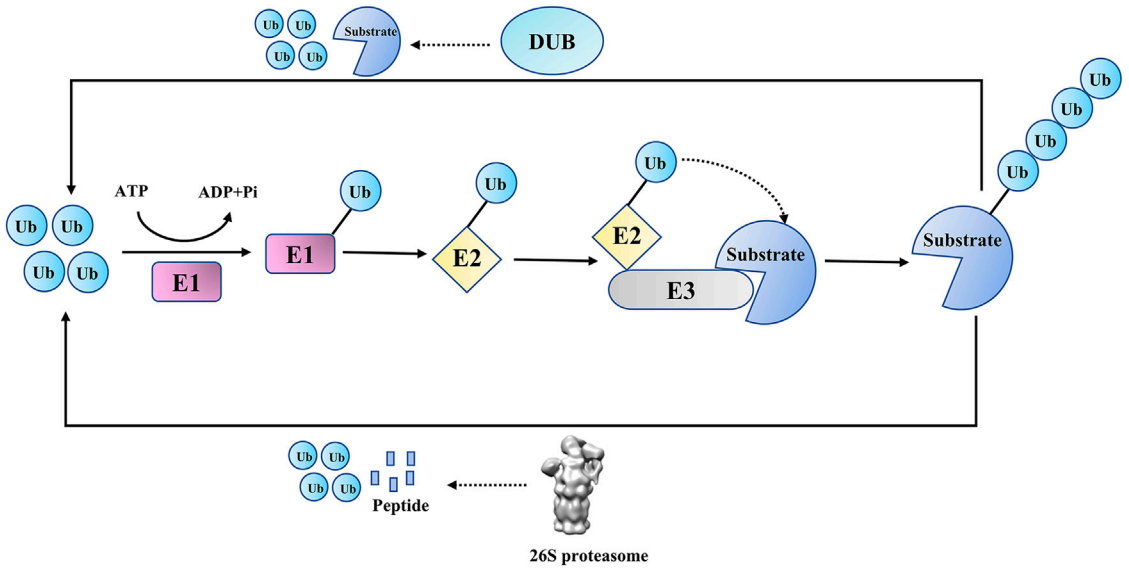


FIGURE 1 | Ubiquitin proteasome pathway and deubiquitination.

other processes, by deubiquitinating substrates α-ketoglutarate dehydrogenase (OGDH) (Han et al., 2016), ATP citrate lyase (ACLY) (Han et al., 2016), vacuolar protein sorting 34 (VPS34) (Xie et al., 2020), topoisomerase IIβ binding protein 1 (TopBP1) (Kim et al., 2021), receptor-associated protein 80 (RAP80) (Li et al., 2017), and ubiquitin like 4A (UBL4A) (Liu et al., 2014). Ample findings prove that USP13 may also promote the initiation or progression of various tumors. For example, the stabilization of microphthalmia-associated transcription factor (MITF) by USP13 was found to be associated with proliferation of melanoma cells (Zhao et al., 2011); USP13 is abnormally overexpressed in ovarian cancer (OVCA) and drives OVCA metabolism to accelerate cell proliferation through deubiquitinating ACLY and OGDH (Han et al., 2016); in glioblastoma, USP13 promotes the proliferation of glioma stem cells (GSCs) by antagonizing E3 ubiquitin ligase F-box and leucine-rich repeat protein 14 (FBXL14), which inhibits the ubiquitination and degradation of pro-oncogene c-Myc (Fang et al., 2017); in non-small-cell lung cancer (NSCLC), downregulation of USP13 impedes the growth of NSCLC model cells A549 and H226 via suppressing AKT/MAPK signaling pathway (Wu et al., 2019); in colorectal tumor cells, USP13 has been identified as a microRNA-135b24 target that promotes colorectal tumor cell proliferation and glycolysis (Zhang et al., 2013).

Incompatible with the above findings, the recombinant expression of USP13 exhibits only weak deubiquitination enzyme activity *in vitro* (Liu et al., 2011; Zhang et al., 2011). To decipher its activation mechanism for interpretating the paradoxical phenomena, determining USP13 structure has attracted considerable interest over the past few years. Albeit the structures of full-length USP13, as well as its catalytic structural domain have not been obtained, structures of several functional domains are determined (Liu et al., 2011; Zhang et al., 2011; Liu et al., 2014; Han et al., 2016). Given the implications of USP13 in tumorigenesis, seeking compounds that modulate the USP13 emerges an active area of research and achieves impressive progress, with multiple selective compounds being identified successively by both research institutions and pharmaceutical companies (Liu et al., 2011; Liu et al., 2021a).

In this review, we discuss recent advances in our understanding of the physiological roles, structure, and USP13-related diseases. In addition, the appealing stories regarding a range of representative small-molecule inhibitors are listed to help track their evolution.

Structure and Activation Mechanism of USP13

The *usp13* gene is located on human chromosome 3q26.2–q26.3, which encodes USP13, also known as isopeptidase T-3 (Zhang et al., 2011; Timms et al., 1998). USP13 was first identified by Timms *et al.* and consisted of 863 amino acids (Timms et al., 1998). USP13 shares approximately 80% sequence similarity with USP5(Zhang et al., 2011). They have the same domain architecture, including the N-terminal domain, Zinc finger (ZnF) domain (amino acids 209–281), and USP catalytic domain (amino acids 336–861), between the C-box and H-box (including a two-UBA insertion) (Zhang et al., 2011; Ning et al., 2020) (**Figures 2A,D**). The N-terminal residues of USP13 might be essential for physical interaction with other proteins, which could be exemplified by interaction of the N-terminus of USP13 with myeloid cell leukemia sequence 1 (MCL1), a core member of the anti-apoptotic B cell lymphoma 2 (BCL-2) family of proteins (Zhang et al., 2018). As the ZnF domain is generally considered to be a ubiquitin binding site, USP5-ZnF recognizes the C-terminal glycine motif of free Ub chains and activates deubiquitination, while USP13-ZnF domain is unable to bind Ub, although the

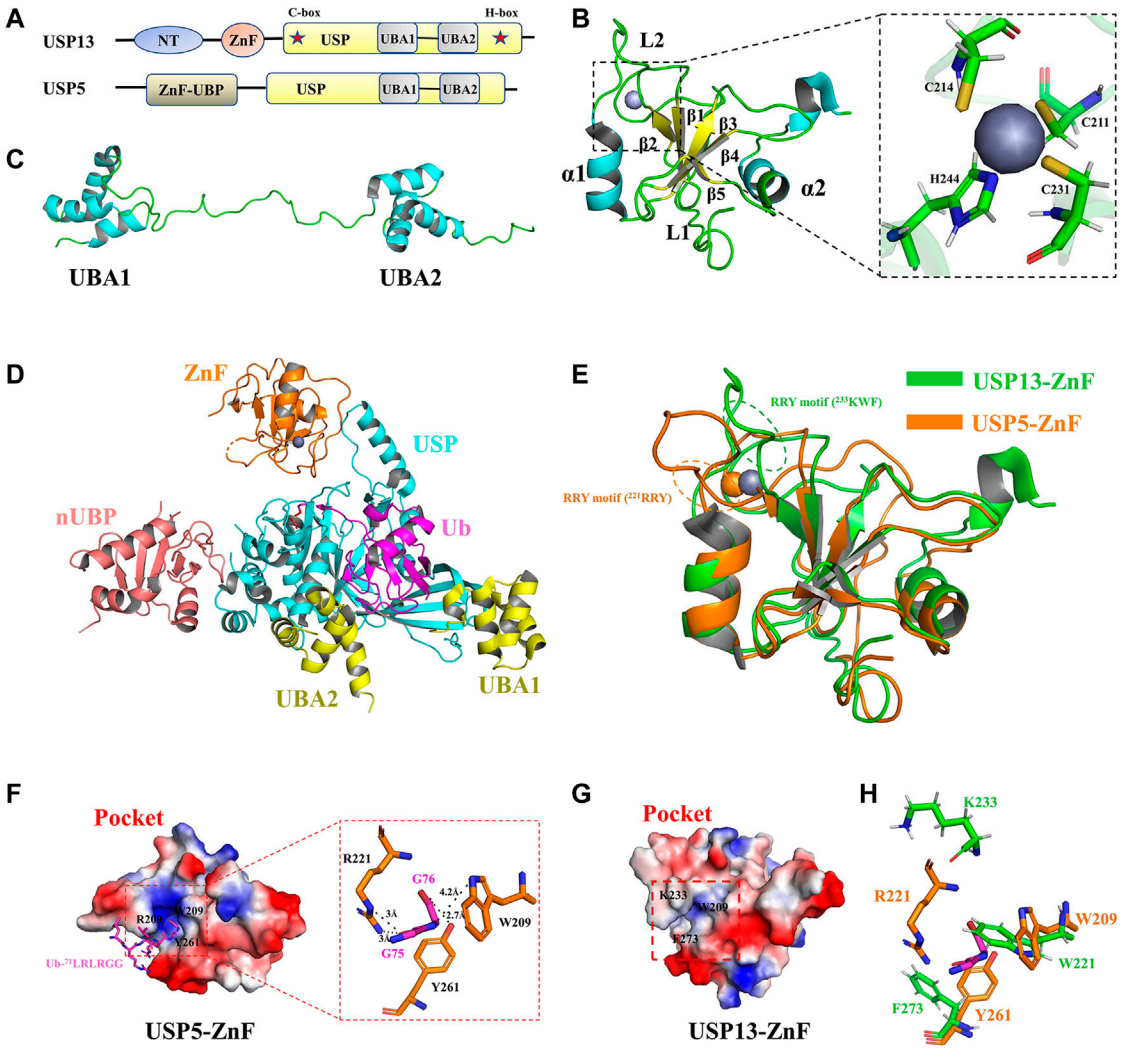


FIGURE 2 | Structure of USP13 and comparison with USP5-ZnF. **(A)** Domain structure of USP13 and USP5. **(B)** Structure of USP13-ZnF (PDB 2L80), in which the α helix is blue, the β sheet is yellow, the loop is green, and the zinc ion is gray. The close-up view shows that the zinc nucleus coordinates with the peptide chain in C3H mode. **(C)** Structure of USP13-UBA (PDB 2LBC), in which the α helix is blue and the loop is green. **(D)** Structure of ubiquitin-USP5 complex (3IHP). nUBP is pink, ZnF is orange, the USP catalytic domain is blue and UBA12 is yellow. Ubiquitin is purple. **(E)** Comparison of the structure between USP13-ZnF and USP5-ZnF (PDB 2G43), USP13-ZnF and USP5-ZnF are green and orange, respectively. **(F)** Electrostatic surface of the Ubiquitin-USP5-ZnF complex (PDB 2G45). Ubiquitin glycine motif (^{71}LRLRGG, purple) is inserted into the ubiquitin binding pocket of USP5-ZNF, and it can be seen from the close-up view that ubiquitin G75 and G76 form hydrogen bonds interact with W209, R221, and Y261 on ZnF. **(H)** Electrostatic surface of the USP13-ZnF. **(G)** The residues of the combined Ub-G75 and Ub-G76 on USP5 (orange) are displayed in sticks, and the corresponding residues in USP13 (green) are displayed in sticks.

sequences of the ZnF domains from these two USPs are homologous (Zhang et al., 2011; Reyes-Turcu et al., 2006) (**Figure 2B**, **Supplementary Figure S1**). The USP13 catalytic domain contains a conserved C-box and H-box including a two-UBA insertion (Timms et al., 1998; Zhang et al., 2011). Although the experimental results showed that USP13-UBA could bind ubiquitin, USP13 still exhibited only weak deubiquitination enzyme activity, which is incompatible with the findings that USP13 can deubiquitinate various important substrates implicated in disease and tumor development (Liu et al., 2011; Zhang et al., 2011). It is assumed that USP13 is constitutively in a state of self-inhibition, whereas it can be activated when it is modified or interacts with other proteins. However, the structure of USP13 catalytic domain has not been available until now, at large impeding interpretation of its active mechanism at the atomic level.

Fortunately, the USP13-ZnF domain and the tandem UBA domain have been obtained using NMR (Zhang et al., 2011). In 2011, Hu et al. reported the solution structure of USP13-ZnF (PDB 2L80). As shown in **Figure 2B**, USP13-ZnF contains only one zinc nucleus that coordinates with the peptide chain in C3H mode. The USP13-ZnF domain consists of five anti-parallel β sheets and two α helices located on both sides with a flexible loop connecting them (named Loop 2, L2). In contrast with

USP13-ZnF domain, USP5-ZnF binds Ub with a comparatively high affinity, echoing the distinct structures from two ZnF domains (Reyes-Turcu et al., 2006). Structural comparison revealed no significant difference (RMSD = 1.47) between USP13-ZnF and USP5-ZnF, but there were some slight differences between the two USPs (**Figure 2E**). Firstly, USP13-ZnF and USP5-ZnF bind Ub with distinct pockets: the pocket USP13-ZnF appears shallower and harbors few positive charges than that of USP5-ZnF, which is not conducive to the binding of Ub glycine (**Figures 2F,G**); the L2 regions in the two ZnF domains are moderately different, and Arg-Arg-Try motif (RRY motif) in L2 regions are likely associated with ubiquitin binding (**Figure 2E**). In addition, W209, R221 and Y261 on USP5 form hydrogen bonds with Ub-75G and Ub-76G, which are key residues for Ub binding (**Figure 2F**). However, it is speculated from the structural alignment (**Figure 2H**, **Supplementary Figure S1**) that W221, K233, and F273 corresponding to USP13 are not completely conservative, especially since K233 is offset from the pocket (K233 at L2 region) (Zhang et al., 2011). In conclusion, USP13-ZnF failed to bind Ub for two reasons: one is distribution of the binding pocket charge; and the other is change of conserved ubiquitin-binding residues.

Both USP13 and USP5 contain tandem UBA domains inserted between the C-box and H-box, and UBA contains the very conserved Ub binding motif Met-Gly-Phe (MGF) (Timms et al., 1998; Raasi et al., 2005). In 2011, Hu et al. reported the solution structure of USP13-UBA (PDB 2LBC). Structural analysis demonstrated that UBA consists of three α-helices, and there was no direct interaction between the two UBAs, which were linked by a long loop (**Figure 2C**). Albeit no ubiquitin-bound UBA structure is resolved, sequence alignment revealed that USP13-UBA contains an MGF motif that presumably can bind Ub (**Supplementary Figure S1**), consistent with results from pull-down and ITC experiments (Zhang et al., 2011). In addition, NMR titration data reflected that M664, F666, M739, and F741 might be the key residues responsible for the binding of USP13-UBA to Ub (Zhang et al., 2011). Except for binding Ub, USP13-UBA2 has also been reported to be required for binding other proteins, such as the E3 ubiquitin ligase glycoprotein 78 (gp78) (Liu et al., 2014).

In spite of the high similarity between USP13 and USP5 both in sequence and domains structures, USP13 recombinant protein exhibits weak deubiquitination activity *in vitro*, dramatically different from its homolog USP5 with high activity both *in vivo* and *in vitro*. In the Ubiquitin-7-amido-4-methylcoumarin (Ub-AMC) hydrolysis experiment, USP5 showed high deubiquitination activity at 1.5 nM, while USP13 only has displayed extremely weak activity until protein concentration increased to 500 nM(Zhang et al., 2011). In the ubiquitin chain hydrolysis experiment, USP5 can hydrolyze into anchored ubiquitin chains one by one from the near end until all ubiquitin chains are cleaved into single ubiquitin chains, and all polyubiquitin chains, Lys-48 and Lys-63 linear ubiquitin chains, can be recognized and cleaved by USP5 (Amerik Ayu et al., 1997; Reyes-Turcu et al., 2006; Reyes-Turcu et al., 2008). However, the experiment proved that USP13 has no hydrolytic activity to Lys-48 and Lys-63 chain diubiquitin but can slowly hydrolyze Lys-63 chain tetraubiquitin to triubiquitin and monoubiquitin (Zhang et al., 2011). Overall, the USP13-ZnF domain cannot bind to Ub to activate USP13, whereas USP13-UBA can bind, which may partially explain the reason why USP13 displays only weak basal deubiquitination enzyme activity: the binding sites of USP13 to Ub are less than that other USP members, thus providing weaker binding affinity and consequent cleavage activity towards ubiquitin chains; There possibly exists constitutive self-inhibition for full-length USP13 supported by the interaction of UBA with ZnF domain, which is hypothesized to be released by recruitment of other proteins or modification, such as phosphorylation. However, no relevant research progress is reported to verify this hypothesis at present.

CELLULAR FUNCTION OF USP13

USP13 in Energy Metabolism

The tricarboxylic acid cycle is the core pathway of energy metabolism and the hub of carbohydrate, lipid and amino acid metabolism, providing precursor molecules for the synthesis of various lipids, non-essential amino acids and nucleotides (Akram, 2014; Han et al., 2016; Salway, 2018). Studies have demonstrated that USP13 can regulate the cellular levels of two key proteins involved in mitochondrial energy metabolism (Han et al., 2016). In normal cells, glucose is converted to acetyl-CoA, entering into the tricarboxylic acid cycle and further generating citric acid (Gameiro and Bell, 2011; Gameiro et al., 2013). Part of citric acid is transported to the cytoplasm, where it is converted to acetyl-CoA by ACLY and eventually supplied for lipid synthesis (Hatzivassiliou et al., 2005). However, in most cases, glutamate intake in tumor cells would markedly ascend in order to provide more intermediates of the tricarboxylic acid cycle, maintaining lipid synthesis (Han et al., 2016). Glutamic acid is converted to α-ketoglutarate by glutaminase and glutamate dehydrogenase, entering into the tricarboxylic acid cycle. Subsequently, α-ketoglutarate is oxidized to succinic acid by OGDH to ensure smooth operation of the tricarboxylic acid cycle (Sun and Denko, 2014; Han et al., 2016) (**Figure 3A**).

Phosphatidylinositol-3-kinase (PI3K)/AKT is a well-recognized signaling pathway related to energy metabolism, and USP13 knockdown is demonstrated to enhance the sensitivity of OVCA cells to AKT inhibitors, implying the role of USP13 in PI3K/AKT-dependent energy metabolism (Hanrahan et al., 2012; Han et al., 2016; Xie et al., 2019). Consistently, ACLY and OGDH were identified as USP13 interacting proteins utilizing mass spectrometry (Han et al., 2016). Meanwhile, protein-binding assay and deubiquitylation experiments demonstrated that USP13 could interact with OGDH or ACLY through N-terminal domain or C-terminal domain, respectively, to remove K48-linked ubiquitin chains from ACLY and OGDH, stabilizing their intracellular protein levels. When WT-USP13 but not CA-USP13 (C345A-USP13 mutation, inactive mutation) was overexpressed, ACLY and OGDH protein concentrations in OVCA cells were upregulated, but mRNA levels were not significantly altered.

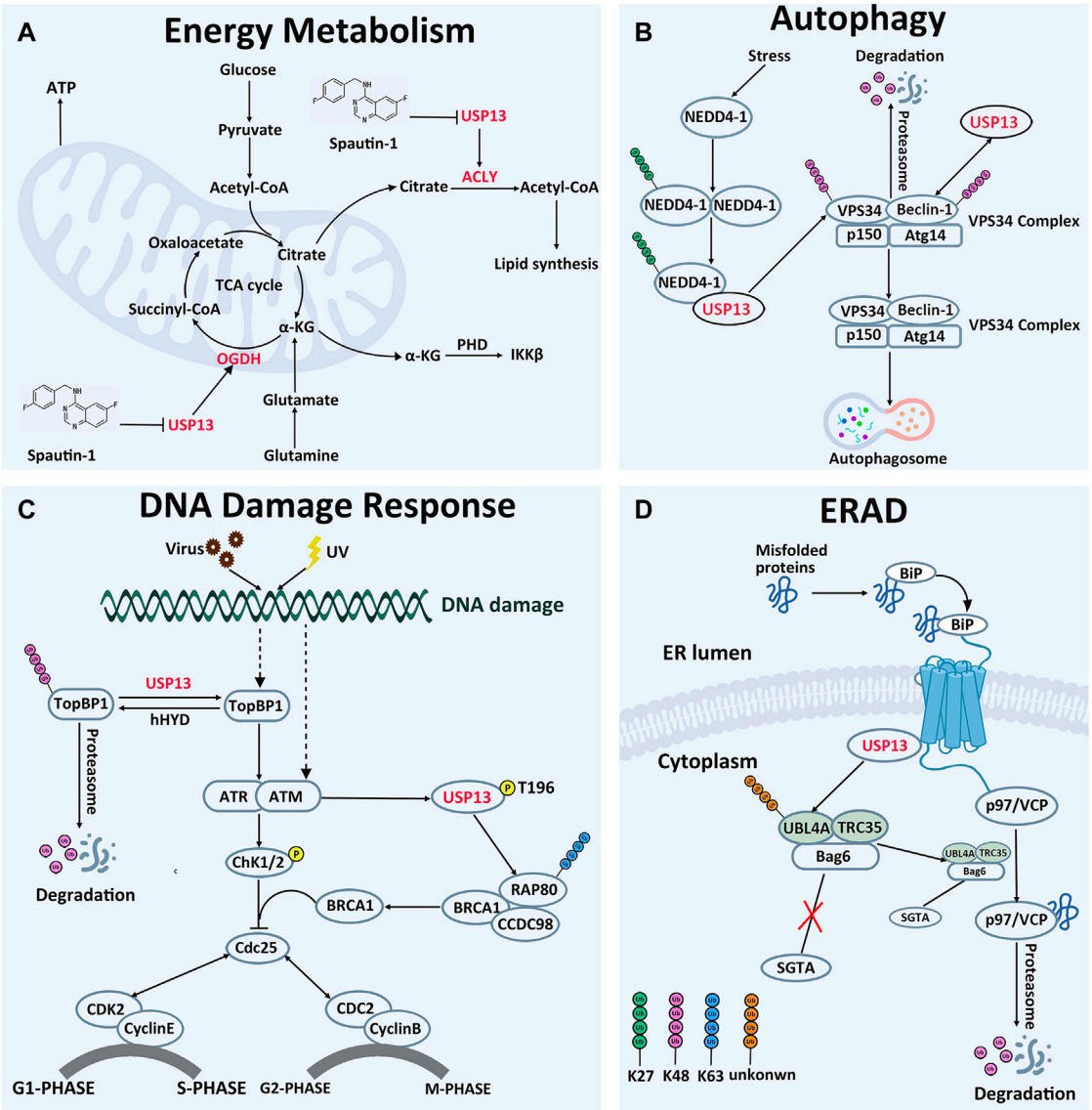


FIGURE 3 | Cellular Function of USP13. **(A)** In energy metabolism, USP13 promotes cell energy metabolism by removing OGDH and ACLY degradation signals. **(B)** In autophagy, USP13 is recruited by auto-ubiquitinated NEDD4-1 to remove VPS34 subunit degradation signal. On the other hand, USP13 deubiquitinated Beclin-1 and removed the degradation signal of Beclin-1. Consequently, the VPS34 complex initiates autophagosome formation. K27-linked Ub chains are green, K48-linked Ub chains are pink. **(C)** In DNA damage reaction, USP13 antagonizes E3 ubiquitin ligase hHYD to remove the TopBP1 degradation signal, then TopBP1 activates the ATR signaling pathway and ultimately activates the G1-S phase checkpoint, making cells remain in the G1 phase. In addition, phosphorylated USP13 by ATM is recruited to DNA damage sites to cleave the other Ub chains to facilitate K63-linked ubiquitin chains, which promotes RAP80 localization and ultimately activates the G2-M phase checkpoint, making cells remain in the G2 phase. K48-linked Ub chains are pink. Phosphorylation is yellow. **(D)** In ERAD, hyper-ubiquitination of UBL4A can induce the cleavage and inactivation of Bag6, causing ERAD inhibition and inhibiting the interaction of UBL4A with SGTA directly. USP13 interacts physically with gp78 and Bag6, removing hyper-ubiquitination of UBL4A, controlling precisely the ERAD process. Unknown-linked Ub chains are orange.

Consistently, tissue microarray detected that USP13 knockdown could reduce the ACLY and OGDH protein levels in cells, reducing the synthesis of fatty acids from the source. As expected, USP13 knockout evidently inhibited tumor cell growth either in OVCA cells or xenograft tumor models in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice (Han et al., 2016). In conclusion, inhibition of the deubiquitination activity of USP13 can impair the energy metabolism of tumor cells, hence providing novel insights into interventions of tumor cells targeting on energy metabolism pathway.

USP13 in Autophagy

Autophagy is a process where cells self-degrade and recycles their intracellular organelles under stress or starvation. Disruption of the autophagy system may trigger the occurrence of tumors and autoimmune diseases (Levine and Kroemer, 2008; Glick et al., 2010; Levy et al., 2017). Data demonstrated that VPS34 PI3K

activity and its protein partners play essential roles in harmonizing both autophagosome initiation and maturation (Liu et al., 2011). VPS34 and Beclin1 are the core components of the VPS34 complexes (Ohashi et al., 2019; Xie et al., 2020; Yang et al., 2021).

In 2011, Yuan *et al.* found that USP13 could interact with the C-terminal domain of Beclin-1 subunit in the VPS34 complex and deubiquitinate Beclin-1, thereby enhancing the stability of the VPS34 complex, which would contribute to the formation of autophagosomes (Liu et al., 2011). Deubiquitination experiments demonstrated that overexpression of USP13 could reduce the ubiquitination level of Beclin-1, and this effect could be counteracted by the USP13 inhibitor spautin-1. Interestingly, Beclin-1 knockout also reduced USP13 protein abundance in turn, indicating the two proteins are regulated reciprocally. In addition, a recent study pronounced another implication of USP13 in modulating autophagy through deubiquitinating VPS34 subunit (Xie et al., 2020). When autophagy occurs, neural precursor cells expressed developmentally downregulated 4-1 (NEDD4-1) would form oligomer and undergo K29-linked auto-ubiquitination at K1279. The auto-ubiquitinated NEDD4-1 can then interact with USP13 and act as a bridge connecting USP13 to VPS34, removing the K48-linked ubiquitin chain on VPS34. Furthermore, it was demonstrated that CA-USP13 is not able to cleave K48-linked ubiquitin chain, suggesting that USP13 deubiquitination activity is necessary to stabilize VPS34 (**Figure 3B**). Thus, modulating USP13 perhaps offers an effective target in the management of diseases brought by disfunction of the autophagy pathways. Consistently, treatment with spautin-1, a selective inhibitor of USP13, protected the brain from cerebral ischemia reperfusion injury through blocking autophagy activation (Liu et al., 2021b). In addition, upregulation of USP13 is proved to attenuate intervertebral disc degeneration (IVDD) through promoting autophagy (Dai et al., 2021).

USP13 in DNA Damage Response

DNA replication is an important process of genetic information transmission, and imperfect replication processes lead to genomic instability, which is a critical cause of tumors (Downs et al., 2007; Anindya, 2020). Stimulus from external radiation, viral infection and other stimuli can trigger DNA damage in cells, initiating the DNA damage repair system to protect the DNA structure from destruction (Li et al., 2017). It is indicated that USP13 can exquisitely adjust several vital proteins involved in the DNA damage response through deubiquitinating them, in degradation-dependent and independent manner (Li et al., 2017; Kim et al., 2021). Here, we take its regulation of RAP80 and TopBP1 for instances to discuss (**Figure 3C**).

RAP80 exerts effects in myriad aspects of DNA damage repair, including cell cycle checkpoint activation and chromatin homologous recombination (Mailand et al., 2007; Silver and Livingston, 2012). It is observed that USP13 deficiency abrogates DNA damage-induced G2/M checkpoint and renders cells sensible to irradiation and treatment of cisplatin in a RAP80-dependent manner, underlying the implications of USP13 in DNA damage repair through modulating RAP80 (Li et al., 2017). The evidence establishes that the binding of RAP80 to K63-linked ubiquitin chain is essential for recruitment of itself and other proteins to DNA damage sites (Kim et al., 2007; Sobhian et al., 2007; Wang et al., 2007; Yan et al., 2007). However, there are approximately 15 sites on RAP80 prone to forming ubiquitin chains, and activation of multiple sites might sterically block modification of RAP80 by K63-linked ubiquitin chain. Following DNA damage, phosphorylated USP13 by ATM is recruited to DNA damage sites to cleave the ubiquitin chains from more than three sites of RAP80 (K75, K90, and K112), releasing their restriction on the K63-linked ubiquitin chain, improving the focus formation of the RAP80-BRCA1 complex, and eventually facilitating DDR. Notably, this function of USP13 depends on deubiquitination activity, since CA-USP13 cannot reduce RAP80 ubiquitination level (Li et al., 2017). Taken together, USP13 has an impact on ubiquitination of RAP80, instead of its protein degradation, to regulate its focus formation and DDR-related function. In addition, depletion of USP13 in OVCA cell line EFO-27 cells sensitized cells to the Poly (ADP-ribose) polymerase (PARP) inhibitor, Olaparib, and incubation with USP13 inhibitor Spautin-1 also conferred EFO-27 cells sensitive to Olaparib. Furthermore, following treatment of Spautin-1 in conjunction with Olaparib, effects on OVCA models are remarkably enhanced, indicating that USP13 may be applied to overcome the chemotherapy resistance of cancer cells (Li et al., 2017).

Alternatively, USP13 can also modulate the ubiquitination level of TopBP1, another key protein implicated in replication stress-related DNA-damage responses (Kim et al., 2021). Following DNA replication stress, TopBP1 is recruited near single-stranded DNA to activate the ATR, thereby regulating the G1-S phase checkpoint (Ma et al., 2020; Kim et al., 2021). In normal cells, TopBP1 is ubiquitinated by the E3 ubiquitin ligase human hyperplastic discs (hHYD) for degradation by the proteasome (Honda et al., 2002). Under DNA damage, the ubiquitination level of TopBP1 was pronouncedly reduced by USP13, accompanied by accumulation of TopBP1 in cells. Protein interaction experiments demonstrated that USP13 could co-immunoprecipitate with endogenous TopBP1, and *in vitro* deubiquitination enzyme experiments showed that WT-USP13 could deubiquitinate TopBP1, while CA-USP13 could not, highlighting the requirement for USP13 ubiquitination activity (Kim et al., 2021). The observations that ATR phosphorylation was reduced in USP13-deficient cells and can be restored by recombinant expression of TopBP1 established that USP13 can regulate DNA replication stress by controlling the degradation of the TopBP1. Importantly, TopBP1 is proved to be correlated with multiple cancers and exerts roles in chemotherapy resistance (Forma et al., 2012; Chowdhury et al., 2014; Lv et al., 2016; Liu et al., 2021c; Laine et al., 2021). Moreover, incubation with USP13 inhibitor spautin-1 reduces survival of OVCA cell lines after replication stress inducing agents, implying that the development of selective USP13 inhibitors is feasible for treatment of these patients of conventional cancer chemotherapy (Kim et al., 2021).

Conclusively, these data illustrate that USP13 can deubiquitinate key proteins engaged in DNA damage response

to induce their dysfunction or degradation, fine-tuning the DNA damage repair system.

USP13 in ERAD

To control protein quality in cells, proteins are strictly monitored in the endoplasmic reticulum. Those proteins that cannot be correctly folded will be degraded by the ERAD pathway, where the misfolded proteins should be moved by the process, named retrotranslocation, from the endoplasmic reticulum across the membrane to the cytosol for ubiquitination by ER-associated ubiquitin conjugating systems (Hirsch et al., 2009; Ye and Rape, 2009). As a recognition signal, the polyubiquitin chains on the substrates can enroll the p97/VCP ATPase and its cofactor Ufd1-Npl4, releasing substrates from the ER membrane into the cytosol (Ye et al., 2001; Flierman et al., 2003). It is reported that USP13 and gp78 are two enzymes with opposing activity, but manipulate in combination the ubiquitination of ER substrates, thus coordinately promoting ERAD (Liu et al., 2014) (**Figure 3D**). Gp78, as one of the well-described E3s in ERAD, plays a master regulator of retrotranslocation, via mediating ubiquitination of many ERAD substrates and interacting with ERAD machinery proteins, such as BCL-2-associated athanogene 6 (Bag6) multiprotein complex (Fang et al., 2001; Song et al., 2005; Jo et al., 2011; Wang et al., 2011; Chen et al., 2012). On the luminal side, a complex containing gp78 can recruit the misfolded proteins recognized by molecular chaperone proteins for ubiquitination and retrotranslocation (Brodsky and McCracken, 1999; Wu and Rapoport, 2018). Bag6 with chaperone "holdase" activity can improve the turnover of retrotranslocated polypeptides through holding them in a soluble state and facilitating the transfer of the substrate from the gp78 containing complex to the proteasome for degradation, owing to the weak interaction of Bag6 with the proteasome (Minami et al., 2010; Wang et al., 2011). UBL4A is one of two Bag6 partners, promotes association of Bag6 with a co-chaperone. Hyper-ubiquitination of UBL4A can induce the cleavage and inactivation of Bag6, causing ERAD inhibition (Chartron et al., 2012; Xu et al., 2012). Therefore, ubiquitination chains on UBL4A are essential for the ERAD pathway. It is reported that USP13 can form a specific interaction with the Bag6 complex via the Bag6 UBL domain, and further remove ubiquitin conjugates from UBL4, hyper-ubiquitination of UBL4 under USP13 knockdown conditions might inhibit the interaction of UBL4 with SGTA directly, and therefore disrupting this functional connection between Bag6 and SGTA (Liu et al., 2014; Chu et al., 2020). Data demonstrated that ubiquitin conjugates on Ubl4A from either USP13 deficient cells or USP13 knockdown cells accumulated more than that on UBL4 from control cells. Similarly, ubiquitinated UBL4A can be significantly reduced after treatment of with recombinant USP13, which can be blocked by the specific DUB inhibitor ubiquitin aldehyde (Ub-Al) (Liu et al., 2014). As a result, USP13 can inhibit the elimination of misfolded proteins by the ERAD pathway. Notably, the impact of deubiquitination enzyme USP13 on ERAD process needs the assistance of gp78, and in turn improves the ubiquitination specificity of gp78 substrates. USP13 interacts physically with gp78 and Bag6, fine-tuning the ubiquitin dynamics of UBL4A in the Bag6 complex. Gp78 adds ubiquitin chains into UBL4A, whereas USP13 antagonizes this activity to limit UBL4A ubiquitination. In conclusion, it appears that USP13 and gp78, these two antagonized enzymes against each other, corporately maintain the balance between ubiquitination and deubiquitination, controlling precisely the ERAD process.

It is noteworthy that USP13 also can act in ERAD downstream of retro-translocation through enhancing the solubility of retrotranslocated substrates (Liu et al., 2014; Chu et al., 2020). It is proved that USP13 knockdown has negative effects on the solubility of several ERAD substrates, including model ERAD-substrate TCRα. This phenomenon is postulated perhaps due to mutual influence between USP13 and Bag6 (Yu et al., 1997; Soetandyo et al., 2010). Lately, USP13 has been reported to deubiquitinate under stress, which is also the substrate of autocrine motility factor receptor (AMFR) E3 ligase, activating CASP3 followed by Bag6 cleavage (Mitchell et al., 2007; Benhar et al., 2008). Consequently, the produced N-terminal Bag6 is converted from an ERAD regulator to an autophagy modulator and apoptosis trigger.

USP13 in Other Cellular Activities

In addition to these cell functions as described above, USP13 is also implicated in many other distinctive cell activities, albeit its regulatory mechanism remains not elucidated clearly. For instance, in non-small-cell lung cancer (NSCLC), downregulation of USP13 inhibits MAPK/AKT signaling (Han et al., 2016). In contrast, in breast cancer cells, silencing USP13 can facilitate AKT phosphorylation by downregulating PTEN level, accompanied by tumor cell proliferation and glycolysis (Zhang et al., 2013). As STING (also known as MITA), a deubiquitination substrate of USP13, is pivotal for host defense against viruses dependent on the NF-κB pathway and USP13 is supposed to be involved in the NF-κB signaling pathway and regulates innate immunity via deubiquitinating STING (Sun et al., 2017). Consistently, it has been reported that deletion of USP13 can activate the NF-κB signaling pathway in response to herpesvirus infection, increasing resistance to the virus (Sun et al., 2017). Moreover, phosphorylation of USP13 at Y708 by CDC-like kinase 3 (CLK3) can facilitate the interaction between USP13 and the proto-oncoprotein c-Myc, further suppressing tumorigenesis (Zhou et al., 2020). Overall, USP13 is capable of affecting various cellular processes, including protein localization or degradation through regulating the ubiquitination levels of multiple protein substrates, thereby the dysfunction of USP13 can relate to a wide variety of diseases, even the occurrence of tumors, which highlight the potency of USP13 as a therapeutic target.

USP13 and Tumors

A growing number of studies have demonstrated that USP13 overexpression is closely related to tumor grade, tumor invasion, chemotherapy resistance and poor prognosis (Liu et al., 2014; Han et al., 2016; Fang et al., 2017; Li et al., 2017; Kim et al., 2021; Liu and Moussa, 2021).

The Cancer Genomics Atlas (TCGA) analysis detected significant overexpression of USP13 in OVCA cells. Immunohistochemical (IHC) assay exhibited USP13

expression levels in OVCA cells upregulated at least 3.7 times, compared with those in normal ovarian tissues (Han et al., 2016). In addition, clinical data showed that USP13 overexpression led to a short survival cycle and poor prognosis for OVCA patients and is closely related to tumor grade. Consistently, knockout or pharmacological inhibition of USP13 impeded tumor cell proliferation and enhanced sensitivity to chemotherapeutic agents in both cell lines and mouse models (Han et al., 2016; Zhang et al., 2018). Mechanistically, USP13 promoted the energy metabolism of tumor cells, and provided precursor substances for the synthesis of sugar, lipids and non-essential amino acids in cancer cells, through deubiquitinating and stabilizing ACLY and OGDH (Han et al., 2016). These are necessary for tumor cell proliferation and invasion. USP13, on the other hand, can deubiquitinate and stabilize MCL1, which is not sensitive to MCL-2 family inhibitors, and render tumor cells highly resistant to BH3-type chemotherapy drugs (Oltersdorf et al., 2005; Delbridge et al., 2016; Kotschy et al., 2016). Since the effect of USP13 on maintaining this resistance, inhibition of USP13 seems likely to be viewed as a practical way to overcome drug resistance in the therapy of OVCA.

The *c-Myc* gene encodes a proto-oncoprotein, a widely recognized transcription factor regulating approximately 10–15% of genes implicated in cell proliferation, differentiation, apoptosis and other processes (Friedman et al., 2017; Habib et al., 2020), and c-Myc mutations are often associated with tumors (Berns et al., 1997; Brodsky and McCracken, 1999; Hermeking et al., 2000; Morrish et al., 2003; Wilson et al., 2004). Recently, it has been reported that USP13 is co-overexpressed with c-Myc in many tumors, such as NSCLC (Wu et al., 2019), cholangiocarcinoma (CAA) (Zhou et al., 2020), GSCs (Fang et al., 2017), and hepatocellular carcinoma (HCC) (Huang et al., 2020). Consistently, knockdown or pharmacological inhibition of USP13 antagonized tumor cell growth. For example, in non-small-cell lung cancer, downregulation of USP13 suppresses ATK/MAPK signaling, reducing c-Myc protein levels and retards tumor growth both in tumor cells and nude mice (Wu et al., 2019). In cholangiocarcinoma, TGF-β signaling triggers the phosphorylation of CLK3, a serine/threonine kinase that directly phosphorylates USP13 at Y708 and facilitates USP13 interaction with c-Myc (Zhou et al., 2020); in GSCs, USP13 can enhance the stability through deubiquitinating c-Myc, activating purine synthesis mediated by c-Myc and inducing the tumorigenesis of GSCs (Fang et al., 2017); in hepatocellular carcinoma, knockdown of USP13 by shRNA can markedly downregulate c-Myc expression, resisting xenograft tumor growth of HCC (Huang et al., 2020). Hence, inhibition of USP13 might be beneficial for related cancer treatment.

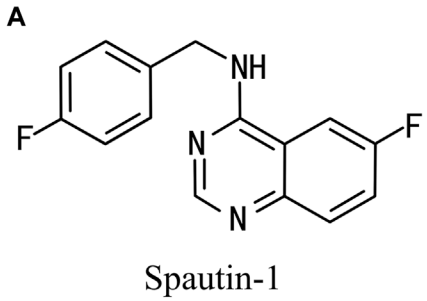
Likewise, USP13 exerts an antitumor role in several types of cancers. For example, USP13 prevents tumor cell growth by deubiquitinating PTEN in breast cancer, OSCC and bladder cancer. It is assumed that overexpression of USP13 can block AKT signaling pathway, suppressing tumor cell proliferation, invasion, and glycolysis through up-regulating PTEN protein levels (Zhang et al., 2013; Xiang et al., 2015; Zhang et al., 2018; Man et al., 2019).

In addition, USP13 is also involved in the development of other diseases and tumors. In cell and animal models, USP13 participates in ubiquitination modifications of key targets in Parkinson's disease, such as tau, α-synuclein and E3 ubiquitin ligase parkin (Liu et al., 2019a; Liu et al., 2019b). In melanoma, MITF is essential for cell proliferation and differentiation via regulating multiple genes transcription. USP13 has been identified as a deubiquitination enzyme of MITF to modulate the ubiquitination level of MITF, affecting the survival of melanoma cells (Zhao et al., 2011). In gastric cancer, the high expression of USP13 is associated with high invasion, contributing to reduced survival rate of patients. It is supposed that USP13 deubiquitinated and stabilized Snail protein, promoting metastasis in gastric cancer cells (Zhang et al., 2022). Collectively, due to its role in a variety of tumors and neurodegenerative diseases, USP13 has emerged as a potential therapeutic target for diverse tumors.

Inhibition of USP13

Owing to the significance of USP13 in the above cellular processes and diseases, especially tumors, to seek and develop high potent inhibitors presumably thus offer an attractive strategy for research and treatment of related diseases targeting USP13. Currently, spautin-1 is a widely acknowledged inhibitor of USP13(Liu et al., 2011; Zhang et al., 2018; Guo et al., 2020) (**Figure 4A**). In 2011, Yuan *et al.* discovered a more efficient autophagy inhibitor, MBCQ, through high-throughput screening. Subsequently, they carried out molecular optimization based on MBCQ and designed many of its derivatives. Among them, C43 is the most superior at selectivity and inhibitory activity, and is named spautin-1 (Liu et al., 2011). Spautin-1 was identified to selectively inhibit the deubiquitination enzymes USP10 and USP13 with an IC50 of 0.6–0.7 μM, and spautin-1 treatment can enhance the ubiquitination-directed degradation of the Beclin1-VSP34 complex and reduce the intracellular concentration of phosphatidylinositol 3-phosphate (PI3P), a crucial component in autophagosome membranes formation (Levine and Kroemer, 2008; Glick et al., 2010; Levy et al., 2017). Remarkably, several studies have successively demonstrated that the use of spautin-1 in combination with chemotherapy can effectively increase tumor cell mortality and attenuate tumor cell migration and xenotransplantation, both in cell models and animal models, suggesting that spautin-1 may be a potential lead compound targeting USP13 (Zhang et al., 2018; Liao et al., 2019; Guo et al., 2020).

More lately, a new study on USP13 inhibitors was reported. Liu *et al.* designed and synthesized six derivatives of spautin-1 (**Figure 4B**), which exhibit higher inhibition efficiency against USP13 (**Figure 4C**) and capability of crossing the blood-brain barrier (Liu et al., 2021a), compared to spautin-1, enabling the development of inhibitors in neurodegenerative diseases. They first treated neuroblastoma SH-SY5Y cells with six inhibitors at a concentration ranging from 1 nM to 1 mM, and detected USP13 activity utilizing ELISA assay. The IC50 values of USP13 for these inhibitors ranged from 0.11 to 2.13 nM. Among them, bK50118-C displays the highest inhibitory efficiency against α-synuclein, although its IC50 is not the smallest. Therefore, BK50118-C is selected for the next ADME research. Conclusively, the new USP13 inhibitor



C

Compound name	IC50 to USP13(nM)
BK50118-A	0.11
BK50118-B	2.13
BK50118-C	0.42
CL3-499	0.29
CL3-512	0.27
CL3-514	0.61
Spautin-1	600-700

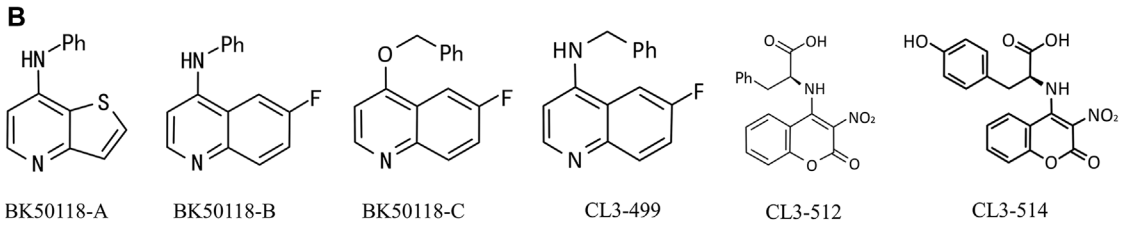


FIGURE 4 | USP13 inhibitors. **(A)** Chemical structure of Spautin-1; **(B)** Spautin-1 derivatives; **(C)** IC50 to USP13 was calculated.

BK50118-C designed by Liu *et al.* is the first USP13 inhibitor that can cross the blood-brain barrier, providing a powerful tool for research on USP13-related neurodegenerative diseases in the future.

CONCLUSION

Given a decisive role of the ubiquitin–proteasome system (UPS) in protein quality control in eukaryotes, UPS disorder is associated with many diseases, even tumors (Ravid and Hochstrasser, 2008; Harrigan et al., 2018). As a member of this system, the deubiquitinating enzyme USP13 participates in many aspects of cellular processes, as result dysregulation of USP13 gives rise to plenty of diseases through deubiquitination of various critical substrate proteins, including OGDH (Han et al., 2016), ACLY (Han et al., 2016), VPS34 (Liu et al., 2011), TopBP1 (Kim et al., 2021), RAP80 (Li et al., 2017), UBL4A (Liu et al., 2014), and STING (Sun et al., 2017), highlighting that USP13 is emerging as appealing targets for the therapy of the diseases. Consistently, knockdown or pharmacological inhibition of USP13 by spautin-1 retards the growth, differentiation and invasion of many tumors, providing a possibility for antagonizing the drug resistance of tumor cells. Furthermore, recent studies have shown that derivatives of spautin-1 display better USP13 inhibition and the ability to cross the blood-brain barrier, which is presumably beneficial for research on USP13-related neurodegenerative disease (Liu et al., 2021a). However, here a few critical issues are raised. Firstly, since the recombinant expression of USP13 only exhibits weak deubiquitination activity *in vitro*, it should be addressed whether it is in a state of self-inhibition *in vivo* and needs to be activated by other proteins, or its local solubility in the cells requires to be increased for activation. In addition, as neither the structure of the USP13 holoenzyme nor its complex structure with substrate proteins or inhibitors has been determined, it is limited for us to decipher its molecular mechanisms in cell activity. In future, the structure and activity regulation mechanism of USP13 remains to be further elaborated. Moreover, much attention should be paid to the validation utilization of USP13 as a drug target in research on the pathogenesis of diseases, in particular tumors.

We anticipate that this manuscript can supply information on the structure, biology and physiology of USP13, particularly its relation with malignant diseases, paving the way for the clinical transfer of USP13 inhibitors to druggable compounds.

AUTHOR CONTRIBUTIONS

FW was the lead PI and refined the manuscript. XL and ZM drafted the manuscript. XL and GY produced all the figures. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China Grants 32071269 and 31961133015 to. FW, 31870791 to ZM and Ensan Fundation 2022009 and National Undergraduates Innovation and Entrepreneurship Training Program of China S202110007124 to ZY.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2022.875124/full#supplementary-material

REFERENCES

Akram, M. (2014). Citric Acid Cycle and Role of its Intermediates in Metabolism. *Cell Biochem Biophys* 68 (3), 475–478. doi:10.1007/s12013-013-9750-1

Amerik Ayu, S. S., Swaminathan, S., Krantz, B. A., Wilkinson, K. D., and Hochstrasser, M. (1997). *In Vivo* disassembly of Free Polyubiquitin Chains by Yeast Ubp14 Modulates Rates of Protein Degradation by the Proteasome. *EMBO J.* 16 (16), 4826–4838. doi:10.1093/emboj/16.16.4826

Anindya, R. (2020). Single-stranded DNA Damage: Protecting the Single-Stranded DNA from Chemical Attack. *DNA Repair* 87, 102804. doi:10.1016/j.dnarep.2020.102804

Benhar, M., Forrester, M. T., Hess, D. T., and Stamler, J. S. (2008). Regulated Protein Denitrosylation by Cytosolic and Mitochondrial Thioredoxins. *Science* 320 (5879), 1050–1054. doi:10.1126/science.1158265

Berns, K., Hijmans, E. M., and Bernards, R. (1997). Repression of C-Myc Responsive Genes in Cycling Cells Causes G1 Arrest through Reduction of Cyclin E/CDK2 Kinase Activity. *Oncogene* 15 (11), 1347–1356. doi:10.1038/sj.onc.1201280

Brodsky, J. L., and McCracken, A. A. (1999). ER Protein Quality Control and Proteasome-Mediated Protein Degradation. *Semin. Cel Develop. Biol.* 10 (5), 507–513. doi:10.1006/scdb.1999.0321

Chartron, J. W., Vandervelde, D. G., and Clemons, W. M., Jr (2012). Structures of the Sgt2/SGTA Dimerization Domain with the Get5/UBL4A UBL Domain Reveal an Interaction that Forms a Conserved Dynamic Interface. *Cel Rep.* 2 (6), 1620–1632. doi:10.1016/j.celrep.2012.10.010

Chen, Z., Du, S., and Fang, S. (2012). gp78: a Multifaceted Ubiquitin Ligase that Integrates a Unique Protein Degradation Pathway from the Endoplasmic Reticulum. *Cpps* 13 (5), 414–424. doi:10.2174/138920312802430590

Cho, J., Park, J., Kim, E. E., and Song, E. J. (2020). Assay Systems for Profiling Deubiquitinating Activity. *Int. J. Mol. Sci.* 21 (16). doi:10.3390/ijms21165638

Chowdhury, P., Lin, G. E., Liu, K., Song, Y., Lin, F.-T., and Lin, W.-C. (2014). Targeting TopBP1 at a Convergent point of Multiple Oncogenic Pathways for Cancer Therapy. *Nat. Commun.* 5, 5476. doi:10.1038/ncomms6476

Chu, Y., Dong, X., Kang, Y., Liu, J., Zhang, T., Yang, C., et al. (2020). The Chaperone BAG6 Regulates Cellular Homeostasis between Autophagy and Apoptosis by Holding LC3B. *iScience* 23 (11), 101708. doi:10.1016/j.isci.2020.101708

Ciechanover, A. (2003). The Ubiquitin Proteolytic System and Pathogenesis of Human Diseases: a Novel Platform for Mechanism-Based Drug Targeting. *Biochem. Soc. Trans.* 31 (2), 474–481. doi:10.1042/bst0310474

Cruz, L., Soares, P., and Correia, M. (2021). Ubiquitin-Specific Proteases: Players in Cancer Cellular Processes. *Pharmaceuticals (Basel)* 14 (9). doi:10.3390/ph14090848

Dai, F., Yu, P., Yu, Z., Jiang, H., Ma, Z., and Liu, J. (2021). Yiqi Huoxue Recipe Delayed Intervertebral Disc Degeneration by Activating Autophagy. *Front. Pharmacol.* 12, 705747. doi:10.3389/fphar.2021.705747

Dandrea, A., and Pellman, D. (1998). Deubiquitinating Enzymes: A New Class of Biological Regulators. *Crit. Rev. Biochem. Mol. Biol.* 33 (5), 337–352. doi:10.1080/10409239891204251

Delbridge, A. R. D., Grabow, S., Strasser, A., and Vaux, D. L. (2016). Thirty Years of BCL-2: Translating Cell Death Discoveries into Novel Cancer Therapies. *Nat. Rev. Cancer* 16 (2), 99–109. doi:10.1038/nrc.2015.17

Dittmar, G., and Winklhofer, K. F. (2019). Linear Ubiquitin Chains: Cellular Functions and Strategies for Detection and Quantification. *Front. Chem.* 7, 915. doi:10.3389/fchem.2019.00915

Downs, J. A., Nussenzweig, M. C., and Nussenzweig, A. (2007). Chromatin Dynamics and the Preservation of Genetic Information. *Nature* 447 (7147), 951–958. doi:10.1038/nature05980

Fang, S., Ferrone, M., Yang, C., Jensen, J. P., Tiwari, S., and Weissman, A. M. (2001). The Tumor Autocrine Motility Factor Receptor, Gp78, Is a Ubiquitin Protein Ligase Implicated in Degradation from the Endoplasmic Reticulum. *Proc. Natl. Acad. Sci. U.S.A.* 98 (25), 14422–14427. doi:10.1073/pnas.251401598

Fang, X., Zhou, W., Wu, Q., Huang, Z., Shi, Y., Yang, K., et al. (2017). Deubiquitinase USP13 Maintains Glioblastoma Stem Cells by Antagonizing FBXL14-Mediated Myc Ubiquitination. *J. Exp. Med.* 214 (1), 245–267. doi:10.1084/jem.20151673

Flierman, D., Ye, Y., Dai, M., Chau, V., and Rapoport, T. A. (2003). Polyubiquitin Serves as a Recognition Signal, rather Than a Ratcheting Molecule, during Retrotranslocation of Proteins across the Endoplasmic Reticulum Membrane. *J. Biol. Chem.* 278 (37), 34774–34782. doi:10.1074/jbc.m303360200

Forma, E., Krzeslak, A., Bernaciak, M., Romanowicz-Makowska, H., and Brys, M. (2012). Expression of TopBP1 in Hereditary Breast Cancer. *Mol. Biol. Rep.* 39 (7), 7795–7804. doi:10.1007/s11033-012-1622-z

Friedman, M., Levin, C. E., and Henika, P. R. (2017). Addition of Phytochemical-Rich Plant Extracts Mitigate the Antimicrobial Activity of Essential Oil/wine Mixtures against *Escherichia coli* O157:H7 but Not against *Salmonella enterica*. *Food Control* 73, 562–565. doi:10.1016/j.foodcont.2016.09.002

Gameiro, C. M., and Bell, P. A. (2011). Reductive Glutamine Metabolism by IDH1 Mediates Lipogenesis under Hypoxia. *Nature* 481 (7381), 380–384. doi:10.1038/nature10602

Gameiro, P. A., Yang, J., Metelo, A. M., Pérez-Carro, R., Baker, R., Wang, Z., et al. (2013). *In Vivo* HIF-Mediated Reductive Carboxylation Is Regulated by Citrate Levels and Sensitizes VHL-Deficient Cells to Glutamine Deprivation. *Cel Metab.* 17 (3), 372–385. doi:10.1016/j.cmet.2013.02.002

Glick, D., Barth, S., and Macleod, K. F. (2010). Autophagy: Cellular and Molecular Mechanisms. *J. Pathol.* 221 (1), 3–12. doi:10.1002/path.2697

Guo, J., Zhang, J., Liang, L., Liu, N., Qi, M., Zhao, S., et al. (2020). Potent USP10/13 Antagonist Spautin-1 Suppresses Melanoma Growth via ROS-mediated DNA Damage and Exhibits Synergy with Cisplatin. *J. Cel Mol Med* 24 (7), 4324–4340. doi:10.1111/jcmm.15093

Habib, S., Ariatti, M., and Singh, M. (2020). Anti-c-myc RNAi-Based Onconanotherapeutics. *Biomedicines* 8 (12). doi:10.3390/biomedicines8120612

Han, C., Yang, L., Choi, H. H., Baddour, J., Achreja, A., Liu, Y., et al. (2016). Amplification of USP13 Drives Ovarian Cancer Metabolism. *Nat. Commun.* 7, 13525. doi:10.1038/ncomms13525

Hanrahan, A. J., Schultz, N., Westfal, M. L., Sakr, R. A., Giri, D. D., Scarperi, S., et al. (2012). Genomic Complexity and AKT Dependence in Serous Ovarian Cancer. *Cancer Discov.* 2 (1), 56–67. doi:10.1158/2159-8290.cd-11-0170

Harrigan, J. A., Jacq, X., Martin, N. M., and Jackson, S. P. (2018). Deubiquitylating Enzymes and Drug Discovery: Emerging Opportunities. *Nat. Rev. Drug Discov.* 17 (1), 57–78. doi:10.1038/nrd.2017.152

Hatzivassiliou, G., Zhao, F., Bauer, D. E., Andreadis, C., Shaw, A. N., Dhanak, D., et al. (2005). ATP Citrate Lyase Inhibition Can Suppress Tumor Cell Growth. *Cancer Cell* 8 (4), 311–321. doi:10.1016/j.ccr.2005.09.008

Hermeking, H., Rago, C., Schuhmacher, M., Li, Q., Barrett, J. F., Obaya, A. J., et al. (2000). Identification of CDK4 as a Target of C-MYC. *Proc. Natl. Acad. Sci. U.S.A.* 97 (5), 2229–2234. doi:10.1073/pnas.050586197

Hirsch, C., Gauss, R., Horn, S. C., Neuber, O., and Sommer, T. (2009). The Ubiquitylation Machinery of the Endoplasmic Reticulum. *Nature* 458 (7237), 453–460. doi:10.1038/nature07962

Honda, Y., Tojo, M., Matsuzaki, K., Anan, T., Matsumoto, M., Ando, M., et al. (2002). Cooperation of HECT-Domain Ubiquitin Ligase hHYD and DNA Topoisomerase II-Binding Protein for DNA Damage Response. *J. Biol. Chem.* 277 (5), 3599–3605. doi:10.1074/jbc.m104347200

Huang, J., Gu, Z. L., Chen, W., Xu, Y. Y., and Chen, M. (2020). Knockdown of Ubiquitin-specific Peptidase 13 Inhibits Cell Growth of Hepatocellular Carcinoma by Reducing c-Myc Expression. *Kaohsiung J. Med. Sci.* 36 (8), 615–621. doi:10.1002/kjm2.12209

Jo, Y., Sguigna, P. V., and Debose-Boyd, R. A. (2011). Membrane-associated Ubiquitin Ligase Complex Containing Gp78 Mediates Sterol-Accelerated Degradation of 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase. *J. Biol. Chem.* 286 (17), 15022–15031. doi:10.1074/jbc.m110.211326

Kim, H., Chen, J., and Yu, X. (2007). Ubiquitin-Binding Protein RAP80 Mediates BRCA1-dependent DNA Damage Response. *Science* 316 (5828), 1202–1205. doi:10.1126/science.1139621

Kim, W., Zhao, F., Gao, H., Qin, S., Hou, J., Deng, M., et al. (2021). USP13 Regulates the Replication Stress Response by Deubiquitinating TopBP1. *DNA Repair* 100, 103063. doi:10.1016/j.dnarep.2021.103063

Komander, D., Clague, M. J., and Urbé, S. (2009). Breaking the Chains: Structure and Function of the Deubiquitinases. *Nat. Rev. Mol. Cel Biol* 10 (8), 550–563. doi:10.1038/nrm2731

Kotschy, A., Szlavik, Z., Murray, J., Davidson, J., Maragno, A. L., Le Toumelin-Braizat, G., et al. (2016). The MCL1 Inhibitor S63845 Is Tolerable and Effective

in Diverse Cancer Models. *Nature* 538 (7626), 477–482. doi:10.1038/nature19830

Kwasna, D., Abdul Rehman, S. A., Natarajan, J., Matthews, S., Madden, R., De Cesare, V., et al. (2018). Discovery and Characterization of ZUFSP/ZUP1, a Distinct Deubiquitinase Class Important for Genome Stability. *Mol. Cel* 70 (1), 150–164. doi:10.1016/j.molcel.2018.02.023

Laine, A., Nagelli, S. G., Farrington, C., Butt, U., Cvrljevic, A. N., Vainonen, J. P., et al. (2021). CIP2A Interacts with TopBP1 and Drives Basal-like Breast Cancer Tumorigenesis. *Cancer Res.* 81 (16), 4319–4331. doi:10.1158/0008-5472.can-20-3651

Levine, B., and Kroemer, G. (2008). Autophagy in the Pathogenesis of Disease. *Cell* 132 (1), 27–42. doi:10.1016/j.cell.2007.12.018

Levy, J. M. M., Towers, C. G., and Thorburn, A. (2017). Targeting Autophagy in Cancer. *Nat. Rev. Cancer* 17 (9), 528–542. doi:10.1038/nrc.2017.53

Li, Y., Luo, K., Yin, Y., Wu, C., Deng, M., Li, L., et al. (2017). USP13 Regulates the RAP80-BRCA1 Complex Dependent DNA Damage Response. *Nat. Commun.* 8, 15752. doi:10.1038/ncomms15752

Liao, Y., Guo, Z., Xia, X., Liu, Y., Huang, C., Jiang, L., et al. (2019). Inhibition of EGFR Signaling with Spautin-1 Represents a Novel Therapeutics for Prostate Cancer. *J. Exp. Clin. Cancer Res.* 38 (1), 157. doi:10.1186/s13046-019-1165-4

Liu, H., Zhao, Z., Wu, T., Zhang, Q., Lu, F., Gu, J., et al. (2021). Inhibition of Autophagy-dependent Pyroptosis Attenuates Cerebral Ischaemia/reperfusion Injury. *J. Cel Mol Med* 25 (11), 5060–5069. doi:10.1111/jcmm.16483

Liu, J., Xia, H., Kim, M., Xu, L., Li, Y., Zhang, L., et al. (2011). Beclin1 Controls the Levels of P53 by Regulating the Deubiquitination Activity of USP10 and USP13. *Cell* 147 (1), 223–234. doi:10.1016/j.cell.2011.08.037

Liu, K., Graves, J. D., Lin, F.-T., and Lin, W.-C. (2021). Overexpression of TopBP1, a Canonical ATR/Chk1 Activator, Paradoxically Hinders ATR/Chk1 Activation in Cancer. *J. Biol. Chem.* 296, 100382. doi:10.1016/j.jbc.2021.100382

Liu, X., Balaraman, K., Lynch, C. C., Hebron, M., Wolf, C., and Moussa, C. (2021). Novel Ubiquitin Specific Protease-13 Inhibitors Alleviate Neurodegenerative Pathology. *Metabolites* 11 (9). doi:10.3390/metabo11090622

Liu, X., Hebron, M. L., Mulki, S., Wang, C., Lekah, E., Ferrante, D., et al. (2019). Ubiquitin Specific Protease 13 Regulates Tau Accumulation and Clearance in Models of Alzheimer's Disease. *Jad* 72 (2), 425–441. doi:10.3233/jad-190635

Liu, X., Hebron, M., Shi, W., Lonskaya, I., and Moussa, C. E.-H. (2019). Ubiquitin Specific Protease-13 Independently Regulates Parkin Ubiquitination and Alpha-Synuclein Clearance in Alpha-Synucleinopathies. *Hum. Mol. Genet.* 28 (4), 548–560. doi:10.1093/hmg/ddy365

Liu, X., and Moussa, C. (2021). Regulatory Role of Ubiquitin Specific Protease-13 (USP13) in Misfolded Protein Clearance in Neurodegenerative Diseases. *Neuroscience* 460, 161–166. doi:10.1016/j.neuroscience.2021.02.004

Liu, Y., Soetandyo, N., Lee, J. G., Liu, L., Xu, Y., Clemons, W. M., et al. (2014). USP13 Antagonizes Gp78 to Maintain Functionality of a Chaperone in ER-Associated Degradation. *Elife* 3, e01369. doi:10.7554/eLife.01369

Luan, B., Huang, X., Wu, J., Mei, Z., Wang, Y., Xue, X., et al. (2016). Structure of an Endogenous Yeast 26S Proteasome Reveals Two Major Conformational States. *Proc. Natl. Acad. Sci. U.S.A.* 113 (10), 2642–2647. doi:10.1073/pnas.1601561113

Lv, Y., Yanan, H., Yu, X., Liu, R., Zhang, S., Zheng, X., et al. (2016). TopBP1 Contributes to the Chemoresistance in Non-small Cell Lung Cancer through Upregulation of P53. *Dddt* 10, 3053–3064. doi:10.2147/dddt.s90705

Ma, M., Rodriguez, A., and Sugimoto, K. (2020). Activation of ATR-Related Protein Kinase upon DNA Damage Recognition. *Curr. Genet.* 66 (2), 327–333. doi:10.1007/s00294-019-01039-w

Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., et al. (2007). RNF8 Ubiquitylates Histones at DNA Double-Strand Breaks and Promotes Assembly of Repair Proteins. *Cell* 131 (5), 887–900. doi:10.1016/j.cell.2007.09.040

Man, X., Piao, C., Lin, X., Kong, C., Cui, X., and Jiang, Y. (2019). USP13 Functions as a Tumor Suppressor by Blocking the NF-kB-Mediated PTEN Downregulation in Human Bladder Cancer. *J. Exp. Clin. Cancer Res.* 38 (1), 259. doi:10.1186/s13046-019-1262-4

Minami, R., Hayakawa, A., Kagawa, H., Yanagi, Y., Yokosawa, H., and Kawahara, H. (2010). BAG-6 Is Essential for Selective Elimination of Defective Proteasomal Substrates. *J. Cel Biol* 190 (4), 637–650. doi:10.1083/jcb.200908092

Mitchell, D. A., Morton, S. U., Fernhoff, N. B., and Marletta, M. A. (2007). Thioredoxin Is Required for S-Nitrosation of Procaspase-3 and the Inhibition of Apoptosis in Jurkat Cells. *Proc. Natl. Acad. Sci. U.S.A.* 104 (28), 11609–11614. doi:10.1073/pnas.0704898104

Morrish, F., Giedt, C., and Hockenbery, D. (2003). c-MYC Apoptotic Function Is Mediated by NRF-1 Target Genes. *Genes Dev.* 17 (2), 240–255. doi:10.1101/gad.1032503

Ning, F., Xin, H., Liu, J., Lv, C., Xu, X., Wang, M., et al. (2020). Structure and Function of USP5: Insight into Physiological and Pathophysiological Roles. *Pharmacol. Res.* 157, 104557. doi:10.1016/j.phrs.2019.104557

Ohashi, Y., Tremel, S., and Williams, R. L. (2019). VPS34 Complexes from a Structural Perspective. *J. Lipid Res.* 60 (2), 229–241. doi:10.1194/jlr.r089490

Oltersdorf, T., Elmore, S. W., Shoemaker, A. R., Armstrong, R. C., Augeri, D. J., Belli, B. A., et al. (2005). An Inhibitor of Bcl-2 Family Proteins Induces Regression of Solid Tumours. *Nature* 435 (7042), 677–681. doi:10.1038/nature03579

Raasi, S., Varadan, R., Fushman, D., and Pickart, C. M. (2005). Diverse Polyubiquitin Interaction Properties of Ubiquitin-Associated Domains. *Nat. Struct. Mol. Biol.* 12 (8), 708–714. doi:10.1038/nsmb962

Ravid, T., and Hochstrasser, M. (2008). Diversity of Degradation Signals in the Ubiquitin-Proteasome System. *Nat. Rev. Mol. Cel Biol* 9 (9), 679–689. doi:10.1038/nrm2468

Reyes-Turcu, F. E., Horton, J. R., Mullally, J. E., Heroux, A., Cheng, X., and Wilkinson, K. D. (2006). The Ubiquitin Binding Domain ZnF UBP Recognizes the C-Terminal Diglycine Motif of Unanchored Ubiquitin. *Cell* 124 (6), 1197–1208. doi:10.1016/j.cell.2006.02.038

Reyes-Turcu, F. E., Shanks, J. R., Komander, D., and Wilkinson, K. D. (2008). Recognition of Polyubiquitin Isoforms by the Multiple Ubiquitin Binding Modules of Isopeptidase T. *J. Biol. Chem.* 283 (28), 19581–19592. doi:10.1074/jbc.m800947200

Salway, J. G. (2018). The Krebs Uric Acid Cycle: A Forgotten Krebs Cycle. *Trends Biochem. Sci.* 43 (11), 847–849. doi:10.1016/j.tibs.2018.04.012

Silver, D. P., and Livingston, D. M. (2012). Mechanisms of BRCA1 Tumor Suppression: Figure 1. *Cancer Discov.* 2 (8), 679–684. doi:10.1158/2159-8290.cd-12-0221

Sippl, W., Collura, V., and Colland, F. (2011). Ubiquitin-specific Proteases as Cancer Drug Targets. *Future Oncol.* 7 (5), 619–632. doi:10.2217/fon.11.39

Sobhian, B., Shao, G., Lilli, D. R., Culhane, A. C., Moreau, L. A., Xia, B., et al. (2007). RAP80 Targets BRCA1 to Specific Ubiquitin Structures at DNA Damage Sites. *Science* 316 (5828), 1198–1202. doi:10.1126/science.1139516

Soetandyo, N., Wang, Q., Ye, Y., and Li, L. (2010). Role of Intramembrane Charged Residues in the Quality Control of Unassembled T-Cell Receptor Alpha-Chains at the Endoplasmic Reticulum. *J. Cel Sci* 123 (Pt 7), 1031–1038. doi:10.1242/jcs.059758

Song, B.-L., Sever, N., and Debose-Boyd, R. A. (2005). Gp78, a Membrane-Anchored Ubiquitin Ligase, Associates with Insig-1 and Couples Sterol-Regulated Ubiquitination to Degradation of HMG CoA Reductase. *Mol. Cel* 19 (6), 829–840. doi:10.1016/j.molcel.2005.08.009

Sun, H., Zhang, Q., Jing, Y.-Y., Zhang, M., Wang, H.-Y., Cai, Z., et al. (2017). USP13 Negatively Regulates Antiviral Responses by Deubiquitinating STING. *Nat. Commun.* 8, 15534. doi:10.1038/ncomms15534

Sun, R. C., and Denko, N. C. (2014). Hypoxic Regulation of Glutamine Metabolism through HIF1 and SIAH2 Supports Lipid Synthesis that Is Necessary for Tumor Growth. *Cel Metab.* 19 (2), 285–292. doi:10.1016/j.cmet.2013.11.022

Timms, K. M. M. a. a-L., Ansari-Lari, M. A., Morris, W., Brown, S. N., and Gibbs, R. A. (1998). The Genomic Organization of Isopeptidase T-3 (ISOT-3), a New Member of the Ubiquitin Specific Protease Family (UBP). *Gene* 217 (1-2), 101–106. doi:10.1016/s0378-1119(98)00341-2

Wang, B., Matsuoka, S., Ballif, B. A., Zhang, D., Smogorzewska, A., Gygi, S. P., et al. (2007). Abraxas and RAP80 Form a BRCA1 Protein Complex Required for the DNA Damage Response. *Science* 316 (5828), 1194–1198. doi:10.1126/science.1139476

Wang, Q., Liu, Y., Soetandyo, N., Baek, K., Hegde, R., and Ye, Y. (2011). A Ubiquitin Ligase-Associated Chaperone Holdase Maintains Polypeptides in Soluble States for Proteasome Degradation. *Mol. Cel* 42 (6), 758–770. doi:10.1016/j.molcel.2011.05.010

Wang, Y., and Wang, F. (2021). Post-translational Modifications of Deubiquitinating Enzymes: Expanding the Ubiquitin Code. *Front. Pharmacol.* 12, 685011. doi:10.3389/fphar.2021.685011

Wilson, A., Murphy, M. J., Oskarsson, T., Kaloulis, K., Bettess, M. D., Oser, G. M., et al. (2004). c-Myc Controls the Balance between Hematopoietic Stem Cell Self-Renewal and Differentiation. *Genes Dev.* 18 (22), 2747–2763. doi:10.1101/gad.313104

Wu, X., and Rapoport, T. A. (2018). Mechanistic Insights into ER-Associated Protein Degradation. *Curr. Opin. Cel Biol.* 53, 22–28. doi:10.1016/j.ceb.2018.04.004

Wu, Y., Zhang, Y., Liu, C., Zhang, Y., Wang, D., Wang, S., et al. (2019). Amplification of USP13 Drives Non-small Cell Lung Cancer Progression Mediated by AKT/MAPK Signaling. *Biomed. Pharmacother.* 114, 108831. doi:10.1016/j.biopha.2019.108831

Xiang, S., Fang, J., Wang, S., Deng, B., and Zhu, L. (2015). MicroRNA-135b Regulates the Stability of PTEN and Promotes Glycolysis by Targeting USP13 in Human Colorectal Cancers. *Oncol. Rep.* 33 (3), 1342–1348. doi:10.3892/or.2014.3694

Xie, W., Jin, S., Wu, Y., Xian, H., Tian, S., Liu, D.-A., et al. (2020). Auto-ubiquitination of NEDD4-1 Recruits USP13 to Facilitate Autophagy through Deubiquitinating VPS34. *Cel Rep.* 30 (8), 2807–2819. doi:10.1016/j.celrep.2020.01.088

Xie, Y., Shi, X., Sheng, K., Han, G., Li, W., Zhao, Q., et al. (2019). PI3K/Akt Signaling Transduction Pathway, Erythropoiesis and Glycolysis in Hypoxia (Review). *Mol. Med. Rep.* 19 (2), 783–791. doi:10.3892/mmr.2018.9713

Xu, Y., Cai, M., Yang, Y., Huang, L., and Ye, Y. (2012). SGTA Recognizes a Noncanonical Ubiquitin-like Domain in the Bag6-Ubl4A-Trc35 Complex to Promote Endoplasmic Reticulum-Associated Degradation. *Cel Rep.* 2 (6), 1633–1644. doi:10.1016/j.celrep.2012.11.010

Yan, J., Kim, Y.-S., Yang, X.-P., Li, L.-P., Liao, G., Xia, F., et al. (2007). The Ubiquitin-Interacting Motif-Containing Protein RAP80 Interacts with BRCA1 and Functions in DNA Damage Repair Response. *Cancer Res.* 67 (14), 6647–6656. doi:10.1158/0008-5472.can-07-0924

Yang, G., Song, W., Postoak, J. L., Chen, J., Martinez, J., Zhang, J., et al. (2021). Autophagy-related Protein PIK3C3/VPS34 Controls T Cell Metabolism and Function. *Autophagy* 17 (5), 1193–1204. doi:10.1080/15548627.2020.1752979

Ye, Y., Meyer, H. H., and Rapoport, T. A. (2001). The AAA ATPase Cdc48/p97 and its Partners Transport Proteins from the ER into the Cytosol. *Nature* 414 (6864), 652–656. doi:10.1038/414652a

Ye, Y., and Rape, M. (2009). Building Ubiquitin Chains: E2 Enzymes at Work. *Nat. Rev. Mol. Cel Biol* 10 (11), 755–764. doi:10.1038/nrm2780

Yu, H., Kaung, G., Kobayashi, S., and Kopito, R. R. (1997). Cytosolic Degradation of T-Cell Receptor Alpha Chains by the Proteasome. *J. Biol. Chem.* 272 (33), 20800–20804. doi:10.1074/jbc.272.33.20800

Yuan, T., Yan, F., Ying, M., Cao, J., He, Q., Zhu, H., et al. (2018). Inhibition of Ubiquitin-specific Proteases as a Novel Anticancer Therapeutic Strategy. *Front. Pharmacol.* 9, 1080. doi:10.3389/fphar.2018.01080

Zhang, J., Zhang, P., Wei, Y., Piao, H.-l., Wang, W., Maddika, S., et al. (2013). Deubiquitylation and Stabilization of PTEN by USP13. *Nat. Cel Biol* 15 (12), 1486–1494. doi:10.1038/ncb2874

Zhang, S., Zhang, M., Jing, Y., Yin, X., Ma, P., Zhang, Z., et al. (2018). Deubiquitinase USP13 Dictates MCL1 Stability and Sensitivity to BH3 Mimetic Inhibitors. *Nat. Commun.* 9 (1), 215. doi:10.1038/s41467-017-02693-9

Zhang, T., Zheng, J., Qiao, L., and Zhao, W. (2022). Deubiquitinase USP13 Promotes the Epithelial-Mesenchymal Transition and Metastasis in Gastric Cancer by Maintaining Snail Protein. *Pathol. Res. Pract.* 229, 153705. doi:10.1016/j.prp.2021.153705

Zhang, Y.-H., Zhou, C.-J., Zhou, Z.-R., Song, A.-X., and Hu, H.-Y. (2011). Domain Analysis Reveals that a Deubiquitinating Enzyme USP13 Performs Non-activating Catalysis for Lys63-Linked Polyubiquitin. *PLoS One* 6 (12), e29362. doi:10.1371/journal.pone.0029362

Zhao, X., Fiske, B., Kawakami, A., Li, J., and Fisher, D. E. (2011). Regulation of MITF Stability by the USP13 Deubiquitinase. *Nat. Commun.* 2, 414. doi:10.1038/ncomms1421

Zhou, Q., Lin, M., Feng, X., Ma, F., Zhu, Y., Liu, X., et al. (2020). Targeting CLK3 Inhibits the Progression of Cholangiocarcinoma by Reprogramming Nucleotide Metabolism. *J. Exp. Med.* 217 (8). doi:10.1084/jem.20191779

Conflict of Interest: 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

GLOSSARY

DUB deubiquitination

USP13 ubiquitin-specific protease 13

ERAD endoplasmic reticulum-associated degradation

E1 ubiquitin-activating enzyme

E2 ubiquitin-conjugating enzyme

E3 ubiquitin ligase

LUBAC linear ubiquitin chain assembly complex

USPs ubiquitin-specific proteases

OTUs ovarian tumor proteases

UCHs ubiquitin C-terminal hydrolase

MJDs Machado–Josephin domain-containing proteases

MINDYs motifs interacting with the ubiquitin-containing novel DUB family

JAMMs JAB1, MPN, MOV34 family

ZUP1 zinc finger containing Ub peptidase 1

OGDH α-ketoglutarate dehydrogenase

ACLY ATP citrate lyase

VPS34 vacuolar protein sorting 34

TopBP1 topoisomerase IIβ binding protein 1

RAP80 receptor-associated protein 80

Ubl4A ubiquitin like 4A

MITF microphthalmia-associated transcription factor

FBXL14 F-box and leucine-rich repeat protein 14

MAPK mitogen-activated protein kinase

ZnF Zinc finger

PTEN phosphatase and tensin homolog deleted on chromosome ten

UBA ubiquitin-associated

MCL1 myeloid cell leukemia sequence 1

BCL-2 B cell lymphoma 2

NMR Nuclear Magnetic Resonance

ITC isothermal titration calorimetry

gp78 glycoprotein 78

Ub-AMC Ubiquitin-7-amido-4- methylcoumarin

PI3K Phosphatidylinositol-3-kinase

OVCA ovarian cancer

CA-USP13 C345A-USP13 mutation

NOD/SCID nonobese diabetic/severe combined immunodeficiency

NEDD4-1 neural precursor cell expressed developmentally downregulated 4-1

IVDD intervertebral disc degeneration

DDR DNA damage repair

BRCA1 breast cancer 1

PARP Poly (ADP-ribose) polymerase

hHYD human hyperplastic discs

VCP valosin-containing protein

Ufd1 ubiquitin fusion degradation 1

Npl4 nuclear protein localization protein 4

Bag6 BCL-2-associated athanogen 6

Ub-Al ubiquitin aldehyde

AMFR autocrine motility factor receptor

NSCLC non-small-cell lung cancer

STING Stimulator of interferon genes

CLK3 CDC-like kinase 3

TCGA The Cancer Genomics Atlas

IHC Immunohistochemical

CAA cholangiocarcinoma

GSCs glioma stem cells

HCC hepatocellular carcinoma

OSCC oral squamous cell carcinoma

PI3P phosphatidylinositol 3-phosphate

UPS ubiquitin–proteasome system.

frontiers | Frontiers in Cell and Developmental Biology

MINI REVIEW
published: 05 May 2022
doi: 10.3389/fcell.2022.890121

Association Between Neddylation and Immune Response

*Jiali Zhu, Feng Chu, Meirong Zhang, Wenhuan Sun and Fangfang Zhou**

Institutes of Biology and Medical Science, Soochow University, Suzhou, China

Neddylation is a ubiquitin-like post-translational protein modification. It occurs *via* the activation of the neural precursor cell expressed, developmentally downregulated protein 8 (NEDD8) by three enzymes: activating enzyme, conjugating enzyme, and ligase. NEDD8 was first isolated from the mouse brain in 1992 and was initially considered important for the development and differentiation of the central nervous system. Previously, the downregulation of neddylation was associated with some human diseases, such as neurodegenerative disorders and cancers. In recent years, neddylation has also been proven to be pivotal in various processes of the human immune system, including the regulation of inflammation, bacterial infection, viral infection, and T cell function. Additionally, NEDD8 was found to act on proteins that can affect viral transcription, leading to impaired infectivity. Here, we focused on the influence of neddylation on the innate and adaptive immune responses.

Keywords: neddylation, NEDD8, immune response, innate immune cells, anti-viral pathway

OPEN ACCESS

Edited by:
Zhenghong Lin,
Chongqing University, China

Reviewed by:
Xiaohua Yan,
Nanchang University, China
Chenghua Li,
Sichuan University, China

****Correspondence:***
Fangfang Zhou
zhoufangfang@suda.edu.cn

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *05 March 2022*
Accepted: *30 March 2022*
Published: *05 May 2022*

Citation:
Zhu J, Chu F, Zhang M, Sun W and Zhou F (2022) Association Between Neddylation and Immune Response. Front. Cell Dev. Biol. 10:890121. doi: 10.3389/fcell.2022.890121

INTRODUCTION

Neddylation is a form of post-translational protein modifications (PTMs) in which the ubiquitin-like protein neural precursor cell expressed, developmentally downregulated protein 8 (NEDD8) binds to the target protein *via* a process similar to ubiquitination (Yu et al., 2019; Zhao et al., 2021). It was reported that the sequences of NEDD8 and ubiquitin are 59% identical (**Figure 1A**) (Enchev et al., 2015). NEDD8 was first isolated from the mouse brain, (Kumar et al., 1992). The first identified substrate was Cdc53, which is a yeast cullin (Gao et al., 2006). To date, the best-studied neddylation substrates are those from the largest ubiquitin E3 ligase family called cullin-RING ligases (CRLs) (Enchev et al., 2015), which are activated by conformational changes at the C-terminal (Duda et al., 2008; Mohanty et al., 2021). NEDD8 has also been found to act on other substrates, known as non-cullin proteins, to impact gene expression, cell survival, organ development, and stress response (Enchev et al., 2015). Once CRLs are activated, various cellular substrates participate in innate immune responses, cell cycle regulation, and cytoskeleton modeling (Mohanty et al., 2021).

In 1998, Cullin3 (Cul3) and Cul-4A were observed to be highly expressed in cultured colon cancer cells and primary breast cancer (Chen et al., 1998; Du et al., 1998). Elevated expression of NEDD8 was then observed in various human tumor cell lines, including leukemia cells and HeLa (Hori et al., 1999). Both these findings confirmed that neddylation is relevant to cancer progression. Some neddylated non-cullin proteins, such as the neddylated tumor suppressor phosphatase and tensin homolog (PTEN) and breast cancer-associated protein 3 (BCA3) have been shown to be cancer promoters (Mo et al., 2016; Xie et al., 2021). In 2009, it was found that inhibition of the activating enzyme (E1) of neddylation using MLN4924 (Pevonedistat) can suppress tumor progression, and it is currently undergoing clinical trials, in combination with chemotherapies, against various types of malignant tumors (Soucy et al., 2009; Xie et al., 2021) (NCT04090736, NCT03745352). There is a

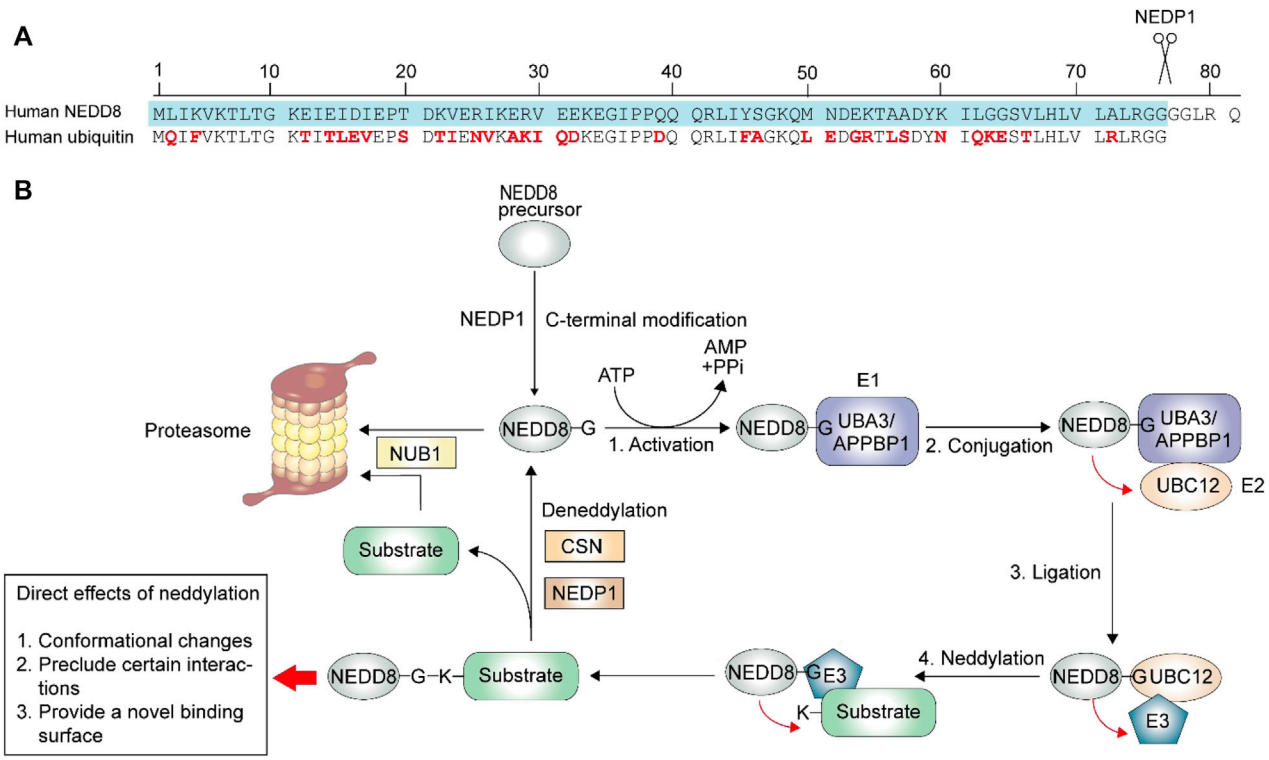


FIGURE 1 | Neddylation process. The neddylation process is similar to ubiquitination, with a three-step enzymatic reaction. **(A)** The maturation of the NEDD8 is a C-terminal hydrolytic activity by NEDP1. The ubiquitin contains 76 amino acids, and the different residues between matured NEDD8 (blue) and ubiquitin is highlighted in red. **(B)** After maturation, NEDD8 binds to the activating enzyme E1 in an ATP-dependent manner. NEDD8 is then accepted by conjugating enzyme E2. Finally, E2 transfers NEDD8 to ligase E3, which then links the glycine of NEDD8 and the lysine residues of its substrates *via* an isopeptide bond. The following deneddylation process deconjugates NEDD8 from the neddylated substrate. And after deconjugation, NUB1 (Negative regulator of ubiquitin like proteins 1) directs NEDD8 and substrate (like CRLs) to proteasome for degradation. The direct effects of neddylation on its substrates include three parts: conformational changes, preclude certain interactions and provide a novel binding surface.

large body of evidence demonstrating that MLN4924 functions as a tumor inhibitor by triggering DNA-damage responses, cell cycle arrest, apoptosis, autophagy, and alteration of mitochondrial function (Soucy et al., 2009; Zhao et al., 2012; Zhou et al., 2019).

During the past decade, the connection between neddylation and immunity has been investigated, describing the importance of this type of PTM in controlling immune responses and immune-related diseases. In this review, we summarize the neddylation process and its regulatory effects on innate and adaptive immunity.

NEDDYLATION PROCESS

The primary product of the *NEDD8* gene is a NEDD8 precursor, which needs to be modified to expose the C-terminal glycine before it acts on its targets (Enchev et al., 2015). NEDD8 precursor contains 81 amino acids (**Figure 1A**), and NEDD8 protease 1 (NEDP1, also known as the human deneddylase 1, DEN1), is involved in NEDD8 precursor processing (**Figure 1B**). After the proteolytic process, the NEDD8 activating enzyme (NAE, E1) facilitates NEDD8 activation in an ATP-dependent manner, and a high-energy intermediate is produced. Following this, the conjugating enzyme (E2) accepts and transfers NEDD8 to ligase (E3), which ensures specific conjugation between activated NEDD8 and its target protein (Wu et al., 2003; Rabut and Peter, 2008). Similar to ubiquitin, the final attachment occurs *via* an isopeptide linkage between conserved C-terminal glycine 76 of NEDD8 and the lysine residue of its substrates (Rabut and Peter, 2008; Watson et al., 2011). Previous studies have identified APPBP1-UBA3 (amyloid β precursor protein binding protein 1-ubiquitin activating enzyme 3) complex as E1, UBC12/UBE2M (ubiquitin conjugating enzyme E2 M) and UBE2F as E2, RING-box protein 1 (RBX1)/ regulator of cullins 1 (ROC1), RBX2/ROC2, and murine double minute 2 (MDM2) as E3 (Rabut and Peter, 2008; Li et al., 2019). Interestingly, some enzymes involved in neddylation also participate in the ubiquitination process, like MDM2 (Brooks and Gu, 2006), and the ubiquitin RING-class E3 component is not only the target of NEDD8 but also serves as E3 in the neddylation pathway (Huang et al., 2004). The following deneddylation is to separate the conjugated NEDD8 and substrate, during which the COP9 signalosome (CSN) and NEDP1 serve as deneddylase (Rabut and Peter, 2008).

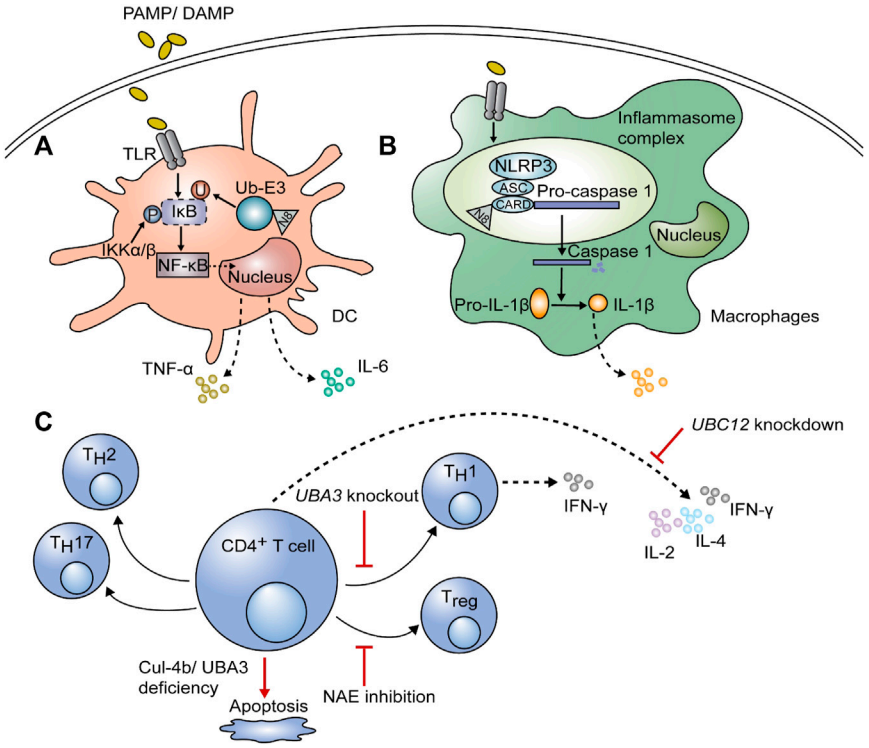


FIGURE 2 | The function of neddylation on immune cells. After stimulation with pathogen-associated molecular patterns or damage-associated molecular patterns (PAMPs or DAMPs), neddylation is required to induce proinflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), in dendritic cells (DCs) and macrophages. **(A)** After ubiquitination and degradation of inhibitor κB (IκB), nuclear factor (NF)-κB translocates into the nucleus and initiates gene expression; this process is dependent on the neddylation of ubiquitin E3 ligase. **(B)** The NLR family pyrin domain containing 3 (NLRP3) in the inflammasome complex of macrophages can be neddylated at the caspase recruitment domain (CARD), leading to self-cleavage of pro-caspase-1 and maturation of IL-1β, thus affecting inflammation. **(C)** For adaptive immune cells, inhibition of neddylation by knockdown of *UBC12* blocks cytokine secretion by $CD4^+$ T cells, including that of IL-2, IL-4, and IFN-γ. Knockout of *UBA3* can downregulate interferon (IFN)-γ-producing T_H1 cells and even result in apoptosis. NEDD8-activating enzyme (NAE) inhibition can regulate the polarization of $CD4^+$ T cells with lower T_{reg} differentiation and a shift towards the T_H1 phenotype. The deficiency of Cul-4b also has negative effects on $CD4^+$ T cell survival.

NEDDYLATION AND INNATE IMMUNE CELLS

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) involved in the innate immunity. Once they are stimulated by pathogens, the inhibitor κB (IκB) is phosphorylated by IκB kinase (IKKβ or α), and then ubiquitinated and degraded by the E3 ligase complex. CRL-1 is a component of the E3 ligase complex, which consists of Cul1, RING box protein (SAG), and S-phase kinase-associated protein 1 (SKP1) (Emanuele et al., 2011). Ubiquitination and degradation of IκB are dependent on the neddylation of Cul1 at the C-terminal lysine residue *via* conjugation to activated NEDD8 (**Figure 2A**) (Xirodimas, 2008). Upon degradation of IκB, nuclear factor-κB (NF-κB) is activated; it translocates into the nucleus, leading to an increase in the transcription and secretion of proinflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Mathewson et al., 2013; Jiang et al., 2021). Besides, neddylation is of great significance for survival of DCs. On the one hand, it was confirmed that knockdown of the key genes in neddylation pathway (*Cullin-1*, *Cullin-5*, *SENP8*, and *NEDD8*) can promote apoptosis and autophagy in *Mycobacterium tuberculosis* antigen stimulated DCs (Singhal et al., 2012; Chadha et al., 2015). On the other hand, Mathewson et al. found that inhibition of neddylation by MLN4924 treatment for 24 h or knockdown of *SAG* impairs the function of DCs without affecting the MAPK/ERK (Mitogen-activated protein kinase/Extracellular signal-related kinase) pathway and cell viability (Mathewson et al., 2013). However, long term MLN4924 admission was proven to reduce the number of APCs significantly, including DCs and macrophages, whereas showed minimal effect on T cells and B cells (Pai et al., 2017). Therefore, the effects of MLN4924 on survival of DCs is time-dependent. Importantly, MLN4924 was noticed to sensitize apoptosis and necroptosis of monocytes and immature DCs (iDCs) induced by TNF, and this effect is closely correlates with the suppressed expression of A20 (a ubiquitination editing enzyme), cellular inhibitor of apoptosis protein 2 (cIAP2), TNF receptor associated factor 1 (TRAF1) and FLIP (FLICE inhibitory protein), which provide cells resistance to TNF-induced cell death (El-Mesery et al., 2015).

Same as in DCs, lipopolysaccharide (LPS)-induced proinflammatory cytokine production can be suppressed by MLN4924, inhibiting ubiquitination and degradation of IκBα, thus impairing nuclear translocation of NF-κB (Chang et al., 2012). Besides, the NOD-like receptor family pyrin domain containing 3 (NLRP3)/apoptosis-associated speck-like protein (ASC) is associated with pro-caspase-1 *via* the caspase recruitment domain (CARD) in inflammasome complex. The activity of NEDD8 on CARD is necessary for pro-caspase-1 to self-cleave into caspase-1 (**Figure 2B**), which is followed by the maturation of pro-IL -1β to IL-1β (Segovia et al., 2015; Zhou et al., 2019; Swanson et al., 2019; Jiang et al., 2021). Watahiki et al. also found that MLN4924 treatment prevents LPS-stimulated *Il1b* gene expression, thus could be a new strategy for inflammatory diseases (Watahiki et al., 2020). Except for affecting inflammatory responses, MLN4924 also promotes polarization towards M2 macrophages (Asare et al., 2017). The neddylation pathway was found to be activated during methicillin-resistant *Staphylococcus aureus* (MRSA) infection, providing protection through the NEDD8-Cullin3-Nrf2-ROS axis and increased reactive oxygen species (ROS) in mouse peritoneal macrophages (Xiu et al., 2021). Moreover, neddylation inhibition was proved to decrease macrophage tumor infiltration through chemotactic cytokine ligand 2 (CCL2) reduction, thus modulates the tumor microenvironment and could be a potent cancer therapy (Zhou et al., 2019; Meerang et al., 2020). Besides, CCL5 accumulation due to MLN4924 led to M2 macrophage infiltration, and exacerbates chronic pancreatitis (Lin et al., 2021). Apart from functional effects, neddylation also matters in survival of macrophages. It was reported that partial treatment using MLN4924 diminishes TNF-α and IL-6 induced by LPS without impairing cell viability, while persistent treatment inhibited cell proliferation because of G2 cell-cycle arrest and apoptosis in RAW264.7 macrophages (Li et al., 2013). This phenomenon is mainly due to blockade of cullin neddylation, leading to inactivation of CRL E3 ligase, accumulation of cell-cycle inhibitory CRL substrates (including Wee1, p21, and p27) and induction of DNA damage (Li et al., 2013). Cycle-inhibiting factor homolog in *Burkholderia pseudomallei* (CHBP) is a bacterial deamidase effector, which recognizes the host NEDD8 and catalyzes its deamidation and triggers macrophage-specific apoptosis but preserves integrity of cell membrane (Yao et al., 2012). Hence, CHBP may has similar effect like MLN4924 which needs further research.

Neutrophils, a type of polymorphonuclear leukocytes, are myeloid lineage cells that are recruited to specific sites as the first line of innate immune responses against pathogens (Kolaczkowska and Kubes, 2013). It has been reported that neutrophil and monocyte counts in the blood increased because of MLN4924 treatment (Asare et al., 2017). Earlier study claimed that MLN4924 treatment inhibits neutrophil function of TNF-α, IL-6, and IL-1β production in a dose-dependent manner by suppressing NF-κB signaling pathway (Jin et al., 2018). Xiong et al. found that SAG deficiency dramatically increases the levels of TNF-α, but did not influence the translocation of NF-κB in neutrophils, differing from the effects observed in macrophages (Xiong et al., 2018). Since SAG is one of the targets of NEDD8 it is worth investigating whether or how neddylation acts on neutrophils.

These results suggest that neddylation can regulate the secretion of proinflammatory cytokines and proliferation in innate immune cells, as well as other aspects, such as migration and polarization of macrophages. Since adequate evidence proved that neddylation inhibition by either gene (*Cullin-1*, *Cullin-5*, *SENP8*, and *NEDD8*) knockdown or MLN4924 can result in death of innate immune cells, when MLN4924 is involved in the therapeutic treatment, like cancer treatment, the patient's immune system needs to be closely monitored.

NEDDYLATION AND ANTI-VIRAL PATHWAYS

Zhao et al. used mouse models with myeloid deficiency in UBA3 or NEDD8 to study the effects of neddylation on the response against RNA virus, and they found UBA3 absence results in impaired IFN-β (interferon-β) as well as IFN-α production in myeloid dendritic cells (mDCs), proposing that myeloid neddylation is required to induce IFN production upon Sendai virus (SeV) infection (Zhao et al., 2021). Previous studies on zebrafish demonstrated that both interferon regulatory factor 3 (IRF3) and IRF7 are potential substrates of neddylation during spring viremia of carp virus (SVCV) infection and can activate anti-viral responses (Yu et al., 2019). They also claimed that neddylation inhibition increases zebrafish sensitivity to SVCV infection. And tests about SeV infection showed similar results, demonstrating that during infection, NEDD8 directly targets the C-terminal lysine residues of IRF7 (**Figure 3**) and partially improves its transcription by inhibiting its dimerization with the IFN-α repressor, IRF5 (Zhao et al., 2021). Through neddylation, induction of type I IFN by RNA virus is promoted, especially that of IFN-α. And research about MLN4924 demonstrated that neddylation is necessary for IRF3 to bind to the IFN-β promoter during SeV infection in HEK-293T, but the exact mechanism remains unclear (Song et al., 2016). Different from that, another study on SeV claimed that degradation of IRF3 is due to C-terminal phosphorylation by polyubiquitinated TANK-binding kinase 1 (TBK1), which is induced by a neddylated cullin-based ubiquitin ligase (Bibeau-Poirier et al., 2006). Although neddylation of IRF3 and IRF7 has been confirmed in different species, its function in the key factors in the innate immune pathway, such as melanoma differentiation-associated protein 5 (MDA5), mitochondrial antiviral-signaling protein (MAVS), and TBK1 has not yet been understood (Yu et al., 2019).

The DNA sensor, cyclic GMP-AMP synthase (cGAS), plays a fundamental role in viral DNA recognition. After cGAS is activated by combining with cytosolic DNA, ATP, and GTP and then converted into the cyclic GMP-AMP (cGAMP), the stimulator of interferon genes (STING) is activated, then triggers the following immune responses (Li et al., 2013; Wang et al., 2014; Hopfner and Hornung, 2020). The NEDD8 E3 ligase Rnf111 (Arkadia, or ring finger 111) has been shown to neddylate cGAS at numerous lysine

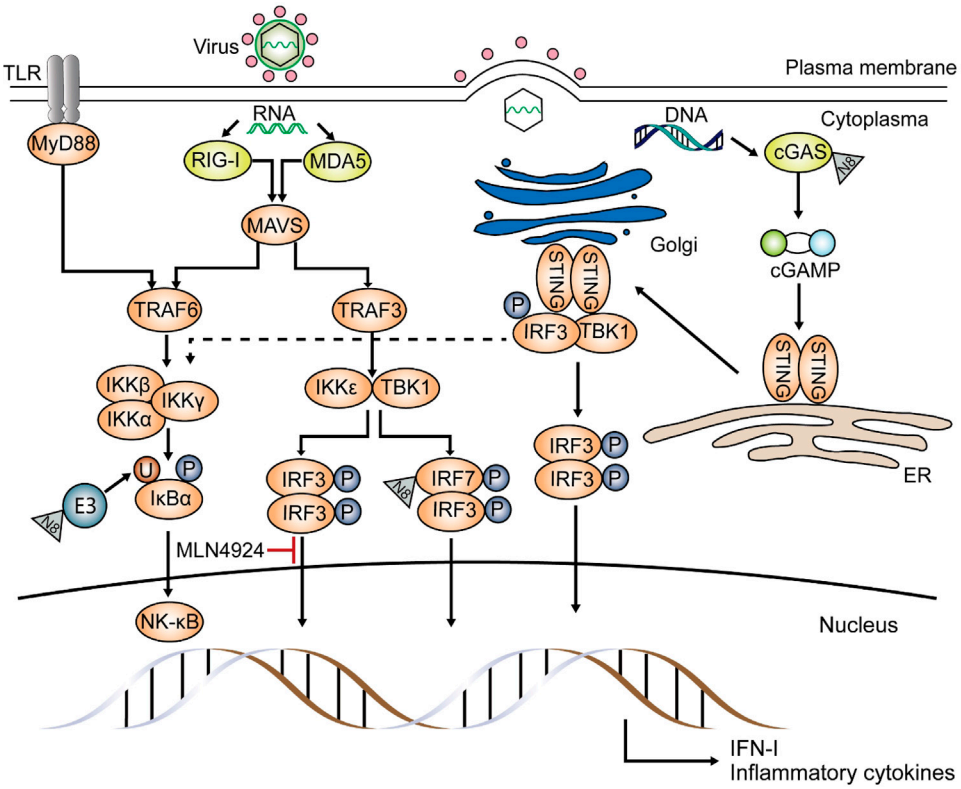


FIGURE 3 | Effects of neddylation on the anti-viral pathway. Neddylation is pivotal in the antiviral pathway, inducing inflammatory cytokines and type I-IFN. Upon infection by RNA viruses, such as Sendai virus (SeV), NEDD8 either acts on ubiquitin E3 ligase and affects NF-κB translocation or binds to IRF7 directly and promotes inflammatory cytokine and type I IFN production. Moreover, neddylation is involved in the conjugation of interferon regulatory factor 3 (IRF3) and the IFN-β promoter, and this was proved using MLN4924. If invaders are DNA viruses, such as herpes simplex virus, NEDD8 targets the DNA sensor cyclic GMP-AMP synthase (cGAS) and converts it to cGAMP. cGAMP then attaches to the stimulator of interferon genes (STING) and activates TANK-binding kinase 1 (TBK1) and IRF3, activating the type I-IFN gene. STING signaling also results in the expression of inflammatory cytokines through the NF-κB pathway (dashed line). Other proteins involved in the innate antiviral pathway have not yet been reported as substrates of neddylation.

sites upon herpes simplex virus 1 (HSV-1) infection (**Figure 3**), promoting its dimerization and DNA-binding capacity (Li et al., 2021). This research also proposed that neddylation inhibition by MLN4924 or deficiency of UBE2M or Rnf111 can weaken the stimulation of cGAS-STING. Another study demonstrated that MLN4924 treatment impairs HSV-1-induced NF-κB activation; this phenomenon can only be detected in the early phase of infection without affecting the activation of IRF3 and becomes inefficient in the later phase (Zhang et al., 2016).

Aside from acting directly on the anti-viral pathway, studies have demonstrated that the life cycle of viruses, including human immunodeficiency virus (HIV), Influenza A virus (IAV), and Hepatitis B virus (HBV), can be regulated by neddylation, mostly by targeting the replication process (Dias et al., 2009; Stanley et al., 2012; Liu et al., 2017). For example, the viral infectivity factor (Vif) of HIV needs UBE2F, the neddylation E2, to counteract the cytidine deaminases A3G. Hence, neddylation inhibition, using MLN4924 or knockdown of UBE2F, can suppress HIV replication (Stanley et al., 2012). HIV-2 viral protein X (Vpx) mediates depletion of the restriction factor SAM domain and HD domain-containing protein 1 (SAMHD1) *via* CRL4 (DCAF1) E3 ligase, and impaired neddylation can block this pathway, thus interfered HIV infection (Nekorchuk et al., 2013; Wei et al., 2014; Wang et al., 2017). The M1 protein and polymerase basic protein 2 (PB2, the component of RNA-dependent RNA polymerase) of IAV can be neddylated, causing reduced stability and inhibition of IAV replication (Dias et al., 2009; Zhang et al., 2017; Li et al., 2020). As for HBV, a double-stranded DNA virus, it was showed that neddylation of HBV regulatory X protein (HBx) at residues K91 and K95 by MDM2 can improve its stability and chromatin localization, thereby favoring viral replication (Liu et al., 2017). Recent research also found that HBV replication can be suppressed by NEDD8 knockdown and MLN4924 admission (Abounouh et al., 2022). In addition, MLN4924 treatment can restrain both its replication and antigen production. This is mediated by the activation of the ERK to inhibit necessary transcriptional factors, including hepatocyte nuclear factor 1 α (HNF1α), CCAAT/enhancer-binding protein (C/EBPα), and HNF4α (Xie et al., 2021). Furthermore, neddylation was noted to be required for other viruses such as Human enteroviruses and Kaposi's sarcoma-associated herpesvirus (KSHV), making MLN4924 an promising anti-viral treatment (Hughes et al., 2015; Chang et al., 2017; Zhang et al., 2021).

There is ample evidence showing that neddylation is sometimes required to fight against viral invasion, but there

are conflicting results. One report claimed that NEDD8 knockdown does not affect LPS- or SeV-induced IFN-β production in HeLa, HEK-293T, and THP-1 cells (Song et al., 2016), differing from the results in mDC mentioned above. And considering that pretreatment of HEK-293T with MLN4924 can inhibit IRF3 bind to IFN-β promoter during SeV infection, it is reasonable to assume that NEDD8 deficiency affects IFN-β production could be different in different cell lines, and MLN4924 suppresses IFN-β production in a neddylation-independent manner, but both assumptions need more evidence. Therefore, whether neddylation can benefit type I IFN production when facing virus infection and whether neddylation blockade by MLN4924 is the ideal therapeutic method for virus infection should be researched further.

NEDDYLATION AND ADAPTIVE IMMUNITY

Neddylation was also shown to regulate adaptive immunity as well as innate immunity (**Figure 2C**). Knockdown of *UBC12* in $CD4^+$ T cells results in impaired cell proliferation; suppressed production of cytokines, including IL-2, IL-4, and IFN-γ; and activation of ERK (Jin et al., 2013). Decreased IFN-γ-producing T_H1 cells were also detected in *UBA3* knockout mice, causing less resistance to early phase parasitic infection by *Plasmodium yoelii* 17XNL (Cheng et al., 2018). They also proved that neddylation is necessary for T cell survival by suppressing mitochondria-dependent apoptosis induced by B-cell lymphoma-2 (Bcl-2). T-cell-specific, *SAG* genetic knockout animal shows normal mature T cell development, but their T cells show significantly declined activation, proliferation and T-effector cytokine release. And MLN4924 treatment showed similar *in vitro* and *in vivo* results (Mathewson et al., 2016). MLN4924 inhibits the NEDD8-activating enzyme, which then regulate T cell polarization in chronic lymphocytic leukemia (CLL) patients with lower T_{reg} differentiation and a shift to the T_H1 phenotype but increased production of IFN-γ (Best et al., 2021). Recently, it was shown that neddylated Cul-4b is more abundant after T cell activation, and it is necessary to maintain the survival rate of effector $CD4^+$ T cells. Since Cul-4b lacking $CD4^+$ T cells are not capable of repairing DNA damage, they are more likely to undergo apoptosis (Dar et al., 2021). Taken together, neddylation is an indispensable process for T cells to function properly and survive.

For B cells, it has been reported that neddylation acts on CRLs and disrupts the NF-κB pathway in CLL B-cells. Using MLN4924 treatment, the BCL-2 homology 3-only protein (including Bim and Noxa) expression is induced in CLL cells, followed by cell apoptosis and reduced drug resistance (Godbersen et al., 2014). Alkylating agents can further promote MLN4924-induced DNA damage and apoptosis of CLL cells (Paiva et al., 2015). The effects of neddylation on B cells remain largely unknown and require further investigation.

As stated in the previous section, the inhibitor MLN4924 is a potential anti-tumor treatment, but it was found to have negative effects in treating glioblastoma (Zhou et al., 2019). Although MLN4924 can slow down tumor growth in glioblastoma, it can also elevate T-cell negative regulator programmed death-ligand 1 (PD-L1) expression by inhibiting SKP1-Cul1-F-box and WD repeat domain-containing 7 (FBXW7) activity, then lead to impaired T cell killing ability. Another study found that MLN4924 can cause impaired NEDD8-dependent clearance of misfolded proteins in dMMR/MSI tumors (deficient DNA mismatch repair/microsatellite instability tumors), and by combination with anti-PD1, potent synergistic activity was achieved and tumor immune microenvironment was tested to be altered since the number of cytotoxic T cells and conventional $CD4^+$ T cells increased whereas regulatory T cells reduced (McGrail et al., 2020).

In summary, current studies of neddylation and adaptive immunity are mostly relevant to cancer. The inhibition of neddylation can disrupt cytokines production and survival of T/B cells. MLN4924 has beneficial effects in anti-tumor therapy and autoimmune diseases, but it can also impair patients' immune responses, making them more vulnerable to infections. Therefore, the utilization MLN4924 should be strictly monitored and studied further.

DISCUSSION

As a type of PTM, neddylation plays a vital role in the innate and adaptive immune responses. The neddylation process is required for immune cells to function and survive, and it is indispensable in the anti-viral pathway. And the neddylation inhibitor MLN4924 is recognized as a novel and promising cancer therapeutic strategy. However, some questions still need further research.

Firstly, although neddylation was discovered decades ago, its function and mechanism in the innate immunity and the basis of NEDD8 activation remain largely unknown. Secondly, the function of neddylation after viral infection and subsequent IFN production is debated. Thirdly, MLN4924 can suppress replication of some virus (HIV and HBV), but this treatment can also impair the anti-viral response, thus we need to learn how to balance the dual effects of MLN4924.

Once we assure these questions, the regulatory mechanisms of neddylation will be clarified and provide sound theoretical basis for the utilization of MLN4924, shedding light on treatment of cancer, viral infection and other related diseases.

AUTHOR CONTRIBUTIONS

JZ drafted the manuscript; FC discussed the concept of the manuscript; MZ and WS drew the figures; FZ contributed to the writing and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by a special program from the Chinese National Natural Science Funds (82041009 and 32125016 to FZ), Jiangsu National Science Foundation (19KJA550003 to FZ).

ACKNOWLEDGMENTS

We would like to appreciate all those who helped us during the writing of this review.

REFERENCES

Abounouh, K., Kayesh, M. E. H., Altawalah, H., Kitab, B., Murakami, S., Ogawa, S., et al. (2022). Blocking Neddylation Elicits Antiviral Effect against Hepatitis B Virus Replication. *Mol. Biol. Rep.* 49 (1), 403–412. doi:10.1007/s11033-021-06886-w

Asare, Y., Ommer, M., Azombo, F. A., Alampour-Rajabi, S., Sternkopf, M., Sanati, M., et al. (2017). Inhibition of Atherogenesis by the COP9 Signalosome Subunit 5 *In Vivo*. *Proc. Natl. Acad. Sci. U S A.* 114 (13), E2766–E2775. doi:10.1073/pnas.1618411114

Best, S., Lam, V., Liu, T., Bruss, N., Kittai, A., Danilova, O. V., et al. (2021). Immunomodulatory Effects of Pevonedistat, a NEDD8-Activating Enzyme Inhibitor, in Chronic Lymphocytic Leukemia-Derived T Cells. *Leukemia* 35 (1), 156–168. doi:10.1038/s41375-020-0794-0

Bibeau-Poirier, A., Gravel, S.-P., Clément, J.-F., Rolland, S., Rodier, G., Coulombe, P., et al. (2006). Involvement of the IκB Kinase (IKK)-Related Kinases Tank-Binding Kinase 1/IKKi and Cullin-Based Ubiquitin Ligases in IFN Regulatory Factor-3 Degradation. *J. Immunol.* 177 (8), 5059–5067. doi:10.4049/jimmunol.177.8.5059

Brooks, C. L., and Gu, W. (2006). p53 Ubiquitination: Mdm2 and beyond. *Mol. Cel* 21 (3), 307–315. doi:10.1016/j.molcel.2006.01.020

Chadha, A., Mehto, S., Selvakumar, A., Vashishta, M., Kamble, S. S., Popli, S., et al. (2015). Suppressive Role of Neddylation in Dendritic Cells during *Mycobacterium tuberculosis* Infection. *Tuberculosis* 95 (5), 599–607. doi:10.1016/j.tube.2015.05.014

Chang, F.-M., Reyna, S. M., Granados, J. C., Wei, S.-J., Innis-Whitehouse, W., Maffi, S. K., et al. (2012). Inhibition of Neddylation Represses Lipopolysaccharide-Induced Proinflammatory Cytokine Production in Macrophage Cells. *J. Biol. Chem.* 287 (42), 35756–35767. doi:10.1074/jbc.m112.397703

Chang, P. J., Chen, L. W., Chen, L. Y., Hung, C. H., Shih, Y. J., and Wang, S. S. (2017). Effects of the NEDD8-Activating Enzyme Inhibitor MLN4924 on Lytic Reactivation of Kaposi's Sarcoma-Associated Herpesvirus. *J. Virol.* 91 (19). doi:10.1128/JVI.00505-17

Chen, L. C., Manjeshwar, S., Lu, Y., Moore, D., Ljung, B. M., Kuo, W. L., et al. (1998). The Human Homologue for the *Caenorhabditis elegans* Cul-4 Gene Is Amplified and Overexpressed in Primary Breast Cancers. *Cancer Res.* 58 (16), 3677–3683.

Cheng, Q., Liu, J., Pei, Y., Zhang, Y., Zhou, D., Pan, W., et al. (2018). Neddylation Contributes to CD4+ T Cell-Mediated Protective Immunity against Blood-Stage Plasmodium Infection. *Plos Pathog.* 14 (11), e1007440. doi:10.1371/journal.ppat.1007440

Dar, A. A., Sawada, K., Dybas, J. M., Moser, E. K., Lewis, E. L., Park, E., et al. (2021). The E3 Ubiquitin Ligase Cul4b Promotes CD4+ T Cell Expansion by Aiding the Repair of Damaged DNA. *Plos Biol.* 19 (2), e3001041. doi:10.1371/journal.pbio.3001041

Dias, A., Bouvier, D., Crépin, T., McCarthy, A. A., Hart, D. J., Baudin, F., et al. (2009). The Cap-Snatching Endonuclease of Influenza Virus Polymerase Resides in the PA Subunit. *Nature* 458 (7240), 914–918. doi:10.1038/nature07745

Du, M., Sansores-Garcia, L., Zu, Z., and Wu, K. K.-y. (1998). Cloning and Expression Analysis of a Novel Salicylate Suppressible Gene, Hs-CUL-3, a Member of cullin/Cdc53 Family. *J. Biol. Chem.* 273 (38), 24289–24292. doi:10.1074/jbc.273.38.24289

Duda, D. M., Borg, L. A., Scott, D. C., Hunt, H. W., Hammel, M., and Schulman, B. A. (2008). Structural Insights into NEDD8 Activation of Cullin-RING Ligases: Conformational Control of Conjugation. *Cell* 134 (6), 995–1006. doi:10.1016/j.cell.2008.07.022

El-Mesery, M., Seher, A., Stühmer, T., Siegmund, D., and Wajant, H. (2015). MLN4924 Sensitizes Monocytes and Maturing Dendritic Cells for TNF-dependent and -independent Necroptosis. *Br. J. Pharmacol.* 172 (5), 1222–1236. doi:10.1111/bph.12998

Emanuele, M. J., Elia, A. E. H., Xu, Q., Thoma, C. R., Izhar, L., Leng, Y., et al. (2011). Global Identification of Modular Cullin-RING Ligase Substrates. *Cell* 147 (2), 459–474. doi:10.1016/j.cell.2011.09.019

Enchev, R. I., Schulman, B. A., and Peter, M. (2015). Protein Neddylation: beyond Cullin-RING Ligases. *Nat. Rev. Mol. Cel Biol* 16 (1), 30–44. doi:10.1038/nrm3919

Gao, F., Cheng, J., Shi, T., and Yeh, E. T. H. (2006). Neddylation of a Breast Cancer-Associated Protein Recruits a Class III Histone Deacetylase that Represses NFκB-dependent Transcription. *Nat. Cel Biol* 8 (10), 1171–1177. doi:10.1038/ncb1483

Godbersen, J. C., Humphries, L. A., Danilova, O. V., Kebbekus, P. E., Brown, J. R., Eastman, A., et al. (2014). The Nedd8-Activating Enzyme Inhibitor MLN4924 Thwarts Microenvironment-Driven NF-Kb Activation and Induces Apoptosis in Chronic Lymphocytic Leukemia B Cells. *Clin. Cancer Res.* 20 (6), 1576–1589. doi:10.1158/1078-0432.ccr-13-0987

Hopfner, K.-P., and Hornung, V. (2020). Molecular Mechanisms and Cellular Functions of cGAS-STING Signalling. *Nat. Rev. Mol. Cel Biol* 21 (9), 501–521. doi:10.1038/s41580-020-0244-x

Hori, T., Osaka, F., Chiba, T., Miyamoto, C., Okabayashi, K., Shimbara, N., et al. (1999). Covalent Modification of All Members of Human Cullin Family Proteins by NEDD8. *Oncogene* 18 (48), 6829–6834. doi:10.1038/sj.onc.1203093

Huang, D. T., Walden, H., Duda, D., and Schulman, B. A. (2004). Ubiquitin-like Protein Activation. *Oncogene* 23 (11), 1958–1971. doi:10.1038/sj.onc.1207393

Hughes, D. J., Wood, J. J., Jackson, B. R., Baquero-Pérez, B., and Whitehouse, A. (2015). NEDDylation Is Essential for Kaposi's Sarcoma-Associated Herpesvirus Latency and Lytic Reactivation and Represents a Novel Anti-KSHV Target. *Plos Pathog.* 11 (3), e1004771. doi:10.1371/journal.ppat.1004771

Jiang, Y., Li, L., Li, Y., Liu, G., Hoffman, R. M., and Jia, L. (2021). Neddylation Regulates Macrophages and Implications for Cancer Therapy. *Front. Cel Dev. Biol.* 9, 681186. doi:10.3389/fcell.2021.681186

Jin, H.-s., Liao, L., Park, Y., and Liu, Y.-C. (2013). Neddylation Pathway Regulates T-Cell Function by Targeting an Adaptor Protein Shc and a Protein Kinase Erk Signaling. *Proc. Natl. Acad. Sci. U.S.A.* 110 (2), 624–629. doi:10.1073/pnas.1213819110

Jin, J., Jing, Z., Ye, Z., Guo, L., Hua, L., Wang, Q., et al. (2018). MLN4924 Suppresses Lipopolysaccharide-Induced Proinflammatory Cytokine Production in Neutrophils in a Dose-dependent Manner. *Oncol. Lett.* 15 (5), 8039–8045. doi:10.3892/ol.2018.8333

Kolaczkowska, E., and Kubes, P. (2013). Neutrophil Recruitment and Function in Health and Inflammation. *Nat. Rev. Immunol.* 13 (3), 159–175. doi:10.1038/nri3399

Kumar, S., Tomooka, Y., and Noda, M. (1992). Identification of a Set of Genes with Developmentally Down-Regulated Expression in the Mouse Brain. *Biochem. Biophysical Res. Commun.* 185 (3), 1155–1161. doi:10.1016/0006-291x(92)91747-e

Li, C., Zhang, L., Qian, D., Cheng, M., Hu, H., Hong, Z., et al. (2021). RNF111-facilitated Neddylation Potentiates cGAS-Mediated Antiviral Innate Immune Response. *Plos Pathog.* 17 (3), e1009401. doi:10.1371/journal.ppat.1009401

Li, L., Kang, J., Zhang, W., Cai, L., Wang, S., Liang, Y., et al. (2019). Validation of NEDD8-Conjugating Enzyme UBC12 as a New Therapeutic Target in Lung Cancer. *EBioMedicine* 45, 81–91. doi:10.1016/j.ebiom.2019.06.005

Li, L., Liu, B., Dong, T., Lee, H. W., Yu, J., Zheng, Y., et al. (2013a). Neddylation Pathway Regulates the Proliferation and Survival of Macrophages. *Biochem. Biophysical Res. Commun.* 432 (3), 494–498. doi:10.1016/j.bbrc.2013.02.028

Li, X.-D., Wu, J., Gao, D., Wang, H., Sun, L., and Chen, Z. J. (2013b). Pivotal Roles of cGAS-cGAMP Signaling in Antiviral Defense and Immune Adjuvant Effects. *Science* 341 (6152), 1390–1394. doi:10.1126/science.1244040

Li, Y., Chai, W., Min, J., Ye, Z., Tong, X., Qi, D., et al. (2020). Neddylation of M1 Negatively Regulates the Replication of Influenza A Virus. *J. Gen. Virol.* 101 (12), 1242–1250. doi:10.1099/jgv.0.001503

Lin, Y., Chen, Y., Feng, W., Hua, R., Zhang, J., Huo, Y., et al. (2021). Neddylation Pathway Alleviates Chronic Pancreatitis by Reducing HIF1α-CCL5-dependent Macrophage Infiltration. *Cell Death Dis* 12 (3), 273. doi:10.1038/s41419-021-03549-3

Liu, N., Zhang, J., Yang, X., Jiao, T., Zhao, X., Li, W., et al. (2017). HDM2 Promotes NEDDylation of Hepatitis B Virus HBx to Enhance its Stability and Function. *J. Virol.* 91 (16). doi:10.1128/JVI.00340-17

Mathewson, N. D., Fujiwara, H., Wu, S.-R., Toubai, T., Oravecz-Wilson, K., Sun, Y., et al. (2016). SAG/Rbx2-Dependent Neddylation Regulates T-Cell Responses. *Am. J. Pathol.* 186 (10), 2679–2691. doi:10.1016/j.ajpath.2016.06.014

Mathewson, N., Toubai, T., Kapeles, S., Sun, Y., Oravecz-Wilson, K., Tamaki, H., et al. (2013). Neddylation Plays an Important Role in the Regulation of Murine and Human Dendritic Cell Function. *Blood* 122 (12), 2062–2073. doi:10.1182/blood-2013-02-486373

McGrail, D. J., Garnett, J., Yin, J., Dai, H., Shih, D. J. H., Lam, T. N. A., et al. (2020). Proteome Instability Is a Therapeutic Vulnerability in Mismatch Repair-

Deficient Cancer. *Cancer Cell* 37 (3), 371–386 e312. doi:10.1016/j.ccell.2020.01.011

Meerang, M., Kreienbühl, J., Orlowski, V., Müller, S. L. C., Kirschner, M. B., and Opitz, I. (2020). Importance of Cullin4 Ubiquitin Ligase in Malignant Pleural Mesothelioma. *Cancers (Basel)* 12 (11). doi:10.3390/cancers12113460

Mo, Z., Zhang, Q., Liu, Z., Lauer, J., Shi, Y., Sun, L., et al. (2016). Neddylation Requires Glycyl-tRNA Synthetase to Protect Activated E2. *Nat. Struct. Mol. Biol.* 23 (8), 730–737. doi:10.1038/nsmb.3250

Mohanty, P., Chatterjee, K. S., and Das, R. (2021). NEDD8 Deamidation Inhibits Cullin RING Ligase Dynamics. *Front. Immunol.* 12, 695331. doi:10.3389/fimmu.2021.695331

Nekorchuk, M. D., Sharifi, H. J., Furuya, A. K., Jellinger, R., and de Noronha, C. M. (2013). HIV Relies on Neddylation for Ubiquitin Ligase-Mediated Functions. *Retrovirology* 10, 138. doi:10.1186/1742-4690-10-138

Pai, C.-C. S., Khuat, L. T., Chen, M., Murphy, W. J., and Abedi, M. (2017). Therapeutic Effects of a NEDD8-Activating Enzyme Inhibitor, Pevonedistat, on Sclerodermatous Graft-Versus-Host Disease in Mice. *Biol. Blood Marrow Transplant.* 23 (1), 30–37. doi:10.1016/j.bbmt.2016.10.022

Paiva, C., Godbersen, J. C., Berger, A., Brown, J. R., and Danilov, A. V. (2015). Targeting Neddylation Induces DNA Damage and Checkpoint Activation and Sensitizes Chronic Lymphocytic Leukemia B Cells to Alkylating Agents. *Cel Death Dis* 6–e1807. doi:10.1038/cddis.2015.161

Rabut, G., and Peter, M. (2008). Function and Regulation of Protein Neddylation. *EMBO Rep.* 9 (10), 969–976. doi:10.1038/embor.2008.183

Segovia, J. A., Tsai, S.-Y., Chang, T.-H., Shil, N. K., Weintraub, S. T., Short, J. D., et al. (2015). Nedd8 Regulates Inflammasome-dependent Caspase-1 Activation. *Mol. Cel Biol* 35 (3), 582–597. doi:10.1128/mcb.00775-14

Singhal, J., Agrawal, N., Vashishta, M., Priya, N. G., Tiwari, B. K., Singh, Y., et al. (2012). Suppression of Dendritic Cell-Mediated Responses by Genes in Calcium and Cysteine Protease Pathways during *Mycobacterium tuberculosis* Infection. *J. Biol. Chem.* 287 (14), 11108–11121. doi:10.1074/jbc.m111.300319

Song, H., Huai, W., Yu, Z., Wang, W., Zhao, J., Zhang, L., et al. (2016). MLN4924, a First-In-Class NEDD8-Activating Enzyme Inhibitor, Attenuates IFN-β Production. *J.I.* 196 (7), 3117–3123. doi:10.4049/jimmunol.1501752

Soucy, T. A., Smith, P. G., Milhollen, M. A., Berger, A. J., Gavin, J. M., Adhikari, S., et al. (2009). An Inhibitor of NEDD8-Activating Enzyme as a New Approach to Treat Cancer. *Nature* 458 (7239), 732–736. doi:10.1038/nature07884

Stanley, D. J., Bartholomeeusen, K., Crosby, D. C., Kim, D. Y., Kwon, E., Yen, L., et al. (2012). Inhibition of a NEDD8 Cascade Restores Restriction of HIV by APOBEC3G. *Plos Pathog.* 8 (12), e1003085. doi:10.1371/journal.ppat.1003085

Swanson, K. V., Deng, M., and Ting, J. P.-Y. (2019). The NLRP3 Inflammasome: Molecular Activation and Regulation to Therapeutics. *Nat. Rev. Immunol.* 19 (8), 477–489. doi:10.1038/s41577-019-0165-0

Wang, H., Guo, H., Su, J., Rui, Y., Zheng, W., Gao, W., et al. (2017). Inhibition of Vpx-Mediated SAMHD1 and Vpr-Mediated Host Helicase Transcription Factor Degradation by Selective Disruption of Viral CRL4 (DCAF1) E3 Ubiquitin Ligase Assembly. *J. Virol.* 91 (9). doi:10.1128/JVI.00225-17

Wang, Q., Liu, X., Cui, Y., Tang, Y., Chen, W., Li, S., et al. (2014). The E3 Ubiquitin Ligase AMFR and INSIG1 Bridge the Activation of TBK1 Kinase by Modifying the Adaptor STING. *Immunity* 41 (6), 919–933. doi:10.1016/j.immuni.2014.11.011

Watahiki, A., Shimizu, K., Hoshikawa, S., Chiba, M., Kitamura, H., Egusa, H., et al. (2020). Lipin-2 Degradation Elicits a Proinflammatory Gene Signature in Macrophages. *Biochem. Biophysical Res. Commun.* 524 (2), 477–483. doi:10.1016/j.bbrc.2020.01.119

Watson, I. R., Irwin, M. S., and Ohh, M. (2011). NEDD8 Pathways in Cancer, Sine Quibus Non. *Cancer Cell* 19 (2), 168–176. doi:10.1016/j.ccr.2011.01.002

Wei, W., Guo, H., Liu, X., Zhang, H., Qian, L., Luo, K., et al. (2014). A First-In-Class NAE Inhibitor, MLN4924, Blocks Lentiviral Infection in Myeloid Cells by Disrupting Neddylation-dependent Vpx-Mediated SAMHD1 Degradation. *J. Virol.* 88 (1), 745–751. doi:10.1128/jvi.02568-13

Wu, K., Yamoah, K., Dolios, G., Gan-Erdene, T., Tan, P., Chen, A., et al. (2003). DEN1 Is a Dual Function Protease Capable of Processing the C Terminus of Nedd8 and Deconjugating Hyper-Neddylated CUL1. *J. Biol. Chem.* 278 (31), 28882–28891. doi:10.1074/jbc.m302888200

Xie, M., Guo, H., Lou, G., Yao, J., Liu, Y., Sun, Y., et al. (2021a). Neddylation Inhibitor MLN4924 Has anti-HBV Activity via Modulating the ERK-HNF1α-C/EBPα-HNF4α axis. *J. Cel Mol Med* 25 (2), 840–854. doi:10.1111/jcmm.16137

Xie, P., Peng, Z., Chen, Y., Li, H., Du, M., Tan, Y., et al. (2021b). Neddylation of PTEN Regulates its Nuclear Import and Promotes Tumor Development. *Cell Res* 31 (3), 291–311. doi:10.1038/s41422-020-00443-z

Xiong, X., Mathewson, N. D., Li, H., Tan, M., Fujiwara, H., Li, H., et al. (2018). SAG/RBX2 E3 Ubiquitin Ligase Differentially Regulates Inflammatory Responses of Myeloid Cell Subsets. *Front. Immunol.* 9, 2882. doi:10.3389/fimmu.2018.02882

Xirodimas, D. P. (2008). Novel Substrates and Functions for the Ubiquitin-like Molecule NEDD8. *Biochem. Soc. Trans.* 36 (Pt 5), 802–806. doi:10.1042/BST0360802

Xiu, H., Peng, Y., Huang, X., Gong, J., Yang, J., Cai, J., et al. (2021). Neddylation Alleviates Methicillin-Resistant *Staphylococcus aureus* Infection by Inducing Macrophage Reactive Oxygen Species Production. *J.I.* 207 (1), 296–307. doi:10.4049/jimmunol.2001167

Yao, Q., Cui, J., Wang, J., Li, T., Wan, X., Luo, T., et al. (2012). Structural Mechanism of Ubiquitin and NEDD8 Deamidation Catalyzed by Bacterial Effectors that Induce Macrophage-specific Apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* 109 (50), 20395–20400. doi:10.1073/pnas.1210831109

Yu, G., Liu, X., Tang, J., Xu, C., Ouyang, G., and Xiao, W. (2019). Neddylation Facilitates the Antiviral Response in Zebrafish. *Front. Immunol.* 10, 1432. doi:10.3389/fimmu.2019.01432

Zhang, T., Ye, Z., Yang, X., Qin, Y., Hu, Y., Tong, X., et al. (2017). NEDDylation of PB2 Reduces its Stability and Blocks the Replication of Influenza A Virus. *Sci. Rep.* 7, 43691. doi:10.1038/srep43691

Zhang, X., Ye, Z., Pei, Y., Qiu, G., Wang, Q., Xu, Y., et al. (2016). Neddylation Is Required for Herpes Simplex Virus Type I (HSV-1)-Induced Early Phase Interferon-Beta Production. *Cell Mol Immunol* 13 (5), 578–583. doi:10.1038/cmi.2015.35

Zhang, Z., Guo, H., Wang, J., Li, Y., Gao, Y., Liu, Q., et al. (2021). Inhibition of the Neddylation Pathway Suppresses Enterovirus Replication. *Virol. Sin.* 36 (6), 1664–1667. doi:10.1007/s12250-021-00427-2

Zhao, M., Zhang, Y., Yang, X., Jin, J., Shen, Z., Feng, X., et al. (2021). Myeloid Neddylation Targets IRF7 and Promotes Host Innate Immunity against RNA Viruses. *Plos Pathog.* 17 (9), e1009901. doi:10.1371/journal.ppat.1009901

Zhao, Y., Xiong, X., Jia, L., and Sun, Y. (2012). Targeting Cullin-RING Ligases by MLN4924 Induces Autophagy via Modulating the HIF1-REDD1-TSC1-mTORC1-DEPTOR axis. *Cel Death Dis* 3, e386. doi:10.1038/cddis.2012.125

Zhou, L., Jiang, Y., Liu, X., Li, L., Yang, X., Dong, C., et al. (2019a). Promotion of Tumor-Associated Macrophages Infiltration by Elevated Neddylation Pathway via NF-Kb-CCL2 Signaling in Lung Cancer. *Oncogene* 38 (29), 5792–5804. doi:10.1038/s41388-019-0840-4

Zhou, Q., Li, H., Li, Y., Tan, M., Fan, S., Cao, C., et al. (2019b). Inhibiting Neddylation Modification Alters Mitochondrial Morphology and Reprograms Energy Metabolism in Cancer Cells. *JCI Insight* 4 (4). doi:10.1172/jci.insight.121582

Zhou, S., Zhao, X., Yang, Z., Yang, R., Chen, C., Zhao, K., et al. (2019c). Neddylation Inhibition Upregulates PD-L1 Expression and Enhances the Efficacy of Immune Checkpoint Blockade in Glioblastoma. *Int. J. Cancer* 145 (3), 763–774. doi:10.1002/ijc.32379

Conflict of Interest: 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 | Frontiers in Cell and Developmental Biology

REVIEW
published: 11 May 2022
doi: 10.3389/fcell.2022.833086

Pathophysiology of Primary Cilia: Signaling and Proteostasis Regulation

*Emanuela Senatore, Rosa Iannucci, Francesco Chiuso, Rossella Delle Donne, Laura Rinaldi and Antonio Feliciello**

Department of Molecular Medicine and Medical Biotechnology, University Federico II, Naples, Italy

Primary cilia are microtubule-based, non-motile sensory organelles present in most types of growth-arrested eukaryotic cells. They are transduction hubs that receive and transmit external signals to the cells in order to control growth, differentiation and development. Mutations of genes involved in the formation, maintenance or disassembly of ciliary structures cause a wide array of developmental genetic disorders, also known as ciliopathies. The primary cilium is formed during G1 in the cell cycle and disassembles at the G2/M transition. Following the completion of the cell division, the cilium reassembles in G1. This cycle is finely regulated at multiple levels. The ubiquitin-proteasome system (UPS) and the autophagy machinery, two main protein degradative systems in cells, play a fundamental role in cilium dynamics. Evidence indicate that UPS, autophagy and signaling pathways may act in synergy to control the ciliary homeostasis. However, the mechanisms involved and the links between these regulatory systems and cilium biogenesis, dynamics and signaling are not well defined yet. Here, we discuss the reciprocal regulation of signaling pathways and proteolytic machineries in the control of the assembly and disassembly of the primary cilium, and the impact of the derangement of these regulatory networks in human ciliopathies.

Keywords: ubiquitin, signaling, cAMP, PKA, autophagy, proteasome, E3 ligase

OPEN ACCESS

Edited by:
Zhenghong Lin,
Chongqing University, China

Reviewed by:
Francesc R. Garcia-Gonzalo,
Autonomous University of Madrid, Spain
Manuela Morleo,
Telethon Institute of Genetics and Medicine (TIGEM), Italy
Yenniffer Ávalos,
University of Santiago, Chile

****Correspondence:***
Antonio Feliciello
feliciel@unina.it

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *10 December 2021*
Accepted: *21 April 2022*
Published: *11 May 2022*

Citation:
Senatore E, Iannucci R, Chiuso F, Delle Donne R, Rinaldi L and Feliciello A (2022) Pathophysiology of Primary Cilia: Signaling and Proteostasis Regulation. Front. Cell Dev. Biol. 10:833086. doi: 10.3389/fcell.2022.833086

INTRODUCTION

The primary cilium is a non-motile, thin, microtubule-based organelle protruding from the apical membrane of most eukaryotic cells. Primary cilia act as antennae that receive and transmit extracellular signals into the cells, thus regulating a variety of biological functions, such as development, differentiation, growth and metabolism (Ishikawa and Marshall, 2011). The cilium consists of a basal body, a transition zone and an axoneme. The basal body derives from the differentiation of the mother centriole of the centrosome in G0 phase-arrested cells. The transition zone between basal body and axoneme acts as a gate that controls the entry and exit of cargoes within the ciliary compartment. The axoneme, also known as axial filament, is a cytoskeletal structure formed by nine doublets of microtubules surrounded by the ciliary membrane, contiguous with the cell membrane (Gerdes et al., 2009). Components of the axoneme undergo post-translational modifications that contribute to the dynamics and the stability of the cilium. The most important ciliary modification is represented by tubulin acetylation that stabilizes the axonemal structure (Wloga et al., 2017).

Primary cilia formation is strictly dependent on the cell cycle phase. Growth-arrested cells are mostly ciliated, while re-entry into the cell cycle following growth factor or hormone stimulation induces cilium resorption (Plotnikova et al., 2009). In the G0 phase of the cell cycle, vesicles

generated from Golgi, named distal appendages vesicles (DAVs), are transported near the distal appendages of the mother centriole. The EH domain containing protein 1 (EHD1) promotes the fusion of DAVs in a large ciliary vesicle that incapsulates the distal appendages of the mother centriole (Lu et al., 2015). Under the ciliary vesicle, two microtubules of each centriolar triplets start to elongate, generating the ciliary axoneme. Contextually with extension of microtubules, the ciliary vesicle elongates by fusing with Rab-8 positive vesicles. The nascent cilium migrates to the plasma membrane and fuse with it, linking the two compartments (Sánchez and Dynlacht, 2016). This mechanism of cilium assembly is typical of mesenchymal cells. In epithelial cells, primary cilia formation occurs at the cells surface, in a process termed alternative route. A predominant role in this mechanism is played by the midbody, a microtubules structure surrounded by membrane, whose remnants localize at the apical surface of epithelial cells after cytokinesis. In G0 phase, when the midbody remnant (MBR) is close to the centrosome at the apical surface, patches of MBR membrane localize near the centrosome and generate the ciliary membrane (Labat-de-Hoz et al., 2021).

Since ribosomes are absent in primary cilia, ciliary proteins are synthesized into the cytoplasm and imported into the cilium mainly through the intraflagellar transport system (IFT), a multimeric complex machinery involved in anterograde/retrograde transport of cargoes along the entire length of cilium. The heterotrimeric KIF3A/KIF3B/KAP kinesin-2 is the principal motor of the IFT-B complex that regulates the movement of cargoes from the ciliary base to the tip (anterograde transport). Instead, the cytoskeletal motor protein dynein drives the IFT-A complex that controls the transport of cargoes from the tip to the ciliary base (retrograde transport) (Webb et al., 2020). Although distinct roles were initially identified, it has been recently demonstrated that IFT-A and IFT-B can participate to both anterograde and retrograde transport (Liem et al., 2012; Kobayashi et al., 2021). Mutations of genes involved in the formation, maintenance, disassembly and trafficking of primary cilium often cause developmental genetic disorders, known as ciliopathies. The principal clinical features of ciliopathies include retinal degeneration, kidney cysts formation, polydactyly, intellectual disability and skeletal defects (Badano et al., 2006; Fliegauf et al., 2007; Reiter and Leroux, 2017).

The formation and the stability of primary cilia are finely regulated by the ubiquitin-proteasome system (UPS) and the autophagy machinery, two important degradative systems operating in all eukaryotic cells. Moreover, different signaling pathways generated at- or directed to- the primary cilium operate through UPS and/or autophagy to control ciliary dynamics. Understanding how UPS and autophagy machineries influence primary ciliogenesis and identifying the mechanisms involved will lead to the identification of relevant therapeutic targets for ciliopathies and proliferative disorders, including cancer, in which ciliary pathways are often deregulated (Han et al., 2009; Moser et al., 2009; Kobayashi et al., 2017; Liu et al., 2018; Eguether and Hahne, 2018; Han et al., 2018; Peixoto et al., 2020b).

This review will focus on cilia regulation by UPS and autophagy machineries, by analyzing the dynamic interplay between these degradative systems and signaling pathways in the control of primary cilium.

REGULATION OF PRIMARY CILIA BY THE UBIQUITIN-PROTEASOME SYSTEM

Proteolysis *via* the ubiquitin-proteasome system is a fundamental homeostatic mechanism that cells use to control a variety of biological functions, including differentiation, growth, development and metabolism (Dikic, 2017). The pathway works by marking with ubiquitin-like proteins (UBLs) several substrates, which will be targeted to degradation by the proteasome or to specific compartments or to relevant biological partners. In the first reaction, ubiquitin is activated through the formation of a thioester bond between its C-terminal region and the cysteine residue in the active site of E1 enzyme in an ATP-dependent reaction. Eventually, the ubiquitin moiety is transferred to the E2 conjugating enzyme. The E3 ubiquitin ligases are enzymes that mediate the transfer of ubiquitin molecules to the final target substrate. Three distinct families of E3 ubiquitin ligases have been characterized: 1) RING E3 ligases that transfer ubiquitin moieties directly from E2 to a lysine residue of the specific substrate; 2) HECT E3 ligases that bind and transfer the ubiquitin molecules from the cysteine to a lysine residue on the substrate; 3) RING-between-RING (RBR) E3 ligases with two RING domains, one interacting with the E2-ubiqutin complex and the other one binding on a cysteine residue the ubiquitin, which will be transferred to the substrate (Husnjak and Dikic, 2012; Smit and Sixma, 2014). Recently, a new class of E3 ligases, named RING-cys-relay (RCR), has been identified. RCR E3 ligases contain a RING domain that interacts with E2 enzymes and a tandem cysteine domain (TC) that transfers ubiquitin moieties to a threonine residue present on the substrate (Pao et al., 2018; Mabbitt et al., 2020). Substrates of the UPS can be mono, poly or multi-mono ubiquitylated. In the mono-ubiquitylation reaction, a single ubiquitin moiety is added to a specific lysine residue of the protein. Instead, poly-ubiquitylation requires the addition of ubiquitin chains, linear or branched, to a lysine residue. In multi-mono-ubiquitylation reactions single molecules of ubiquitin are covalently attached to different lysines in the substrate (Callis, 2014). The location of lysine residue on ubiquitin involved in poly-ubiquitylation reactions determines the fate of the target protein. Thus, ubiquitylation at lysine 11 or lysine 48 is linked to proteolysis, whereas ubiquitylation at lysine 63 mostly regulates the targeting or activity of the ubiquitylated protein. Similarly, monoubiquitylation, multi-monoubiquitylation and branched polyubiquitylation are related to non-proteolytic functions such as endocytosis, DNA repair, signal transduction, protein interaction, localization and activity (Haglund and Dikic, 2005; Sadowski et al., 2012). Proteins degradation occurs through the 26S proteasome, a multiprotein complex that catalyzes degradation of ~80% of all cellular proteins (Bard et al., 2018). Protein ubiquitylation is a reversible process, since deubiquitylating enzymes (DUBs) can remove the ubiquitin chains from the substrates (Wilkinson, 2009) (**Figure 1**). A

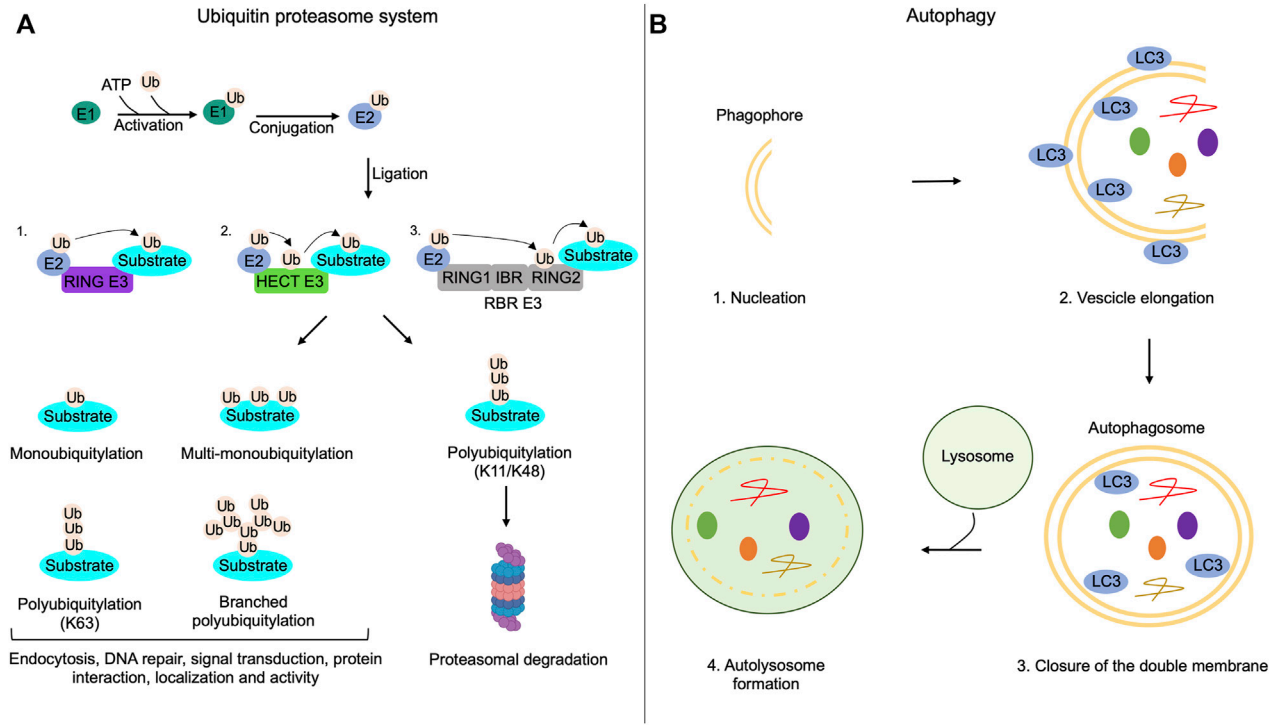


FIGURE 1 | Ubiquitin-proteasome system and autophagy mechanisms. **(A)** The addition of ubiquitin moieties to substrates occurs through three sequential enzymatic reactions: binding of ubiquitin to E1 enzymes in an ATP-dependent reaction (activation); transferring of ubiquitin to E2 enzymes (conjugation); covalent attachment of the ubiquitin moiety to the target substrate by E3 enzymes (ligation). RING-E3 ligases directly transfer ubiquitin from E2 enzymes to the substrate; HECT-E3 ligases bind ubiquitin and then transfer it to the substrate; RING between RING ligases (RBR) interact with E2, bind the ubiquitin on a cysteine residue of the RING domain and then transfer it to the substrates. Substrates of the UPS can be monoubiquitylated, multi-monoubiquitylated or polyubiquitylated. Polyubiquitylations can be characterized by the addition of linear or branched ubiquitin chains. Monoubiquitylations, multi-monoubiquitylations, branched polyubiquitylations and linear polyubiquitylations that involve lysine 63 (K63) of ubiquitin regulate non-proteolytic functions of modified proteins, such as endocytosis, DNA repair, signal transduction, protein interaction, localization and activity. Polyubiquitylation that involves lysine 11 (K11) or lysine 48 (K48) on the ubiquitin is generally linked to proteolysis of the substrate through the proteasome. **(B)** Autophagy initiates with the formation of a double membrane named phagophore (nucleation); the phagophore elongates and sequestrates cargoes recognized by LC3 (vesicle elongation); the closure of the double membrane generates the autophagosome (closure of the double membrane); autophagosomes, then, fuse with lysosomes and both the inner membrane of autophagosomes and sequestered cargoes are lysed (autolysosome formation).

role of UPS in ciliary activities has been originally postulated and then experimentally addressed by proteomic studies and network-based approaches that identified different elements of the ubiquitin system (activating ubiquitin enzymes, E3 ligases and DUBs) as components of primary cilium (Ishikawa et al., 2012; Amato et al., 2014). In recent years, a growing list of ciliary proteins, as substrates of the ubiquitin system, supports a fundamental role of UPS in the regulation of cilium biogenesis and dynamics.

UBIQUITIN-PROTEASOME SYSTEM PROMOTES CILIA ASSEMBLY AND ELONGATION

A human genome wide RNAi screening identified components of UPS that control the stability of ciliary proteins, such as IFT88 and CPAP, required for cilium assembly (Kim et al., 2016) (**Figure 2**). The analysis identified UBR5, an E3 ubiquitin-protein ligase and component of the N-end rule degradation pathway, as an important regulator of cilia biogenesis. Thus, UBR5 ubiquitylates the centrosome and spindle pole-associated protein 1 (CSPP1), a protein located at centrosome/centriolar satellites and ciliary axoneme, that is mutated in Joubert syndrome (Patzke et al., 2010; Tuz et al., 2014). In Human embryonic kidney cells (HEK293) and hTERT-immortalized retinal pigment epithelial cells (hTERT-RPE), the non-proteolytic ubiquitylation of CSPP1 by UBR5 regulates centrosomal localization of the protein, necessary for primary cilium assembly (Shearer et al., 2018). UPS also controls cilium assembly by promoting degradation of the inhibitors of ciliogenesis. Cullin1 (Cul1), a core component of Skip1-Cullin-F-box (SCF) E3 ubiquitin ligase complexes, ubiquitylates and degrades Dishvelled 2 (Dvl2), an inhibitor of cilium biogenesis (Cardozo and Pagano, 2004). Phosphorylation of Dvl2 by Wnt5a stabilizes the human enhancer of filamentation one protein (HEF1/Cas-L/NEDD9) and induces its binding to aurora A kinase (AurA). The HEF1/AurA complex at basal body phosphorylates and activates the Histone deacetylase 6 (HDAC6), which deacetylases tubulin favoring cilium

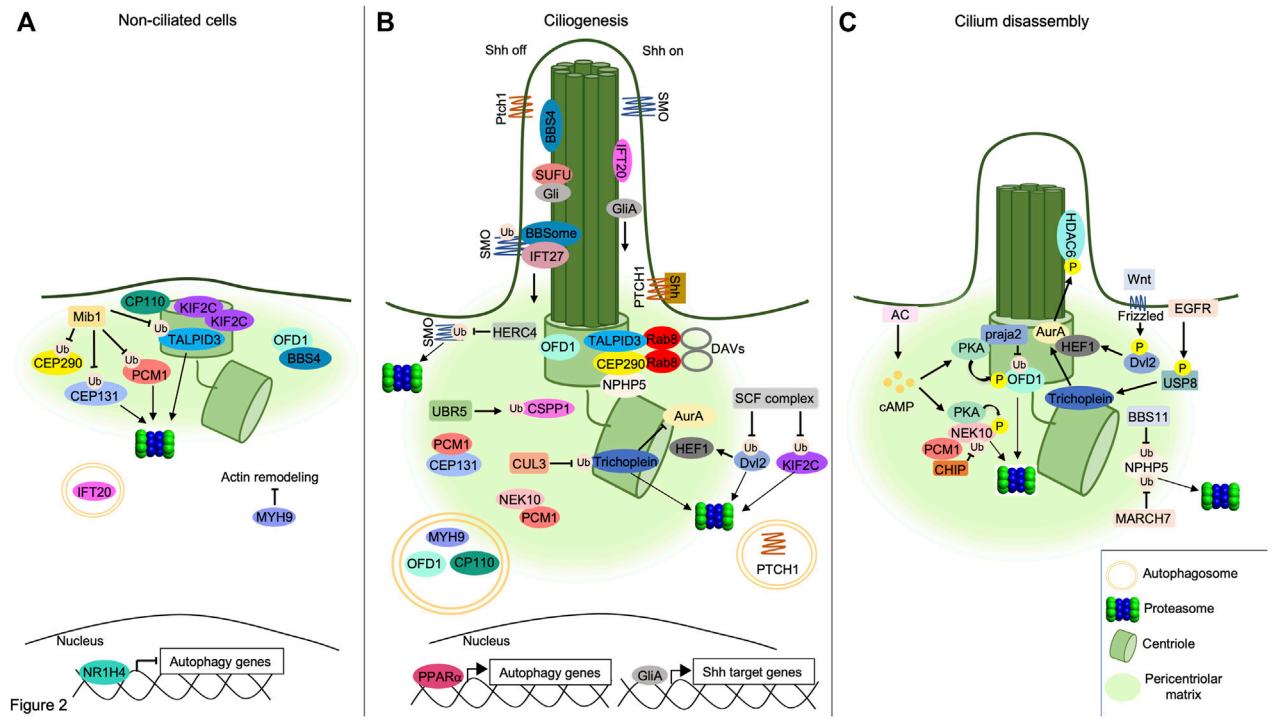


Figure 2

FIGURE 2 | Interplay between UPS/autophagy and signaling pathways in cilia dynamics. **(A)** UPS and autophagy inhibit ciliogenesis. The E3 ligase MIB1 ubiquitylates PCM1, TALPID3 and CEP131, targeting them to the proteasome, while ubiquitylation of CEP290 by MIB1 prevents its binding to TALPID3, inhibiting the formation of DAVs. OFD1 retains BBS4 at pericentriolar satellites and prevents BBSome assembly. Accumulation of KIF2C and CP110 at basal body, inhibition of acting remodeling by MYH9 and proteolysis of IFT20 by autophagy prevent cilium elongation. The inhibition of autophagy genes by the nuclear factor NR1H4 is indicated below. **(B)** Positive role of UPS and autophagy in ciliogenesis. Centrosomal TALPID3/CEP290/Rab8 complex supports DAVs formation. UBR5 ubiquitylates and targets CSPP1 to pericentriolar satellites. Multiprotein complexes assembled by PCM1, CEP131 and NEK10 at pericentriolar matrix contribute to cilium formation. SCF E3 ligase complex ubiquitylates and degrades Dvl2, destabilizing HEF1. Similarly, CUL3 ubiquitylates and degrades Trichoplein, thus inhibiting AurA. NPHP5 accumulates at centrosome forming complexes with centrosomal CEP290 and TALPID3. Pericentriolar OFD1 is degraded through the autophagy pathway, allowing BBS4 to relocalize at ciliary compartment. Similarly, autophagic degradation of MYH9 leads to actin remodeling underlying to axoneme formation. CP110 is also degraded through autophagy leading to axoneme growth. PPARα promotes the transcription of autophagy genes. In absence of Shh ligand (Shh off), SMO is ubiquitylated by an unknown ciliary ligase and transported out of cilium by the IFT27/BBSome complex. Here, SMO is ubiquitylated and degraded by the E3 ligase HERC4. SUFU sequestrates Gli proteins within the cilium. Following binding to Shh ligand (Shh on), PTCH1 receptor exits out of cilium and is degraded through autophagy. Transport of SMO within the cilium activates GliA proteins and induces Shh-dependent nuclear gene transcription. **(C)** Cilium disassembly induced by proteolytic machineries. NPHP5 is ubiquitylated and delocalized by BBS11 or degraded by MARCH7. Phosphorylation of Dvl2 by Wnt5a stabilizes HEF1 and induces its binding to AurA. Localization of HEF1/AurA complex at basal body induces phosphorylation and activation of HDAC6. Following EGFR stimulation, USP8 deubiquitylates and activates Trichoplein which, in turn, stimulates AurA. Increase of cAMP levels by AC activates PKA. Phosphorylation by PKA primes OFD1 to ubiquitylation and proteolysis through a praja2-proteasome pathway. Similarly, PKA phosphorylates the pro-ciliogenic kinase NEK10, promoting its ubiquitylation by CHIP and consequent proteasomal degradation.

disassembly (Pugacheva et al., 2007; Lee et al., 2012). In HEK293T and hTERT-RPE, Cul1-mediated proteolysis of Dvl2 inhibits HDAC6 activity, thus supporting ciliogenesis (Kim et al., 2021). SCF complexes also regulate cilium formation by controlling the stability of the kinesin family member 2c (KIF2C). In growing cells, KIF2C induces depolymerization of microtubules and consequent cilium disassembly (Miyamoto et al., 2015). Ubiquitin-dependent proteolysis of KIF2C by the Cullin adaptor F-Box and WD repeat domain containing 5 (FBXW5) positively contributes to the assembly of primary cilium. In human retinal pigment epithelial cells (RPE1), downregulation of FBXW5 induces accumulation of KIF2C at the basal body and impairs ciliogenesis (Schweiggert et al., 2021).

The elongation of the axonemal structure in ciliogenesis is regulated by UPS. In proliferating cells, trichoplein/mitostatin (TpMs), a keratin-binding protein often downregulated in epithelial cancers, localizes at mother and daughter centrioles and contributes to the activation of AurA (Inoko et al., 2012). Activated AurA induces deacetylation of tubulin, *via* HDAC6, and cilium disassembly (Pugacheva et al., 2007). When RPE1 cells complete the mitotic cycle, trichoplein is degraded by ubiquitin-dependent proteolysis initiated by Cullin 3 (CUL3) RING E3 ligase including also the potassium channel tetramerization domain containing 17 (KCTD17). Proteolysis of trichoplein inactivates AurA and promotes ciliary axoneme elongation (Kasahara et al., 2014). Interestingly, KCTD17-induced trichoplein degradation can be counteracted by the ubiquitin carboxyl-terminal hydrolase 8 (USP8) that deubiquitylates trichoplein. Moreover, USP8 hydrolase is stimulated and stimulates EGF receptor (EGFR) by regulating its polyubiquitylation (Berlin et al., 2010). Once activated, USP8 counteracts KCTD17-mediated ubiquitylation of trichoplein,

thus suppressing primary ciliogenesis in EGF-stimulated cells (Kasahara et al., 2018).

DYNAMIC REGULATION OF CILIUM DISASSEMBLY BY UBIQUITIN-PROTEASOME SYSTEM

Depending on the role of ubiquitin substrates, UPS can also inhibit the formation of primary cilia. Mindbomb E3 ubiquitin ligase protein 1 (MIB1) is an E3 ligase that inhibits initial steps of cilium formation by ubiquitylating different substrates located at centrosome/pericentriolar satellites, including pericentriolar matrix protein 1 (PCM1). This acts as a scaffold protein for components of pericentriolar satellites, including CEP131 and CEP290, thus playing a fundamental role in ciliogenesis (Woodruff et al., 2014). In human osteosarcoma cells (U2OS) and RPE1 cells, ubiquitylation of PCM1 and associated partners by MIB1 inhibits cilium formation (Villumsen et al., 2013). MIB1 also plays a role in the regulation of Rab8-containing DAVs that are essential for the transition from mother centriole to basal bodies. Thus, TALPID3 gene, mutated in a mild form of Joubert syndrome, encodes for a centrosomal protein required for ciliogenesis and Sonic Hedgehog (Shh) signaling pathway. TALPID3 localizes at distal end of centrioles where it forms a multiprotein complex including CP110, CEP290 and Rab8A. The TALPID3 complex promotes formation of ciliary vesicles at centrioles that eventually fuse with secondary vesicles to form the ciliary membrane around the assembling axoneme (Kobayashi et al., 2014). Under growing conditions in HEK293T cells, ubiquitylation of CEP290 and TALPID3 by MIB1 inhibits the DAVs formation at the centrosome. In growth-arrested RPE1 cells, PCM1 retains MIB1 at pericentriolar satellites, preventing TALPID3 ubiquitylation and leading to cilia formation (Wang et al., 2016). In HeLa cells, ubiquitylation of PCM1 by MIB1 is counteracted by the ubiquitin carboxyl-terminal hydrolase 9X (USP9X), a deubiquitylating enzyme that protects PCM1 from proteolysis, maintaining the integrity of centriolar satellites (Han et al., 2019).

UPS also controls the disassembly of primary cilia. A fundamental role in this process is played by nephrocystin 5 (NPHP5), a gene product mutated in Senior-Løken syndrome, a ciliopathy disease characterized by congenital amaurosis (Leber congenital amaurosis) and nephronophthisis (Stone et al., 2011). During interphase, NPHP5 is located at the centrosome and interacts with CEP290, positively controlling the integrity of BBSome, a conserved multiprotein complex that controls trafficking of cargoes and receptors within the primary cilium (Barbelanne et al., 2013, 2015). In G2/M phase transition in HEK293 and RPE1 cells, ubiquitylation of NPHP5 by the E3 ubiquitin ligase TRIM32/BBS11 induces delocalization of the protein from centrosome and consequent cilium disassembly. Similarly, a MARCH family member of membrane-bound E3 ubiquitin ligase (MARCH7) ubiquitylates and degrades NPHP5 promoting cilia loss. As expected, the activity of both E3 ligases, TRIM32 and MARCH7, is counteracted by the deubiquitylase USP9X. In fact, in growth-arrested conditions, USP9X is recruited at the centrosome, deubiquitylates NPHP5 and prevents its degradation and cilium disassembly (Das et al., 2017).

DYSREGULATION OF UBIQUITIN-PROTEASOME SYSTEM IN CILIOPATHY DISORDERS

Given the crucial role of UPS in primary ciliogenesis, it is important to consider the potential impact of UPS dysregulation in the pathogenesis of human ciliopathies. A direct link between dysfunctional UPS and ciliopathies has been established by identifying loss-of function mutations of RPGRIP1L (RPGRIP1 like) that are causally linked to Joubert syndrome and Meckel syndrome (Wiegering et al., 2018). In normal cells, RPGRIP1L protein localizes at transition zone of primary cilium and here it controls the ciliary targeting of components of the proteasome, such as PSMD2 protein (Gerhardt et al., 2015). Loss of function mutation of RPGRIP1L impairs the proteasomal activity at ciliary compartment, thus contributing to ciliopathy phenotype. Similarly, the loss of BBS4 and OFD1, two ciliopathy-related proteins, decreases localization of proteasomal subunits at the centrosome, causing the accumulation of Sonic Hedgehog and Notch signaling mediators that are normally degraded by UPS. Accordingly, the overexpression of proteasomal subunits or the chemical activation of the proteasome partially restores the signaling defects in BBS4 and OFD1 deleted Zebrafish (Liu et al., 2014). Furthermore, a genome wide RNAi screening identified the deubiquitylating enzyme USP35 as a genetic suppressor of Bardet-Biedl syndrome 4 (BBS4). Inactivation of USP35 in a zebrafish model of BBS4 ciliopathy, rescues different ciliary defects, such as impaired convergent and extension movements during gastrulation, renal tubule convolution and retinal degeneration, thus ameliorating the phenotype(s) of BBS4-depleted animals (Tsai et al., 2019).

These results suggest a primary remarkable role of UPS dysregulation in the onset of ciliopathy disorders and highlight potential therapeutic targets to restore primary cilium functions by locally modulating UPS activity.

AUTOPHAGY CONTROL OF CILIOGENESIS

Autophagy is an intracellular self-degradative process that is essential for the maintenance of the cellular energetic balance in response to nutrient stress or to eliminate dysfunctional proteins and organelles, playing a fundamental role in different physiological conditions, such as development, tissue homeostasis, metabolic adaptation and signaling, immunity, inflammation and elimination of microorganisms. Derangement of the autophagy pathway is linked to a variety of human disorders (Mizushima, 2005; Klionsky et al., 2021). Under nutrients supplementation, basal level of the autophagy machinery supports the turnover of organelles and proteins. During nutrients deprivation, autophagy is rapidly activated to maintain the appropriate energetic supply for all cellular activities

(Levine and Kroemer, 2008). Autophagy is a multistep process that includes: 1) nucleation of autophagic vesicles (phagophores); 2) vesicles elongation with sequestration of cargoes; 3) closure of the double membrane; 4) fusion of formed autophagosomes to lysosomes. In the fused vesicles, both the inner membrane of autophagosomes and cargoes are enzymatically and chemically eliminated (**Figure 1**) (Levine et al., 2011). The autophagy machinery is driven by the sequential activation of a variety of gene products originally identified in yeast, named ATGs, that are highly conserved in mammalian genome. The initiation of autophagic process is controlled by signaling pathways involving the mechanistic target of rapamycin (mTOR kinase) and AMP-activated protein kinase (AMPK) that oppositely control autophagy through direct phosphorylation of Unc-51 Like Autophagy Activating Kinase 1 (ULK1) at distinct sites (Kim et al., 2011). For many years, autophagy has been considered as a bulk sequestration system. However, it is now well established that autophagy profoundly contributes to different aspects of cellular homeostasis by selectively removing cytoplasmic materials, such as protein aggregates, damaged organelles and invading pathogens (Zaffagnini and Martens, 2016).

Cargoes targeted to the autophagy machinery are recognized by ATG8 family members (LC3 and GABARAP proteins) through direct interaction with LC3-interacting regions (LIRs) present in a variety of target proteins or *via* receptors, such as p62 (also known as Sequestosome 1, SQSTM1), a multifunctional adapter protein that recognizes ubiquitinated cargoes and facilitates their elimination by the degradative pathway (Pankiv et al., 2007; Birgisdottir et al., 2013). Considering that both autophagy and ciliogenesis occur under conditions of nutrients deprivation, a functional link between both processes has been originally supposed and subsequently supported by several lines of evidence (**Figure 2**). Thus, signaling pathways activated at ciliary compartment, such as Sonic Hedgehog, stimulate the autophagy process through the regulation of autophagy-related proteins located at the base of primary cilium. Inhibiting ciliogenesis negatively impacts on the autophagy pathway. Moreover, ATG proteins have been identified as resident of the ciliary compartment and have been implicated in the regulation of ciliary signaling and activities (Pampliega et al., 2013; Yamamoto and Mizushima, 2021). In growing cells, basal activation of autophagy machinery inhibits cilia formation, at least in part, through the autophagic degradation of IFT20, a component of the IFT system involved in cilium assembly. Hence, the inhibition of the autophagy pathway induces cilia extension and signaling. In contrast, under nutrients deprivation, IFT20 protein is stabilized and accumulates in the ciliary compartment, positively contributing to ciliogenesis (Pampliega et al., 2013). This positive effect of autophagy on cilium biogenesis has been, at least in part, mechanistically linked to proteolysis of OFD1, a protein localized at the centrosome/basal body and pericentriolar satellites. In growing cells, OFD1 restraints ciliogenesis by retaining BBS4, a component of the BBSome, at pericentriolar satellites. Starvation-induced activation of autophagy machinery promotes targeted proteolysis of the pericentriolar pool of OFD1, with consequent release of BBS4 and BBSome formation, a prerequisite for the onset of ciliogenesis (Tang et al., 2013). Moreover, in serum-deprived mouse embryonic fibroblasts (MEFs), autophagy promotes primary cilia formation, at least in part, through the degradation of CP110. CP110 is a centrosomal protein that suppresses ciliogenesis by capping the distal ends of both centrioles. During starvation, the autophagic degradation of CP110 mediated by NudC-like protein 2 (NudCL2), a selective autophagy receptor at the mother centriole, induces cilium formation. This mechanism was confirmed also in Zebrafish models, where depletion of NudCL2 generates a ciliary phenotype that is rescued by CP110 depletion (Liu et al., 2021).

The differential effects of autophagy on ciliogenesis can be explained by the use of different cellular contexts and experimental models. Thus, in retinal cells, autophagy promotes ciliogenesis, whereas in renal cells it inhibits cilia elongation. In serum-deprived human kidney 2 (HK2) cells, induction of autophagy by rapamycin or Tat-Beclin1 peptide treatment significantly reduces cilia length, suggesting that autophagy inhibits cilia formation. A potential mechanism concerns VPS39, a component of the HOPS complex involved in the fusion of lysosomes with autophagosomes vacuoles. In kidney cells, VPS39 controls localization of IFT20 and OFD1 at pericentriolar satellites, negatively regulating cilia elongation. Genetic silencing of VPS39, by reducing OFD1 levels at pericentriolar satellites and promoting recruitment of IFT20 at ciliary structures, induces cilia overgrowth. This effect was reversed by concomitant activation of the autophagy machinery, supporting the notion that in renal cells autophagy prevents cilia elongation by controlling OFD1 and IFT20 localization at pericentriolar satellites (Iaconis et al., 2020). A similar role for autophagy in ciliogenesis has been described in epithelial cells of the respiratory tract. In these cells, the shortening of cilia length by cigarette smoking, a process termed "ciliophagy," can be prevented by heterozygous knockout of the autophagic gene beclin1 (Lam et al., 2013). The autophagy-induced shortening of primary cilia is mediated by HDAC6, a deacetylating enzyme involved both, in cilia loss and maturation of autophagosome (Lee et al., 2010; Gradilone et al., 2013). Downregulation of HDAC6 restores cilia length in mice exposed to cigarette smoking (Lam et al., 2013). This mechanism has been observed also in cholangiocarcinoma cells, where the inhibition of either HDAC6 or autophagy machinery increases cilium length. Mechanistically, HDAC6-mediated deacetylation of ciliary proteins favors their ubiquitylation and consequent recognition by autophagy receptors, such as NBR1 and CALCOCO2, that target them to autophagic degradation, thus reducing cilium length (Peixoto et al., 2020a). An involvement of the autophagy machinery in the control of ciliogenesis in airway epithelial cells has been recently described. Thus, AMPK activation promotes autophagic degradation of KIF19A, a microtubule-depolymerizing kinesin located at the cilia that is required for ciliary length control, and cilia disassembly. This mechanism is counteracted by adenylate cyclase 6 (AC6) that inhibits AMPK binding to KIF19A, preventing cilia disassembly. Accordingly, AC6 knock-out airway cilia are deficient in kinesin KIF19A and show

abnormal length and function (Arora et al., 2020). Altogether, these data suggest that autophagy exerts differential effects on ciliogenesis depending on cell types and players involved.

An additional layer of complexity in the interplay between autophagy and ciliogenesis is represented by myosin heavy chain 9 (MYH9)/myosinIIA, a suppressor of actin dynamics and negative regulator of primary ciliogenesis (Rao et al., 2014). During autophagy, NIMA-related kinase 9 (NEK9), by employing its LIR domain, binds MYH9 and facilitates its elimination through the autophagy machinery (Yamamoto et al., 2021). NEK9 also controls the stability of the pericentriolar pool of OFD1, most likely acting through an unidentified adaptor-independent mechanism. This hypothesis was supported by identification of LIR domains on OFD1 protein that directly interact with autophagosomal LC3/GABARAP proteins, mediating the elimination of the protein through the autophagy machinery (Morleo et al., 2021). By promoting the autophagic removal of MYH9 and OFD1 protein complexes, NEK9 restores actin remodeling and promotes cilium elongation (Yamamoto et al., 2021).

Peroxisome proliferator-activated receptors (PPARα) and nuclear receptor subfamily one group H member 4 (NR1H4) regulate ciliogenesis through autophagy. PPARα is activated under fasted conditions and positively regulates the transcription of genes involved in the autophagic pathway (Lee et al., 2014). Treatment with PPARα ligand in different mammalian cell lines induces cilia formation, both in normal and serum-deprived conditions, and these effects are abrogated in ATG7 knockout mouse embryonic fibroblasts (MEFs) (Liu et al., 2018). Conversely, NR1H4 inhibits autophagy by repressing the transcription of autophagic genes (Seok et al., 2014). As expected, treatment with NR1H4 ligand in serum-deprived cells reduces primary ciliogenesis, whereas genetic silencing of NR1H4 promotes ciliogenesis, even under normal medium conditions. These studies demonstrate that transcriptional regulation of autophagic genes by nuclear receptors PPARα and NR1H4 controls ciliary dynamics (Liu et al., 2018b).

Another important aspect of the reciprocal regulation between ciliogenesis and autophagy is represented by the primary cilia-autophagy-NRF2 (PAN) axis, originally identified in human embryonic stem cells (hESC). Thus, during neuroectodermal differentiation, the ciliogenesis leads to the activation of autophagy machinery that, in turn, inhibits the nuclear factor erythroid 2-related factor 2 (NRF2), promoting the neuroectodermal differentiation (Jang et al., 2016). The function of PAN axis has been demonstrated also in fibroblasts, in which the inhibition of ciliogenesis leads to upregulation of NRF2 activity that can be rescued by autophagy-activating mTOR inhibitors (Martin-Hurtado et al., 2019).

AUTOPHAGY AND CILIOPATHIES

Dysregulation of autophagy pathway can contribute to the onset of ciliopathies, thus representing a valuable therapeutic target for ciliary disorders. The pathogenic role of autophagy in human ciliopathies emerged from studies on polycystic kidney disease (PKD), the most common form of renal cystic genetic disorder associated with alterations of primary cilia (Kathem et al., 2014). In PKD, there is an impairment of autophagy machinery mostly due to altered fusion between autophagosomes and lysosomes vesicles (Belibi et al., 2011). The impaired autophagic flux has been reproduced in zebrafish mutants for PKD1, as well as in mouse and human PKD1-null kidney epithelial cells. In these models, downregulation of the core autophagy protein ATG5 increases cysts growth (Zhu et al., 2017). Conversely, activation of autophagy machinery by a specific inducer Beclin-1 peptide markedly attenuates the cystic phenotype and ameliorates the kidney function (Chang et al., 2017; Zhu et al., 2017). These data indicate that the use of chemical modulators of autophagic pathway represents a valuable strategy for the treatment of ciliopathy disorders.

Although the involvement of autophagy in the onset of autosomal dominant polycystic kindey disease (ADPKD) has been largely demonstrated, only modulators of mTOR and AMPK activity have been tested in clinical studies for the treatment of ADPKD. Rapamycin and other mTOR inhibitors are very effective in experimental studies (Tao et al., 2005; Shillingford et al., 2006, 2010; Wahl et al., 2006). However, in clinical trials they showed loss of efficacy in the progression of renal impairment (Serra et al., 2010; Walz et al., 2010; Lin et al., 2019). Preclinical studies have demonstrated also the efficiency of metformin, an activator of AMPK, in ADPKD treatment (Takiar et al., 2011; Chang et al., 2017; Pastor-Soler et al., 2022). Recently, metformin was tested also in randomized clinical trials in which it has been shown that it is safe but slightly reduces renal impairment (Seliger et al., 2018; Perrone et al., 2021).

In addition to PKD, other ciliopathies have been causally linked to altered autophagy. RPGRIP1L gene mutations are associated with different ciliopathies mostly due to impairment of protein degradation and protein processing by UPS (Gerhardt et al., 2015). However, drug-induced restoration of proteasomal activity does not completely rescue the ciliary phenotype induced by RPGRIP1L loss, suggesting that this protein works through a different mechanism in regulating ciliary activity. Evidence revealed that RPGRIP1L absence, thus, impairs autophagy at ciliary compartment by increasing the activation of MTOR complex 1 (MTORC1) pathway and the levels of OFD1 at the base of cilium. Inhibition of MTORC1 activity by rapamycin restores both the autophagic flux and the cilia length in RPGRIP1L-null MEFs, without affecting proteasomal activity. This data indicates that RPGRIP1L independently regulates both autophagic and proteasomal activities to control ciliogenesis (Struchtrup et al., 2018). A R998Q mutation of serine/threonine-protein kinase VPS15, a PI3K regulator and component of the autophagic machinery, has been identified in family members affected by shorter cilia. VPS15 forms a complex with Golgin GM130 and regulates the trafficking of intraflagellar protein IFT20 from Golgi apparatus to ciliary compartment. Mutations of VPS15 retain IFT20 at Golgi membranes, impairing IFT20-dependent transport of membrane proteins from cis-Golgi to ciliary compartment, thereby reducing cilium elongation (Stoetzel et al., 2016). The

transport of IFT20 to the primary cilium is also regulated by the autophagy protein ATG16L1. Thus, following serum deprivation, ATG16L1 and IFT20 form a stable complex that is co-transported from the Golgi apparatus to the ciliary compartment. In absence of ATG16L1, IFT20 accumulates in the Golgi apparatus, causing aberrant ciliary structures (Boukhalfa et al., 2021).

A mechanistic link between human ciliopathies and deregulated autophagy machinery has been recently described. Oral-Facial-Digital type I syndrome (OFDI), a ciliopathy disorder caused by mutations of OFD1 gene, is characterized by upregulation of the autophagy pathway. Mechanistically, evidence indicates that OFD1 protein acts as an autophagy receptor that participates in an "autophagy self-regulated mechanism" that promotes autophagic elimination of ATG13, a component of the ULK1 autophagy initiation complex. By removing ATG13, OFD1 limits excessive activation of the autophagy machinery. Accordingly, inhibition of autophagy in mouse models of OFDI ameliorates polycystic kidney, a typical clinical manifestation of the disease (Morleo et al., 2021).

Autophagy alterations causing defective ciliogenesis have been correlated also to non- hereditary neurological diseases, such as focal malformations of cortical development (FMCDs). This is a pediatric intractable epileptic disorder characterized by cortical dyslamination, focal cortical dysplasia (FCD) and hemimegaloencephaly (HME). Activating somatic mutations of mTOR kinase have been identified and causally linked to FMCDs. Mechanistically, uncontrolled mTOR activation leads to defective autophagy, accumulation of OFD1 at the pericentriolar satellites and inhibition of neuronal ciliogenesis. Disrupted ciliogenesis affects Wnt pathway, essential for neuronal polarization, and leads to cortical dyslamination typical of FMCDs (Park et al., 2018). Altogether, the data indicate an important role of autophagy in ciliary dynamics and signaling, and suggest the possibility to target the autophagic machinery to treat different forms of ciliopathies.

CROSSTALK OF UBIQUITIN-PROTEASOME SYSTEM, AUTOPHAGY AND CILIARY PATHWAYS

Primary cilia act as transduction hubs that transmit extracellular signals into cell body. For this function, primary cilia are considered as "the cell's antenna" that sense, transmit and regulate signaling pathways involved in essential aspects of cellular homeostasis and organ development. Different membrane receptors, including GPCRs, and signaling molecules have been identified as resident of primary cilia (Wheway et al., 2018). Trafficking of the receptors and associated partners throughout the cilium subserves as a mechanism to finely control the rate and magnitude of cilium-based signaling pathways. Reciprocal regulation between signaling pathways, UPS and autophagy machinery in the control of ciliary dynamics has been described. Once activated, GPCRs are rapidly transported out of the cilium by the BBSome complex (Ye et al., 2018). BBSome-mediated removal of GPCRs requires K63 ubiquitylation of the receptor by a β-Arrestin-mediated mechanism (Shinde et al., 2020). The ubiquitin system, thus, acts as a general control mechanism for ciliary signaling pathways.

Sonic Hedgehog (Shh) is the most important signaling pathway operating within the primary cilium and plays a fundamental role in development, regeneration and organ homeostasis. Derangement of Shh pathway is causally associated to ciliopathy disorders and cancer (Dafinger et al., 2011; Hynes et al., 2014; Sasai et al., 2019). Shh pathway operates within intact primary cilia and most of Shh components are located within the ciliary compartment. Under resting conditions, the tumor suppressor membrane receptor Patched (PTCH1) inhibits the accumulation of smoothened (SMO), a class frizzled GPCR and component of Shh, in the ciliary compartment (Rohatgi et al., 2007). In these conditions, suppressor of fused protein (SUFU) sequestrates the Shh transcription effectors Gli proteins in the cilium. Here, at ciliary base, PKA phosphorylation primes Gli proteins processing into transcriptional repressors. The binding of Shh ligand to PTCH1 activates SMO that translocates to ciliary compartment, inducing the release of unprocessed, active Gli proteins from SUFU, allowing them to migrate into the nucleus and activate the transcription of target genes (Corbit et al., 2005; Tukachinsky et al., 2010).

UPS plays a fundamental role in the regulation of the Shh pathway at different levels. It controls PKA-dependent processing of Gli2 and Gli3 proteins in the repressive forms. Similarly, other components of Shh pathway are regulated by the ubiquitin system (Gerhardt et al., 2016). The regulation of Shh pathway by UPS also impacts on cilia dynamics. Thus, the ubiquitin carboxyl-terminal hydrolase 14 (USP14), by regulating the stability of KIF7, a member of kinesin-4 family and essential regulator of microtubule dynamics and Shh pathway, inhibits ciliogenesis and cilia elongation. Pharmacological inhibition of USP14 restores Shh activity and ciliogenesis in PKD1 mutant MEFs (Massa et al., 2019). UPS also controls the ciliary localization of SMO. In absence of ligand, ubiquitylated SMO exits from the cilium through the IFT system and BBSome complex. Inhibition of ubiquitylation allows SMO accumulation in the ciliary compartment, even in the absence of Shh ligand (Desai et al., 2020). Moreover, SMO levels are regulated by UPS. HECT and RLD domain containing E3 ligase 4 (HERC4) ubiquitylates SMO promoting its degradation through the proteasome, as well as *via* lysosomes. Shh stimulation or HERC4 inactivation inhibits HERC4/SMO complex formation and prevents SMO proteolysis, thus activating downstream pathways (Jiang et al., 2019). Deubiquitylation of SMO by the ubiquitin carboxyl-terminal hydrolase isozyme 5 (UCHL5/UCH37) also positively regulates Shh signaling (Zhou et al., 2018). All these studies suggest that targeting UPS constitutes a powerful strategy to modulate Shh pathway at the ciliary compartment, restoring deranged ciliary signaling in ciliopathy disorders.

Shh pathway is also controlled by autophagy. Following ligand stimulation, PTCH1 undergoes to polyubiquitylation and proteolysis, promoting accumulation of SMO at ciliary compartment. Blocking macroautophagy, by preventing PTCH1 ubiquitylation, reduces the transport of SMO to the

cilium and impairs Shh pathway (Yang et al., 2021). Reciprocal regulation between autophagy and Shh in the control of cilium dynamics and activity has been described (Li et al., 2012; Petralia et al., 2013; Wang et al., 2013). Serum-deprived Gli2-knockout NIH3T3 cells show longer cilia and increased autophagic flux. This lengthening of primary cilia is caused by increased autophagic degradation of pericentriolar OFD1. Blocking the autophagy machinery rescues ciliary length in $Gli2^{-/-}$ cells, indicating that Gli2 controls cilia elongation through autophagy (Hsiao et al., 2018). Furthermore, $Gli2^{-/-}$ cells display a significant delay in cell cycle re-entry that can be rescued by concomitant downregulation of KIF3A, supporting the existence of a functional link between autophagy, cilium length and cell proliferation (Hsiao et al., 2018).

Shh can also induce ciliogenesis through a non-canonical pathway involving the SMO-mediated activation of LKB1/AMPK pathway, a positive regulator of autophagy. Thus, in LKB1 knockdown cells, stimulation with Shh fails to induce ciliogenesis and this phenotype can be rescued by treatment with autophagy activators (Akhshi and Trimble, 2021). Evidence indicates that Shh regulates early steps of autophagic process. Activation of autophagy by nutrients deprivation requires the synthesis and accumulation of components of the autophagic machinery at the base of primary cilium through a mechanism involving the IFT system and Hh signaling. Interfering with the IFT compromises autophagy and this can be rescued by Gli2 overexpression (Pampliega et al., 2013). These studies indicate that Shh pathway controls ciliogenesis and cilia dynamics by positively regulating autophagy, which in a feed-back loop controls ciliary Shh signaling. Considering that dysregulation of Shh signaling constitutes a common feature in several ciliopathies, the data strongly support the use of modulators of the autophagy pathway as therapeutic strategy for the treatment of these disorders.

UPS can also regulate ciliary pathways by modulating the stability of tyrosine kinase receptors located within the cilium, such as platelet-derived growth factor receptor α (PDGFRα). During cilium elongation, localization of PDGFRα at ciliary compartment is required for local activation of the receptor by PDGF-AA ligand. Once activated, ciliary PDGFRα undergoes to ubiquitylation and internalization through a mechanism controlled by ciliary IFT20/E3 ligase Cbl complexes. The receptor internalization limits the downstream activation of the cascade that might otherwise lead to overgrowth of primary cilia (Schmid et al., 2018).

THE IMPACT OF cAMP-REGULATED PROTEOLYTIC SYSTEMS ON PRIMARY CILIA

cAMP is an ancient second messenger that mediates the biological responses to a variety of hormones and neurotransmitters, controlling metabolism, differentiation, cell growth, development, and synaptic activities. The main effector of cAMP is protein kinase A (PKA). PKA is a tetrameric holoenzyme composed of two regulatory (R) and two catalytic (PKAc, C) subunits. Ligand-induced activation of dedicated GPCRs at plasma membrane activates the adenylate cyclase which in turn synthesizes cAMP. The binding of cAMP to R subunits dissociates PKA holoenzyme and releases free active C subunits. Phosphorylation of cellular substrates by C subunit controls most of the cAMP functions inside cells (Taylor et al., 2013; Newton et al., 2016; Rinaldi et al., 2019). PKA holoenzyme is compartmentalized at discrete intracellular sites by direct binding to A-Kinase Anchor Proteins (AKAPs). AKAPs operate as transduceosomes that assemble components of cAMP generating systems (receptors and adenylate cyclase), effector enzymes (PKA and Epac) and attenuating enzymes (cAMP-phosphodiesterases and protein phosphatases), creating intracellular sites where distinct signaling pathways converge and focus, optimizing the biological response to a given stimulus (Carlucci et al., 2008; Lignitto et al., 2011; Yang and McKnight, 2015; Jones et al., 2016; Rinaldi et al., 2017, 2018; Bucko et al., 2019).

Primary cilia, functioning as transduction hubs, are enriched of receptors, including GPCRs, that possess a ciliary localization sequence that allow their transport from Golgi to ciliary membrane (Berbari et al., 2008). Different components of the cAMP pathway have been identified within the primary cilium, including AKAP/PKA complexes, adenylates cyclases, phosphodiesterares (PDEs) and phosphatases (Barzi et al., 2010; Choi et al., 2011; Mick et al., 2015). Ciliary cAMP-PKA axis plays an inhibitory role in the Shh pathway finely controlling the proteasomal processing of the transcription activators Gli2 and Gli3 to transcriptional repressors (Pan et al., 2009; Chen et al., 2011; Niewiadomski et al., 2014). PKA-regulated UPS system is involved in the regulation of cilium biogenesis and dynamics (Pal and Mukhopadhyay, 2015; Bachmann et al., 2016; Tschaikner et al., 2020). Never in mitosis A (NIMA)-related kinase 10 (NEK10) is a member of mitotic kinases that regulates mitogenesis, cilium biogenesis and airway mucociliary clearance (Fry et al., 2012; Porpora et al., 2018; Chivukula et al., 2020). Genetic inactivation of NEK10 affects primary ciliogenesis in mammalian cells and medaka fish development. Moreover, germline inactivating mutations of NEK10 have been causally linked to a human ciliopathy syndrome characterized by pathological airway dilation, impaired mucociliary clearance and bronchiectasis (Chivukula et al., 2020).

An interplay between PKA, NEK10 and UPS pathway has been established. Thus, in course of cAMP stimulation, PKA phosphorylates NEK10 and induces its ubiquitylation and degradation mediated by the E3 ligase chaperone-assisted C-terminus of Hsc70-interacting protein (CHIP/Stub1). By reducing NEK10 levels, PKA-CHIP complex leads to cilia resorption (Porpora et al., 2018). This regulatory system is lost in autosomal recessive spinocerebellar ataxia-16 (SCAR16) patients fibroblasts carrying germline inactivating mutations of CHIP (Porpora et al., 2018). cAMP, acting through the UPS, also controls the stability of OFD1. In serum deprived condition, membrane stimulation of cAMP synthesis induces PKA phosphorylation of OFD1. Phosphorylated OFD1 is rapidly ubiquitylated by E3 ligase praja2 and degraded by the proteasome. Proteolysis of OFD1 markedly reduces bulk levels

of the protein and impairs primary cilium elongation and medaka fish development. This mechanism is defective in an OFD1 variant carrying the patient mutation E97G (Senatore et al., 2021).

These data indicate an important role of the cAMP/UPS pathway in cilia dynamics and development, with potential pathogenic for ciliopathy disorders. Recently, heterozygous variants of catalytic subunits of PKA have been identified in individuals affected by a multiple congenital malformation syndrome characterized by cardiac defects, postaxial polydactyly and alterations of Shh pathway, suggesting a fundamental role of cAMP-PKA pathway in the pathogenesis of ciliopathies (Palencia-Campos et al., 2020).

Further studies are needed to better elucidate how UPS and autophagy are involved in the several signaling pathways that occurs at the primary cilia, mostly because understanding these molecular mechanisms could be useful for the study and the treatment of both ciliopathies and proliferative disorders that are often accompanied by alterations of ciliary dynamics (Eguether and Hahne, 2018).

CONCLUDING REMARKS

Primary cilium is a sensory non-motile organelle that receives, transmits and integrates signaling inputs from extracellular microenvironment to intracellular compartment, playing a major role in key cellular activities. Derangement of ciliary activities contributes to the pathogenesis of human genetic and proliferative disorders. Cilium biogenesis, dynamics and functions are finely regulated by UPS and autophagy, and components of the proteolytic machineries are closely associated to ciliary compartment. By controlling localization and levels of ciliary proteins, UPS and autophagy regulate key aspects of cilium biology. These degradative systems are timely and spatially regulated by signaling pathways generated at the ciliary compartment or by distantly located receptors at non-ciliary membranes. Integration of signaling inputs and degradative pathways is expected to finely modulate ciliary activities in response to changes of extracellular microenvironment. Further studies are needed to identify additional, relevant ciliary substrates of UPS/autophagy pathways and to elucidate the molecular mechanisms by which autophagy and UPS differentially regulates ciliogenesis and dynamics in different cell types or under changed metabolic needs. Similarly, the differential regulation of cilium dynamics by signaling pathways activated at different intracellular sites, such as cAMP cascade, needs further investigation.

Understanding the complex regulation of primary cilium by UPS and autophagy machineries, and dissecting the signaling events controlling cilium biogenesis and dynamics will pave the way for the development of novel therapeutic approaches based on selective targeting of these degradative systems in genetic and proliferative disorders.

AUTHOR CONTRIBUTIONS

ES and AF wrote the article with contributions from RI, LR, FC, RD, and ES prepared the figures.

FUNDING

This work was supported by the "Associazione Italiana per la Ricerca sul Cancro" (No. AIRC, IG 2018-ID22062), the Italian Ministry of University and Research (No. PON 2018; Per.Med.Net) and the European Regional Development Fund-POR Campania FESR 2014/2020 (Rare.Plat.Net) to AF. The authors apologize to those whose work was not cited owing to space limitations.

REFERENCES

Akhshi, T., and Trimble, W. S. (2021). A Non-canonical Hedgehog Pathway Initiates Ciliogenesis and Autophagy. *J. Cell Biol.* 220, e202004179. doi:10.1083/jcb.202004179

Amato, R., Morleo, M., Giaquinto, L., di Bernardo, D., and Franco, B. (2014). A Network-Based Approach to Dissect the Cilia/centrosome Complex Interactome. *BMC Genomics* 15, 658. doi:10.1186/1471-2164-15-658

Arora, K., Lund, J. R., Naren, N. A., Zingarelli, B., and Naren, A. P. (2020). AC6 Regulates the Microtubule-Depolymerizing Kinesin KIF19A to Control Ciliary Length in Mammals. *J. Biol. Chem.* 295, 14250–14259. doi:10.1074/jbc.RA120.013703

Bachmann, V. A., Mayrhofer, J. E., Ilouz, R., Tschaikner, P., Raffeiner, P., Röck, R., et al. (2016). Gpr161 Anchoring of PKA Consolidates GPCR and cAMP Signaling. *Proc. Natl. Acad. Sci. U.S.A.* 113, 7786–7791. doi:10.1073/pnas.1608061113

Badano, J. L., Mitsuma, N., Beales, P. L., and Katsanis, N. (2006). The Ciliopathies: an Emerging Class of Human Genetic Disorders. *Annu. Rev. Genom. Hum. Genet.* 7, 125–148. doi:10.1146/annurev.genom.7.080505.115610

Barbelanne, M., Hossain, D., Chan, D. P., Peränen, J., and Tsang, W. Y. (2015). Nephrocystin Proteins NPHP5 and Cep290 Regulate BBSome Integrity, Ciliary Trafficking and Cargo Delivery. *Hum. Mol. Genet.* 24, 2185–2200. doi:10.1093/hmg/ddu738

Barbelanne, M., Song, J., Ahmadzai, M., and Tsang, W. Y. (2013). Pathogenic NPHP5 Mutations Impair Protein Interaction with Cep290, a Prerequisite for Ciliogenesis. *Hum. Mol. Genet.* 22, 2482–2494. doi:10.1093/hmg/ddt100

Bard, J. A. M., Goodall, E. A., Greene, E. R., Jonsson, E., Dong, K. C., and Martin, A. (2018). Structure and Function of the 26S Proteasome. *Annu. Rev. Biochem.* 87, 697–724. doi:10.1146/annurev-biochem-062917-011931

Barzi, M., Berenguer, J., Menendez, A., Alvarez-Rodriguez, R., and Pons, S. (2010). Sonic-hedgehog-mediated Proliferation Requires the Localization of PKA to the Cilium Base. *J. Cell Sci.* 123, 62–69. doi:10.1242/jcs.060020

Belibi, F., Zafar, I., Ravichandran, K., Segvic, A. B., Jani, A., Ljubanovic, D. G., et al. (2011). Hypoxia-inducible Factor-1α (HIF-1α) and Autophagy in Polycystic Kidney Disease (PKD). *Am. J. Physiology-Renal Physiology* 300, F1235–F1243. doi:10.1152/ajprenal.00348.2010

Berbari, N. F., Johnson, A. D., Lewis, J. S., Askwith, C. C., and Mykytyn, K. (2008). Identification of Ciliary Localization Sequences within the Third Intracellular Loop of G Protein-Coupled Receptors. *MBoC* 19, 1540–1547. doi:10.1091/mbc.e07-09-0942

Berlin, I., Schwartz, H., and Nash, P. D. (2010). Regulation of Epidermal Growth Factor Receptor Ubiquitination and Trafficking by the USP8·STAM Complex. *J. Biol. Chem.* 285, 34909–34921. doi:10.1074/jbc.M109.016287

Birgisdottir, Å. B., Lamark, T., and Johansen, T. (2013). The LIR Motif - Crucial for Selective Autophagy. *J. Cell Sci.* 126, 3237–3247. doi:10.1242/jcs.126128

Boukhalfa, A., Roccio, F., Dupont, N., Codogno, P., and Morel, E. (2021). The Autophagy Protein ATG16L1 Cooperates with IFT20 and INPP5E to Regulate the Turnover of Phosphoinositides at the Primary Cilium. *Cell Rep.* 35, 109045. doi:10.1016/j.celrep.2021.109045

Bucko, P. J., Lombard, C. K., Rathbun, L., Garcia, I., Bhat, A., Wordeman, L., et al. (2019). Subcellular Drug Targeting Illuminates Local Kinase Action. *Elife* 8, e52220. doi:10.7554/eLife.52220

Callis, J. (2014). The Ubiquitination Machinery of the Ubiquitin System. *Arabidopsis Book* 12, e0174. doi:10.1199/tab.0174

Cardozo, T., and Pagano, M. (2004). The SCF Ubiquitin Ligase: Insights into a Molecular Machine. *Nat. Rev. Mol. Cell Biol.* 5, 739–751. doi:10.1038/nrm1471

Carlucci, A., Lignitto, L., and Feliciello, A. (2008). Control of Mitochondria Dynamics and Oxidative Metabolism by cAMP, AKAPs and the Proteasome. *Trends Cell Biol.* 18, 604–613. doi:10.1016/j.tcb.2008.09.006

Chang, M.-Y., Ma, T.-L., Hung, C.-C., Tian, Y.-C., Chen, Y.-C., Yang, C.-W., et al. (2017). Metformin Inhibits Cyst Formation in a Zebrafish Model of Polycystin-2 Deficiency. *Sci. Rep.* 7, 7161. doi:10.1038/s41598-017-07300-x

Chen, Y., Yue, S., Xie, L., Pu, X.-h., Jin, T., and Cheng, S. Y. (2011). Dual Phosphorylation of Suppressor of Fused (Sufu) by PKA and GSK3β Regulates its Stability and Localization in the Primary Cilium. *J. Biol. Chem.* 286, 13502–13511. doi:10.1074/jbc.M110.217604

Chivukula, R. R., Montoro, D. T., Leung, H. M., Yang, J., Shamseldin, H. E., Taylor, M. S., et al. (2020). A Human Ciliopathy Reveals Essential Functions for NEK10 in Airway Mucociliary Clearance. *Nat. Med.* 26, 244–251. doi:10.1038/s41591-019-0730-x

Choi, Y.-H., Suzuki, A., Hajarnis, S., Ma, Z., Chapin, H. C., Caplan, M. J., et al. (2011). Polycystin-2 and Phosphodiesterase 4C Are Components of a Ciliary A-Kinase Anchoring Protein Complex that Is Disrupted in Cystic Kidney Diseases. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10679–10684. doi:10.1073/pnas.1016214108

Corbit, K. C., Aanstad, P., Singla, V., Norman, A. R., Stainier, D. Y. R., and Reiter, J. F. (2005). Vertebrate Smoothened Functions at the Primary Cilium. *Nature* 437, 1018–1021. doi:10.1038/nature04117

Dafinger, C., Liebau, M. C., Elsayed, S. M., Hellenbroich, Y., Boltshauser, E., Korenke, G. C., et al. (2011). Mutations in KIF7 Link Joubert Syndrome with Sonic Hedgehog Signaling and Microtubule Dynamics. *J. Clin. Invest.* 121, 2662–2667. doi:10.1172/JCI43639

Das, A., Qian, J., and Tsang, W. Y. (2017). USP9X Counteracts Differential Ubiquitination of NPHP5 by MARCH7 and BBS11 to Regulate Ciliogenesis. *PLoS Genet.* 13, e1006791. doi:10.1371/journal.pgen.1006791

Desai, P. B., Stuck, M. W., Lv, B., and Pazour, G. J. (2020). Ubiquitin Links Smoothened to Intraflagellar Transport to Regulate Hedgehog Signaling. *J. Cell Biol.* 219, e201912104. doi:10.1083/jcb.201912104

Dikic, I. (2017). Proteasomal and Autophagic Degradation Systems. *Annu. Rev. Biochem.* 86, 193–224. doi:10.1146/annurev-biochem-061516-044908

Eguether, T., and Hahne, M. (2018). Mixed Signals from the Cell's Antennae: Primary Cilia in Cancer. *EMBO Rep.* 19, e46589. doi:10.15252/embr.201846589

Fliegauf, M., Benzing, T., and Omran, H. (2007). When Cilia Go Bad: Cilia Defects and Ciliopathies. *Nat. Rev. Mol. Cell Biol.* 8, 880–893. doi:10.1038/nrm2278

Fry, A. M., O'Regan, L., Sabir, S. R., and Bayliss, R. (2012). Cell Cycle Regulation by the NEK Family of Protein Kinases. *J. Cell Sci.* 125, 4423–4433. doi:10.1242/jcs.111195

Gerdes, J. M., Davis, E. E., and Katsanis, N. (2009). The Vertebrate Primary Cilium in Development, Homeostasis, and Disease. *Cell* 137, 32–45. doi:10.1016/j.cell.2009.03.023

Gerhardt, C., Lier, J. M., Burmühl, S., Struchtrup, A., Deutschmann, K., Vetter, M., et al. (2015). The Transition Zone Protein Rpgrip1l Regulates Proteasomal Activity at the Primary Cilium. *J. Cell Biol.* 210, 1027–1045. doi:10.1083/jcb.201408060

Gerhardt, C., Wiegering, A., Leu, T., and Rüther, U. (2016). Control of Hedgehog Signalling by the Cilia-Regulated Proteasome. *JDB* 4, 27. doi:10.3390/jdb4030027

Gradilone, S. A., Radtke, B. N., Bogert, P. S., Huang, B. Q., Gajdos, G. B., and LaRusso, N. F. (2013). HDAC6 Inhibition Restores Ciliary Expression and Decreases Tumor Growth. *Cancer Res.* 73, 2259–2270. doi:10.1158/0008-5472.CAN-12-2938

Haglund, K., and Dikic, I. (2005). Ubiquitylation and Cell Signaling. *EMBO J.* 24, 3353–3359. doi:10.1038/sj.emboj.7600808

Han, K.-J., Wu, Z., Pearson, C. G., Peng, J., Song, K., and Liu, C.-W. (2019). Deubiquitinase USP9X Maintains Centriolar Satellite Integrity by Stabilizing Pericentriolar Material 1 Protein. *J. Cell Sci.* 132, jcs221663. doi:10.1242/jcs.221663

Han, S. J., Jung, J. K., Im, S.-S., Lee, S.-R., Jang, B.-C., Park, K. M., et al. (2018). Deficiency of Primary Cilia in Kidney Epithelial Cells Induces Epithelial to Mesenchymal Transition. *Biochem. Biophysical Res. Commun.* 496, 450–454. doi:10.1016/j.bbrc.2018.01.079

Han, Y.-G., Kim, H. J., Dlugosz, A. A., Ellison, D. W., Gilbertson, R. J., and Alvarez-Buylla, A. (2009). Dual and Opposing Roles of Primary Cilia in Medulloblastoma Development. *Nat. Med.* 15, 1062–1065. doi:10.1038/nm.2020

Hsiao, C.-J., Chang, C.-H., Ibrahim, R. B., Lin, I.-H., Wang, C.-H., Wang, W.-J., et al. (2018). Gli2 Modulates Cell Cycle Re-entry through Autophagy-Mediated Regulation on the Length of Primary Cilia. *J. Cell Sci.* 131, jcs221218. doi:10.1242/jcs.221218

Husnjak, K., and Dikic, I. (2012). Ubiquitin-binding Proteins: Decoders of Ubiquitin-Mediated Cellular Functions. *Annu. Rev. Biochem.* 81, 291–322. doi:10.1146/annurev-biochem-051810-094654

Hynes, A. M., Giles, R. H., Srivastava, S., Eley, L., Whitehead, J., Danilenko, M., et al. (2014). Murine Joubert Syndrome Reveals Hedgehog Signaling Defects as a Potential Therapeutic Target for Nephronophthisis. *Proc. Natl. Acad. Sci. U.S.A.* 111, 9893–9898. doi:10.1073/pnas.1322373111

Iaconis, D., Crina, C., Brillante, S., Indrieri, A., Morleo, M., and Franco, B. (2020). The HOPS Complex Subunit VPS39 Controls Ciliogenesis through Autophagy. *Hum. Mol. Genet.* 29, 1018–1029. doi:10.1093/hmg/ddaa029

Inoko, A., Matsuyama, M., Goto, H., Ohmuro-Matsuyama, Y., Hayashi, Y., Enomoto, M., et al. (2012). Trichoplein and Aurora A Block Aberrant Primary Cilia Assembly in Proliferating Cells. *J. Cell Biol.* 197, 391–405. doi:10.1083/jcb.201106101

Ishikawa, H., and Marshall, W. F. (2011). Ciliogenesis: Building the Cell's Antenna. *Nat. Rev. Mol. Cell Biol.* 12, 222–234. doi:10.1038/nrm3085

Ishikawa, H., Thompson, J., Yates, J. R., and Marshall, W. F. (2012). Proteomic Analysis of Mammalian Primary Cilia. *Curr. Biol.* 22, 414–419. doi:10.1016/j.cub.2012.01.031

Jang, J., Wang, Y., Lalli, M. A., Guzman, E., Godshalk, S. E., Zhou, H., et al. (2016). Primary Cilium-Autophagy-Nrf2 (PAN) Axis Activation Commits Human Embryonic Stem Cells to a Neuroectoderm Fate. *Cell* 165, 410–420. doi:10.1016/j.cell.2016.02.014

Jiang, W., Yao, X., Shan, Z., Li, W., Gao, Y., and Zhang, Q. (2019). E3 Ligase Herc4 Regulates Hedgehog Signalling through Promoting Smoothened Degradation. *J. Mol. Cell Biol.* 11, 791–803. doi:10.1093/jmcb/mjz024

Jones, B. W., Deem, J., Younts, T. J., Weisenhaus, M., Sanford, C. A., Slack, M. C., et al. (2016). Targeted Deletion of AKAP7 in Dentate Granule Cells Impairs Spatial Discrimination. *Elife* 5, e20695. doi:10.7554/eLife.20695

Kasahara, K., Aoki, H., Kiyono, T., Wang, S., Kagiwada, H., Yuge, M., et al. (2018). EGF Receptor Kinase Suppresses Ciliogenesis through Activation of USP8 Deubiquitinase. *Nat. Commun.* 9, 758. doi:10.1038/s41467-018-03117-y

Kasahara, K., Kawakami, Y., Kiyono, T., Yonemura, S., Kawamura, Y., Era, S., et al. (2014). Ubiquitin-proteasome System Controls Ciliogenesis at the Initial Step of Axoneme Extension. *Nat. Commun.* 5, 5081. doi:10.1038/ncomms6081

Kathem, S., Mohieldin, A. M., M. Mohieldin, A., and M. Nauli, S. (2013). The Roles of Primary Cilia in Polycystic Kidney Disease. *AIMS Mol. Sci.* 1, 27–46. doi:10.3934/molsci.2013.1.27

Kim, J. H., Ki, S. M., Joung, J.-G., Scott, E., Heynen-Genel, S., Aza-Blanc, P., et al. (2016). Genome-wide Screen Identifies Novel Machineries Required for Both Ciliogenesis and Cell Cycle Arrest upon Serum Starvation. *Biochimica Biophysica Acta (BBA) - Mol. Cell Res.* 1863, 1307–1318. doi:10.1016/j.bbamcr.2016.03.021

Kim, J., Kundu, M., Viollet, B., and Guan, K.-L. (2011). AMPK and mTOR Regulate Autophagy through Direct Phosphorylation of Ulk1. *Nat. Cell Biol.* 13, 132–141. doi:10.1038/ncb2152

Kim, S.-O., Cho, K. S., Kim, B. Y., and Lee, K. H. (2021). Cullin 1 (CUL1) Promotes Primary Ciliogenesis through the Induction of Ubiquitin-proteasome-dependent Dvl2 Degradation. *IJMS* 22, 7572. doi:10.3390/ijms22147572

Klionsky, D. J., Petroni, G., Amaravadi, R. K., Baehrecke, E. H., Ballabio, A., Boya, P., et al. (2021). Autophagy in Major Human Diseases. *EMBO J.* 40, e108863. doi:10.15252/embj.2021108863

Kobayashi, T., Ishida, Y., Hirano, T., Katoh, Y., and Nakayama, K. (2021). Cooperation of the IFT-A Complex with the IFT-B Complex Is Required for Ciliary Retrograde Protein Trafficking and GPCR Import. *MBoC* 32, 45–56. doi:10.1091/mbc.E20-08-0556

Kobayashi, T., Kim, S., Lin, Y.-C., Inoue, T., and Dynlacht, B. D. (2014). The CP110-Interacting Proteins Talpid3 and Cep290 Play Overlapping and Distinct Roles in Cilia Assembly. *J. Cell Biol.* 204, 215–229. doi:10.1083/jcb.201304153

Kobayashi, T., Nakazono, K., Tokuda, M., Mashima, Y., Dynlacht, B. D., and Itoh, H. (2017). HDAC 2 Promotes Loss of Primary Cilia in Pancreatic Ductal Adenocarcinoma. *EMBO Rep.* 18, 334–343. doi:10.15252/embr.201541922

Labat-de-Hoz, L., Rubio-Ramos, A., Casares-Arias, J., Bernabé-Rubio, M., Correas, I., and Alonso, M. A. (2021). A Model for Primary Cilium Biogenesis by Polarized Epithelial Cells: Role of the Midbody Remnant and Associated Specialized Membranes. *Front. Cell Dev. Biol.* 8, 622918. doi:10.3389/fcell.2020.622918

Lam, H. C., Cloonan, S. M., Bhashyam, A. R., Haspel, J. A., Singh, A., Sathirapongsasuti, J. F., et al. (2013). Histone Deacetylase 6-mediated Selective Autophagy Regulates COPD-Associated Cilia Dysfunction. *J. Clin. Invest.* 123, 5212–5230. doi:10.1172/JCI69636

Lee, J.-Y., Koga, H., Kawaguchi, Y., Tang, W., Wong, E., Gao, Y.-S., et al. (2010). HDAC6 Controls Autophagosome Maturation Essential for Ubiquitin-Selective Quality-Control Autophagy. *EMBO J.* 29, 969–980. doi:10.1038/emboj.2009.405

Lee, J. M., Wagner, M., Xiao, R., Kim, K. H., Feng, D., Lazar, M. A., et al. (2014). Nutrient-sensing Nuclear Receptors Coordinate Autophagy. *Nature* 516, 112–115. doi:10.1038/nature13961

Lee, K. H., Johmura, Y., Yu, L.-R., Park, J.-E., Gao, Y., Bang, J. K., et al. (2012). Identification of a Novel Wnt5a-Ck1ε-Dvl2-Plk1-Mediated Primary Cilia Disassembly Pathway. *EMBO J.* 31, 3104–3117. doi:10.1038/emboj.2012.144

Levine, B., and Kroemer, G. (2008). Autophagy in the Pathogenesis of Disease. *Cell* 132, 27–42. doi:10.1016/j.cell.2007.12.018

Levine, B., Mizushima, N., and Virgin, H. W. (2011). Autophagy in Immunity and Inflammation. *Nature* 469, 323–335. doi:10.1038/nature09782

Li, H., Li, J., Li, Y., Singh, P., Cao, L., Xu, L.-j., et al. (2012). Sonic Hedgehog Promotes Autophagy of Vascular Smooth Muscle Cells. *Am. J. Physiology-Heart Circulatory Physiology* 303, H1319–H1331. doi:10.1152/ajpheart.00160.2012

Liem, K. F., Ashe, A., He, M., Satir, P., Moran, J., Beier, D., et al. (2012). The IFT-A Complex Regulates Shh Signaling through Cilia Structure and Membrane Protein Trafficking. *J. Cell Biol.* 197, 789–800. doi:10.1083/jcb.201110049

Lignitto, L., Carlucci, A., Sepe, M., Stefan, E., Cuomo, O., Nisticò, R., et al. (2011). Control of PKA Stability and Signalling by the RING Ligase Praja2. *Nat. Cell Biol.* 13, 412–422. doi:10.1038/ncb2209

Lin, C.-H., Chao, C.-T., Wu, M.-Y., Lo, W.-C., Lin, T.-C., and Wu, M.-S. (2019). Use of Mammalian Target of Rapamycin Inhibitors in Patient with Autosomal Dominant Polycystic Kidney Disease: an Updated Meta-Analysis. *Int. Urol. Nephrol.* 51, 2015–2025. doi:10.1007/s11255-019-02292-1

Liu, H., Kiseleva, A. A., and Golemis, E. A. (2018a). Ciliary Signalling in Cancer. *Nat. Rev. Cancer* 18, 511–524. doi:10.1038/s41568-018-0023-6

Liu, M., Zhang, W., Li, M., Feng, J., Kuang, W., Chen, X., et al. (2021). NudCL2 Is an Autophagy Receptor that Mediates Selective Autophagic Degradation of CP110 at Mother Centrioles to Promote Ciliogenesis. *Cell Res.* 31, 1199–1211. doi:10.1038/s41422-021-00560-3

Liu, Y. P., Tsai, I.-C., Morleo, M., Oh, E. C., Leitch, C. C., Massa, F., et al. (2014). Ciliopathy Proteins Regulate Paracrine Signaling by Modulating Proteasomal Degradation of Mediators. *J. Clin. Invest.* 124, 2059–2070. doi:10.1172/JCI71898

Liu, Z.-q., Lee, J. N., Son, M., Lim, J.-Y., Dutta, R. K., Maharjan, Y., et al. (2018b). Ciliogenesis Is Reciprocally Regulated by PPARA and NR1H4/FXR through Controlling Autophagy *In Vitro* and *In Vivo*. *Autophagy* 14, 1–17. doi:10.1080/15548627.2018.1448326

Lu, Q., Insinna, C., Ott, C., Stauffer, J., Pintado, P. A., Rahajeng, J., et al. (2015). Early Steps in Primary Cilium Assembly Require EHD1/EHD3-dependent Ciliary Vesicle Formation. *Nat. Cell Biol.* 17, 228–240. doi:10.1038/ncb3109

Mabbitt, P. D., Loreto, A., Déry, M.-A., Fletcher, A. J., Stanley, M., Pao, K.-C., et al. (2020). Structural Basis for RING-Cys-Relay E3 Ligase Activity and its Role in Axon Integrity. *Nat. Chem. Biol.* 16, 1227–1236. doi:10.1038/s41589-020-0598-6

Martin-Hurtado, A., Martin-Morales, R., Robledinos-Antón, N., Blanco, R., Palacios-Blanco, I., Lastres-Becker, I., et al. (2019). NRF2-dependent Gene Expression Promotes Ciliogenesis and Hedgehog Signaling. *Sci. Rep.* 9, 13896. doi:10.1038/s41598-019-50356-0

Massa, F., Tammaro, R., Prado, M. A., Cesana, M., Lee, B.-H., Finley, D., et al. (2019). The Deubiquitinating Enzyme Usp14 Controls Ciliogenesis and Hedgehog Signaling. *Hum. Mol. Genet.* 28, 764–777. doi:10.1093/hmg/ddy380

Mick, D. U., Rodrigues, R. B., Leib, R. D., Adams, C. M., Chien, A. S., Gygi, S. P., et al. (2015). Proteomics of Primary Cilia by Proximity Labeling. *Dev. Cell* 35, 497–512. doi:10.1016/j.devcel.2015.10.015

Miyamoto, T., Hosoba, K., Ochiai, H., Royba, E., Izumi, H., Sakuma, T., et al. (2015). The Microtubule-Depolymerizing Activity of a Mitotic Kinesin Protein KIF2A Drives Primary Cilia Disassembly Coupled with Cell Proliferation. *Cell Rep.* 10, 664–673. doi:10.1016/j.celrep.2015.01.003

Mizushima, N. (2005). The Pleiotropic Role of Autophagy: from Protein Metabolism to Bactericide. *Cell Death Differ.* 12, 1535–1541. doi:10.1038/sj.cdd.4401728

Morleo, M., Brillante, S., Formisano, U., Ferrante, L., Carbone, F., Iaconis, D., et al. (2021). Regulation of Autophagosome Biogenesis by OFD1-mediated Selective Autophagy. *EMBO J.* 40. doi:10.15252/embj.2020105120

Moser, J. J., Fritzler, M. J., and Rattner, J. B. (2009). Primary Ciliogenesis Defects Are Associated with Human Astrocytoma/glioblastoma Cells. *BMC Cancer* 9, 448. doi:10.1186/1471-2407-9-448

Newton, A. C., Bootman, M. D., and Scott, J. D. (2016). Second Messengers. *Cold Spring Harb. Perspect. Biol.* 8, a005926. doi:10.1101/cshperspect.a005926

Niewiadomski, P., Kong, J. H., Ahrends, R., Ma, Y., Humke, E. W., Khan, S., et al. (2014). Gli Protein Activity Is Controlled by Multisite Phosphorylation in Vertebrate Hedgehog Signaling. *Cell Rep.* 6, 168–181. doi:10.1016/j.celrep.2013.12.003

Pal, K., and Mukhopadhyay, S. (2015). Primary Cilium and Sonic Hedgehog Signaling during Neural Tube Patterning: Role of GPCRs and Second Messengers. *Devel Neurobio* 75, 337–348. doi:10.1002/dneu.22193

Palencia-Campos, A., Aoto, P. C., Machal, E. M. F., Rivera-Barahona, A., Soto-Bielicka, P., Bertinetti, D., et al. (2020). Germline and Mosaic Variants in PRKACA and PRKACB Cause a Multiple Congenital Malformation Syndrome. *Am. J. Hum. Genet.* 107, 977–988. doi:10.1016/j.ajhg.2020.09.005

Pampliega, O., Orhon, I., Patel, B., Sridhar, S., Díaz-Carretero, A., Beau, I., et al. (2013). Functional Interaction between Autophagy and Ciliogenesis. *Nature* 502, 194–200. doi:10.1038/nature12639

Pan, Y., Wang, C., and Wang, B. (2009). Phosphorylation of Gli2 by Protein Kinase A Is Required for Gli2 Processing and Degradation and the Sonic Hedgehog-Regulated Mouse Development. *Dev. Biol.* 326, 177–189. doi:10.1016/j.ydbio.2008.11.009

Pankiv, S., Clausen, T. H., Lamark, T., Brech, A., Bruun, J.-A., Outzen, H., et al. (2007). p62/SQSTM1 Binds Directly to Atg8/LC3 to Facilitate Degradation of Ubiquitinated Protein Aggregates by Autophagy. *J. Biol. Chem.* 282, 24131–24145. doi:10.1074/jbc.M702824200

Pao, K.-C., Wood, N. T., Knebel, A., Rafie, K., Stanley, M., Mabbitt, P. D., et al. (2018). Activity-based E3 Ligase Profiling Uncovers an E3 Ligase with Esterification Activity. *Nature* 556, 381–385. doi:10.1038/s41586-018-0026-1

Park, S. M., Lim, J. S., Ramakrishina, S., Kim, S. H., Kim, W. K., Lee, J., et al. (2018). Brain Somatic Mutations in MTOR Disrupt Neuronal Ciliogenesis, Leading to Focal Cortical Dyslamination. *Neuron* 99, 83–97. e7. doi:10.1016/j.neuron.2018.05.039

Pastor-Soler, N. M., Li, H., Pham, J., Rivera, D., Ho, P.-Y., Mancino, V., et al. (2022). Metformin Improves Relevant Disease Parameters in an Autosomal Dominant Polycystic Kidney Disease Mouse Model. *Am. J. Physiology-Renal Physiology* 322, F27–F41. doi:10.1152/ajprenal.00298.2021

Patzke, S., Redick, S., Warsame, A., Murga-Zamalloa, C. A., Khanna, H., Doxsey, S., et al. (2010). CSPP Is a Ciliary Protein Interacting with Nephrocystin 8 and Required for Cilia Formation. *MBoC* 21, 2555–2567. doi:10.1091/mbc.e09-06-0503

Peixoto, E., Jin, S., Thelen, K., Biswas, A., Richard, S., Morleo, M., et al. (2020a). HDAC6-dependent Ciliophagy Is Involved in Ciliary Loss and

Cholangiocarcinoma Growth in Human Cells and Murine Models. *Am. J. Physiology-Gastrointestinal Liver Physiology* 318, G1022–G1033. doi:10.1152/ajpgi.00033.2020

Peixoto, E., Richard, S., Pant, K., Biswas, A., and Gradilone, S. A. (2020b). The Primary Cilium: Its Role as a Tumor Suppressor Organelle. *Biochem. Pharmacol.* 175, 113906. doi:10.1016/j.bcp.2020.113906

Perrone, R. D., Abebe, K. Z., Watnick, T. J., Althouse, A. D., Hallows, K. R., Lalama, C. M., et al. (2021). Primary Results of the Randomized Trial of Metformin Administration in Polycystic Kidney Disease (TAME PKD). *Kidney Int.* 100, 684–696. doi:10.1016/j.kint.2021.06.013

Petralia, R. S., Schwartz, C. M., Wang, Y.-X., Kawamoto, E. M., Mattson, M. P., and Yao, P. J. (2013). Sonic Hedgehog Promotes Autophagy in Hippocampal Neurons. *Biol. Open* 2, 499–504. doi:10.1242/bio.20134275

Plotnikova, O. V., Pugacheva, E. N., and Golemis, E. A. (2009). Primary Cilia and the Cell Cycle. *Methods Cell Biol.* 94, 137–160. doi:10.1016/S0091-679X(08)94007-3

Porpora, M., Sauchella, S., Rinaldi, L., Delle Donne, R., Sepe, M., Torres-Quesada, O., et al. (2018). Counterregulation of cAMP-Directed Kinase Activities Controls Ciliogenesis. *Nat. Commun.* 9, 1224. doi:10.1038/s41467-018-03643-9

Pugacheva, E. N., Jablonski, S. A., Hartman, T. R., Henske, E. P., and Golemis, E. A. (2007). HEF1-dependent Aurora A Activation Induces Disassembly of the Primary Cilium. *Cell* 129, 1351–1363. doi:10.1016/j.cell.2007.04.035

Rao, Y., Hao, R., Wang, B., and Yao, T.-P. (2014). A Mec17-Myosin II Effector Axis Coordinates Microtubule Acetylation and Actin Dynamics to Control Primary Cilium Biogenesis. *PLoS One* 9, e114087. doi:10.1371/journal.pone.0114087

Reiter, J. F., and Leroux, M. R. (2017). Genes and Molecular Pathways Underpinning Ciliopathies. *Nat. Rev. Mol. Cell Biol.* 18, 533–547. doi:10.1038/nrm.2017.60

Rinaldi, L., Delle Donne, R., Borzacchiello, D., Insabato, L., and Feliciello, A. (2018). The Role of Compartmentalized Signaling Pathways in the Control of Mitochondrial Activities in Cancer Cells. *Biochimica Biophysica Acta (BBA) - Rev. Cancer* 1869, 293–302. doi:10.1016/j.bbcan.2018.04.004

Rinaldi, L., Delle Donne, R., Catalanotti, B., Torres-Quesada, O., Enzler, F., Moraca, F., et al. (2019). Feedback Inhibition of cAMP Effector Signaling by a Chaperone-Assisted Ubiquitin System. *Nat. Commun.* 10, 2572. doi:10.1038/s41467-019-10037-y

Rinaldi, L., Sepe, M., Delle Donne, R., Conte, K., Arcella, A., Borzacchiello, D., et al. (2017). Mitochondrial AKAP1 Supports mTOR Pathway and Tumor Growth. *Cell Death Dis.* 8–e2842. doi:10.1038/cddis.2017.241

Rohatgi, R., Milenkovic, L., and Scott, M. P. (2007). Patched1 Regulates Hedgehog Signaling at the Primary Cilium. *Science* 317, 372–376. doi:10.1126/science.1139740

Sadowski, M., Suryadinata, R., Tan, A. R., Roesley, S. N. A., and Sarcevic, B. (2012). Protein Monoubiquitination and Polyubiquitination Generate Structural Diversity to Control Distinct Biological Processes. *IUBMB Life* 64, 136–142. doi:10.1002/iub.589

Sánchez, I., and Dynlacht, B. D. (2016). Cilium Assembly and Disassembly. *Nat. Cell Biol.* 18, 711–717. doi:10.1038/ncb3370

Sasai, N., Toriyama, M., and Kondo, T. (2019). Hedgehog Signal and Genetic Disorders. *Front. Genet.* 10, 1103. doi:10.3389/fgene.2019.01103

Schmid, F. M., Schou, K. B., Vilhelm, M. J., Holm, M. S., Breslin, L., Farinelli, P., et al. (2018). IFT20 Modulates Ciliary PDGFRα Signaling by Regulating the Stability of Cbl E3 Ubiquitin Ligases. *J. Cell Biol.* 217, 151–161. doi:10.1083/jcb.201611050

Schweiggert, J., Habeck, G., Hess, S., Mikus, F., Beloshistov, R., Meese, K., et al. (2021). SCF Fbxw5 Targets Kinesin-13 Proteins to Facilitate Ciliogenesis. *EMBO J.* 40. doi:10.15252/embj.2021107735

Seliger, S. L., Abebe, K. Z., Hallows, K. R., Miskulin, D. C., Perrone, R. D., Watnick, T., et al. (2018). A Randomized Clinical Trial of Metformin to Treat Autosomal Dominant Polycystic Kidney Disease. *Am. J. Nephrol.* 47, 352–360. doi:10.1159/000488807

Senatore, E., Chiuso, F., Rinaldi, L., Intartaglia, D., Delle Donne, R., Pedone, E., et al. (2021). The TBC1D31/praja2 Complex Controls Primary Ciliogenesis through PKA-directed OFD1 Ubiquitylation. *EMBO J.* 40, e106503. doi:10.15252/embj.2020106503

Seok, S., Fu, T., Choi, S.-E., Li, Y., Zhu, R., Kumar, S., et al. (2014). Transcriptional Regulation of Autophagy by an FXR-CREB axis. *Nature* 516, 108–111. doi:10.1038/nature13949

Serra, A. L., Poster, D., Kistler, A. D., Krauer, F., Raina, S., Young, J., et al. (2010). Sirolimus and Kidney Growth in Autosomal Dominant Polycystic Kidney Disease. *N. Engl. J. Med.* 363, 820–829. doi:10.1056/NEJMoa0907419

Shearer, R. F., Frikstad, K.-A. M., McKenna, J., McCloy, R. A., Deng, N., Burgess, A., et al. (2018). The E3 Ubiquitin Ligase UBR5 Regulates Centriolar Satellite Stability and Primary Cilia. *MBoC* 29, 1542–1554. doi:10.1091/mbc.E17-04-0248

Shillingford, J. M., Murcia, N. S., Larson, C. H., Low, S. H., Hedgepeth, R., Brown, N., et al. (2006). The mTOR Pathway Is Regulated by Polycystin-1, and its Inhibition Reverses Renal Cystogenesis in Polycystic Kidney Disease. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5466–5471. doi:10.1073/pnas.0509694103

Shillingford, J. M., Piontek, K. B., Germino, G. G., and Weimbs, T. (2010). Rapamycin Ameliorates PKD Resulting from Conditional Inactivation of Pkd1. *Jasn* 21, 489–497. doi:10.1681/ASN.2009040421

Shinde, S. R., Nager, A. R., and Nachury, M. V. (2020). Ubiquitin Chains Earmark GPCRs for BBSome-Mediated Removal from Cilia. *J. Cell Biol.* 219, e202003020. doi:10.1083/jcb.202003020

Smit, J. J., and Sixma, T. K. (2014). RBR E3-ligases at Work. *EMBO Rep.* 15, 142–154. doi:10.1002/embr.201338166

Stoetzel, C., Bär, S., De Craene, J.-O., Scheidecker, S., Etard, C., Chicher, J., et al. (2016). A Mutation in VPS15 (PIK3R4) Causes a Ciliopathy and Affects IFT20 Release from the Cis-Golgi. *Nat. Commun.* 7, 13586. doi:10.1038/ncomms13586

Stone, E. M., Cideciyan, A. V., Aleman, T. S., Scheetz, T. E., Sumaroka, A., Ehlinger, M. A., et al. (2011). Variations in NPHP5 in Patients with Nonsyndromic Leber Congenital Amaurosis and Senior-Loken Syndrome. *Arch. Ophthalmol.* 129, 81–87. doi:10.1001/archophthalmol.2010.330

Struchtrup, A., Wiegering, A., Stork, B., Rüther, U., and Gerhardt, C. (2018). The Ciliary Protein RPGRIP1L Governs Autophagy Independently of its Proteasome-Regulating Function at the Ciliary Base in Mouse Embryonic Fibroblasts. *Autophagy* 14, 567–583. doi:10.1080/15548627.2018.1429874

Takiar, V., Nishio, S., Seo-Mayer, P., King, J. D., Li, H., Zhang, L., et al. (2011). Activating AMP-Activated Protein Kinase (AMPK) Slows Renal Cystogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 2462–2467. doi:10.1073/pnas.1011498108

Tang, Z., Lin, M. G., Stowe, T. R., Chen, S., Zhu, M., Stearns, T., et al. (2013). Autophagy Promotes Primary Ciliogenesis by Removing OFD1 from Centriolar Satellites. *Nature* 502, 254–257. doi:10.1038/nature12606

Tao, Y., Kim, J., Schrier, R. W., and Edelstein, C. L. (2005). Rapamycin Markedly Slows Disease Progression in a Rat Model of Polycystic Kidney Disease. *Jasn* 16, 46–51. doi:10.1681/ASN.2004080660

Taylor, S. S., Zhang, P., Steichen, J. M., Keshwani, M. M., and Kornev, A. P. (2013). PKA: Lessons Learned after Twenty Years. *Biochimica Biophysica Acta (BBA) - Proteins Proteomics* 1834, 1271–1278. doi:10.1016/j.bbapap.2013.03.007

Tsai, I.-C., Adams, K. A., Tzeng, J. A., Shennib, O., Tan, P. L., and Katsanis, N. (2019). Genome-wide Suppressor Screen Identifies USP35/USP38 as Therapeutic Candidates for Ciliopathies. *JCI Insight* 4, e130516. doi:10.1172/jci.insight.130516

Tschaikner, P., Enzler, F., Torres-Quesada, O., Aanstad, P., and Stefan, E. (2020). Hedgehog and Gpr161: Regulating cAMP Signaling in the Primary Cilium. *Cells* 9, 118. doi:10.3390/cells9010118

Tukachinsky, H., Lopez, L. V., and Salic, A. (2010). A Mechanism for Vertebrate Hedgehog Signaling: Recruitment to Cilia and Dissociation of SuFu-Gli Protein Complexes. *J. Cell Biol.* 191, 415–428. doi:10.1083/jcb.201004108

Tuz, K., Bachmann-Gagescu, R., O'Day, D. R., Hua, K., Isabella, C. R., Phelps, I. G., et al. (2014). Mutations in CSPP1 Cause Primary Cilia Abnormalities and Joubert Syndrome with or without Jeune Asphyxiating Thoracic Dystrophy. *Am. J. Hum. Genet.* 94, 62–72. doi:10.1016/j.ajhg.2013.11.019

Villumsen, B. H., Danielsen, J. R., Povlsen, L., Sylvestersen, K. B., Merdes, A., Beli, P., et al. (2013). A New Cellular Stress Response that Triggers Centriolar Satellite Reorganization and Ciliogenesis. *EMBO J.* 32, 3029–3040. doi:10.1038/emboj.2013.223

Wahl, P. R., Serra, A. L., Le Hir, M., Molle, K. D., Hall, M. N., and Wüthrich, R. P. (2006). Inhibition of mTOR with Sirolimus Slows Disease Progression in Han: SPRD Rats with Autosomal Dominant Polycystic Kidney Disease (ADPKD). *Nephrol. Dial. Transpl.* 21, 598–604. doi:10.1093/ndt/gfi181

Walz, G., Budde, K., Mannaa, M., Nürnberger, J., Wanner, C., Sommerer, C., et al. (2010). Everolimus in Patients with Autosomal Dominant Polycystic Kidney Disease. *N. Engl. J. Med.* 363, 830–840. doi:10.1056/NEJMoa1003491

Wang, L., Lee, K., Malonis, R., Sanchez, I., and Dynlacht, B. D. (2016). Tethering of an E3 Ligase by PCM1 Regulates the Abundance of Centrosomal KIAA0586/Talpid3 and Promotes Ciliogenesis. *eLife* 5, e12950. doi:10.7554/eLife.12950

Wang, Y., Han, C., Lu, L., Magliato, S., and Wu, T. (2013). Hedgehog Signaling Pathway Regulates Autophagy in Human Hepatocellular Carcinoma Cells. *Hepatology* 58, 995–1010. doi:10.1002/hep.26394

Webb, S., Mukhopadhyay, A. G., and Roberts, A. J. (2020). Intraflagellar Transport Trains and Motors: Insights from Structure. *Seminars Cell & Dev. Biol.* 107, 82–90. doi:10.1016/j.semcdb.2020.05.021

Wheway, G., Nazlamova, L., and Hancock, J. T. (2018). Signaling through the Primary Cilium. *Front. Cell Dev. Biol.* 6, 8. doi:10.3389/fcell.2018.00008

Wiegering, A., Rüther, U., and Gerhardt, C. (2018). The Ciliary Protein Rpgrip1l in Development and Disease. *Dev. Biol.* 442, 60–68. doi:10.1016/j.ydbio.2018.07.024

Wilkinson, K. D. (2009). DUBs at a Glance. *J. Cell Sci.* 122, 2325–2329. doi:10.1242/jcs.041046

Wloga, D., Joachimiak, E., Louka, P., and Gaertig, J. (2017). Posttranslational Modifications of Tubulin and Cilia. *Cold Spring Harb. Perspect. Biol.* 9, a028159. doi:10.1101/cshperspect.a028159

Woodruff, J. B., Wueseke, O., and Hyman, A. A. (2014). Pericentriolar Material Structure and Dynamics. *Phil. Trans. R. Soc. B* 369, 20130459. doi:10.1098/rstb.2013.0459

Yamamoto, Y., Chino, H., Tsukamoto, S., Ode, K. L., Ueda, H. R., and Mizushima, N. (2021). NEK9 Regulates Primary Cilia Formation by Acting as a Selective Autophagy Adaptor for MYH9/myosin IIA. *Nat. Commun.* 12, 3292. doi:10.1038/s41467-021-23599-7

Yamamoto, Y., and Mizushima, N. (2021). Autophagy and Ciliogenesis. *JMA J.* 4, 207–215. doi:10.31662/jmaj.2021-0090

Yang, L., and McKnight, G. S. (2015). Hypothalamic PKA Regulates Leptin Sensitivity and Adiposity. *Nat. Commun.* 6, 8237. doi:10.1038/ncomms9237

Yang, X., Jin, N., Wang, Y., Yao, Y., Wang, Y., Li, T., et al. (2021). Macroautophagy Supports Sonic Hedgehog Signaling by Promoting Patched1 Degradation. *Biochimica Biophysica Acta (BBA) - Mol. Cell Res.* 1868, 119124. doi:10.1016/j.bbamcr.2021.119124

Ye, F., Nager, A. R., and Nachury, M. V. (2018). BBSome Trains Remove Activated GPCRs from Cilia by Enabling Passage through the Transition Zone. *J. Cell Biol.* 217, 1847–1868. doi:10.1083/jcb.201709041

Zaffagnini, G., and Martens, S. (2016). Mechanisms of Selective Autophagy. *J. Mol. Biol.* 428, 1714–1724. doi:10.1016/j.jmb.2016.02.004

Zhou, Z., Yao, X., Pang, S., Chen, P., Jiang, W., Shan, Z., et al. (2018). The Deubiquitinase UCHL5/UCH37 Positively Regulates Hedgehog Signaling by Deubiquitinating Smoothened. *J. Mol. Cell Biol.* 10, 243–257. doi:10.1093/jmcb/mjx036

Zhu, P., Sieben, C. J., Xu, X., Harris, P. C., and Lin, X. (2017). Autophagy Activators Suppress Cystogenesis in an Autosomal Dominant Polycystic Kidney Disease Model. *Hum. Mol. Genet.* 26, ddw376–172. doi:10.1093/hmg/ddw376

Conflict of Interest: 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

GLOSSARY

MIB1 mindbomb E3 ubiquitin ligase protein 1
PCM1 pericentriolar matrix protein 1
UBR5 ubiquitin protein ligase E3 component N-recognin 5
DAVs distal appendages vesicles
BBS4 Bardet-Biedl syndrome 4
OFD1 oral-facial-digital syndrome 1
MYH9 myosin heavy chain 9
IFT20 intraflagellar transport protein 20
NR1H4 nuclear receptor subfamily 1 group H member 4
CSPP1 centrosome and spindle pole-associated protein 1
NEK10 never in mitosis A related kinase 10
SCF Skip1-Cullin-F-box
KIF2c kinesin family member 2c
Dvl2 dishvelled 2
HEF1 human enhancer of filamentation 1 protein
CUL3 cullin 3
PPARα peroxisome proliferator-activated receptor
Shh Sonic Hedgehog; Smo, smoothened
IFT27 intraflagellar transport protein 27
HERC4 HECT and RLD domain containing E3 ligase 4
SUFU suppressor of fused protein
PTCH1 patched 1
GliA activated Gli
NPHP5 nephrocystin 5
BBS11 Bardet-Biedl syndrome 11
MARCH7 membrane associated ring-CH-type finger 7
AurA aurora A kinase
USP8 ubiquitin carboxyl-terminal hydrolase 8
EGFR EGF receptor
AC adenylate cyclase
PKA protein kinase A
CHIP C-terminus of Hsc70-interacting protein
Ub ubiquitin
K48 lysine 48
K63 lysine 63
K11 lysine 11
LC3 microtubule-associated protein 1A/1B-light chain
RBR RING-between-RING

frontiers | Frontiers in Cell and Developmental Biology

MINI REVIEW
published: 12 May 2022
doi: 10.3389/fcell.2022.889166

Emerging Role of Ubiquitin-Specific Protease 19 in Oncogenesis and Cancer Development

*Fabiana Alejandra Rossi and Mario Rossi**

Genómica Funcional y Ciencia de Datos, Instituto de Investigaciones en Medicina Traslacional (IIMT), CONICET-Universidad Austral, Buenos Aires, Argentina

Ubiquitination and ubiquitin-like post-translational modifications control the activity and stability of different tumor suppressors and oncoproteins. Hence, regulation of this enzymatic cascade offers an appealing scenario for novel antineoplastic targets discovery. Among the different families of enzymes that participate in the conjugation of Ubiquitin, deubiquitinating enzymes (DUBs), responsible for removing ubiquitin or ubiquitin-like peptides from substrate proteins, have attracted increasing attention. In this regard, increasing evidence is accumulating suggesting that the modulation of the catalytic activity of DUBs represents an attractive point of therapeutic intervention in cancer treatment. In particular, different lines of research indicate that USP19, a member of the DUBs, plays a role in the control of tumorigenesis and cancer dissemination. This review aims at summarizing the current knowledge of USP19 wide association with the control of several cellular processes in different neoplasms, which highlights the emerging role of USP19 as a previously unrecognized prognosis factor that possesses both positive and negative regulation activities in tumor biology. These observations indicate that USP19 might represent a novel putative pharmacologic target in oncology and underscores the potential of identifying specific modulators to test in clinical settings.

Keywords: PTMs, ubiquitination, DUBs, USP19, tumorigenesis, metastasis

OPEN ACCESS

Edited by:
Long Zhang,
Zhejiang University, China

Reviewed by:
Simon Sipen Wing,
McGill University, Canada

****Correspondence:***
Mario Rossi
mrossi-conicet@austral.edu.ar

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *03 March 2022*
Accepted: *26 April 2022*
Published: *12 May 2022*

Citation:
Rossi FA and Rossi M (2022) Emerging Role of Ubiquitin-Specific Protease 19 in Oncogenesis and Cancer Development. Front. Cell Dev. Biol. 10:889166. doi: 10.3389/fcell.2022.889166

INTRODUCTION

Following translation, proteins can undergo several posttranslational modifications (PTMs) to modulate their activity, such as phosphorylation, methylation, glycosylation, acetylation, sumoylation and ubiquitination. These modifications represent a very important component in the physiological regulation of different pathways, including protein degradation, DNA repair activity, gene regulation and signal transduction, among others (Millar et al., 2019). Since growth regulatory proteins that drive tumorigenesis are modified by PTMs (Krueger and Srivastava, 2006), understating the mechanisms by which these modifications regulate oncogenic, or tumor suppressive pathways is of great relevance to restrain their effects upon pathological scenarios (Konstantinopoulos et al., 2007).

Moreover, the alteration in the levels and functionality of the components comprising the pathways responsible for the different PTMs, is related to different pathologies, including cancer (Xu et al., 2018; Sharma et al., 2019; Chen et al., 2020; Vellosillo and Minguez, 2021). In particular, ubiquitin-related PTMs are under active study as their dysregulation has been linked with the onset and progression of different oncological disorders (Reinstein and Ciechanover, 2006; Shi and Grossman, 2010).

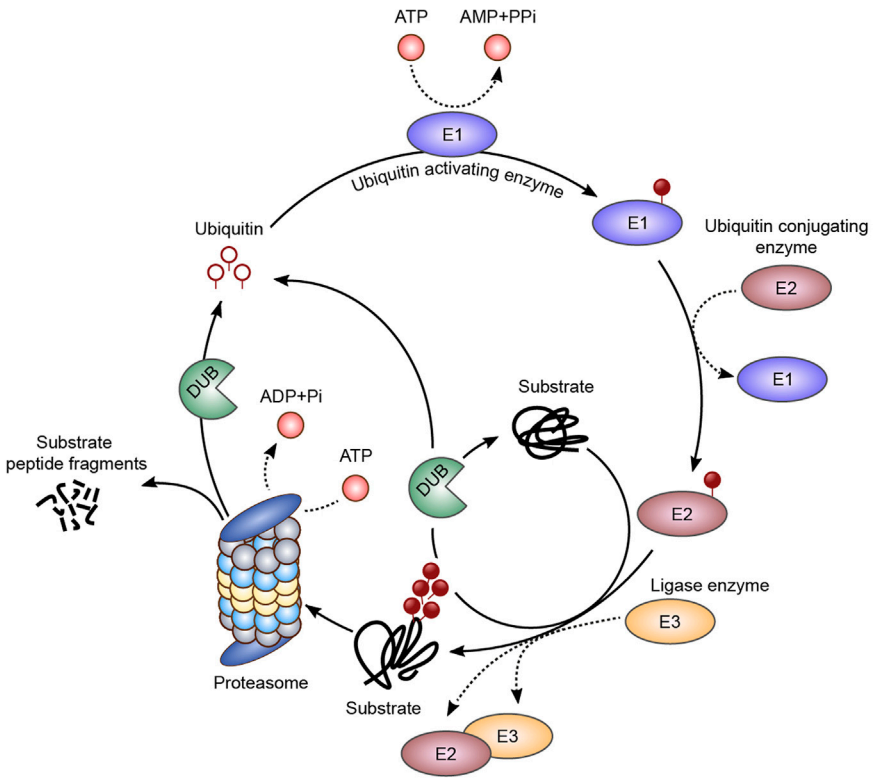


FIGURE 1 | Ubiquitination pathway. The ubiquitin molecule is activated by an E1 ubiquitin activating enzyme, in an ATP-dependent step, and a thioester intermediate is formed (E1-S-ubiquitin). The ubiquitin molecule is then transferred to an E2 conjugating enzyme (E2-S-ubiquitin), and then to the final substrate by an E3 ligase. Ubiquitin bound as monomers or polymers with different topologies are associated with different biological outputs, such as regulation of enzymatic activity, localization, protein-protein interactions, among others. Sequential ubiquitin conjugations form a polyubiquitin chain on the substrate, which can be recognized and degraded by the 26S proteasome. The deubiquitinating enzymes (DUBs) are responsible for the ubiquitin molecules recycling and chain editing.

UBIQUITINATION

Ubiquitination is the covalent attachment of ubiquitin (an 8-kDa 76 amino-acid molecule) to target proteins, and it plays crucial roles in the regulation of target proteins activity, stability, subcellular localization and trafficking, and interaction with other proteins (Damgaard, 2021). Therefore, this modification affects a great number of biological processes (Mevissen and Komander, 2017).

Protein ubiquitination is a tightly regulated process which involves the activity of two groups of enzymes, namely, E1/E2/E3 ligases and deubiquitinating enzymes (DUBs) (**Figure 1**).

The attachment of ubiquitination moieties to target proteins is catalyzed by the sequential action of a ubiquitin ATP-dependent activating enzyme (E1), which transfers the ubiquitin molecule to a ubiquitin conjugating enzyme (E2) by trans-thiolation, and by a ubiquitin ligase (E3), which provide substrate specificity to ubiquitin conjugation (Ciechanover, 1994; Hershko and Ciechanover, 1998; Komander and Rape, 2012).

This modification can occur as ubiquitin monomers or polymer chains, and since the ubiquitin molecule contains eight ubiquitination sites (seven internal lysine residues -Lys 6, 11, 27, 29, 33, 48 and 63- and a primary amine at the N-terminus), various types of ubiquitin chains with different length and shape might form (Akutsu et al., 2016; Yau and Rape, 2016; Dwane et al., 2017; Kwon and Ciechanover, 2017; Ohtake and Tsuchiya, 2017).

Furthermore, the ubiquitin molecule is subject to other PTMs such as phosphorylation, acetylation (Ohtake et al., 2015; Wauer et al., 2015; Huguenin-Dezot et al., 2016), and modification with ubiquitin-like proteins such as interferon (IFN)-stimulated gene 15 (ISG15) (Fan et al., 2015) and small ubiquitin-related modifier (SUMO) (Lamoliatte et al., 2013). Therefore, these modifications broaden the ubiquitin code versatility, as they affect not only ubiquitin interactions but also the formation and topology of the polyubiquitin chain.

The nature of the ubiquitin chain determines the outcome of the substrate protein, and different molecular signals are induced in the cell (Ikeda and Dikic, 2008; Sadowski and Sarcevic, 2010), affecting biological processes such as protein stability through proteasome degradation, DNA repair and replication, signal transduction, gene regulation, molecule trafficking and endocytosis, etc. (Hershko and Ciechanover, 1998; Haglund and Dikic, 2005; Komander and Rape, 2012; Yau and Rape, 2016).

The deubiquitinating enzymes are proteases that reverse the modification of proteins by a single ubiquitin or ubiquitin-like protein, and remodel polyubiquitin/ubiquitin-like chains on target proteins. They hydrolyze the isopeptide bond between

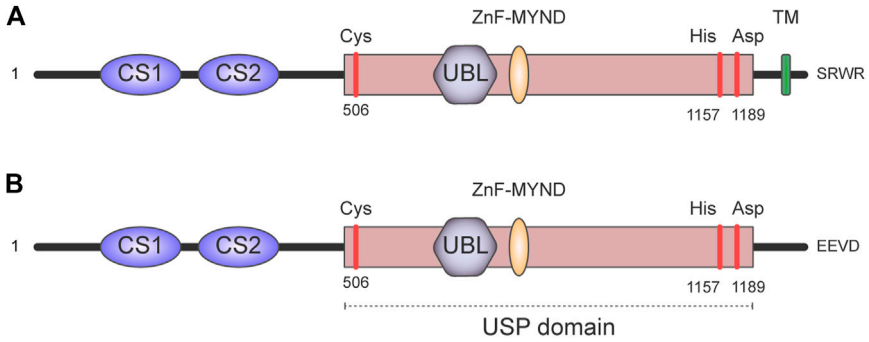


FIGURE 2 | Domain architecture of USP19. It contains two CHORD-SGT1 domains (namely CS1 and CS2) at its N-terminus and a large USP domain with a ubiquitin-like domain (UBL) and myeloid translocation protein 8, Nervy protein, Deaf-1 zinc finger (MYND Zn-finger). The positions of the amino acids Cys, His, and Asp in the catalytic triad are indicated in red. There are multiple USP19 isoforms generated by alternative splicing. In particular, alternative splicing of the last exon generates isoforms with a cytoplasmic localization or isoforms anchored to the endoplasmic reticulum. This schematic depicts: **(A)** the USP19 isoform that contains a transmembrane (TM) domain which anchors USP19 to the endoplasmic reticulum. **(B)** The soluble USP19 isoform has a relatively hydrophilic region and an EEVD motif in the C-terminus instead.

the ubiquitin and the substrate residue of either the target protein or another ubiquitin molecule (Komander et al., 2009; Komander and Rape, 2012). The human genome encodes nearly 100 DUBs, each with distinct substrate specificities and catalytic properties, which confer high precision upon ubiquitin chains processing (Komander et al., 2009; Mevissen and Komander, 2017). Consequently, individual DUBs likely confer specific actions (Komander et al., 2009; Huang and Dixit, 2016) and pharmacological modulation of their catalytic activity should lead to desired outcomes upon physiological or pharmacological scenarios.

Based on sequence and structural similarities, DUBs have been classified into seven families: Ubiquitin-specific proteases (USPs), Ubiquitin C-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado-Joseph (Josephin) domain (MJD) proteases, Jab1/MPN domain-associated metallo-iso-peptidases (JAMM/MPM+), Zinc finger UB-specific proteases (ZUP/ZUFSP), and monocyte chemotactic protein-induced proteins (MCPIP). Except for the JAMMs, which are zinc-dependent metalloproteases, the remaining families are cysteine proteases (Reyes-Turcu et al., 2009; Hanpude et al., 2015; Mevissen and Komander, 2017; Kwasna et al., 2018).

GENERAL PROPERTIES OF USP19

Human ubiquitin-specific protease 19 (USP19) is a modular deubiquitinating enzyme that belongs to the largest family of DUBs, the USPs (Nijman et al., 2005; Reyes-Turcu et al., 2009). This family is characterized by the presence of a highly conserved USP catalytic domain fold (Hu et al., 2002; Hu et al., 2005; Avvakumov et al., 2006; Renatus et al., 2006; Komander et al., 2008), which holds two well-conserved motifs (Cys and His boxes), each containing the critical residues for the enzymatic activity. Moreover, USP19 contains two CHORD-SGT1/P23 domains (namely CS1 and CS2) at its N-terminus, which are relevant for the interaction with other proteins, as well as for the intra-molecular inhibition and regulation of the catalytic core (Xue et al., 2020) (**Figure 2**).

USP19 presents different isoforms generated by alternative splicing, and the most distinctive feature—structurally and functionally–is that some of them have a cytoplasmic localization, while others have a transmembrane domain that serves as anchorage to the endoplasmic reticulum (Hassink et al., 2009) (**Figure 2**).

Like other DUBs, USP19 is covalently modified by PTMs such as phosphorylation and ubiquitination, which affect its activity and half-life, respectively (Matsuoka et al., 2007; Velasco et al., 2013).

Functionally, USP19 has mainly been associated with protein quality control and cellular homeostasis (Hassink et al., 2009; Lee et al., 2014; Wiles et al., 2015; He et al., 2016; He et al., 2017), muscle development (Combaret et al., 2005; Sundaram et al., 2009; Wiles et al., 2015), and it has been shown that it controls the half-life of several proteins such as HIF1-α (Altun et al., 2012), BECN1 (Cui et al., 2016), TGFßRI (Zhang et al., 2012), TRAF3 (Gu et al., 2017), HRD1 (Harada et al., 2016), TAK1 (Lei et al., 2019), KPC1 (Lu et al., 2009), c-IAPs one and 2 (Mei et al., 2011), HDAC1/2 (Wu et al., 2017), COROA2 (Lim et al., 2016), LRP6 (Perrody et al., 2016) and MARCH6 (Nakamura et al., 2014), therefore affecting cellular processes relevant in tumorigenesis such as DNA damage repair, apoptosis, the TGF-β Pathway, hypoxia and angiogenesis, immunity, proliferation, ERAD and autophagy.

THE ROLE OF USP19 IN CANCER MALIGNANCY

Disrupted regulation of protein ubiquitination is a trigger of cancer, among other diseases. Not surprisingly, alterations in the levels of the ubiquitination cascade components -including the DUBs-have been associated with multiple neoplasms (Shi and Grossman, 2010; Deng et al., 2020; Sun et al., 2020).

In the last couple of years, increasing evidence has begun to demonstrate that USP19 is associated with tumor progression and that it represents a novel prognostic factor for the outcome of several malignant diseases. In particular, it has been shown that USP19 plays both positive and negative roles in the onset and development of diverse neoplasms, in a tissue-specific manner. Consequently, in the following paragraphs, results denoting USP19 relevance in different signaling pathways regulating cell proliferation and cell-cycle progression, as well as tumor growth and metastasis will be presented, therefore unveiling the importance of conducting extensive studies to further the study of USP19's dual role in tumorigenesis under different molecular scenarios, and to establish its significance as a potential new target for the clinical treatment of cancer.

USP19 as a DUB Negatively Regulating Tumorigenesis

A couple of recent papers presented results indicating that USP19 negatively affected proliferation and migration in clear cell renal cell (Hu et al., 2020) and serous ovarian carcinomas (Kang et al., 2021).

Hu and others utilized clear cells renal cancer (ccRCC) cell lines *in vitro* and demonstrated that overexpression of USP19 levels negatively affected migration and proliferation, and the opposite occurred upon USP19 silencing. They validated their results using *in vivo* models and observed that USP19 downregulation promoted tumor growth in a xenograft model. Moreover, they conducted *in silico* analyses and observed that USP19 mRNA levels were significantly lower in ccRCC than normal tissues, and that low USP19 expression was associated with disease progression and poor prognostic outcomes in a The Cancer Genome Atlas (TCGA) cohort of patients (Hu et al., 2020). These results were consistent with a previous work by Liu and collaborators, who performed an *in silico* analysis and observed that isoform uc003cvz.3, which is mainly localized in the cytoplasm, serves as an indicator of poor outcome in patients with advanced stage ccRCC (Liu et al., 2013).

Similarly, Kang et al. applied a machine learning model on RNA-sequencing data from 51 patients who received conventional therapies for high-grade serous ovarian carcinoma (HGSC) and identified USP19 and RPL23 as candidate prognostic markers. Specifically, they showed that patients with lower USP19 or higher RPL23 mRNA levels had worse prognoses and they validated their model using publicly available data from the TCGA (Kang et al., 2021). They also observed that USP19 levels positively correlated with TOP3B and XRN2, which regulate genome instability (Kang et al., 2021). Based on this observation, and considering that USP19 interacts with and deubiquitinates HDAC1/2 in order to regulate DNA damage repair and chromosomal stability (Wu et al., 2017) and that both ccRCC and HGSC are characterized by high genomic instability, it is plausible that USP19-mediated deubiquitination of key regulators associated with DSB repair or genome instability might be responsible for the worse prognosis observed in ccRCC and HGSC patients with low USP19 levels (Kang et al., 2021).

In addition, Shahriyari L and collaborators (Shahriyari et al., 2019) described the existence of a correlation between the expression of USP19, RBM15B and the tumor suppressor gene BAP1 (BRCA1 associated protein-1) in different type of cancers. All three genes are in proximity of the 3p21 tumor suppressor region, which is commonly altered in many cancers, suggesting that USP19 could play a functional role in BAP1 molecular mechanism of action or its alteration could be a byproduct of chromosomal rearrangement affecting other genes. Although further characterization is required, this observation highlights the potential of USP19 as a putative prognostic biomarker in different cancers.

USP19 Positively Regulates Tumor Growth and Metastasis

Opposite to the role of USP19 as a tumor suppressor, recent work has also established that antagonism of USP19 expression conferred a prominent antiproliferative and antitumorigenic response in diverse neoplasms: Ewing sarcoma, gastric, breast and colorectal cancers (Gierisch et al., 2019; Dong et al., 2020; Rossi et al., 2021; Zhu et al., 2021), suggesting pro-tumorigenic roles in these tissues.

Ewing sarcoma is the second most common pediatric bone and soft tissue tumor, which is characterized by the presence of a chimeric oncoprotein, EWS-FLI1, due to a genetic translocation between chromosomes 22 and 11 (Desmaze et al., 1997). Gierisch and collaborators demonstrated that this protein, which maintains tumor cells survival, is regulated by USP19 in a post translational manner, and dependent on its catalytic activity (Gierisch et al., 2019). Downregulation of USP19 levels resulted in a reduction of EWS-FLI1 levels, hence decreasing tumor cells growth and colony formation capability, whereas the opposite occurred upon USP19 (TM isoform) overexpression. Using *in vivo* experiments, the authors demonstrated that tumor growth was delayed when USP19 levels were reduced.

On the other hand, Dong and others analyzed USP19 relevance in gastric cancer (Dong et al., 2020). Their results revealed that USP19 TM isoform overexpression enhanced cell proliferation and exhibited anti-apoptotic properties, as well as it increased cells migration and spreading capabilities *in vitro*; the opposite was observed upon USP19 silencing (multiple isoforms). Furthermore, they showed that increased USP19 (TM isoform) levels enhanced MMP2/MMP9 protein expression and enzyme activity, and that genetic alteration of USP19 levels affected tumorigenesis using *in vivo* models. Finally, using a cohort of 212 gastric cancer patients, the authors observed that USP19 expression was significantly increased in gastric cancer tissues, compared to normal gastric tissues, and the high level of USP19 expression was positively correlated with a poorer prognosis.

Similarly, our group analyzed USP19 clinical significance in breast cancer (Rossi et al., 2021). We demonstrated that USP19 positively regulates breast tumor cells migration and invasion *in vitro*, and that genetic silencing reduces cells motility, whereas its overexpression increases migratory and invasive capabilities—dependent on USP19's catalytic activity and ER localization. Our results also indicated that USP19 does not

affect breast cancer cells proliferation in two dimensions, in concordance with Lu and collaborators (Lu et al., 2011), but significantly modulates proliferation and invasion if cells are grown embedded in extracellular matrix proteins and basement membrane proteins. *In vivo* experiments showed that USP19 silencing reduces tumorigenicity and delays tumor onset and growth, and the opposite was observed upon wild type USP19 overexpression (but not when overexpressing a catalytically dead mutant, or a cytoplasmic version of USP19). Using experimental metastasis assays, we verified that USP19 silencing reduces cells' ability to engraft in secondary tissues, and using *in silico* approaches and TCGA data, we demonstrated that the Wnt pathway is activated in patient samples expressing high levels of USP19. In concordance with these results, we observed a positive correlation between USP19 and LRP6 levels (a Wnt pathway coreceptor). Functional analysis on USP19 overexpressing cells indicated that LRP6 silencing reverted migratory and invasive phenotypes, possibly as a downstream USP19 effector. Finally, we conducted a retrospective analysis on early breast cancer patients which revealed that USP19 expression levels correlated with poor outcome and reduced distant metastasis free survival, hence serving as a prognostic factor in early breast cancer patients.

Lastly, a very recent publication by Zhu and collaborators studied USP19 pertinence in colorectal carcinogenesis (Zhu et al., 2021). Their work showed that ERK2 signaling is responsible for lipid synthesis mediated by cytoplasmic-localized malic enzyme 1 (ME1) phosphorylation, which is overexpressed in a variety of cancers (including colorectal cancer). USP19-mediated ME1 stabilization is enhanced by phosphorylation, generating oncogenic phenotypes, and either USP19 deletion or a point mutation in ME1 protein that prevents ubiquitination, represses colorectal carcinogenesis. Of note, USP19 catalytic activity is necessary to ensure ME1 stabilization. Finally, the authors showed that the USP19-ME1 signaling axis is dysregulated in human colorectal cancer samples, and that USP19 is upregulated during colorectal carcinogenesis pathogenesis and spontaneous tumor development.

Supplementary Table S1 summarizes USP19 relevance in different cancers, and whether is catalytic activity or specific isoform is important in each type of neoplasm.

CONCLUDING REMARKS

Various studies have linked USP19 to different cancers, and either its overexpression or silencing may dysregulate the function of several proteins with oncogenic or tumor-suppressive properties, which in the long run may impact on the onset and development of tumors. Since USP19 has different isoforms, and divergent effects have been observed in different cancers, it is plausible to assume that this difference could be explained by the effect these isoforms exert on differing substrates. Moreover, USP19 is a fundamental deubiquitinase with pivotal roles in several cellular processes related to tumorigenesis, including DNA damage repair (Wu et al., 2017), apoptosis (Mei et al., 2011), the TGF-β Pathway (Zhang et al., 2012), hypoxia and angiogenesis (Altun et al., 2012; Boscaro et al., 2020), immunity (Cui et al., 2016; Jin et al., 2016; Gu et al., 2017; Lei et al., 2019; Wu et al., 2019; Liu et al., 2021), proliferation (Lu et al., 2009), ERAD (Hassink et al., 2009) and autophagy (Cui et al., 2016). Given its versatility, USP19's role on tumorigenesis and metastasis might also be determined by a combinatorial effect on diverse signaling pathways rather than a specific substrate. In this respect, more studies should be performed to analyze the association of USP19 with cancer-related signaling pathways and putative targets, regulatory mechanisms affecting its expression and to search for molecular alterations shared by tumors across different tissues and new targets to better understand how USP19 is affecting cell survival and cellular homeostasis.

Taken together, the findings described here implicate USP19 as a previously unrecognized target for the development of novel therapeutic alternatives for cancer treatments.

AUTHOR CONTRIBUTIONS

FR and MR designed the article, and acquired, analyzed, and interpreted data for writing the article.

FUNDING

This work was supported by the grants UA2020 from Universidad Austral awarded to FR and MR. MR was funded by grant PICT 2018-03688 from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2022.889166/full#supplementary-material

REFERENCES

Akutsu, M., Dikic, I., and Bremm, A. (2016). Ubiquitin Chain Diversity at a Glance. *J. Cell Sci.* 129, 875–880. doi:10.1242/jcs.183954

Altun, M., Zhao, B., Velasco, K., Liu, H., Hassink, G., Paschke, J., et al. (2012). Ubiquitin-specific Protease 19 (USP19) Regulates Hypoxia-Inducible Factor 1α (HIF-1α) during Hypoxia. *J. Biol. Chem.* 287, 1962–1969. doi:10.1074/jbc.m111.305615

Avvakumov, G. V., Walker, J. R., Xue, S., Finerty, P. J., Mackenzie, F., Newman, E. M., et al. (2006). Amino-terminal Dimerization, NRDP1-Rhodanese Interaction, and Inhibited Catalytic Domain Conformation of the Ubiquitin-specific Protease 8 (USP8). *J. Biol. Chem.* 281, 38061.doi:10.1074/jbc.m606704200

Boscaro, C., Carotti, M., Albiero, M., Trenti, A., Fadini, G. P., Trevisi, L., et al. (2020). Non-genomic Mechanisms in the Estrogen Regulation of Glycolytic Protein Levels in Endothelial Cells. *FASEB J.* 34, 12768. doi:10.1096/fj.202001130r

Chen, L., Liu, S., and Tao, Y. (2020). Regulating Tumor Suppressor Genes: Post-translational Modifications. *Sig Transduct. Target Ther.* 5, 90. doi:10.1038/s41392-020-0196-9

Ciechanover, A. (1994). The Ubiquitin-Proteasome Proteolytic Pathway. *Cell* 79, 13–21. doi:10.1016/0092-8674(94)90396-4

Combaret, L., Adegoke, O. A. J., Bedard, N., Baracos, V., Attaix, D., and Wing, S. S. (2005). USP19 Is a Ubiquitin-specific Protease Regulated in Rat Skeletal Muscle during Catabolic States. *Am. J. Physiology-Endocrinology Metabolism* 288, E693–E700. doi:10.1152/ajpendo.00281.2004

Cui, J., Jin, S., and Wang, R.-F. (2016). The BECN1-USP19 axis Plays a Role in the Crosstalk between Autophagy and Antiviral Immune Responses. *Autophagy* 12, 1210–1211. doi:10.1080/15548627.2016.1173801

Damgaard, R. B. (2021). The Ubiquitin System: from Cell Signalling to Disease Biology and New Therapeutic Opportunities. *Cell Death Differ.* 28, 423–426. doi:10.1038/s41418-020-00703-w

Deng, L., Meng, T., Chen, L., Wei, W., and Wang, P. (2020). The Role of Ubiquitination in Tumorigenesis and Targeted Drug Discovery. *Sig Transduct. Target Ther.* 5, 11. doi:10.1038/s41392-020-0107-0

Desmaze, C., Brizard, F., Turc-Carel, C., Melot, T., Delattre, O., Thomas, G., et al. (1997). Multiple Chromosomal Mechanisms Generate an EWS/FLI1 or an EWS/ERG Fusion Gene in Ewing Tumors. *Cancer Genet. Cytogenet.* 97, 12–19. doi:10.1016/s0165-4608(96)00326-3

Dong, Z., Guo, S., Wang, Y., Zhang, J., Luo, H., Zheng, G., et al. (2020). USP19 Enhances MMP2/MMP9-Mediated Tumorigenesis in Gastric Cancer. *Onco Targets Ther.* 13, 8495–8510. doi:10.2147/ott.s240543

Dwane, L., Gallagher, W. M., Ní Chonghaile, T., and O'Connor, D. P. (2017). The Emerging Role of Non-traditional Ubiquitination in Oncogenic Pathways. *J. Biol. Chem.* 292, 3543–3551. doi:10.1074/jbc.r116.755694

Fan, J.-B., Arimoto, K.-l., Motamedchaboki, K., Yan, M., Wolf, D. A., and Zhang, D.-E. (2015). Identification and Characterization of a Novel ISG15-Ubiquitin Mixed Chain and its Role in Regulating Protein Homeostasis. *Sci. Rep.* 5, 12704. doi:10.1038/srep12704

Gierisch, M. E., Pedot, G., Walser, F., Lopez-Garcia, L. A., Jaaks, P., Niggli, F. K., et al. (2019). USP19 Deubiquitinates EWS-FLI1 to Regulate Ewing Sarcoma Growth. *Sci. Rep.* 9, 951. doi:10.1038/s41598-018-37264-5

Gu, Z., Shi, W., Zhang, L., Hu, Z., and Xu, C. (2017). USP19 Suppresses Cellular Type I Interferon Signaling by Targeting TRAF3 for Deubiquitination. *Future Microbiol.* 12, 767–779. doi:10.2217/fmb-2017-0006

Haglund, K., and Dikic, I. (2005). Ubiquitylation and Cell Signaling. *EMBO J.* 24, 3353–3359. doi:10.1038/sj.emboj.7600808

Hanpude, P., Bhattacharya, S., Dey, A. K., and Maiti, T. K. (2015). Deubiquitinating Enzymes in Cellular Signaling and Disease Regulation. *IUBMB Life* 67, 544–555. doi:10.1002/iub.1402

Harada, K., Kato, M., and Nakamura, N. (2016). USP19-Mediated Deubiquitination Facilitates the Stabilization of HRD1 Ubiquitin Ligase. *Int. J. Mol. Sci.* 17. doi:10.3390/ijms17111829

Hassink, G. C., Zhao, B., Sompallae, R., Altun, M., Gastaldello, S., Zinin, N. V., et al. (2009). The ER-resident Ubiquitin-specific Protease 19 Participates in the UPR and Rescues ERAD Substrates. *EMBO Rep.* 10, 755–761. doi:10.1038/embor.2009.69

He, W.-T., Xue, W., Gao, Y.-G., Hong, J.-Y., Yue, H.-W., Jiang, L.-L., et al. (2017). HSP90 Recognizes the N-Terminus of Huntingtin Involved in Regulation of Huntingtin Aggregation by USP19. *Sci. Rep.* 7, 14797. doi:10.1038/s41598-017-13711-7

He, W.-T., Zheng, X.-M., Zhang, Y.-H., Gao, Y.-G., Song, A.-X., van der Goot, F. G., et al. (2016). Cytoplasmic Ubiquitin-specific Protease 19 (USP19) Modulates Aggregation of Polyglutamine-Expanded Ataxin-3 and Huntingtin through the HSP90 Chaperone. *PLoS One* 11, e0147515. doi:10.1371/journal.pone.0147515

Hershko, A., and Ciechanover, A. (1998). The Ubiquitin System. *Annu. Rev. Biochem.* 67, 425–479. doi:10.1146/annurev.biochem.67.1.425

Hu, M., Li, P., Li, M., Li, W., Yao, T., Wu, J., et al. (2002). Crystal Structure of a UBP-Family Deubiquitinating Enzyme in Isolation and in Complex with Ubiquitin Aldehyde. *Cell* 111, 1041–1054. doi:10.1016/s0092-8674(02)01199-6

Hu, M., Li, P., Song, L., Jeffrey, P. D., Chenova, T. A., Wilkinson, K. D., et al. (2005). Structure and Mechanisms of the Proteasome-Associated Deubiquitinating Enzyme USP14. *EMBO J.* 24, 3747–3756. doi:10.1038/sj.emboj.7600832

Hu, W., Su, Y., Fei, X., Wang, X., Zhang, G., Su, C., et al. (2020). Ubiquitin Specific Peptidase 19 Is a Prognostic Biomarker and Affect the Proliferation and Migration of Clear Cell Renal Cell Carcinoma. *Oncol. Rep.* 43, 1964–1974. doi:10.3892/or.2020.7565

Huang, X., and Dixit, V. M. (2016). Drugging the Undruggables: Exploring the Ubiquitin System for Drug Development. *Cell Res.* 26, 484–498. doi:10.1038/cr.2016.31

Huguenin-Dezot, N., De Cesare, V., Peltier, J., Knebel, A., Kristaryianto, Y. A., Rogerson, D. T., et al. (2016). Synthesis of Isomeric Phosphoubiquitin Chains Reveals that Phosphorylation Controls Deubiquitinase Activity and Specificity. *Cell Rep.* 16, 1180–1193. doi:10.1016/j.celrep.2016.06.064

Ikeda, F., and Dikic, I. (2008). Atypical Ubiquitin Chains: New Molecular Signals. 'Protein Modifications: Beyond the Usual Suspects' Review Series. *EMBO Rep.* 9, 536–542. doi:10.1038/embor.2008.93

Jin, S., Tian, S., Chen, Y., Zhang, C., Xie, W., Xia, X., et al. (2016). USP 19 Modulates Autophagy and Antiviral Immune Responses by Deubiquitinating Beclin-1. *EMBO J.* 35, 866–880. doi:10.15252/embj.201593596

Kang, H., Choi, M. C., Kim, S., Jeong, J. Y., Kwon, A. Y., Kim, T. H., et al. (2021). USP19 and RPL23 as Candidate Prognostic Markers for Advanced-Stage High-Grade Serous Ovarian Carcinoma. *Cancers (Basel)* 13, 976. doi:10.3390/cancers13163976

Komander, D., Clague, M. J., and Urbé, S. (2009). Breaking the Chains: Structure and Function of the Deubiquitinases. *Nat. Rev. Mol. Cell Biol.* 10, 550–563. doi:10.1038/nrm2731

Komander, D., Lord, C. J., Scheel, H., Swift, S., Hofmann, K., Ashworth, A., et al. (2008). The Structure of the CYLD USP Domain Explains its Specificity for Lys63-Linked Polyubiquitin and Reveals a B Box Module. *Mol. Cell* 29, 451–464. doi:10.1016/j.molcel.2007.12.018

Komander, D., and Rape, M. (2012). The Ubiquitin Code. *Annu. Rev. Biochem.* 81, 203–229. doi:10.1146/annurev-biochem-060310-170328

Konstantinopoulos, P. A., Karamouzis, M. V., and Papavassiliou, A. G. (2007). Post-translational Modifications and Regulation of the RAS Superfamily of GTPases as Anticancer Targets. *Nat. Rev. Drug Discov.* 6, 541–555. doi:10.1038/nrd2221

Krueger, K. E., and Srivastava, S. (2006). Posttranslational Protein Modifications: Current Implications for Cancer Detection, Prevention, and Therapeutics. *Mol. Cell. Proteomics* 5, 1799–1810. doi:10.1074/mcp.r600009-mcp200

Kwasna, D., Abdul Rehman, S. A., Natarajan, J., Matthews, S., Madden, R., De Cesare, V., et al. (2018). Discovery and Characterization of ZUFSP/ZUP1, a Distinct Deubiquitinase Class Important for Genome Stability. *Mol. Cell* 70, 150–164. doi:10.1016/j.molcel.2018.02.023

Kwon, Y. T., and Ciechanover, A. (2017). The Ubiquitin Code in the Ubiquitin-Proteasome System and Autophagy. *Trends Biochem. Sci.* 42, 873–886. doi:10.1016/j.tibs.2017.09.002

Lamoliatte, F., Bonneil, E., Durette, C., Caron-Lizotte, O., Wildemann, D., Zerweck, J., et al. (2013). Targeted Identification of SUMOylation Sites in Human Proteins Using Affinity Enrichment and Paralog-specific Reporter Ions. *Mol. Cell. Proteomics* 12, 2536–2550. doi:10.1074/mcp.m112.025569

Lee, J.-G., Kim, W., Gygi, S., and Ye, Y. (2014). Characterization of the Deubiquitinating Activity of USP19 and its Role in Endoplasmic Reticulum-Associated Degradation. *J. Biol. Chem.* 289, 3510–3517. doi:10.1074/jbc.m113.538934

Lei, C.-Q., Wu, X., Zhong, X., Jiang, L., Zhong, B., and Shu, H.-B. (2019). USP19 Inhibits TNF-α- and IL-1β-Triggered NF-Kb Activation by Deubiquitinating TAK1. *J. Immunol.* 203, 259–268. doi:10.4049/jimmunol.1900083

Lim, K.-H., Choi, J.-H., Park, J.-H., Cho, H.-J., Park, J.-J., Lee, E.-J., et al. (2016). Ubiquitin Specific Protease 19 Involved in Transcriptional Repression of Retinoic Acid Receptor by Stabilizing CORO2A. *Oncotarget* 7, 34759. doi:10.18632/oncotarget.8976

Liu, Q., Zhao, S., Su, P. F., and Yu, S. (2013). Gene and Isoform Expression Signatures Associated with Tumor Stage in Kidney Renal Clear Cell Carcinoma. *BMC Syst. Biol.* 7 (Suppl. 5), S7–S11. doi:10.1186/1752-0509-7-S5-S7

Liu, T., Wang, L., Liang, P., Wang, X., Liu, Y., Cai, J., et al. (2021). USP19 Suppresses Inflammation and Promotes M2-like Macrophage Polarization by Manipulating NLRP3 Function via Autophagy. *Cell Mol. Immunol.* 18, 2431–2442. doi:10.1038/s41423-020-00567-7

Lu, Y., Adegoke, O. A. J., Nepveu, A., Nakayama, K. I., Bedard, N., Cheng, D., et al. (2009). USP19 Deubiquitinating Enzyme Supports Cell Proliferation by

Stabilizing KPC1, a Ubiquitin Ligase for P27 Kip1. *Mol. Cell Biol.* 29, 547–558. doi:10.1128/mcb.00329-08

Lu, Y., Bedard, N., Chevalier, S., and Wing, S. S. (2011). Identification of Distinctive Patterns of USP19-Mediated Growth Regulation in Normal and Malignant Cells. *PLoS One* 6, e15936. doi:10.1371/journal.pone.0015936

Matsuoka, S., Ballif, B. A., Smogorzewska, A., McDonald, E. R., Hurov, K. E., Luo, J., et al. (2007). ATM and ATR Substrate Analysis Reveals Extensive Protein Networks Responsive to DNA Damage. *Science* 316, 1160–1166. doi:10.1126/science.1140321

Mei, Y., Hahn, A. A., Hu, S., and Yang, X. (2011). The USP19 Deubiquitinase Regulates the Stability of C-IAP1 and C-IAP2. *J. Biol. Chem.* 286, 35380. doi:10.1074/jbc.m111.282020

Mevissen, T. E. T., and Komander, D. (2017). Mechanisms of Deubiquitinase Specificity and Regulation. *Annu. Rev. Biochem.* 86, 159–192. doi:10.1146/annurev-biochem-061516-044916

Millar, A. H., Heazlewood, J. L., Giglione, C., Holdsworth, M. J., Bachmair, A., and Schulze, W. X. (2019). The Scope, Functions, and Dynamics of Posttranslational Protein Modifications. *Annu. Rev. Plant Biol.* 70, 119–151. doi:10.1146/annurev-arplant-050718-100211

Nakamura, N., Harada, K., Kato, M., and Hirose, S. (2014). Ubiquitin-specific Protease 19 Regulates the Stability of the E3 Ubiquitin Ligase MARCH6. *Exp. Cell Res.* 328, 207–216. doi:10.1016/j.yexcr.2014.07.025

Nijman, S. M. B., Luna-Vargas, M. P. A., Velds, A., Brummelkamp, T. R., Dirac, A. M. G., Sixma, T. K., et al. (2005). A Genomic and Functional Inventory of Deubiquitinating Enzymes. *Cell* 123, 773–786. doi:10.1016/j.cell.2005.11.007

Ohtake, F., Saeki, Y., Sakamoto, K., Ohtake, K., Nishikawa, H., Tsuchiya, H., et al. (2015). Ubiquitin Acetylation Inhibits Polyubiquitin Chain Elongation. *EMBO Rep.* 16, 192–201. doi:10.15252/embr.201439152

Ohtake, F., and Tsuchiya, H. (2017). The Emerging Complexity of Ubiquitin Architecture. *J. Biochem.* 161, 125–133. doi:10.1093/jb/mvw088

Perrody, E., Abrami, L., Feldman, M., Kunz, B., Urbé, S., and van der Goot, F. G. (2016). Ubiquitin-dependent Folding of the Wnt Signaling Coreceptor LRP6. *eLife* 5, 19083. doi:10.7554/eLife.19083

Reinstein, E., and Ciechanover, A. (2006). Narrative Review: Protein Degradation and Human Diseases: the Ubiquitin Connection. *Ann. Intern Med.* 145, 676–684. doi:10.7326/0003-4819-145-9-200611070-00010

Renatus, M., Parrado, S. G., D'Arcy, A., Eidhoff, U., Gerhartz, B., Hassiepen, U., et al. (2006). Structural Basis of Ubiquitin Recognition by the Deubiquitinating Protease USP2. *Structure* 14, 1293–1302. doi:10.1016/j.str.2006.06.012

Reyes-Turcu, F. E., Ventii, K. H., and Wilkinson, K. D. (2009). Regulation and Cellular Roles of Ubiquitin-specific Deubiquitinating Enzymes. *Annu. Rev. Biochem.* 78, 363–397. doi:10.1146/annurev.biochem.78.082307.091526

Rossi, F. A., Enriqué Steinberg, J. H., Calvo Roitberg, E. H., Joshi, M. U., Pandey, A., Abba, M. C., et al. (2021). USP19 Modulates Cancer Cell Migration and Invasion and Acts as a Novel Prognostic Marker in Patients with Early Breast Cancer. *Oncogenesis* 10, 28. doi:10.1038/s41389-021-00318-x

Sadowski, M., and Sarcevic, B. (2010). Mechanisms of Mono- and Poly-Ubiquitination: Ubiquitination Specificity Depends on Compatibility between the E2 Catalytic Core and Amino Acid Residues Proximal to the Lysine. *Cell Div.* 5, 19. doi:10.1186/1747-1028-5-19

Shahriyari, L., Abdel-Rahman, M., and Cebulla, C. (2019). BAP1 Expression Is Prognostic in Breast and Uveal Melanoma but Not Colon Cancer and is Highly Positively Correlated with RBM15B and USP19. *PLoS One* 14, e0211507. doi:10.1371/journal.pone.0211507

Sharma, B. S., Prabhakaran, V., Desai, A. P., Bajpai, J., Verma, R. J., and Swain, P. K. (2019). Post-Translational Modifications (PTMs), From a Cancer Perspective: An Overview. *Oncogen* 2, 1–12. doi:10.35702/onc.10012

Shi, D., and Grossman, S. R. (2010). Ubiquitin Becomes Ubiquitous in Cancer: Emerging Roles of Ubiquitin Ligases and Deubiquitinases in Tumorigenesis and as Therapeutic Targets. *Cancer Biol. Ther.* 10, 737–747. doi:10.4161/cbt.10.8.13417

Sun, T., Liu, Z., and Yang, Q. (2020). The Role of Ubiquitination and Deubiquitination in Cancer Metabolism. *Mol. Cancer* 19, 146. doi:10.1186/s12943-020-01262-x

Sundaram, P., Pang, Z., Miao, M., Yu, L., and Wing, S. S. (2009). USP19-deubiquitinating Enzyme Regulates Levels of Major Myofibrillar Proteins in L6 Muscle Cells. *Am. J. Physiology-Endocrinology Metabolism* 297, E1283. doi:10.1152/ajpendo.00409.2009

Velasco, K., Zhao, B., Callegari, S., Altun, M., Liu, H., Hassink, G., et al. (2013). An N-Terminal SIAH-Interacting Motif Regulates the Stability of the Ubiquitin Specific Protease (USP)-19. *Biochem. Biophysical Res. Commun.* 433, 390–395. doi:10.1016/j.bbrc.2013.02.094

Vellosillo, P., and Minguez, P. (2021). A Global Map of Associations between Types of Protein Posttranslational Modifications and Human Genetic Diseases. *iScience* 24, 102917. doi:10.1016/j.isci.2021.102917

Wauer, T., Swatek, K. N., Wagstaff, J. L., Gladkova, C., Pruneda, J. N., Michel, M. A., et al. (2015). Ubiquitin Ser65 Phosphorylation Affects Ubiquitin Structure, Chain Assembly and Hydrolysis. *EMBO J.* 34, 307–325. doi:10.15252/embj.201489847

Wiles, B., Miao, M., Coyne, E., Larose, L., Cybulsky, A. V., and Wing, S. S. (2015). USP19 Deubiquitinating Enzyme Inhibits Muscle Cell Differentiation by Suppressing Unfolded-Protein Response Signaling. *Mol. Biol. Cell* 26, 913–923. doi:10.1091/mbc.e14-06-1129

Wu, M., Tu, H.-Q., Chang, Y., Tan, B., Wang, G., Zhou, J., et al. (2017). USP19 Deubiquitinates HDAC1/2 to Regulate DNA Damage Repair and Control Chromosomal Stability. *Oncotarget* 8, 2197–2208. doi:10.18632/oncotarget.11116

Wu, X., Lei, C., Xia, T., Zhong, X., Yang, Q., and Shu, H.-B. (2019). Regulation of TRIF-Mediated Innate Immune Response by K27-Linked Polyubiquitination and Deubiquitination. *Nat. Commun.* 10, 4115. doi:10.1038/s41467-019-12145-1

Xu, H., Wang, Y., Lin, S., Deng, W., Peng, D., Cui, Q., et al. (2018). PTMD: A Database of Human Disease-Associated Post-translational Modifications. *Genomics, Proteomics Bioinforma.* 16, 244–251. doi:10.1016/j.gpb.2018.06.004

Xue, W., Zhang, S.-X., He, W.-T., Hong, J.-Y., Jiang, L.-L., and Hu, H.-Y. (2020). Domain Interactions Reveal Auto-Inhibition of the Deubiquitinating Enzyme USP19 and its Activation by HSP90 in the Modulation of Huntingtin Aggregation. *Biochem. J.* 477, 4295–4312. doi:10.1042/bcj20200536

Yau, R., and Rape, M. (2016). The Increasing Complexity of the Ubiquitin Code. *Nat. Cell Biol.* 18, 579–586. doi:10.1038/ncb3358

Zhang, L., Zhou, F., Drabsch, Y., Gao, R., Snaar-Jagalska, B. E., Mickanin, C., et al. (2012). USP4 Is Regulated by AKT Phosphorylation and Directly Deubiquitylates TGF-β Type I Receptor. *Nat. Cell Biol.* 14, 717–726. doi:10.1038/ncb2522

Zhu, Y., Gu, L., Lin, X., Zhou, X., Lu, B., Liu, C., et al. (2021). USP19 Exacerbates Lipogenesis and Colorectal Carcinogenesis by Stabilizing ME1. *Cell Rep.* 37, 110174. doi:10.1016/j.celrep.2021.110174

Conflict of Interest: 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 | Frontiers in Cell and Developmental Biology

ORIGINAL RESEARCH
published: 20 May 2022
doi: 10.3389/fcell.2022.833396

Differential Degradation of TRA2A and PYCR2 Mediated by Ubiquitin E3 Ligase E4B

*Yao Lu[1†], Bo Jiang[2,3†], Kangli Peng[1†], Shasha Li[1], Xiangnan Liu[1], Bufan Wang[1], Yuntian Chen[4], Tiepeng Wang[5] and Bo Zhao[1]**

[1]Engineering Research Center of Cell and Therapeutic Antibody, Ministry of Education, and School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China, [2]Department of Hand and Foot Surgery, The Second Affiliated Hospital of Soochow University, Suzhou, China, [3]State Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou, China, [4]Department of Respiratory and Critical Care Medicine, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [5]National Laboratory of Biomacromolecules, Chinese Academy of Sciences Center for Excellence in Biomacromolecules, Institute of Biophysics, Beijing, China

E4B belongs to the U-box E3 ligase family and functions as either an E3 or an E4 enzyme in protein ubiquitination. Transformer2A (TRA2A) and Pyrroline-5-carboxylate reductase 2 (PYCR2) are related to cancer development and are overexpressed in many cancer cells. The degradation of TRA2A and PYCR2 mediated by the ubiquitin-proteasome system (UPS) has not been reported. This study validated that E4B could ubiquitinate TRA2A and PYCR2 as an E3 ligase both *in vitro* and in the HEK293 cells. E4B mediated the degradation by forming K11- and K48- linked polyubiquitin chains on TRA2A and PYCR2, respectively. E4B regulated the alternative splicing function of TRA2A and affected RSRC2 transcription in the HEK293 cells. Although E4B is highly expressed, it hardly degrades TRA2A and PYCR2 in hepatocellular carcinoma (HCC) cells, suggesting other mechanisms exist for degradation of TRA2A and PYCR2 in the HCC cells. We finally reported that E4B interacted with substrates *via* its variable region.

Keywords: ubiquitin, ubiquitination, degradation, E4B, TRA2A, PYCR2

OPEN ACCESS

Edited by:
Zhenghong Lin,
Chongqing University, China

Reviewed by:
Yanfen Liu,
ShanghaiTech University, China
Ismail,
University of Alberta, Canada

****Correspondence:***
Bo Zhao
bozhao@sjtu.edu.cn

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Signaling, a section of the journal Frontiers in Cell and Developmental Biology

Received: *11 December 2021*
Accepted: *14 April 2022*
Published: *20 May 2022*

Citation:
Lu Y, Jiang B, Peng K, Li S, Liu X, Wang B, Chen Y, Wang T and Zhao B (2022) Differential Degradation of TRA2A and PYCR2 Mediated by Ubiquitin E3 Ligase E4B. Front. Cell Dev. Biol. 10:833396. doi: 10.3389/fcell.2022.833396

INTRODUCTION

The ubiquitin-proteasome system (UPS) is responsible for the selective degradation of short-lived proteins and plays an essential role in biological function regulation in eukaryotic cells (Hershko and Ciechanover, 1998). Substrates are targeted by ubiquitin (Ub) through an E1–E2–E3 enzymatic cascade forming different ubiquitin chains at one or more lysine residues (Komander and Rape, 2012). E4B (also called UBE4B) belongs to the U-box E3 ligase family and contains a unique U-box catalytic domain composed of 70 amino acids (Hatakeyama et al., 2001). Initially, E4B is known for its E4 function in targeting the substrates and elongating the polyubiquitin chains (Koegl et al., 1999). Subsequent studies prove that E4B manifests E3 ligase activity depending on the U-box domain, which is responsible for E2 recognition (Hoppe, 2005). E4B is essential in embryo survival and cardiac and nervous system development during the stages of embryonic development (Kaneko-Oshikawa et al., 2005; Mammen et al., 2011; Wu and Leng, 2011). Intriguingly, the alteration of the gene or protein of E4B is involved in the genesis of neuropathies and various types of cancer. E4B has been found to be either overexpressed or suppressed in different cancer such as breast cancer, hepatocellular carcinoma, glioblastoma, promyelocytic leukemia, colorectal cancer, and neuroblastoma (Krona et al., 2003; Heuze et al., 2008; Wu et al., 2011; Zhang et al., 2014; Zhang

et al., 2016). Additionally, E4B can regulate the p53 level to inhibit cell apoptosis and promote tumor development through two pathways. As an E4, E4B synergistically collaborates with MDM2 to target p53 for its ubiquitination and degradation (Fang et al., 2000; Wu et al., 2011). E4B can negatively regulate the protein levels of phosphorylated p53 at Ser15 and Ser392, independent of MDM2 (Du et al., 2016). So far, the mechanism of how E4B affects tumorigenesis is unclear. Except for p53, there is lacking indepth knowledge on other substrates ubiquitination mediated by E4B. Ufd2p, the homolog of E4B in *S. cerevisiae*, contains an N-terminal variable region, a highly conserved Ub elongating region, and a C-terminal U-box domain (Tu et al., 2007). However, the full-length structure of E4B is still not well characterized due to its complexity in the N-terminal variable region (Nordquist et al., 2010). The interaction of E4B with its ubiquitination substrates is also reported rarely.

In our previous work, we developed an orthogonal ubiquitin transfer (OUT) method to identify the substrates of E4B (Zhao et al., 2012; Bhuripanyo et al., 2018; Zhao et al., 2020). In this method, over 100 proteins were attached by xUb (an Ub mutant) under the transfer of E4B in the HEK293 cells, indicating that these proteins may be the potential ubiquitination substrates of E4B (Bhuripanyo et al., 2018). Among these proteins, Transformer2A (TRA2A) and Pyrroline-5-carboxylate reductase 2 (PYCR2) are closely related to cancer development. TRA2A and TRA2B, different isoforms of TRA2 in humans, are encoded by the Transformer2 gene and participate in the alternative splicing (AS) process of pre-mRNA to produce multiple mature mRNAs (Best et al., 2014). Both TRA2A and TRA2B mediate splicing events to affect tumor progression and drug sensitivity (Hirschfeld et al., 2011; Best et al., 2014; Tieju Liu et al., 2017). TRA2A is overexpressed in the glioma cells and triple-negative breast cancer to promote proliferation, invasion, migration, and epithelial–mesenchymal transition (Tieju Liu et al., 2017; Tan et al., 2018). TRA2A can reduce the level of normal RSRC2 splicing product RSRC2s and increase the expression of RSRC2l, an abnormal mRNA splicing product of RSRC2, which promotes the progression of triple negative breast cancer (Tieju Liu et al., 2017). PYCR2, an enzyme that catalyzes the synthesis of L-proline from Δ1-pyrroline-5-carboxylate (P5C), was reported to promote cancer growth and inhibit apoptosis through multiple approaches, such as regulation of cell cycle and redox homeostasis and promotion of growth signaling pathways (Meng et al., 2017; Escande-Beillard et al., 2020; Li et al., 2021). As metabolic reprogramming has been considered a new sign of cancer in recent years, proline metabolism is believed to be a critical factor in the cancer cell growth (Pavlova and Thompson, 2016). The change of proline expression is the most significant factor in amino acid metabolism in hepatocellular carcinoma (HCC), and PYCR2 is abnormally expressed in the esophageal squamous cell carcinoma (ESCC), indicating that PYCR2 may play a key role in cancer progression (Tang et al., 2018; Sun et al., 2019).

The ubiquitination of TRA2A and PYCR2 has not been reported. In this study, we verified that E4B could ubiquitinate TRA2A and PYCR2 as an E3 ligase both *in vitro* and in the HEK293 cells. E4B mediated the formation of K11- or K48-linked polyubiquitin chains on TRA2A and PYCR2 and induced their degradation by the proteasome. Regulation of E4B affected the alternative splicing function of TRA2A in the HEK293 cells. However, the degradation of TRA2A and PYCR2 in the HCC cells was quite different from that in the HEK293 cells, indicating there are other mechanisms for the degradation of TRA2A and PYCR2 in the HCC cells. By constructing the variants of E4B, we validated that the variable region of E4B is an indispensable domain for the interaction of E4B with its substrates, TRA2A and PYCR2.

MATERIALS AND METHODS

Cell Culture and Reagents

DMEM (10100147) and fetal bovine serum (C11995500BT) were from Gibco. MG132 (HY-13259) and protease-inhibitor cocktail (HY-K0010) were from MCE. Lipofectamine™ 3000 Transfection Reagent (L3000015) was from Invitrogen. Cycloheximide (CHX) and N-ethylmaleimide (NEM) were from Sigma-Aldrich. The following antibodies were from Abcam: anti-E4B (ab126759), anti-ubiquitin (ab134953), anti-HA (ab182009). Anti-GAPDH antibody (60004-1-Ig) and anti-PYCR2 antibody (17146-1-AP) were from Proteintech. Anti-TRA2A antibody (GTX87998) was from GeneTex. Anti-MYC antibody (9B11) was from Cell Signaling Technology. Anti-FLAG (M2) antibody (F1804) and anti-FLAG(M2) affinity gel (A2220) were from Sigma-Aldrich. Goat anti-rabbit IgG-Alexa Fluor 790 antibody (111-655-144) and goat anti-mouse IgG- Alexa Fluor 790 antibody (115-005-072) were from Jackson ImmunoResearch. Protein G Agarose (16-266) was from Merckmilipore. Phenylmethanesulfonyl fluoride (PMSF, ST506) and RIPA Lysis Buffer (P0013C) were from Beyotime. Polyetherimide (AC04L091) was from Life-iLab.

Plasmids and Small Interfering RNA Oligonucleotides

Full-length human TRA2A and PYCR2 were cloned into FLAG-tagged pcDNA 3.1 vector. The full-length Ub was cloned into HA-tagged pcDNA 3.1 vector. The genes of Ub mutants, K11R, K48R, and K63R, were synthesized by GENEWIZ. Myc-tagged full-length human E4B and variants were cloned into pLVX-IRES-mcherry vector. His-tagged TRA2A, PYCR2, Ub, and E4B were cloned into pET-28a + vector for protein expression. All plasmids were verified by sequencing. GIPZ Lentiviral shRNA pGIPZ-shE4B and pGIPZ-empty were from Dharmacon.

Small interfering RNA (siRNA) targeted TRA2A was generated by Sangon Biotech. The sequence of **siTRA2A** was forward: 5′-GCCUCAGUUUGUACACAACTT-3′ and reverse: 5′-GUUGUGUACAAACUGAGGCT-3’.

Recombinant Protein Purification and *In Vitro* Ubiquitination Assay

His and FLAG-tag TRA2A and PYCR2 were transformed into BL21 *E. Coli* cells for protein expression. The cells were grown for 4–8 h at 37°C until the OD was 0.8. IPTG (0.25 μM) was added to

the cells, and the expression was induced at 16°C overnight. The cell cultures were spun down and lysed in lysis buffer (50 mM Tris-Base, 500 mM NaCl, 5 mM imidazole, and pH 8.0). Ni-NTA was incubated with the cell lysates for 2 h to bind the target proteins. The cell lysates were washed several times and the elution was collected to obtain the purified targeted proteins.

To assay ubiquitination of these substrates by E4B, 10 μM FLAG-tagged substrate proteins were incubated with 14 μM Ub, 1 μM E1 (Ube1), 5 μM E2 (UbcH5b), and 1 μM E3 (E4B) in TBS buffer containing 50 mM $MgCl_2$ and 1.5 mM ATP. After a 2-h ubiquitin transferring reaction at room temperature, ubiquitination was detected by immunoblotting with an anti-FLAG antibody.

Cells Culture and Transfection

HEK293, HepG2, and HuH7 were cultured in DMEM supplemented with 10% fetal bovine serum and incubated in a 37°C humidified incubator with 5% CO_2. ShE4B and shctrl cells were cultured in the same culture medium with an extra 0.2 μg/ml puromycin. HEK293 cells were cotransfected with the indicated plasmids using the linear Polyetherimide (PEI) reagent according to the manufacturer's protocol. HEK293 was transfected with a small interfering RNA (siRNA) to knock down TRA2A with Lipo3000 according to the manufacturer's protocol. HepG2 and HuH7 cells were transfected with Lipo3000 according to the manufacturer's protocol.

In Vivo Ubiquitination and Co-Immunoprecipitation

The cells were incubated with 10 μM MG132 for 4 or 6 h before harvest. For normal ubiquitination assay, the cells were lysed with RIPA lysis buffer 1 (50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS) containing protease inhibitor cocktail (PIC) and Phenylmethanesulfonyl fluoride (PSMF) for 48 h after transfection. For the ubiquitin chains assays, an extra 100 μM N-ethylmaleimide (NEM) and 100 μM O-Phenanthroline were added to the RIPA lysis buffer 1. For the interaction of E4B and substrates assays, the cells were lysed with RIPA lysis buffer 2 (50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40) containing protease inhibitor cocktail (PIC) and Phenylmethanesulfonyl fluoride (PSMF) for 48 h after transfection. For the immunoprecipitation, 15 μl anti-FLAG (M2) affinity gel for each sample was washed with 0.5 ml of cold TBS three times, then it was added to 1 mg of cell lysates. Shake all the samples gently at 4°C for 4 h, then centrifuge the resin for 1 min at 7,000×*g*. Wash the beads three times with 0.5 ml of cold TBS-T. After the final wash, remove the wash buffer and add 30 μl TBS and 9 μl 5×SDS loading. Centrifuge the samples at 10,000×*g* for 1 min and boil the samples for 15 min. The ubiquitination of the substrates was assayed by immunoblotting with an anti-Ub antibody under denaturing conditions. The ubiquitination chains of substrates were assayed by immunoblotting with an anti-HA antibody under denaturing conditions. The protein–protein interaction between the substrates and E4B was assayed by immunoblotting with an anti-MYC antibody under nondenaturing conditions.

Degradation of Substrates in HEK293 and HCC Cells

1×10^6 HEK293 cells were seeded into 6-well plates and were transfected with FLAG-tagged substrate plasmids and increasing amounts of pLVX-E4B (0, 0.5, 1, 2, and 3 μg) plasmids by PEI. The cells were lysed with RIPA lysis buffer after transfection for 48 h. The amount of total substrate proteins was determined by immunoblotting with an anti-FLAG antibody. HepG2, HuH7, and HEK293 cells were transfected with increasing pLVX-E4B (0, 1, and 2 μg) plasmids with Lipo3000.

For CHX chase assays, 1×10^6 HEK293 cells were seeded into 6-well plates and were cotransfected with 1.5 μg substrate plasmids and 1 μg pLVX-E4B or 1 μg empty pLVX. Another group was only transfected with 1.5 μg substrate plasmids to shE4B and shctrl, respectively. The cells were harvested 48 h after transfection. To block *de novo* protein synthesis, the cells were treated with Cycloheximide (CHX) (50 μg/ml) and incubated for 0, 2, 4, 6, and 8 h before harvesting the cells. The amount of total substrate proteins was determined by immunoblotting with an anti-FLAG antibody. CHX chase assays were performed in the HEK293 cells that stably expressed anti-E4B shRNA to determine the effect of shE4B on substrate stability.

RNA Extraction and RSRC2 Splicing Assays

2.5×10^5 HEK293 cells were seeded into 12-well plates and were transfected with different amounts of FLAG-tagged TRA2A plasmids or E4B plasmids. The total RNA from 12 well-plates was extracted by 500 μl TRIzol reagent after 48 h transfection. RNA was isolated for subsequent RT-PCR to obtain a cDNA library. GAPDH gene was amplified as a control. The primers used in this study were shown below:

RSRC2 forward: 5′-AGAAAACACAGGAGCCGGAG-3′.
RSRC2 reverse: 5′-TGAGTGACTTCTGCCTCTTGA-3′.
GAPDH forward: 5′-TCAAGAAGGTGGTGAAGCA-3′.
GAPDH reverse: 5′-AAGGTGGAGGAGTGGGT-3′.

Statistical Analysis

A statistical analysis was performed using Graphpad Prism (Graphpad prism 8.0 software, San Diego, CA, United States). All the quantitative data were presented as the mean ± SD. Other statistical analysis was performed using the unpaired Student's t-test. $p < 0.05$ was considered statistically significant.

RESULTS

E4B Mediated TRA2A and PYCR2 Ubiquitination Both *in Vitro* and *in Vivo*

In our previous work, we employed the orthogonal ubiquitin transfer (OUT) to identify the proteins that could be targeted by xUb through an xUb-xE1-xE2-xE4B cascade in the HEK293 cells (Zhao et al., 2012; Bhuripanyo et al., 2018). We believe that these are the potential ubiquitination substrates of E4B. Two important

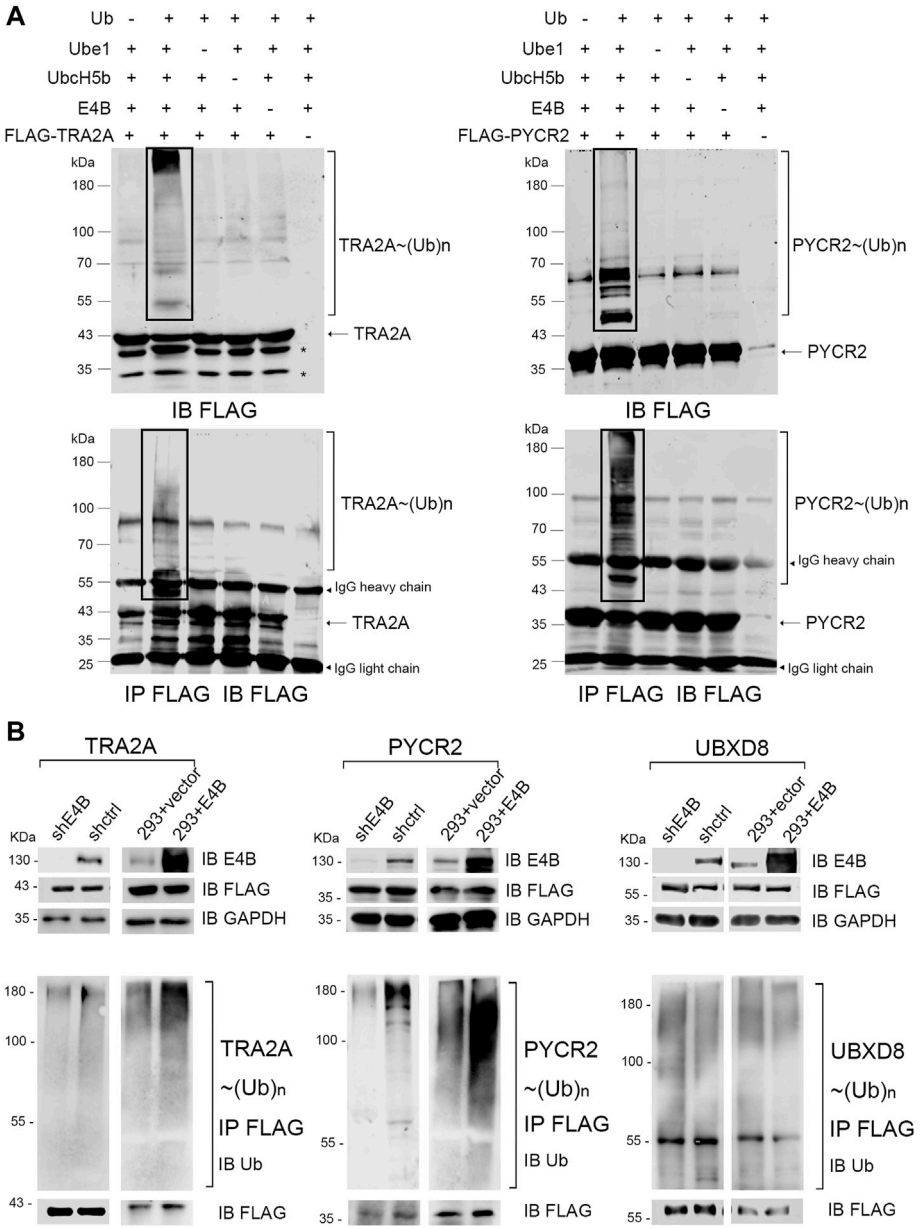


FIGURE 1 | E4B mediated TRA2A and PYCR2 ubiquitination *in vitro* and *in vivo*. **(A)** *In vitro* ubiquitination of TRA2A and PYCR2 through Ube1-UbcH5b-E4B cascade (line 2) and controls without Ub (line 1), E1 (line3), E2 (line 4), E3 (line 5), and FLAG-tagged substate (line 6) were detected with an anti-FLAG antibody. **(B)** Ubiquitination of TRA2A and PYCR2 were detected in the HEK293 cells. One group was stably E4B knockdown HEK293 cell (shE4B) and sh-empty HEK293 cell (shctrl). Another group was the E4B overexpressed HEK293 cell (293 + E4B) and pLVX-empty HEK293 cell (293 + vector). All these groups were transfected with FLAG-tagged TRA2A or PYCR2, respectively, and incubated with MG132 (10 μM) for 4 h before harvesting. The cell lysates were pulled down by immunoprecipitation with an anti-FLAG antibody under a denaturing condition. Ubiquitination of TRA2A and PYCR2 was detected by immunoblotting with an anti-Ub antibody.

proteins: TRA2A and PYCR2, appeared in the OUT pathway based on the MS analysis. Here, we want to verify if TRA2A and PYCR2 are bonafide substrates of E4B. We first expressed and purified FLAG-tagged TRA2A and PYCR2 proteins in *E. coli* cells and established an *in vitro* ubiquitination reaction by mixing with HA-Ub, E1 (Ube1), E2 (UbcH5b), and E3 (E4B) recombinant proteins. Ubiquitination of TRA2A and PYCR2 was detected by immunoblotting with an anti-FLAG antibody. We observed significant polyubiquitination formed on TRA2A and PYCR2 in the presence of all the elements for ubiquitin transfer (**Figure 1A**, line 2). In contrast, neither mono- nor poly-ubiquitination were observed if there was a lack of any enzyme or Ub, or substrates for Ub transfer (**Figure 1A**). To ensure that the ubiquitination was attached to TRA2A and PYCR2, we further used anti-FLAG beads to pull down the substrates in all the groups under a denaturing condition. The

immunoprecipitation results were the same as the ubiquitination in the protein reactions. To show the individual components of the *in vitro* reaction, another transfer assay was designed to detect the HA-Ub attached to components individually with an anti-HA antibody under a nondenaturing condition (**Supplementary Figure S1**). In this assay, HA-Ub could be attached to E1 and E2 to form a thioester bond, while E4B and the substrates can form polyubiquitin chains during the Ub transfer. These results suggested that Ub was attached to TRA2A and PYCR2 depending on the E1-E2-E3 enzymatic transfer and E4B worked as an E3 ligase for the *in vitro* ubiquitination of TRA2A and PYCR2.

We next examined whether TRA2A and PYCR2 could be ubiquitinated by E4B in the HEK293 cells. The HEK293 cells were transfected with a short hairpin RNA against E4B expression to generate a stable E4B knockdown cell line (shRNA) in our previous study. A scramble shRNA was also used to generate the shctrl cells. Meanwhile, the HEK293 cells were transfected with a pLVX-empty vector or pLVX-E4B plasmid to generate the HEK293 + vector (293 + vector) and E4B overexpressed cell line (293 + E4B). E4B knockdown and overexpression groups were both transfected with pcDNA-FLAG-TRA2A and pcDNA-FLAG-PYCR2 plasmids, respectively. Meanwhile, pcDNA-FLAG-UBXD8 was used as a negative control because UBXD8 could not be ubiquitinated by E4B in our previous study (data not shown). The cells were treated with proteasome inhibiter MG132 for 4 h before harvesting. The expression level of E4B was detected with an anti-E4B antibody. FLAG-tagged TRA2A, PYCR2, and UBDX8 were detected with an anti-FLAG antibody. TRA2A, PYCR2, and UBDX8 were pulled down with the anti-FLAG beads and immunoblotted with an anti-Ub antibody to detect the ubiquitination. Significant polyubiquitinations on TRA2A and PYCR2 were observed in 293 + E4B cells compared to 293 + vector cells (**Figure 1B**). In contrast, the ubiquitination of TRA2A and PYCR2 in the shRNA cells decreased compared to that in the shctrl cells. However, there is no difference in ubiquitination of UBDX8 in all the groups, indicating that the ubiquitination of UBDX8 was not affected by E4B. These results showed that TRA2A and PYCR2 could be ubiquitinated by E4B in the HEK293 cells, and the ubiquitination depended on the level of E4B in the HEK293 cells.

E4B Promoted the Degradation of TRA2A and PYCR2 by Forming K11- or K48- Linked Polyubiquitin Chains

Since E4B could ubiquitinate TRA2A and PYCR2 *in vitro* and *in vivo*, we wanted to know whether E4B mediates the degradation of TRA2A and PYCR2. The HEK293 cells were transfected with different amounts of E4B, and the degradation of TRA2A and PYCR2 was detected in two independent experiments simultaneously without MG132 treatment. With the increasing amount of E4B, the protein levels of TRA2A and PYCR2 decreased significantly, indicating that E4B promoted the degradation of TRA2A and PYCR2 (**Figure 2A**). We further performed a cycloheximide (CHX) chase assay to detect the stability of TRA2A and PYCR2. The cells were treated with 50 μg/μL CHX to inhibit protein synthesis and were harvested at 0, 2, 4, 6, and 8 h after the addition of CHX to detect the protein levels of TRA2A and PYCR2 with an anti-FLAG antibody. In the shE4B cells (shE4B), the turnover of TRA2A and PYCR2 was stable even after 8 h treatment with CHX, while TRA2A and PYCR2 were both decreased after 4 h in the shctrl cells. (**Figure 2B**). In the HEK293 + vector groups (293 + vector), which were not transfected with exogenous E4B but pLVX-empty plasmid, the protein levels of TRA2A and PYCR2 decreased after 4 h treatment with CHX (**Figure 2B**). Noticeable turnover was observed in the E4B overexpressed cells (293 + E4B), in which the protein levels of TRA2A and PYCR2 decreased significantly compared to that in the 293 + vector group (**Figure 2B**). These results indicated that TRA2A and PYCR2 are bonafide substrates of E4B. Increasing the expression of E4B in the HEK293 cells accelerated the degradation of TRA2A and PYCR2.

Ub consists of seven Lys (K) residues that can form different types of polyubiquitin chains (Akutsu et al., 2016). Among them, K11- or K48- linked polyubiquitin chains signal protein degradation (Akutsu et al., 2016; Boughton et al., 2020). We wanted to know whether E4B formed K11- or K48- linked polyubiquitin chains on TRA2A and PYCR2. In addition, K63- linked polyubiquitin chain is known as a canonical Ub signal and has been widely studied in inflammatory signaling and NF-κB pathway (Meyer and Rape, 2014). By replacing one Lys (K) to Arg (R) in Ub, we constructed three Ub mutants: K11R, K48R, and K63R. We transfected the HEK293 cells with HA-tagged wtUb and Ub mutants and detected their expression with an anti-HA antibody (**Figure 2C**). Meanwhile, we cotransfected E4B and FLAG-tagged TRA2A and PYCR2 to these HEK293 cells and detected the ubiquitination of TRA2A and PYCR2 with an anti-HA antibody after MG132 (10 μM) treatment for 6 h before harvesting. The results showed that the polyubiquitination formed by K11R and K48R reduced significantly compared to wtUb or K63R (**Figure 2C**). These results validated that E4B formed K11- or K48- linked polyubiquitin chains on TRA2A and PYCR2 and mediated their degradation.

E4B Regulated the Alternative Splicing Function of TRA2A and Affected the Transcription of RSRC2

TRA2A regulates multiple alternative splicing (AS) events by processing the mRNA precursors to mature mRNA (Best et al., 2014). Arginine and serine rich coiled-coil 2 (RSRC2) has been reported to be spliced into two variants by TRA2A at the mRNA level (Tieju Liu et al., 2017). The shorter variant (variant 1, also named RSRC2s) encodes a functional protein, while the longer variant (variant 5, also named RSRC2l) is a nonsense product with an additional exon 4 which includes a stop codon (**Figure 3A**). Overexpression of TRA2A in cells can regulate the alternative splicing of RSRC2 and result in the shift of RSRC2s to RSRC2l (Tieju Liu et al., 2017). We first examined whether the endogenous TRA2A influenced the alternative splicing of RSRC2. We transfected a siTRA2A to HEK293 cells to deplete the endogenous TRA2A and examined the shift of RSRC2. Upon the exhaustion of endogenous TRA2A, the abnormal splicing of RSRC2 (RSRC2l) decreased (**Figure 3B**). Then, the HEK293 cells

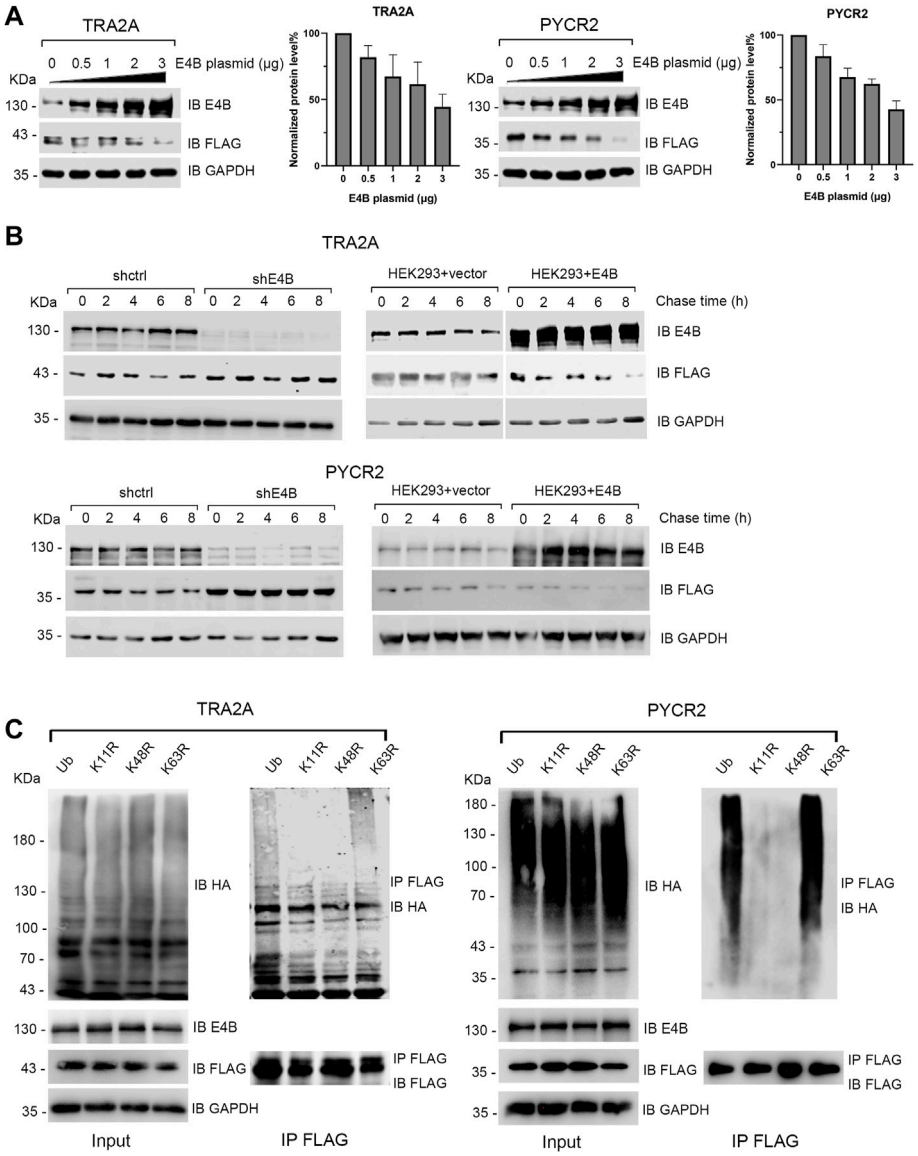


FIGURE 2 | E4B promoted the degradation of TRA2A and PYCR2. **(A)** Increase of E4B accelerated the degradation of TRA2A and PYCR2. The HEK293 cells were transfected with different amounts of E4B plasmid but the same amount of FLAG-TRA2A and FLAG-PYCR2 plasmids, respectively. The protein levels of TRA2A and PYCR2 were detected with an anti-FLAG antibody. Line charts correspond to the WB results. Each sample was measured three times. **(B)** E4B-dependent degradation of TRA2A and PYCR2 assayed by CHX chase. One group was the E4B knockdown HEK293 cells (shE4B) and sh-empty HEK293 cells (shctrl). Another group was the E4B overexpressed HEK293 cells (HEK293 + E4B) and pLVX-empty HEK293 cells (HEK293 + vector). The protein levels of substrates in every group were detected at 0, 2, 4, 6, and 8 h after 50 μg/ml CHX was added to the cells before harvesting. Each sample was measured three times. **(C)** E4B formed K11- and K48-linked polyubiquitin chains on TRA2A and PYCR2. The HEK293 cells were transfected with HA-wtUb (line 1), HA-K11R (line 2), HA-K48R (line 3), and HA-K63R (line4) plasmids. The cells were treated with MG132 for 6 h to ensure the stability of substrates. Ubiquitination of the substrates was detected by immunoblotting with an anti-HA antibody and pulled down FLAG-substrates were detected with an anti-FLAG antibody to equal the amounts.

were transfected with different amounts of TRA2A plasmid, respectively. The total RNA was extracted as the template for reverse transcription PCR (RT-PCR) to generate a cDNA library. We designed a pair of primers for RSRC2 amplification, and a pair of primers for GAPDH amplification was used as a control. The cDNA library was used as the template to amplify both the RSRC2 and GAPDH genes. There are two PCR products amplified: RSRC2s (204 bp), the main product and RSRC2l (282 bp), an abnormal splicing product with an additional exon 4 gene sequence inserted. With the increase of TRA2A, the amount of RSRC2l increased gradually in a dose-dependent manner (**Figure 3C**). These results validated that the abnormal splicing of RSCRC2 happened when the expression of TRA2A increased in the HEK293 cells. To detect whether the degradation of TRA2A mediated by E4B will affect the alternative splicing of RSRC2, the HEK293 cells were cotransfected with E4B and the

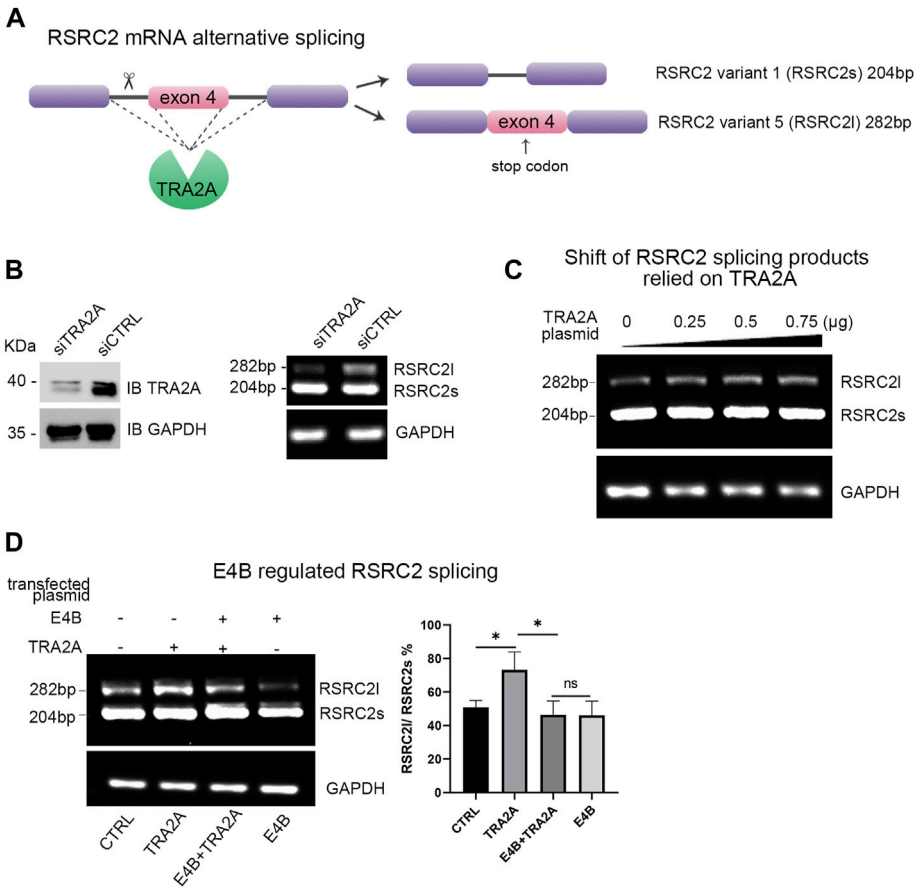


FIGURE 3 | E4B affected alternative splicing of RSRC2. **(A)** Maps of RSRC2 mRNA alternative splicing regulated by TRA2A. There are two splicing variants: RSRC2s and RSRC2l. RSRC2s is the main product while RSRC2l is an abnormal splicing product with an additional exon 4 gene remaining and encoding a stop codon. **(B)** Knockdown TRA2A deceased the abnormal splicing product RSRC2l. Endogenous protein level of TRA2A was exhausted after being transfected with siTRA2A and the total RNA was extracted to generate the cDNA library. RSRC2 gene was amplified by a pair of specific primers and GAPDH gene was amplified as an intracellular control. **(C)** Overexpression of TRA2A induced the shift of RSRC2 splicing products from RSRC2s to RSRC2l. The HEK293 cells were transfected with 0, 0.25, 0.5, and 0.75 μg TRA2A plasmid, respectively, and the total RNA was extracted to generate the cDNA library. RSRC2 gene was amplified by a pair of specific primers and GAPDH gene was amplified as an intracellular control. **(D)** Alternative splicing of RSRC2 was regulated by the expression of TRA2A and E4B. 0.75 μg TRA2A and 1 μg E4B plasmids were transfected into the HEK293 cells in different groups. The bar chart was drawn according to the yields of PCR products. All the experiments were repeated three times. (*$p < 0.5$ vs. control; ns: $p > 0.05$)

PCR products of RSRC2 were detected by the same method. Compared to the control cells, which were transfected with neither E4B nor TRA2A (**Figure 3D**, lane 1), the RSRC2l gene product increased in the cells transfected with TRA2A (**Figure 3D**, lane 2). When transfected with both E4B and TRA2A, the RSRC2l gene product was reduced (**Figure 3D**, lane 3) compared with lane 2. The least RSRC2l gene product was obtained in the cells transfected with E4B but without TRA2A (**Figure 3D**, lane 4). We calculated the ratio of RSRC2l/RSRC2s according to the yields of the PCR products and the results are shown in **Figure 3D** (right panel). The ratio of RSRC2l/RSRC2s in the control cells was about 50% but increased to 70% in the TRA2A overexpressed cells. Nevertheless, the ratio decreased under 40% in the TRA2A overexpressed cells when cotransfected with E4B, indicating that E4B degraded the TRA2A. Compared to the control cells, the ratio in the E4B overexpressed cells was further decreased to 40%, meaning that endogenous TRA2A was degraded by E4B. Taken together, these results suggested that E4B reduced the alternative splicing of RSRC2 and affected its transcription products by mediating TRA2A degradation.

E4B Slightly Mediated Degradation of Endogenous TRA2A and PYCR2 in Hepatocellular Carcinoma Cells

TRA2A and PYCR2 are highly expressed in several cancer tissues and regarded as oncogenes or biomarkers (Chao Liu et al., 2017; Tang et al., 2018). However, E4B is also highly expressed in severe cancer cells. Therefore, we asked an interesting question: why can they overexpress in the same cells simultaneously since E4B induced the degradation of TRA2A and PYCR2? To answer this question, we detected the endogenous protein level of E4B, TRA2A, and PYCR2 in the HCC cell lines HepG2,

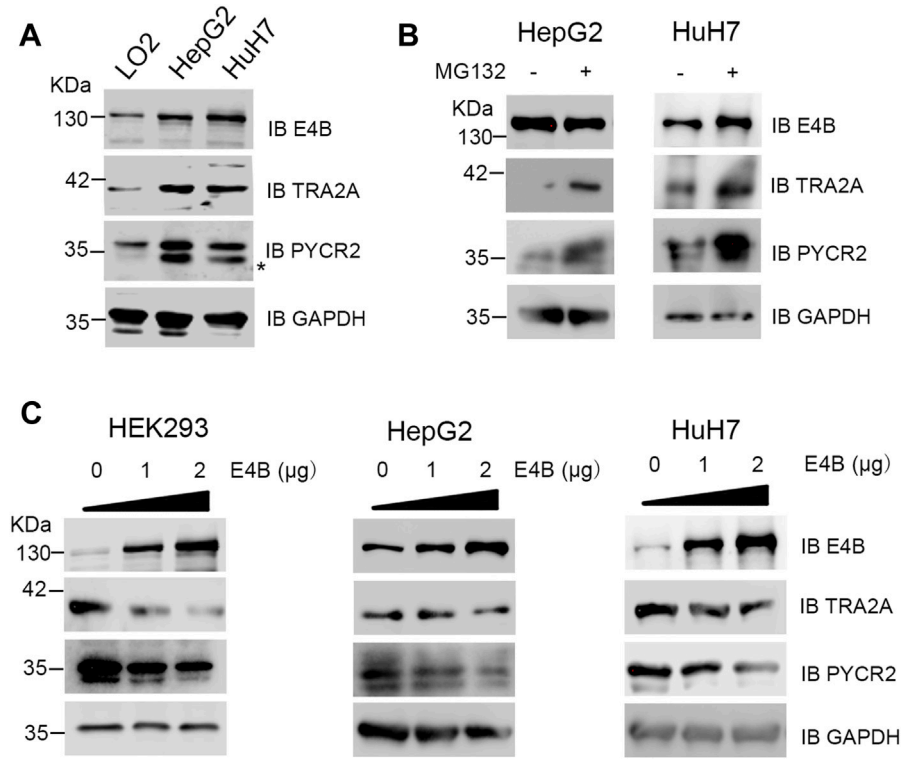


FIGURE 4 | Degradation of endogenous TRA2A and PYCR2 in the HCC cells. **(A)** Expression of endogenous E4B, TRA2A, and PYCR2 in different cell lines. Asterisk might be a nonspecific binding with an anti-PYCR2 antibody. **(B)** Stability of TRA2A and PYCR2 disturbed by MG132 in the HCC cells. 10 μM MG132 was added to the HepG2 and HuH7 cells, and the endogenous expression of TRA2A and PYCR2 was detected with specific antibodies. **(C)** The increase of E4B degraded TRA2A and PYCR2 in the HEK293 cells but slightly in the HepG2 and HuH7 cells. Exogenous E4B was added to the HEK293 and the HCC cells in different amounts, and the degradation of endogenous TRA2A and PYCR2 was detected by specific antibodies.

HuH7, and normal human hepatocyte cell line LO2. Compared to the expression in LO2, the expression of endogenous E4B, TRA2A, and PYCR2 were significantly increased in the HepG2 and HuH7 cells (**Figure 4A**). These results are consistent with what has been reported. We further investigated the stability of TRA2A and PYCR2 in the HCC cell lines. We compared the protein levels in cells with or without MG132 treatment and found that the expression of both TRA2A and PYCR2 increased significantly when the cells were treated with MG132 (**Figure 4B**), demonstrating that the stability of TRA2A and PYCR2 depended on proteasome-related degradation. Considering other E3 ligases in the HepG2 and HuH7 cells, we could not judge whether the degradation of TRA2A and PYCR2 was related to E4B. To further study the role of E4B in the degradation of endogenous TRA2A and PYCR2, the HepG2, HuH7, and HEK293 cells were transfected with different amounts of exogenous E4B and the protein levels of TRA2A and PYCR2 were detected with specific antibodies. Consistent with the results in **Figure 2A**, the endogenous TRA2A and PYCR2 were significantly decreased with the increasing amount of E4B. To our surprise, even transfected with 2 μg E4B, both TRA2A and PYCR2 were slightly decreased in the HepG2 and HuH7 cells (**Figure 4C**). These results conflicted with the results in HEK293 cells, indicating that E4B mediated the degradation of endogenous TRA2A and PYCR2 slightly in the HCC cells. Further study is underway to reveal the degradation mechanism of TRA2A and PYCR2 in the HCC cells.

E4B Interacted With Substrates *via* its Variable Region

E4B contains a U-box domain, which is regarded as the domain for the binding with Ub-E2 conjugate (Hatakeyama et al., 2001). However, few reports showed how E4B interacts with its substrates. Ufd2p, the homolog of E4B in *S. cerevisiae,* catalyzed K29-linked polyubiquitin chain elongation *via* its two N-terminal loops located in the variable region (Chao Liu et al., 2017). In our study, we wanted to know which domain of E4B interacts with TRA2A and PYCR2. The HEK293 cells were cotransfected with the exogenous full-length E4B and FLAG-tagged substrates (TRA2A or PYCR2), and a co-immunoprecipitation assay was performed to identify the interaction between the full-length E4B with TRA2A or PYCR2. The HEK293 cell transfected with the full-length E4B and FLAG-tagged pcDNA empty vector (without the gene of TRA2A or PYCR2 inserted) was used as a control. The cell lysates were pulled down with anti-FLAG beads, and the interaction of

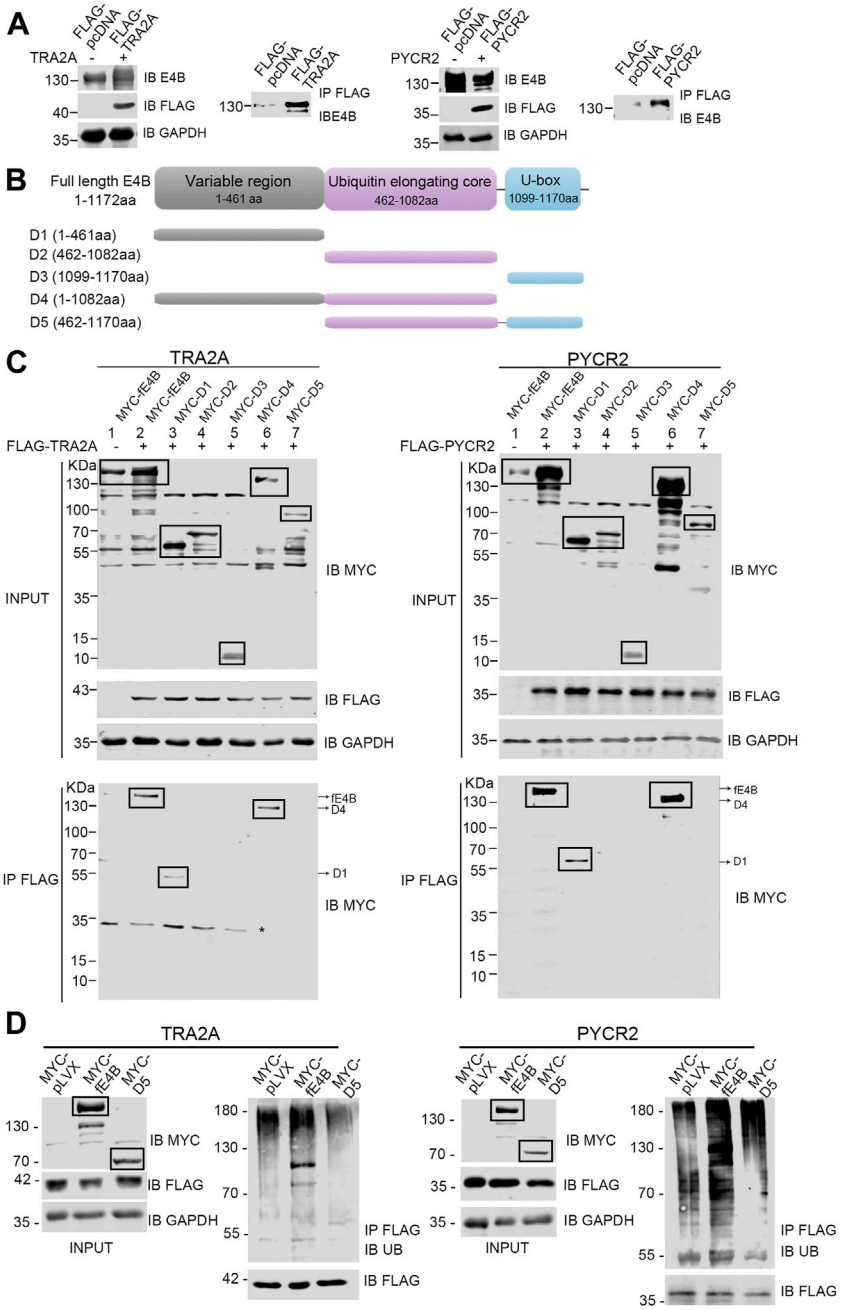


FIGURE 5 | E4B interacted with substrates *via* its variable region. **(A)** Interaction between the full-length E4B and TRA2A and PYCR2. HEK293 cells were transfected with the full-length E4B (fE4B) with or without the transfection of exogenous FLAG-TRA2A and FLAG-PYCR2. Co-immunoprecipitation was carried out by an anti-FLAG antibody and the interaction was detected by an anti-E4B antibody. **(B)** Construction of E4B variants. Full-length E4B consists of a variable region (1-461 aa), a ubiquitin elongating core, and a U-box domain. Based on its sequence and structure, five truncated variants including D1 (1-461aa), D2 (462-1082aa), D3 (1099-1170aa), D4 (1-1082aa), and D5 (462-1170aa) were constructed. **(C)** Interaction between E4B truncated variants and FLAG-TRA2A and FLAG-PYCR2. The HEK293 cells were transfected with MYC-tagged full-length E4B and five variants are treated with MG132 (10 μM) for 4 h before harvesting. TRA2A and PYCR2 were pulled down with an anti-FLAG antibody and the interaction with E4B was detected with an anti-MYC antibody. Due to the nonspecific binding of the anti-MYC antibody, the targeted bands were boxed. Asterisk might be a nonspecific binding with anti-MYC antibody upon a nondenaturing condition. **(D)** Ubiquitination of TRA2A and PYCR2 was promoted by fE4B but not D5. The HEK293 cells were transfected with MYC-pLVX vector (control), MYC-fE4B, and MYC-D5, respectively. The ubiquitination of TRA2A and PYCR2 was detected by an anti-Ub antibody after pull-down by an anti-FLAG antibody upon a denaturing condition. The cells were treated with MG132 (10 μM) for 4 h before harvesting.

substrates with E4B was detected by an anti-E4B antibody. The results showed that the full-length E4B could interact with either TRA2A or PYCR2 (**Figure 5A**), consistent with the result that E4B worked as an E3 in the ubiquitination of TRA2A and PYCR2.

E4B consists of three domains: a variable region (1-461 aa) at the N-terminus, a ubiquitin elongating core (462-1,082 aa), and a U-box domain (1,099-1,170 aa) at the C-terminus (**Figure 5B**). To further study the structure–function relationship between E4B and substrate, we constructed five MYC-tagged E4B truncated variants containing different domains based on the E4B sequence. We named these variants D1 (1-461 aa), D2 (462-1,082 aa), D3 (1,099-1,170 aa), D4 (1-1,082 aa), and D5 (462-1,170 aa), as shown in **Figure 5B** and detected the interaction with FLAG-tagged TRA2A or PYCR2 in the HEK293 cells. The full-length E4B (fE4B), D1, and D4 showed interaction with either TRA2A or PYCR2 (**Figure 5C**). D5, the variant that lacks variable region but contains ubiquitin elongating core and U-box domain, could not be recognized by TRA2A and PYCR2 (**Figure 5C**). These results indicated that the variable region is an indispensable domain for recognition of E4B with its substrate.

Although D5 did not show the interaction with TRA2A or PYCR2, it contains the U-box domain and can bind to Ub-E2 conjugate. We wanted to examine whether D5 would enhance the ubiquitination of TRA2A and PYCR2. The HEK293 cells were transfected with MYC-tagged pLVX plasmid, MYC-tagged fE4B, and MYC-tagged D5, respectively. Meanwhile, FLAG-tagged TRA2A or PYCR2 were cotransfected to all the groups. Ubiquitination was detected by immunoprecipitation of TRA2A or PYCR2 with anti-FLAG beads and immunoblotting with an anti-Ub antibody. Compared to the control cells (pLVX), the cells transfected with fE4B showed a significant increase of ubiquitination on TRA2A and PYCR2. However, the cells transfected with D5 did not increase the ubiquitination of TRA2A and PYCR2 (**Figure 5D**). Taken together, the variable region of E4B plays a key role in the interaction and recognition of substrates. Deleting the variable region of E4B will abolish the ubiquitination of its substrates.

DISCUSSION

E4B is known as a ubiquitin E4 enzyme and most research focused on its polyubiquitin elongation function on its substrates such as p53, ataxin-3, and Yap8 (Matsumoto et al., 2004; Wu et al., 2011; Ferreira et al., 2015). In our previous work, we engineered an orthogonal ubiquitin pathway (OUT) and identified over 100 potential substrates of E4B in the HEK293 cells (Zhao et al., 2012; Bhuripanyo et al., 2018). We found that TRA2A and PYCR2 are in the list but did not validate whether TRA2A and PYCR2 are bonafide substrates of E4B. TRA2A is highly expressed in breast cancer, glioma, and liver cancer, while PYCR2 is regarded as a prognostic biomarker in the HBV-related HCC (Tieju Liu et al., 2017; Tan et al., 2018; Gao et al., 2019). However, ubiquitination of TRA2A and PYCR2 has not been reported. In this study, we reported that E4B could mediate the ubiquitination of TRA2A and PYCR2 *in vitro* and HEK293 cells as an E3 ligase. Further study demonstrated that E4B degraded TRA2A and PYCR2 by forming K11- and K48- linked polyubiquitin chains on substrates. The overexpressed E4B affected alternative splicing of RSRC2 by mediating the degradation of TRA2A in the HEK293 cells (**Figure 3D**).

Since both E4B and its substrates, TRA2A and PYCR2, are highly expressed in many cancer tissues and cells, we wanted to know whether E4B can degrade TRA2A and PYCR2 efficiently in the same type of cancer cells. Intriguingly, although E4B was overexpressed in the HepG2 and HuH7 cells, it hardly degraded endogenous TRA2A and PYCR2, even transfected with exogenous E4B (**Figure 4C**). These results conflicted with those in the HEK293 cells, suggesting that the mechanism of degradation of TRA2A and PYCR2 mediated by E4B is different in cancer cells. For example, deubiquitinating enzymes (DUBs) play a role in cleaving the Ub chain and decreasing the ubiquitination of substrates in the cancer cells. It has been reported that USP2a, USP7, USP21, and USP22 are highly expressed in the HCC tissue or cells and promote tumor development (Cai et al., 2015; Li et al., 2018; Ling et al., 2020; Xiong et al., 2021). However, further study is needed to reveal the mechanism in the HCC cells.

So far, it is unknown of the E4B structure and how E4B interacts with its substrates. Ufd2, the yeast homolog of E4B, consists of a highly variable N-terminal region, a core region domain, and a U-box domain (Tu et al., 2007). Based on the structural similarity, we constructed five E4B truncated variants and investigated the interaction between different domains of E4B and substrates. In addition to wtE4B, only D1 and D4 variants showed interaction with TRA2A and PYCR2, indicating that E4B was bound to its substrates *via* its variable region. These results are consistent with those of a recent study on Ufd2. In their work, Ufd2 was bound to the substrate GFP-Ub *via* its two N-terminal loops located in the highly variable region and played as an E4 enzyme *in vitro* (Chao Liu et al., 2017).

In conclusion, our study reported the ubiquitination and degradation of TRA2A and PYCR2 in different cell lines. The variable region of E4B was indispensable to interact with its substrates. The degradation of TRA2A and PYCR2 in the HEK293 cells was quite different from that in the HCC cells. These results revealed other mechanisms associated with the ubiquitination of TRA2A and PYCR2 in the HCC cells. The mechanism of why E4B does not degrade TRA2A and PYCR2 effectively needs further study in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

BZ conceived the idea and designed the experiments. YL, KP, SL, XL, and BW performed experiments. BJ, YC, and TW provided

technical guidance or intellectual input. BJ provided funding support. YL, SL, and BZ wrote and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

FUNDING

This work was supported by grants from the Natural Science Foundation of China (31770921 and 31971187) and Science and Technology Commission of Shanghai Municipality Project (20JC1411200), Technology and Planning Program of Suzhou (SYS2019063), Project of State Key Laboratory of Radiation Medicine and Protection, Soochow University (GZK1202133), and Medical Application project of Nuclear Technology in Subject Construction Lifting Project of the Second Affiliated Hospital of Soochow University (XKTJ-HRC2021008).

ACKNOWLEDGMENTS

We thank Ronggui Hu at the Chinese Academy of Sciences for providing HuH7 cell lines and Yunsheng Yuan at Shanghai Jiao Tong University for providing LO2 and HepG2 cell lines. We also thank all members of the Zhao Lab at Shanghai Jiao Tong University for their helpful discussions in this work.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2022.833396/full#supplementary-material

Supplementary Figure S1 | Ubiquitin transfer assay of E1, E2, E3, and substrates. E1, E2, E3, and substrates were sequentially added into the mixed reaction to show each component by detecting the HA-Ub complex with an anti-HA antibody upon a nondenaturing condition. The ubiquitination of TRA2A and PYCR2 was also detected with an anti-FLAG antibody upon a denaturing condition.

REFERENCES

Akutsu, M., Dikic, I., and Bremm, A. (2016). Ubiquitin Chain Diversity at a Glance. *J. Cel Sci.* 129, 875–880. doi:10.1242/jcs.183954

Best, A., James, K., Dalgliesh, C., Hong, E., Kheirolahi-Kouhestani, M., Curk, T., et al. (2014). Human Tra2 Proteins Jointly Control a CHEK1 Splicing Switch Among Alternative and Constitutive Target Exons. *Nat. Commun.* 5, 4760. doi:10.1038/ncomms5760

Bhuripanyo, K., Wang, Y., Liu, X., Zhou, L., Liu, R., Duong, D., et al. (2018). Identifying the Substrate Proteins of U-Box E3s E4B and CHIP by Orthogonal Ubiquitin Transfer. *Sci. Adv.* 4, e1701393. doi:10.1126/sciadv.1701393

Boughton, A. J., Krueger, S., and Fushman, D. (2020). Branching via K11 and K48 Bestows Ubiquitin Chains with a Unique Interdomain Interface and Enhanced Affinity for Proteasomal Subunit Rpn1. *Structure* 28, 29–43. doi:10.1016/j.str.2019.10.008

Cai, J.-B., Shi, G.-M., Dong, Z.-R., Ke, A.-W., Ma, H.-H., Gao, Q., et al. (2015). Ubiquitin-specific Protease 7 Accelerates p14(ARF) Degradation by Deubiquitinating Thyroid Hormone Receptor-Interacting Protein 12 and Promotes Hepatocellular Carcinoma Progression. *Hepatology* 61, 1603–1614. doi:10.1002/hep.27682

Du, C., Wu, H., and Leng, R. P. (2016). UBE4B Targets Phosphorylated P53 at Serines 15 and 392 for Degradation. *Oncotarget* 7, 2823–2836. doi:10.18632/oncotarget.6555

Escande-Beillard, N., Loh, A., Saleem, S. N., Kanata, K., Hashimoto, Y., Altunoglu, U., et al. (2020). Loss of PYCR2 Causes Neurodegeneration by Increasing Cerebral Glycine Levels via SHMT2. *Neuron* 107, 82–94. doi:10.1016/j.neuron.2020.03.028

Fang, S., Jensen, J. P., Ludwig, R. L., Vousden, K. H., and Weissman, A. M. (2000). Mdm2 Is a RING finger-dependent Ubiquitin Protein Ligase for Itself and P53. *J. Biol. Chem.* 275, 8945–8951. doi:10.1074/jbc.275.12.8945

Ferreira, R. T., Menezes, R. A., and Rodrigues-Pousada, C. (2015). E4-Ubiquitin Ligase Ufd2 Stabilizes Yap8 and Modulates Arsenic Stress Responses Independent of the U-Box Motif. *Biol. Open* 4, 1122–1131. doi:10.1242/bio.010405

Gao, Q., Zhu, H., Dong, L., Shi, W., Chen, R., Song, Z., et al. (2019). Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. *Cell* 179, 1240. doi:10.1016/j.cell.2019.10.038

Hatakeyama, S., Yada, M., Matsumoto, M., Ishida, N., and Nakayama, K.-I. (2001). U Box Proteins as a New Family of Ubiquitin-Protein Ligases. *J. Biol. Chem.* 276, 33111–33120. doi:10.1074/jbc.m102755200

Hershko, A., and Ciechanover, A. (1998). The Ubiquitin System. *Annu. Rev. Biochem.* 67, 425–479. doi:10.1146/annurev.biochem.67.1.425

Heuzé, M. L., Lamsoul, I., Moog-Lutz, C., and Lutz, P. G. (2008). Ubiquitin-mediated Proteasomal Degradation in normal and Malignant Hematopoiesis. *Blood Cell Mol. Dis.* 40, 200–210. doi:10.1016/j.bcmd.2007.07.011

Hirschfeld, M., Jaeger, M., Buratti, E., Stuani, C., Grueneisen, J., Gitsch, G., et al. (2011). Expression of Tumor-Promoting Cyr61 Is Regulated by hTRA2-Beta1 and Acidosis. *Hum. Mol. Genet.* 20, 2356–2365. doi:10.1093/hmg/ddr128

Hoppe, T. (2005). Multiubiquitylation by E4 Enzymes: 'one Size' Doesn't Fit All. *Trends Biochem. Sci.* 30, 183–187. doi:10.1016/j.tibs.2005.02.004

Kaneko-Oshikawa, C., Nakagawa, T., Yamada, M., Yoshikawa, H., Matsumoto, M., Yada, M., et al. (2005). Mammalian E4 Is Required for Cardiac Development and Maintenance of the Nervous System. *Mol. Cel. Biol.* 25, 10953–10964. doi:10.1128/mcb.25.24.10953-10964.2005

Koegl, M., Hoppe, T., Schlenker, S., Ulrich, H. D., Mayer, T. U., and Jentsch, S. (1999). A Novel Ubiquitination Factor, E4, Is Involved in Multiubiquitin Chain Assembly. *Cell* 96, 635–644. doi:10.1016/s0092-8674(00)80574-7

Komander, D., and Rape, M. (2012). The Ubiquitin Code. *Annu. Rev. Biochem.* 81, 203–229. doi:10.1146/annurev-biochem-060310-170328

Krona, C., Ejeskär, K., Abel, F., Kogner, P., Bjelke, J., Björk, E., et al. (2003). Screening for Gene Mutations in a 500 Kb Neuroblastoma Tumor Suppressor Candidate Region in Chromosome 1p; Mutation and Stage-specific Expression in UBE4B/UFD2. *Oncogene* 22, 2343–2351. doi:10.1038/sj.onc.1206324

Li, W., Cui, K., Prochownik, E. V., and Li, Y. (2018). The Deubiquitinase USP21 Stabilizes MEK2 to Promote Tumor Growth. *Cell Death Dis.* 9, 482. doi:10.1038/s41419-018-0523-z

Li, Y., Bie, J., Song, C., Liu, M., and Luo, J. (2021). PYCR, a Key Enzyme in Proline Metabolism, Functions in Tumorigenesis. *Amino Acids* 53, 1841–1850. doi:10.1007/s00726-021-03047-y

Ling, S., Shan, Q., Zhan, Q., Ye, Q., Liu, P., Xu, S., et al. (2020). USP22 Promotes Hypoxia-Induced Hepatocellular Carcinoma Stemness by a HIF1α/USP22 Positive Feedback Loop upon TP53 Inactivation. *Gut* 69, 1322–1334. doi:10.1136/gutjnl-2019-319616

Liu, C., Liu, W., Ye, Y., and Li, W. (2017). Ufd2p Synthesizes Branched Ubiquitin Chains to Promote the Degradation of Substrates Modified with Atypical Chains. *Nat. Commun.* 8, 14274. doi:10.1038/ncomms14274

Liu, T., Sun, H., Zhu, D., Dong, X., Liu, F., Liang, X., et al. (2017). TRA2A Promoted Paclitaxel Resistance and Tumor Progression in Triple-Negative Breast Cancers via Regulating Alternative Splicing. *Mol. Cancer Ther.* 16, 1377–1388. doi:10.1158/1535-7163.mct-17-0026

Mammen, A. L., Mahoney, J. A., St. Germain, A., Badders, N., Taylor, J. P., Rosen, A., et al. (2011). A Novel Conserved Isoform of the Ubiquitin Ligase UFD2a/UBE4B Is Expressed Exclusively in Mature Striated Muscle Cells. *PLoS One* 6, e28861. doi:10.1371/journal.pone.0028861

Matsumoto, M., Yada, M., Hatakeyama, S., Ishimoto, H., Tanimura, T., Tsuji, S., et al. (2004). Molecular Clearance of Ataxin-3 Is Regulated by a Mammalian E4. *EMBO J.* 23, 659–669. doi:10.1038/sj.emboj.7600081

Meng, L., Donti, T., Xia, F., Niu, Z., Al Shamsi, A., Hertecant, J., et al. (2017). Homozygous Variants in Pyrroline-5-Carboxylate Reductase 2 (PYCR2) in Patients with Progressive Microcephaly and Hypomyelinating Leukodystrophy. *Am. J. Med. Genet.* 173, 460–470. doi:10.1002/ajmg.a.38049

Meyer, H.-J., and Rape, M. (2014). Enhanced Protein Degradation by Branched Ubiquitin Chains. *Cell* 157, 910–921. doi:10.1016/j.cell.2014.03.037

Nordquist, K. A., Dimitrova, Y. N., Brzovic, P. S., Ridenour, W. B., Munro, K. A., Soss, S. E., et al. (2010). Structural and Functional Characterization of the Monomeric U-Box Domain from E4B. *Biochemistry* 49, 347–355. doi:10.1021/bi901620v

Pavlova, N. N., and Thompson, C. B. (2016). The Emerging Hallmarks of Cancer Metabolism. *Cel Metab.* 23, 27–47. doi:10.1016/j.cmet.2015.12.006

Sun, C., Li, T., Song, X., Huang, L., Zang, Q., Xu, J., et al. (2019). Spatially Resolved Metabolomics to Discover Tumor-Associated Metabolic Alterations. *Proc. Natl. Acad. Sci. U.S.A.* 116, 52–57. doi:10.1073/pnas.1808950116

Tan, Y., Hu, X., Deng, Y., Yuan, P., Xie, Y., and Wang, J. (2018). TRA2A Promotes Proliferation, Migration, Invasion and Epithelial Mesenchymal Transition of Glioma Cells. *Brain Res. Bull.* 143, 138–144. doi:10.1016/j.brainresbull.2018.10.006

Tang, L., Zeng, J., Geng, P., Fang, C., Wang, Y., Sun, M., et al. (2018). Global Metabolic Profiling Identifies a Pivotal Role of Proline and Hydroxyproline Metabolism in Supporting Hypoxic Response in Hepatocellular Carcinoma. *Clin. Cancer Res.* 24, 474–485. doi:10.1158/1078-0432.ccr-17-1707

Tu, D., Li, W., Ye, Y., and Brunger, A. T. (2007). Structure and Function of the Yeast U-Box-Containing Ubiquitin Ligase Ufd2p. *Proc. Natl. Acad. Sci. U.S.A.* 104, 15599–15606. doi:10.1073/pnas.0701369104

Wu, H., and Leng, R. P. (2011). UBE4B, a Ubiquitin Chain Assembly Factor, Is Required for MDM2-Mediated P53 Polyubiquitination and Degradation. *Cell Cycle* 10, 1912–1915. doi:10.4161/cc.10.12.15882

Wu, H., Pomeroy, S. L., Ferreira, M., Teider, N., Mariani, J., Nakayama, K. I., et al. (2011). UBE4B Promotes Hdm2-Mediated Degradation of the Tumor Suppressor P53. *Nat. Med.* 17, 347–355. doi:10.1038/nm.2283

Xiong, B., Huang, J., Liu, Y., Zou, M., Zhao, Z., Gong, J., et al. (2021). Ubiquitin-specific Protease 2a Promotes Hepatocellular Carcinoma Progression via Deubiquitination and Stabilization of RAB1A. *Cell Oncol.* 44, 329–343. doi:10.1007/s13402-020-00568-8

Zhang, X.-F., Pan, Q.-Z., Pan, K., Weng, D.-S., Wang, Q.-J., Zhao, J.-J., et al. (2016). Expression and Prognostic Role of Ubiquitination Factor E4B in Primary Hepatocellular Carcinoma. *Mol. Carcinog.* 55, 64–76. doi:10.1002/mc.22259

Zhang, Y., Lv, Y., Zhang, Y., and Gao, H. (2014). Regulation of P53 Level by UBE4B in Breast Cancer. *PLoS One* 9, e90154. doi:10.1371/journal.pone.0090154

Zhao, B., Bhuripanyo, K., Zhang, K., Kiyokawa, H., Schindelin, H., and Yin, J. (2012). Orthogonal Ubiquitin Transfer through Engineered E1-E2 Cascades for Protein Ubiquitination. *Chem. Biol.* 19, 1265–1277. doi:10.1016/j.chembiol.2012.07.023

Zhao, B., Tsai, Y. C., Jin, B., Wang, B., Wang, Y., Zhou, H., et al. (2020). Protein Engineering in the Ubiquitin System: Tools for Discovery and beyond. *Pharmacol. Rev.* 72, 380–413. doi:10.1124/pr.118.015651

Conflict of Interest: 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

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: frontiersin.org/about/contact

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