NONGENOMIC ACTIONS OF THYROID HORMONES IN CANCER

EDITED BY: Osnat Ashur-Fabian, Paul J. Davis, Sandra Incerpi and Shaker A. Mousa

PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-406-4
DOI 10.3389/978-2-88963-406-4

About Frontiers

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

Frontiers Journal Series

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

Dedication to Quality

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

What are Frontiers Research Topics?

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

NONGENOMIC ACTIONS OF THYROID HORMONES IN CANCER

Topic Editors:
Osnat Ashur-Fabian, Sackler Faculty of Medicine, Tel Aviv University, Israel
Paul J. Davis, Albany Medical College, United States
Sandra Incerpi, Roma Tre University, Italy
Shaker A. Mousa, Albany College of Pharmacy and Health Sciences, United States

Citation: Ashur-Fabian, O., Davis, P. J., Incerpi, S., Mousa, S. A., eds. (2020). Nongenomic Actions of Thyroid Hormones in Cancer. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-406-4

Table of Contents

04 ***Editorial: Non Genomic Actions of Thyroid Hormones in Cancer***
Paul J. Davis, Osnat Ashur-Fabian, Sandra Incerpi and Shaker A. Mousa

06 ***Bioactivity of Thyroid Hormone Analogs at Cancer Cells***
Paul J. Davis, Heng-Yuan Tang, Aleck Hercbergs, Hung-Yun Lin, Kelly A. Keating and Shaker A. Mousa

11 ***Tetrac Delayed the Onset of Ocular Melanoma in an Orthotopic Mouse Model***
Osnat Ashur-Fabian, Ofira Zloto, Ina Fabian, Galya Tsarfaty, Martin Ellis, David M. Steinberg, Aleck Hercbergs, Paul J. Davis and Ido Didi Fabian

21 ***Non-genomic Actions of Thyroid Hormones Regulate the Growth and Angiogenesis of T Cell Lymphomas***
Florencia Cayrol, Helena A. Sterle, Maria Celeste Díaz Flaqué, Maria Laura Barreiro Arcos and Graciela A. Cremaschi

33 ***Thyroid Hormones and Cancer: A Comprehensive Review of Preclinical and Clinical Studies***
Eilon Krashin, Agnieszka Piekiełko-Witkowska, Martin Ellis and Osnat Ashur-Fabian

56 ***Molecular Actions of Thyroid Hormone on Breast Cancer Cell Migration and Invasion via Cortactin/N-WASP***
Ivonne Denise Uzair, Jeremias Conte Grand, Marina Ines Flamini and Angel Matias Sanchez

67 ***Tetrac and NDAT Induce Anti-proliferation via Integrin αvβ3 in Colorectal Cancers With Different K-RAS Status***
Yu-Tang Chin, Zong-Rong He, Chi-Long Chen, Hsiao-Ching Chu, Yih Ho, Po-Yu Su, Yu-Chen S. H. Yang, Kuan Wang, Ya-Jung Shih, Yi-Ru Chen, Jens Z. Pedersen, Sandra Incerpi, André Wendindondé Nana, Heng-Yuan Tang, Hung-Yun Lin, Shaker A. Mousa, Paul J. Davis and Jacqueline Whang-Peng

78 ***Corrigendum: Tetrac and NDAT Induce Anti-proliferation via Integrin αvβ3 in Colorectal Cancers With Different K-RAS Status***
Yu-Tang Chin, Zong-Rong He, Chi-Long Chen, Hsiao-Ching Chu, Yih Ho, Po-Yu Su, Yu-Chen S. H. Yang, Kuan Wang, Ya-Jung Shih, Yi-Ru Chen, Jens Z. Pedersen, Sandra Incerpi, André Wendindondé Nana, Heng-Yuan Tang, Hung-Yun Lin, Shaker A. Mousa, Paul J. Davis and Jacqueline Whang-Peng

79 ***Clinical Implications and Impact of Discovery of the Thyroid Hormone Receptor on Integrin αvβ3–A Review***
Aleck Hercbergs

87 ***The Role of Thyroid Hormones in Hepatocyte Proliferation and Liver Cancer***
Fabio Gionfra, Paolo De Vito, Valentina Pallottini, Hung-Yun Lin, Paul J. Davis, Jens Z. Pedersen and Sandra Incerpi

frontiers in Endocrinology

EDITORIAL
published: 04 December 2019
doi: 10.3389/fendo.2019.00847

Editorial: Non Genomic Actions of Thyroid Hormones in Cancer

Paul J. Davis[1]*, *Osnat Ashur-Fabian*[2], *Sandra Incerpi*[3] and *Shaker A. Mousa*[1]

[1] Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, United States,
[2] Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel,
[3] Department of Sciences, University Roma Tre, Rome, Italy

Keywords: integrin αvβ3, angiogenesis, anti-apoptosis, thyroxine, signal transduction, driver genes, tumor microenvironment, tetrac

Editorial on the Research Topic

Non Genomic Actions of Thyroid Hormones in Cancer

The molecular basis of the actions of thyroid hormone requires cellular uptake and liganding of the hormones by specific receptors in the cell nucleus and consequent expression of certain genes (1). That another panel of thyroid hormone receptors might allow iodothyronines to act without entering the cell appeared to be unlikely until a structural protein of the plasma membrane of rapidly dividing endothelial cells and of cancer cells (1, 2) was found to have a discrete receptor for thyroid hormone analogs. This receptor is on the extracellular domain of integrin αvβ3. It enables thyroid hormone stimulation of tumor cell proliferation, of tumor-linked angiogenesis, of tumor cell defense mechanisms, e.g., anti-apoptosis, and, apparently, of chemoresistance and radioresistance (Cayrol et al.; Davis et al.; Krashin et al.). A series of eight publications in a recent issue of *Frontiers in Endocrinology* is devoted to "Integrin αvβ3, non-peptide hormones and cancer" (Ashur-Fabian et al.; Cayrol et al.; Chin et al.; Davis et al.; Gionfra et al.; Hercbergs; Krashin et al.; Uzair et al.).

This previously unrecognized set of mechanisms of actions of thyroid hormones—initiated at the cell surface—has clinical implications (Hercbergs). The principal ligand for the hormone receptor on αvβ3 is L-thyroxine (T4) [(2), Davis et al.]. 3,5,3′-triiodo-L-thyronine (T3) (Uzair et al.) and reverse T3 (rT3) (Davis et al.) may have limited actions at the integrin, but Hercbergs has shown that pharmacological elimination of host T4 and substitution of T3 can serve to arrest tumor growth.

Transcription of an extensive panel of genes may be differentially regulated by T4 at its receptor on αvβ3 of cancer cells (Cayrol et al.; Davis et al.); these include driver genes and genes involved in signal transduction (2), as well as the processes of angiogenesis (Cayrol et al.) and apoptosis [(2), Davis et al.] mentioned above, and epithelial-mesenchymal transition (EMT) (Uzair et al.) and the state of cellular actin (Uzair et al.).

The *Frontiers in Endocrinology* papers on αvβ3 and thyroid hormone also broaden the spectrum of cancers subject to control from this site. The growth of melanoma, particularly that of the eye, may be arrested via tetraiodothyroacetic acid (tetrac), a deaminated T4 analog that inhibits T4 actions at the integrin (Ashur-Fabian et al.). Proliferation of T cell lymphoma cells and angiogenesis at sites of lymphoma xenografts are subject to regulation via αvβ3 (Cayrol et al.). Interestingly, *K-RAS* status (wild vs. mutant) in human colorectal cancer (CRC) cells alters the abundance of heterodimeric αvβ3 protein, but the relatively low threshold for tetrac/integrin activity at the integrin is unchanged by *K-RAS* status and tetrac and chemically modified tetrac are equally effective in mutant and wild-type CRC (Chin et al.).

Edited and reviewed by:
Antonino Belfiore, Department of Clinical and Experimental Medicine, University of Catania, Italy

***Correspondence:**
Paul J. Davis
pdavis.ordwayst@gmail.com

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *17 October 2019*
Accepted: *20 November 2019*
Published: *04 December 2019*

Citation:
Davis PJ, Ashur-Fabian O, Incerpi S and Mousa SA (2019) Editorial: Non Genomic Actions of Thyroid Hormones in Cancer. Front. Endocrinol. 10:847. doi: 10.3389/fendo.2019.00847

It is important to note that normal, non-malignant cells express reduced quantities of αvβ3 and the conformation of the integrin—a reflection of its state of activation (2)—is such that its signaling functions appear to be limited. Integrin αvβ3 is one of the more than two dozen heterodimeric integrins that are structural proteins of the plasma membrane, serving a variety of functions by interacting with proteins and other cells in the immediate microenvironment. The microenvironment specific for αvβ3 is extracellular matrix proteins (vitronectin, fibronectin, osteopontin, etc., containing the Arg-Gly-Asp [RGD] sequence) and various growth factor receptors (1). In a single cell, αvβ3 may also interact with adjacent vascular growth factor receptors. All such interactions in the immediate cellular environment may cause αvβ3 and other integrins to activate intracellular signal transduction pathways with specific downstream consequences in terms of gene expression and cell function. We have not identified other integrins to contain thyroid hormone receptor sites (HY Lin: unpublished observations).

The significance of the recognition of the existence of the receptor for thyroid hormone and perhaps for other non-peptide hormones includes: (1) the receptors are new therapeutic targets for which ligands already exist, e.g., tetrac or chemically modified tetrac as an antagonist of T4; (2) scanning of tumors is feasible with radiolabeled T4, modified structurally so that it does not enter the cells, and (3) drug delivery to tumors with tetrac coupled to a drug-binding nanoparticle, such as poly-lactic-co-glycolic acid, is possible.

In conclusion, it is clear that thyroid hormone as T4 can support cancer growth via cell surface αvβ3. Exploitation of these receptors may have useful therapeutic consequences.

AUTHOR CONTRIBUTIONS

PD drafted the editorial. All authors contributed to manuscript revision, read, and approved the submitted version.

REFERENCES

1. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev.* (2010) 31:139–70. doi: 10.1210/er.2009-0007
2. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol.* (2016) 12:111–21. doi: 10.1038/nrendo.2015.205

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 in Endocrinology

MINI REVIEW
published: 04 December 2018
doi: 10.3389/fendo.2018.00739

Bioactivity of Thyroid Hormone Analogs at Cancer Cells

Paul J. Davis[1,2*], *Heng-Yuan Tang*[2], *Aleck Hercbergs*[3], *Hung-Yun Lin*[4,5,6,7], *Kelly A. Keating*[2] *and Shaker A. Mousa*[2]

[1] Department of Medicine, Albany Medical College, Albany, NY, United States, [2] Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, United States, [3] Department of Radiation Oncology, The Cleveland Clinic, Cleveland, OH, United States, [4] PhD Program for Cancer Molecular Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan, [5] Taipei Cancer Center, Taipei Medical University, Taipei, Taiwan, [6] Traditional Herbal Medicine Research Center of Taipei Medical University Hospital, Taipei Medical University, Taipei, Taiwan, [7] TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei, Taiwan

In the context of genomic thyroid hormone actions in normal (noncancer) cells that involve primary interactions with nuclear thyroid hormone receptors (TRs), L-thyroxine (T4), and 3,3′,5′-triiodo-L-thyronine (reverse T3, rT3) have little bioactivity. In terms of TRs, T4 is a prohormone from which the active nuclear ligand, 3,5,3′-triido-L-thyronine (T3), is generated by deiodination. Deaminated T4 and T3 metabolites have different genomic effects: tetraiodothyroacetic acid (tetrac) is a low grade thyromimetic derivative of T4, whereas triiodothyroacetic acid (triac), the acetic acid metabolite of T3, has substantial thyromimetic activity. In cancer cells, the cell surface receptor for thyroid hormone on integrin αvβ3 mediates non-genomic actions of thyroid hormone analogs. The integrin is expressed in large measure by cancer cells and dividing endothelial cells and has a substantially different panel of responses to thyroid hormone analogs. At αvβ3, T4 is a potent proliferative, anti-apoptotic and pro-angiogenic hormone and is the primary ligand. rT3 may also be proliferative at this site. In contrast, tetrac and triac are antagonists of T4 at αvβ3, but also have anticancer properties at this site that are independent of their effects on the binding of T4.

Keywords: L-thyroxine, tetrac, triac, reverse T3, non-genomic actions

OPEN ACCESS

Edited by:
Roberta Malaguarnera, Università degli studi Magna Græcia di Catanzaro, Italy

Reviewed by:
Rosario Le Moli, Università degli Studi di Catania, Italy
Paolo Ciana, University of Milan, Italy

***Correspondence:**
Paul J. Davis
pdavis.ordwayst@gmail.com

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *24 September 2018*
Accepted: *21 November 2018*
Published: *04 December 2018*

Citation:
Davis PJ, Tang H-Y, Hercbergs A, Lin H-Y, Keating KA and Mousa SA (2018) Bioactivity of Thyroid Hormone Analogs at Cancer Cells. Front. Endocrinol. 9:739. doi: 10.3389/fendo.2018.00739

INTRODUCTION

The concept that the bioactivity of thyroid hormone is expressed by 3,5,3′-triiodo-L-thyronine (T3) has served to identify the critical metabolic and protein synthetic functions of the hormone that are dependent upon interactions of T3 with nuclear thyroid hormone receptors (TRs) in normal cells (1, 2). The T3-TR mechanism of action of thyroid hormone is designated genomic. T3 also has a limited number of effects initiated in cytoplasm or at the plasma membrane that are independent of TRs and thus are non-genomic in mechanism (1). T3 has also been recognized to have specific functions in cancer cells that may depend upon TR mutation (3, 4). The primary thyroid hormone product of the thyroid gland is L-thyroxine (T4), whose status as a prohormone for T3 has been fully appreciated to be defined by deiodinases in a variety of non-thyroid tissues that generate T3 by outer thyroid hormone ring deiodination at the 5' position of the diphenyl ether structure of iodothyronines (5). Inner ring deiodination at the 5 position produces reverse T3 (rT3), which is generally regarded as inactive. Modifications of the alanine side chain of iodothyronines occur naturally at the cellular level, but only to a limited degree, and yields tetraiodothyroacetic acid

(tetrac) and triiodothyroacetic acid (triac), which have some metabolic activities (6).

The appreciation of the existence of a receptor for thyroid hormone analogs on the plasma membrane of cancer and rapidly dividing endothelial cells (1, 7, 8) has enabled the recognition of functions of thyroid hormone analogs that were previously thought to be inactive. The cell surface receptor is on the extracellular domain of integrin αvβ3. The plasma membrane receptor for thyroid hormone has no structural homologies with TRs. At this receptor, T4 promotes cancer cell proliferation, supports anti-apoptosis and enhances angiogenesis because of its presence on endothelial cells (9–11). Tetrac inhibits the actions of T4 at the integrin and in the absence of T4 has a variety of actions on expression of specific genes. That is, T4 and tetrac affect the activities of a number of signal transduction pathways that downstream modulate differentially the transcription of a number of genes (8, 12, 13). The actions of T4 and tetrac at αvβ3 are non-genomic in that they do not directly involve TRs or require hormonal presence in the nucleus. Operationally, however, both genomic and non-genomic actions of thyroid hormones may culminate in specific gene transcription. The receptor on αvβ3 can also control the trafficking of intracellular proteins—including the transfer of cytoplasmic TRs and estrogen receptor-α into the nuclear compartment of cancer cells—and regulate the phosphorylation/activation of TRs (1, 14).

Integrins are a family of two dozen heterodimeric structural proteins of the plasma membrane and are critical to tissue structure and to cell migration. They interact importantly with extracellular matrix proteins and with other cells (15). The receptor for thyroid hormone on αvβ3 is the first small molecule binding site recognized on integrins, but subsequently discrete receptors on αvβ3 have been reported for other small molecules, including dihydrotestosterone (DHT) (16) and the stilbene, resveratrol (17). Integrin αvβ3 is expressed to a limited degree by non-dividing normal, i.e., non-cancer, cells, but the function of such cells does not appear to be affected by any interactions of T4 and the integrin that may occur. This may reflect the ("non-activated") physical state of αvβ3 in non-cancer cells (18). The integrin is functional, however, on the surface of platelets, reflecting the presence of fractions of the plasma membrane of megakaryocytes. T4 has been shown to induce platelet aggregation via αvβ3 (19).

In the succeeding sections, we briefly review the bioactivities of thyroid hormone analogs at the iodothyronine receptor on integrin αvβ3.

BIOACTIVITY OF T4 AT αvβ3

An early demonstration of the non-genomic activity of T4 was its conversion of soluble actin to fibrous actin F-actin (8, 20). This was initially demonstrated in astrocytes and glial cells. The molecular basis of this action of the hormone is incompletely understood, but appears to involve a truncated TRα (TRΔα1) isoform in cytoplasm (8). This isoform does not contain a nuclear localization signal. T3 does not affect the state of actin in cells. Regulation of the state of actin is of obvious importance to both normal and malignant cells.

In 2004, T4 was shown to be pro-angiogenic in the chick choriollantoic membrane (CAM) model. The CAM model has important dependency on αvβ3, and antibody to this integrin blocked the action of T4 on new blood vessel formation. Physiological concentrations of free T4 were shown to be active in this system (21). In this model system, the inhibition of conversion of T4 to T3 did not affect the action of T4 on the state of actin. This action of T4 was shown to be initiated at integrin αvβ3, leading to a series of studies characterizing the receptor on the head of the extracellular domain of the integrin. While integrins are found on both normal cells and malignant cells, αvβ3 is particularly generously expressed by cancer cells and rapidly dividing endothelial cells (8, 15). It and other integrins are very important to cell-cell interactions and cell-extracellular matrix (ECM) proteins that underlie tissue integrity and the orientation of motile cells. As noted above, thyroxine was the first small molecule to be found to bind specifically to αvβ3.

At the integrin, T4 was found to have a number of cancer-relevant functions mediated by αvβ3 (1, 8, 9, 12). These included stimulation of cell proliferation and anti-apoptosis (10). Such properties relied upon activation of signaling pathways [mitogen activated protein kinase [MAPK]/ERK1/2; phosphatidylinositol 3-kinase (PI3K)] that culminated downstream in specific gene transcription (1, 7, 8, 12). In contrast to the genomic actions of T3 that depend on primary intranuclear interactions of T3 with activated TRs, the actions of T4 on cancer cell proliferation, angiogenesis and apoptosis depended on the location of the hormone on the cell surface (1, 8).

Among the genes whose expression is differentially regulated from the plasma membrane and αvβ3 are genes for matrix metalloproteinases, basic fibroblast growth factor (FGF2), hypoxia-inducible factor-1α (HIF-1α), cyclooxygenase-2 (COX-2) and, interestingly, TRα and TRβ; transcription of all of the preceding genes is upregulated by T4 (8). The control of TR gene expression from the cell surface is an example of overlapping non-genomic and genomic effects of thyroid hormone (14). Downregulated is expression of the genes for pro-apoptotic APAF1, CASP3, PMAIP1, and BBC3 (8). The relevance of these genes to cancer cell survival is clear. Tetrac is a naturally occurring derivative of T4 that blocks the binding of T4 (and T3) to the receptor on αvβ3. In the absence of T4, however, tetrac and tetrac that is modified by covalent binding to a nanoparticle or polymer have effects on expression of several hundred genes (12), e.g., angiogenesis-linked vascular endothelial growth factor A (VEGFA), epidermal growth factor receptor (EGFR), cell survival pathway genes *XIAP* and *MCL1* and cell cycle-regulating genes. The latter include genes for multiple cyclins and a cyclin-dependent kinase. Genes relevant to radioresistance and chemoresistance, e.g., p-glycoprotein (*P-gp*) are also affected by tetrac. The expression of all of these genes is decreased by unmodified or modified tetrac, indicating that the modified tetrac compounds have applications as experimental chemotherapeutic agents. Because tetrac is an antagonist of T4, a possible implication of these studies of tetrac is that T4 may be a stimulator of the transcription of this

panel of genes. This possibility has not yet been systematically examined.

BIOACTIVITY OF rT3

The conversion of T4 to rT3, rather than to T3, generates a thyroid hormone analog with no genomic actions. This 5-deiodination process is a function of the action of deiodinase 3 (DIO3, D3) or deiodinase 1 (DIO1, D1) (6). rT3, however, was found a number of years ago to be capable of converting soluble actin to F-actin (20), just as T4 does. In developing mouse cerebellum, astrocytes lacking TRs recover normal actin function with transfection of TRΔα1 (22).

rT3 may also modulate avian lipid metabolism response to epinephrine and steroids (23). The activity of DIO2 (D2) in murine neuroblastoma cells may also be reduced by rT3 (24). Thus, a set of observations in disparate model systems indicates that rT3 has bioactivity. Against this background, we have recently tested rT3 for proliferative activity in glioblastoma cells. We had previously shown that T4 enhances proliferation of several glioma cell lines (25) and that chemically modified tetrac, a T4 antagonist, inhibited the growth of glioblastoma xenografts (26). In the recent studies, the glioblastoma cell line U87MG and two primary cultures of human GBM cells significantly increased their rates of proliferation *in vitro* when exposed to T4, as expected, but also to rT3 (27). These studies must be extended and expanded to include other types of cancer. Confirmation would indicate that conversion of T4 to rT3, rather than to T3, offers cancer cells another thyroid hormone analog support mechanism. Indeed, T3 at physiological concentrations may provide no stimulus to tumor cell proliferation, as a recent clinical study in endstage cancer patients of euthyroid hypothyroxinemia suggests (28). In that study, stabilization or regression of advanced disease was achieved with inhibition of endogenous thyroid hormone production by methimazole and maintenance of the euthyroid state with exogenous T3. Elimination of host T4 production in such patients also minimizes production of rT3.

We can conclude that rT3 has bioactivity and that, possibly, this thyroid hormone analog has proliferative activity on certain cancer cells.

TETRAC AND TRIIODOTHYROACETIC ACID (TRIAC)

In the nucleus, tetrac and triac are thyromimetic (6). Triac has some TRβ-selectivity that has favored its use over tetrac in thyroid hormone-resistant patients to suppress host thyrotropin (TSH) (6), but each agent has been used in this setting. Advantages of the genomic effects of these deaminated derivates of T4 and T3 have also been sought in management of obesity and hyperlipidemia. All such applications involve hormone effects on non-cancer cells.

Because of the heightened expression of αvβ3 in cancer cells, non-genomic actions of tetrac and triac are seen in such cells. Both are anti-proliferative in cancer cells (8). Tetrac has been chemically modified to a nanoparticulate drug (Nanotetrac, NDAT) by covalent coupling to large molecules such as poly-lactic-co-glycolic acid (PLGA) to minimize its access to the intranuclear compartment when the agent is internalized by cells. Tetrac is thyromimetic in the intranuclear compartment (29). Chemically modified tetrac blocks binding of T4 (and T3) to the thyroid hormone receptor on αvβ3, thus eliminating some of the cancer support properties of T4 that were described above. In addition, in the absence of T4, NDAT or tetrac in another formulation in our laboratory in which it is covalently bound to polyethylene glycol (PEG) has actions downstream of the integrin on expression of a large number of cancer-relevant genes (8, 12, 13). The actions are anti-proliferative, pro-apoptotic and anti-angiogenic by multiple mechanisms. Modified tetrac may also impair DNA repair that is important to cancer cell resistance to radiation (30). Finally, by suppressing expression of the *P-gp* gene, modified tetrac may reduce chemoresistance (31), since the plasma membrane P-gp pump exports certain cancer chemotherapeutic drugs (31, 32).

X-irradiation has been shown to activate integrin αvβ3 (18), an effect that is primarily on the β3 monomer and that is thought to contribute to radioresistance (33). This effect is blocked by tetrac (as NDAT).

The actions of triac on cancer cells have been incompletely characterized. It is clear, however, that triac can act at integrin αvβ3 to non-genomically initiate apoptosis in human ovarian cancer cells (34). Triac does not appear to have effects on mitochondria in tumor cells (35). How important genomic effects of triac may be in cancers cells is not known. Triac not surprisingly binds to a genetically modified TRβ that trafficks between cytoplasm and the nucleus in a cancer cell model generated to detect endocrine disrupting chemicals (36), and triac binds to TR in non-cancer cells (37) and has been used clinically to treat certain forms of thyroid hormone resistance because of its facilitated transport across the plasma membrane of normal cells.

It should also be noted that thyroid hormone analogs affect abundance of certain microRNAs (mRNAs) in cancer cells. We have pointed out that Nanotetrac differentially regulates transcription of miR-21 and miR-15A in tumor cells (12, 38) and in so doing is anti-angiogenic and decreases metastatic potential of cancer cells. The effects of T4 on expression of these miRNAs has not yet been examined.

What is apparent is that the thyromimetic properties of deaminated thyroid hormone, particularly tetrac, are importantly overshadowed in cancer cells by its anticancer actions that are expressed via integrin αvβ3. Triac also has anticancer activity at the integrin.

CONCLUSIONS

The existence of integrin αvβ3 on the surface of cancer cells and rapidly dividing endothelial cells provides new insights into the bioactivities of a number of iodothyronines. T4 is a potent

pro-angiogenic hormone with proliferative-enhancing activity in cancer cells, whereas in the context of nuclear TRs, T4 is simply a prohormone for T3. At the thyroid hormone receptor on αvβ3, rT3 may also be biologically active. Finally, deaminated thyroid hormone analogs are weak thyromimetic agents at TRs, but are potent anti-T4 agents in cancer cells and also have a number of anticancer effects that are independent of their activity as blockers of T4 binding to the cell surface receptor.

What about T3 at αvβ3? In higher than physiologic concentrations, T3 can stimulate cancer cell proliferation at the receptor on the integrin (8, 39), but as the induced clinical state of euthyroid hypothyroxinemia in cancer patients suggests, maintenance of euthyroidism exclusively with T3 does not appear to promote tumor growth (28).

The scope of this review is limited by the relatively small number of types of solid tumors that have been studied for possible effects of thyroid hormone analogs. Leukemic cells have not been examined for trophic or antagonistic effects of thyroid hormones. Additional clinical studies are needed of the effects of induction of euthyroid hypothyroxinemia on tumor behavior.

An inference of the discussion of tetrac is the need to test clinically the anticancer efficacy of chemically modified tetrac that has been demonstrated experimentally in cells and in xenografts.

AUTHOR CONTRIBUTIONS

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

FUNDING

The work described here was supported in part by the TMU Research Center of Cancer Translational Medicine from The Featured Area Research Center Program within the framework of the Higher Education Sprout project by the Ministry of Education (MOE) in Taiwan. A grant from NanoPharmaceuticals LLC (Rensselaer, NY, USA) also provided support.

REFERENCES

1. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev.* (2010) 31:139–70. doi: 10.1210/er.2009-0007
2. Vella KR, Hollenberg AN. The actions of thyroid hormone signaling in the nucleus. *Mol Cell Endocrinol.* (2017) 458:127–35. doi: 10.1016/j.mce.2017.03.001
3. Lu C, Cheng SY. Extranuclear signaling of mutated thyroid hormone receptors in promoting metastatic spread in thyroid carcinogenesis. *Steroids* (2011) 76:885–91. doi: 10.1016/j.steroids.2011.03.016
4. Guigon CJ, Cheng SY. Novel non-genomic signaling of thyroid hormone receptors in thyroid carcinogenesis. *Mol Cell Endocrinol.* (2009) 308:63–9. doi: 10.1016/j.mce.2009.01.007
5. Peeters RP, Visser TJ. Metabolism of Thyroid Hormone. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., editors. *Endotext*. South Dartmouth, MA: MDText.com, Inc. (2000)
6. Senese R, Cioffi F, de Lange P, Goglia F, Lanni A. Thyroid: biological actions of "nonclassical" thyroid hormones. *J Endocrinol.* (2014) 221:R1–12. doi: 10.1530/JOE-13-0573
7. Bergh JJ, Lin HY, Lansing L, Mohamed SN, Davis FB, Mousa S, et al. Integrin αVβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* (2005) 146:2864–71. doi: 10.1210/en.2005-0102
8. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol.* (2016) 12:111–21. doi: 10.1038/nrendo.2015.205
9. Mousa SA, Lin HY, Tang HY, Hercbergs A, Luidens MK, Davis PJ. Modulation of angiogenesis by thyroid hormone and hormone analogues: implications for cancer management. *Angiogenesis* (2014) 17:463–9. doi: 10.1007/s10456-014-9418-5
10. Lin HY, Chin YT, Yang YC, Lai HY, Wang-Peng J, Liu LF, et al. Thyroid hormone, cancer, and apoptosis. *Compr Physiol.* (2016) 6:1221–37. doi: 10.1002/cphy.c150035
11. Davis PJ, Sudha T, Lin HY, Mousa SA. Thyroid hormone, hormone analogs, and angiogenesis. *Compr Physiol.* (2015) 6:353–62. doi: 10.1002/cphy.c150011
12. Davis PJ, Glinsky GV, Lin H-Y, Leith JT, Hercbergs A, Tang H-Y, et al. Cancer cell gene expression modulated from plasma membrane integrin αvβ3 by thyroid hormone and nanoparticulate tetrac. *Front Endocrinol.* (2014) 5:240. doi: 10.3389/fendo.2014.00240
13. Glinskii AB, Glinsky GV, Lin HY, Tang HY, Sun M, Davis FB, et al. Modification of survival pathway gene expression in human breast cancer cells by tetraiodothyroacetic acid (tetrac). *Cell Cycle* (2009) 8:3562–70. doi: 10.4161/cc.8.21.9963
14. Hammes SR, Davis PJ. Overlapping nongenomic and genomic actions of thyroid hormone and steroids. *Best Pract Res Clin Endocrinol Metab.* (2015) 29:581–93. doi: 10.1016/j.beem.2015.04.001
15. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer* (2010) 10:9–22. doi: 10.1038/nrc2748
16. Lin HY, Sun M, Lin C, Tang HY, London D, Shih A, et al. Androgen-induced human breast cancer cell proliferation is mediated by discrete mechanisms in estrogen receptor-α-positive and -negative breast cancer cells. *J Steroid Biochem Mol Biol.* (2009) 113:182–8. doi: 10.1016/j.jsbmb.2008.12.010
17. Lin HY, Lansing L, Merillon JM, Davis FB, Tang HY, Shih A, et al. Integrin αVβ3 contains a receptor site for resveratrol. *FASEB J.* (2006) 20:1742–4. doi: 10.1096/fj.06-5743fje
18. Leith JT, Hercbergs A, Kenney S, Mousa SA, Davis PJ. Activation of tumor cell integrin αvβ3 by radiation and reversal of activation by chemically modified tetraiodothyroacetic acid (tetrac). *Endocr Res.* (2018) 43:215–9. doi: 10.1080/07435800.2018.1456550
19. Mousa SS, Davis FB, Davis PJ, Mousa SA. Human platelet aggregation and degranulation is induced *in vitro* by L-thyroxine, but not by 3,5,3'-triiodo-L-thyronine or diiodothyropropionic acid (DITPA). *Clin Appl Thromb Hemost.* (2010) 16:288–93. doi: 10.1177/1076029609348315
20. Leonard JL, Farwell AP. Thyroid hormone-regulated actin polymerization in brain. *Thyroid* (1997) 7:147–51. doi: 10.1089/thy.1997.7.147
21. Davis FB, Mousa SA, O'Connor L, Mohamed S, Lin HY, Cao HJ, et al. Proangiogenic action of thyroid hormone is fibroblast growth factor-dependent and is initiated at the cell surface. *Circ Res.* (2004) 94:1500–6. doi: 10.1161/01.RES.0000130784.90237.4a
22. Flamant F, Samarut J. Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. *Trends Endocrinol Metab.* (2003) 14:85–90. doi: 10.1016/S1043-2760(02)00043-7
23. Bobek S, Sechman A, Niezgoda J, Jacek T. Reverse 3,3',5'-triiodothyronine suppresses increase in free fatty acids in chickens elicited by dexamethasone or adrenaline. *J Vet Med A Physiol Pathol Clin Med.* (2002) 49:121–4. doi: 10.1046/j.1439-0442.2002.00343.x
24. Gavin LA, Moeller M, Shoback D, Cavalieri RR. Reverse T3 and modulators of the calcium messenger system rapidly decrease T4-5'-deiodinase II activity in cultured mouse neuroblastoma cells. *Thyroidology* (1988) 5–12.

25. Davis FB, Tang HY, Shih A, Keating T, Lansing L, Hercbergs A, et al. Acting via a cell surface receptor, thyroid hormone is a growth factor for glioma cells. *Cancer Res.* (2006) 66:7270–5. doi: 10.1158/0008-5472.CAN-05-4365
26. Sudha T, Bharali DJ, Sell S, Darwish NHE, Davis PJ, Mousa SA. Nanoparticulate tetrac inhibits growth and vascularity of glioblastoma xenografts. *Horm. Cancer* (2017) 8:157–65. doi: 10.1007/s12672-017-0293-6
27. Hercbergs A, Mousa SA, Davis PJ. Nonthyroidal illness syndrome and thyroid hormone actions at integrin αvβ3. *J Clin Endocrinol Metab.* (2018) 103:1291–5. doi: 10.1210/jc.2017-01939
28. Hercbergs A, Johnson RE, Ashur-Fabian O, Garfield DH, Davis PJ. Medically induced euthyroid hypothyroxinemia may extend survival in compassionate need cancer patients: an observational study. *Oncologist* (2015) 20:72–6. doi: 10.1634/theoncologist.2014-0308
29. Bharali DJ, Yalcin M, Davis PJ, Mousa SA. Tetraiodothyroacetic acid-conjugated PLGA nanoparticles: a nanomedicine approach to treat drug-resistant breast cancer. *Nanomedicine* (2013) 8:1943–54. doi: 10.2217/nnm.12.200
30. Hercbergs AH, Lin HY, Davis FB, Davis PJ, Leith JT. Radiosensitization and production of DNA double-strand breaks in U87MG brain tumor cells induced by tetraiodothyroacetic acid (tetrac). *Cell Cycle* (2011) 10:352–7. doi: 10.4161/cc.10.2.14641
31. Davis PJ, Incerpi S, Lin HY, Tang HY, Sudha T, Mousa SA. Thyroid hormone and P-glycoprotein in tumor cells. *Biomed Res Int.* (2015) 2015:168427. doi: 10.1155/2015/168427
32. Lin HY, Tang HY, Davis FB, Mousa SA, Incerpi S, Luidens MK, et al. Nongenomic regulation by thyroid hormone of plasma membrane ion and small molecule pumps. *Discov Med.* (2012) 14:199–206.
33. Leith JT, Mousa SA, Hercbergs A, Lin HY, Davis PJ. Radioresistance of cancer cells, integrin αvβ3 and thyroid hormone *Oncotarget* (2018)
34. Shinderman-Maman E, Cohen K, Moskovich D, Hercbergs A, Werner H, Davis PJ, et al. Thyroid hormones derivatives reduce proliferation and induce cell death and DNA damage in ovarian cancer. *Sci Rep.* (2017) 7:16475. doi: 10.1038/s41598-017-16593-x
35. Kvetny J, Bomholt T, Pedersen P, Wilms L, Anthonsen S, Larsen J. Thyroid hormone effect on human mitochondria measured by flow cytometry. *Scand J Clin Lab Invest.* (2009) 69:772–6. doi: 10.3109/00365510903154752
36. Stavreva DA, Varticovski L, Levkova L, George AA, Davis L, Pegoraro G, et al. Novel cell-based assay for detection of thyroid receptor beta-interacting environmental contaminants. *Toxicology* (2016) 368–369:69–79. doi: 10.1016/j.tox.2016.08.012
37. Groeneweg S, Peeters RP, Visser TJ, Visser WE. Triiodothyroacetic acid in health and disease. *J Endocrinol.* (2017) 234:R99–121. doi: 10.1530/JOE-17-0113
38. Mousa SA, Glinsky GV, Lin HY, Ashur-Fabian O, Hercbergs A, Keating KA, et al. Contributions of thyroid hormone to cancer metastasis. *Biomedicines* (2018) 6:E89. doi: 10.3390/biomedicines6030089
39. Lin HY, Landersdorfer CB, London D, Meng R, Lim CU, Lin C, et al. Pharmacodynamic modeling of anti-cancer activity of tetraiodothyroacetic acid in a perfused cell culture system. *PLoS Comput Biol.* (2011) 7:e1001073. doi: 10.1371/journal.pcbi.1001073

Conflict of Interest Statement: Coauthors PJD and SAM have stock holdings in a company that is developing modified tetrac as an anticancer agent and author KAK is a paid consultant of NanoPharmaceuticals LLC.

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

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 08 January 2019
doi: 10.3389/fendo.2018.00775

Tetrac Delayed the Onset of Ocular Melanoma in an Orthotopic Mouse Model

Osnat Ashur-Fabian[1,2], Ofira Zloto[3], Ina Fabian[4], Galya Tsarfaty[5], Martin Ellis[2,6], David M. Steinberg[7], Aleck Hercbergs[8], Paul J. Davis[9,10] and Ido Didi Fabian[3]*

[1] Department of Human Molecular Genetics and Biochemistry, The Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [2] Translational Hemato-Oncology Laboratory, Meir Medical Center, The Hematology Institute and Blood Bank, Kfar-Saba, Israel, [3] Goldschleger Eye Institute, Sheba Medical Center, Affiliated to The Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [4] Department of Cell and Developmental Biology, The Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [5] Department of Diagnostic Imaging, Sheba Medical Center, Ramat Gan, Israel, [6] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [7] Department of Statistics and Operations Research, Tel Aviv University, Tel Aviv, Israel, [8] Department of Radiation Oncology, Cleveland Clinic, Cleveland, OH, United States, [9] Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Albany, NY, United States, [10] Department of Medicine, Albany Medical College, Albany, NY, United States

Ocular melanoma research, the most common primary intraocular malignancy in adults, is hindered by limited *in vivo* models. In a series of experiments using melanoma cells injected intraocularly into mouse eyes, we developed a model for ocular melanoma. Inoculation of 5×10^5 B16F10 cells led to rapid tumor growth, extensive lung metastasis, and limited animal survival, while injection of 10^2 cells was sufficient for intraocular tumors to grow with extended survival. In order to improve tumor visualization, 10^2 melanoma cells (B16F10 or B16LS9) were inoculated into Balb/C albino mouse eyes. These mice developed intraocular tumors that did not metastasize and exhibited extended survival. Next, we studied the therapeutic potential of inhibitor of the thyroid hormones-αvβ3 integrin signaling pathway in ocular melanoma. By utilizing tetraiodothyroacetic acid (tetrac), a thyroid hormone derivative, a delay in tumor onset in the B16F10 (integrin+) arm was observed, compared to the untreated group, while in the B16LS9 cells (integrin–) a similar rate of tumor onset was noticed in both experimental and control groups. In summary, following an optimization process, the mouse ocular melanoma model was developed. The models exhibited an extended therapeutic window and can be utilized as a platform for investigating various drugs and other treatment modalities.

Keywords: melanoma, mouse, αvβ3 integrin, thyroid, tetrac

OPEN ACCESS

Edited by:
Francesco Frasca, Università Degli Studi di Catania, Italy

Reviewed by:
Rosario Le Moli, Università Degli Studi di Catania, Italy
Prathibha Ranganathan, Centre for Human Genetics, India

****Correspondence:***
Osnat Ashur-Fabian
osnataf@gmail.com

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *12 July 2018*
Accepted: *10 December 2018*
Published: *08 January 2019*

Citation:
Ashur-Fabian O, Zloto O, Fabian I, Tsarfaty G, Ellis M, Steinberg DM, Hercbergs A, Davis PJ and Fabian ID (2019) Tetrac Delayed the Onset of Ocular Melanoma in an Orthotopic Mouse Model. Front. Endocrinol. 9:775. doi: 10.3389/fendo.2018.00775

INTRODUCTION

Ocular melanoma is the most common primary intraocular malignancy in adult patients (1, 2). It is estimated that about 50% of patients develop metastatic spread, predominantly to the liver (3, 4). There are no effective treatments and death results in ∼1 year following detection of systemic involvement (5). Although diagnostic and therapeutic tools for the primary ocular tumor have improved significantly over the past 40 years, there has been no change in survival rates (4, 6), emphasizing the need for alternatives to traditional treatments.

Animal models play a significant role in understanding tumor development as well as for developing novel therapeutic approaches in preclinical studies. Efforts have been made to generate ocular melanoma animal models that are suitable for uveal melanoma and its distinctive metastatic behavior (7). However, ocular melanoma research is still hindered by limited *in vivo* models and development is needed of a model that may provide a therapeutic window for preclinical evaluation of experimental treatments.

Thyroid hormones have been shown to influence tumor growth and angiogenesis in a variety of cancer models (8). These effects are attributed to the non-genomic hormonal effects [reviewed in Cheng et al. (9) and Davis et al. (10, 11)]. One of the mechanisms whereby such non-genomic actions may be mediated is via binding of the thyroid hormone to the extracellular domain of integrin αvβ3 (12), a protein which is overexpressed in an array of cancer types and correlates with disease stage (13). Upon binding, thyroid hormone, primarily L-thyroxine (T_4), induces diverse membrane-initiated intracellular activities [reviewed in Davis et al. (11)], including cell proliferations, mainly via the MAPK pathway. Such mitogenic activities have been shown in various types of cancer cells, including glioma (14), breast cancer (15), hepatocarcinoma (16), thyroid cancer (17), sarcoma (18), tumor-associated vascular cells (19), myeloma (20–22) and ovarian cancer (23). We have recently established that hyperthyroidism shortened survival time in a metastatic ocular melanoma mouse model, while hypothyroidism had a significant protective effect (24). Based on these collective results, we hypothesized that natural thyroid hormone derivatives with low-potency thyromimetic activity at the integrin may be utilized for growth inhibition in ocular melanoma. Such analog includes a deaminated form of T_4, tetraiodothyroacetic acid (tetrac), which possess low hormone activity because of shortening of the side chain on the inner ring (removal of a carbon or amine), resulting in the conversion of propionic acid (thyroid hormone) to acetic acid (tetrac). This transforms the compound from thyroid agonists to antagonist (10). Tetrac has low affinity for the nuclear thyroid hormone receptors, through which the classical genomic actions are initiated by the thyroid hormone and is a low-grade thyromimetic in the nucleus (9). Such low-grade thyromimetic genomic effects of tetrac have been shown in various tissues [e.g., (25–27)] and high rates of liver glucuronidation of triac and tetrac have been thought to explain their low bioactivity (26). In contrast, tetrac is an antagonist of T4 actions at the hormone receptor on the extracellular domain of integrin αvβ3 (11). At the cell surface integrin receptor tetrac was shown to displace thyroid hormones binding and to block αvβ3, resulting in reduced cell proliferation, anti-angiogenesis and reduced anti-apoptotic defense pathways activity in multiple cancer models, including mice and human melanoma (28, 29) and reviewed in Davis et al. (11). This antitumor activity of tetrac is initiated at the integrin and chemical modification of tetrac to prevent its nuclear uptake and thus restrict its action to the receptor on αvβ3 heightens the anticancer activity of tetrac via the membrane receptor.

We herein report the development of novel mouse models of ocular melanomas and the effect of a specific thyroid hormone-integrin antagonist on delaying the onset of tumor growth in such models.

MATERIALS AND METHODS

Reagents

Tetrac (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.04 N KOH 4% propylene glycol (PG) solution to a concentration of 1 mg/1 mL.

Cell Lines

B16F10 mouse melanoma cell line (ATCC, CRL-6475) and B16LS9 (a generous gift from Grossniklaus Hans E, MD, Emory Eye Center, Atlanta, GA, USA) were cultured in RPMI 1,640 medium, supplemented with 10% (v/v) heat inactivated fetal calf serum, 2 mM L-glutamine and antibiotics (penicillin/streptomycin), in a humidified atmosphere of 5% CO_2 at 37°C.

Expression of Integrin αvβ3

The expression of αvβ3 integrin on B16F10 or B16Ls9 melanoma cells was quantified using flow-cytometry. In details, the melanoma cells (100,000 cells) were harvested and labeled with 50 μg/mL PE-αv antibodies (Clone RMV-7, Abcam), and FITC-β3 antibodies (Clone HM beta 3.1, Abcam) in 100 mL phosphate-buffered saline (PBS). Following incubation for 15 min at room temperature, the cells were centrifuged, diluted in PBS, and analyzed by a flow cytometer (MACSQuant, Miltenyi Biotec, Bergisch Gladbach, Germany).

Animals

Study animals were wild-type male C57BL/6 or Balb/C mice aged 8 weeks (Harlan Laboratories Ltd, Ein Kerem, Jerusalem). Mice were maintained under specific pathogen-free conditions and housed under controlled conditions (temperature: 20–24°C; humidity: 60–70%). The mice were acclimated to our vivarium for 1 week prior to their use according to study protocols. Up to 6 animals were housed in a cage under conventional conditions and fed chow and water *ad libitum*. All animal procedures and experiments were conducted with approval and under the supervision of the Institutional Animal Care Committee at Tel-Aviv University, and conformed to recommendations of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Experimental Groups and Inoculation of Tumor Cells

For model optimization in the C57Bl/6 mice, the subretinal space (i.e., the choroid) of each mouse's right eye was first inoculated with aliquots of 5×10^5 B16F10 cells ($n = 8$ mice). Next, the same cells were inoculated at decreasing concentration (10^4, 10^3, and 10^2 cells, total 15 mice, $n = 5$ for each concentration). For assessing the Balb/C Albino mice model, the subretinal space of each mouse's right eye was inoculated with aliquots of

10^2B16F10 or B16LS9 cells/1 μL PBS ($n = 5$ for each cell type), using a transconjunctival approach as previously described (30), allowing the inoculated cells to remain in the eye. Mice were anesthetized with a mixture of ketamine and xylazine (120 mg/kg ketamine, 10 mg/kg xylazine), and the experimental eye was desensitized by a drop of oxybuprocaine (Dr. Fischer, Bnei Barak, Israel). Under a dissecting microscope, a 30-gauge needle was inserted ∼1 mm posterior to the limbus through the conjunctiva and sclera and into the subretinal space. The tip of a 10 μL glass syringe with a 32-gauge blunt needle (Hamilton Co., Bonaduz, Switzerland) was introduced into the subretinal space via the needle track, and a 1 μL suspension of tumor cells was then injected into the eyes of the animals. No cells were inoculated until the needle tip was inside the eye, no tumor cell reflux occurred, and the subconjunctival space remained free of tumor cells. For the final interventional study, the subretinal space of each Balb/C Albino mouse's right eye was inoculated with aliquots of 10^2B16F10 or B16LS9 cells in 1 μL PBS. For each experimental model, mice were given drinking water with 35 μg tetrac per day ($n = 16$ mice in the B16F10 model and $n = 16$ in the B16LS9 model), whereas the control group mice were given only polyethylene glycol dissolved in water ($n = 15$ mice in the B16F10 model and $n = 13$ mice in the B16LS9 model). Drinking water was exchanged on daily basis.

Clinical Follow-Up

Mice were checked daily for clinical evidence of intraocular tumor growth. These signs appeared in the form of intraocular bleeding, turbidity, or both. When any of these signs became evident, the mouse was transferred to a separate cage and followed-up until death. The interval between inoculation of tumor cells and death was defined as the survival time. The interval between inoculation of tumor cells and first clinical evidence of intraocular tumor growth was defined as the inoculation-to-tumor time, and the interval between first clinical evidence of intraocular tumor growth to death was defined as the tumor-to-death time. All of these data were recorded and analyzed. We did not observe an effect of the treatments on animal bodyweight (data not shown), an index of lack of toxicity.

Ultrasound and Doppler Measurements

Following general and local anesthesia, as mentioned above, Aquasonic Clear Ultrasound Gel (Medthechnica Healthcare Solutions, Petah-Tikva, Israel) was applied on the mouse's eye baring the intraocular tumor and tumor dimensions were measured and blood flow recorded using an ultrasound probe [Sequoia 512 (Acuson, Mountain View, California, US)] or Vevo 2100 (VisualSonics, Toronto, Canada). Imaging results were analyzed thereafter.

Computed Tomography Scan

Following general anesthesia, as mentioned above, and after injection of Omnipaque™ (iohexol, GE Healthcare, Tel Aviv, Israel) as contrast into the mouse tail vein, mice underwent a CT scan using a TomoScope® Synergy deivce (CT-Imaging, Erlangen, Germany) focusing on the lungs. Serial images were analyzed for the presence of metastases.

Histopathological and Immunohistochemical Studies

The tumor-bearing eyes of all the inoculated mice and lungs of mice from each experimental group were harvested and sent for pathological and immunohistochemical evaluations. Formalin-fixed, paraffin-embedded sections of the collected specimens were hematoxylin and eosin (H&E) stained for histopathologic assessment. For immunostaining, the slides were warmed to 60°C for 60 min, dewaxed in xylene and rehydrated. Hydrogen peroxide (H_2O_2, 3% in PBS) was used to block endogenous peroxidase activity. After being rinsed in PBS, the sections were incubated for 60 min at room temperature with anti-S100 (Z0311, 1:1,000, Dako, Herzliya, Israel), a melanoma marker or anti β3 integrin antibody (ab75872, Abcam, Cambriodge, UK). Detection was performed with Envision+ System-HRP Labeled Polymer Anti-Rabbit (K4003, Dako). The binding antibody was visualized with chromogen AEC substrate (Invitrogen Corporation). Sections were counterstained with hematoxylin and cover-slipped with an aqueous mounting fluid (Glycerol, Dako). The stained sections were reviewed with a light microscope and analyzed by a pathologist.

Statistics

Analysis of the delay in tumor onset was done using the non-parametric logrank test (Mantel-Cox test). Results were considered statistically significant for a $p < 0.05$.

RESULTS

Optimization of Orthotopic Mouse Ocular Melanoma Models

For the generation of an ocular melanoma mouse model, we used the mouse melanoma B16F10 cell line, given its ability to form intraocular tumors (31–34). By injecting 5×10^5 B16F10 cells into the posterior segment of C57Bl/6 murine eyes ($n = 8$), a tumor occupying the entire intraocular cavity was observed within 5–7 days from inoculation (**Figure 1A**). In addition, ultrasound Doppler evaluation showed blood flow within the mass (**Figure 1B**).

After 10 days, eyes were enucleated and sent for pathological processing and H&E staining. **Figure 1C** depicts characteristic melanoma cells behind the lens, between the pigment epithelium and retina in a representative eye specimen.

As the B16F10 cell line is known to metastasize predominantly to the lungs (35), animal CT scans were performed, clearly showing lung metastasis (**Figure 1D**). Macro-metastasis of B16F10 cells, surrounded by typical lung tissue, is shown in dissected lungs from a representative mouse (**Figure 1E**). Pathological processing and H&E staining of the dissected lungs demonstrated aggregates of large epithelioid melanoma cells, surrounded by typical lung tissue (**Figure 1F**). This mouse model in summary showed that intraocular tumor cell growth was rapid and resulted in extensive lung metastasis and limited survival within about 2 weeks. These findings restricted the applicability of this model tor the evaluation of anti-cancer treatments.

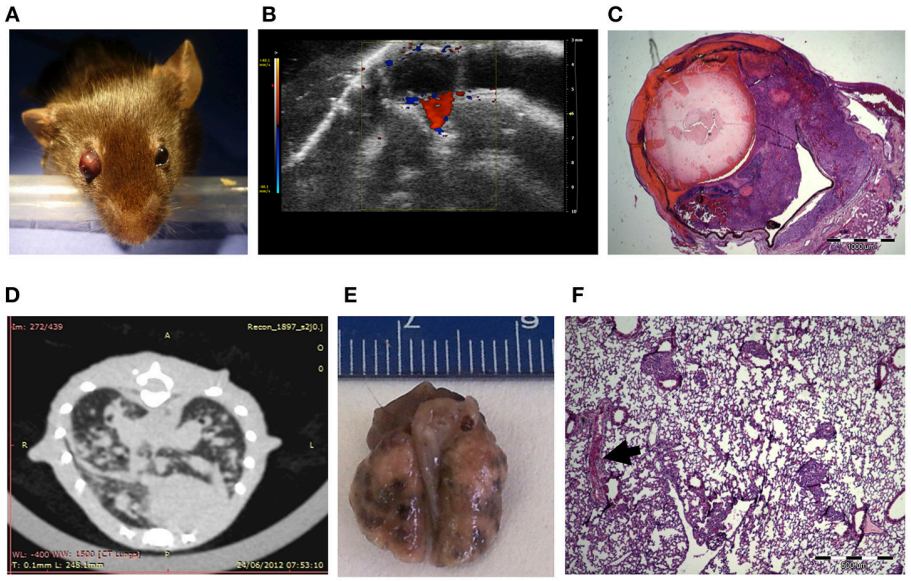

FIGURE 1 | C57Bl/6 mice inoculated with 5×10^5 B16F10 cells **(A)** Buphthalmic (enlarged) right eye filled with tumor. **(B)** Ultrasound Doppler demonstrating blood flow in an intraocular melanoma. **(C)** H&EX2 staining from an enucleated eye. **(D)** CT scan showing lung metastasis. **(E)** Macro metastasis of B16F10 cells in the lung of a representative mouse. **(F)** An aggregate of large epithelioid melanoma cells with expanded cytoplasm, large nuclei and prominent nucleoli within it (arrow), is surrounded by typical lung tissue (H&EX40).

In order to establish a mouse model in which tumor growth rate and metastasis permit a longer therapeutic window, we gradually reduced the intraocular-injected melanoma cell amounts. Aliquots of B16F10 cells were scaled-down to 10^4, 10^3, and eventually 10^2 cell aliquots ($n = 5$ mice in each group) and injected into C57Bl/6 mouse eyes. Based on this preliminary optimization process, the 10^2 cell aliquot was chosen for further use in our experiments because the tumors developed relatively slowly, with a wider therapeutic window. Results indicated that an injection of 10^2 B16F10 cells is sufficient to cause development of a tumor (**Figure 2A**) between 14 and 17 days post inoculation. The enucleated eyes exhibited an intraocular tumor behind the lens (**Figure 2B**). Positive S100 immunostaining confirmed the presence of melanoma cells (**Figure 2C**). Similar to the results with inoculation of 5×10^5 B16F10 cells, in this model, macro metastasis in the lungs were documented by CT (**Figure 2D**) in the dissected lungs (**Figure 2E**), and by H&E staining (**Figure 2F**). Taken together, the inoculation of 10^2 melanoma cells resulted in a mouse model which exhibited slower rate of intraocular tumor growth and metastasis and thus extended the therapeutic window required for pre-clinical anticancer drug evaluations. We have successfully used this model for studying the role of thyroid hormones on ocular melanoma growth (24).

Albino Mouse Ocular Melanoma Model Exhibit Extended Survival and No Tumor Metastasis

One of the limitations of using C57Bl/6 mice as an animal model in this context was its dark eyes, which make it difficult to detect the pigmented intraocular tumors at early stages. We therefore used, as a next step, another mouse strain, the Balb/C albino, which we anticipated would allow better visualization of the intraocular tumors. We repeated the same protocol of inoculating 10^2 melanoma cells into the albino mouse eyes. In the albino mouse studies, an additional mouse melanoma cell line was utilized: B16LS9 ($n = 5$ for each model). While B16F10 cells metastasize primarily to the lungs, the B16LS9 cells are known to spread to the liver when implanted into mice eyes (7, 36). Enlarged eye filled with tumor resulted; starting from 2 weeks following intraocular injections of 10^2 B16F10 cells (**Figure 3A**) or B16LS9 cells (**Figure 3D**). Similarly, the enucleated eyes from both models exhibited intraocular tumor behind the lens, between the pigment epithelium and the retina (**Figures 3B,C,E,F**). Interestingly, inoculation of 10^2 B16F10 or B16LS9 cells in the albino mice did not result in tumor metastasis in the lungs (**Figure 3G**) or liver (**Figure 3H**). These mice continued to thrive for up to 3 months, after which the experiment was terminated, according to our animal ethics protocol.

Tetrac Delayed the Onset of Ocular Melanoma in the Albino Mouse Model

The B16F10 cells serve as a valid platform to examine the thyroid-hormone-αvβ3 axis *in vivo*, due to high expression of this specific integrin (**Supplementary Figures S1A–D**). In contrast, the B16LS9 cells express low levels of αvβ3 (**Supplementary Figures S1E–H**). We have recently established in a B16F10 ocular melanoma model (24) that the hypothyroid environment enhances survival of mice inoculated with the

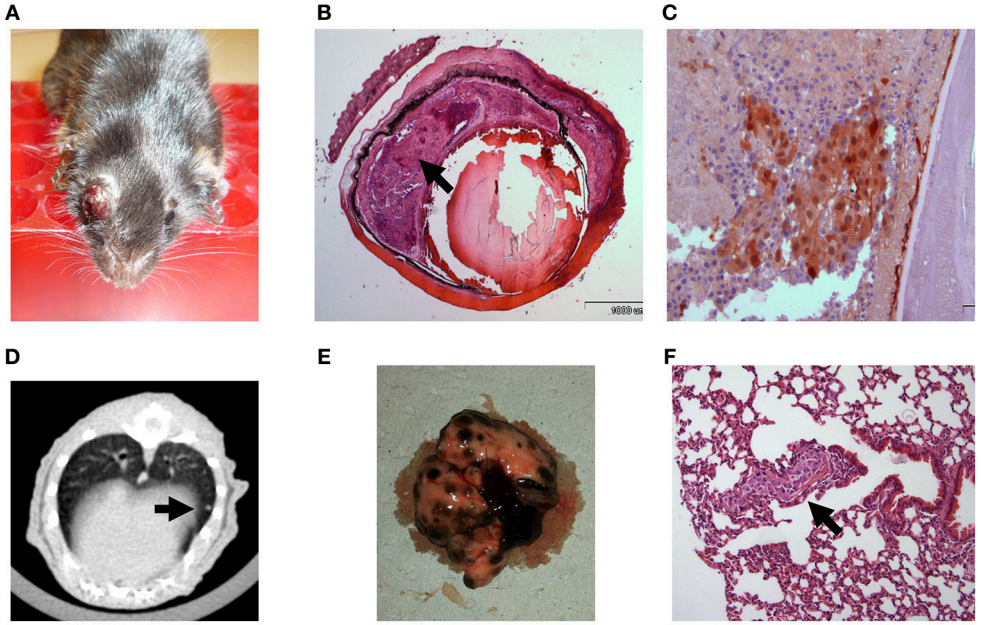

FIGURE 2 | C57Bl/6 mice inoculated with 100 B16F10 cells **(A)** Buphthalmic (enlarged) right eye filled with tumor. **(B)** Enucleated murine eye showing the intraocular tumor located behind the lens, between the pigment epithelium and retina (H&EX2, arrow). **(C)** Tumor cells behind the lens labeled for S100(X20). **(D)** CT scan with lung metastasis **(E)** Macro metastasis in the lungs. **(F)** An aggregate of large epithelioid melanoma cells with expanded cytoplasm, large nuclei and prominent nucleoli within it (arrow), is surrounded by typical lung tissue (H&EX40).

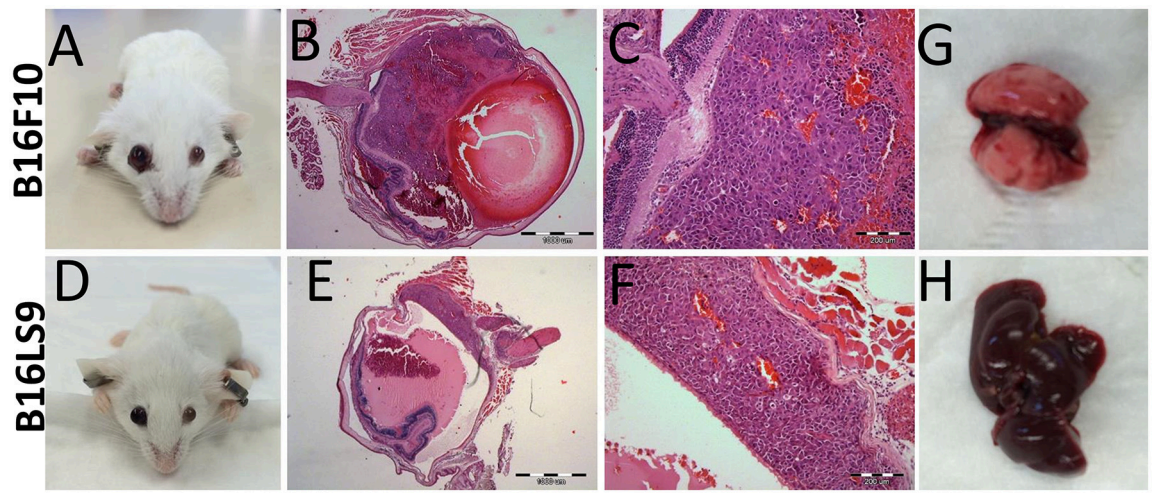

FIGURE 3 | BALB/c White mice inoculated with **(A–C)** 100 B16F10 cells and **(D–F)** 100 B16LS9 cells. **(A,D)** Buphthalmic (enlarged) right eye filled with tumor. **(B,E)** Enucleated murine eye showing the intraocular tumor located behind the lens (H&EX2). **(C,F)** H&EX10 **(G)** Representative lungs from the B16F10 model **(H)** Representative liver from the B16LS9 model.

B16F10 cells, while hyperthyroidism resulted with shorter survival. The unexpected observation that albino mice, when inoculated intraocularly with melanoma cells, do not develop metastasis and exhibit an extended survival, led us to exploit these models to study the potential of thyroid-hormone-αvβ3 inhibitors in delaying the onset of ocular melanoma. One such inhibitor that has been shown in numerous *in vitro* and *in vivo* studies to inhibit thyroid hormone binding to αvβ3 integrin is tetrac and this agent was selected for the next study.

The subretinal space of the right eye of BALB/c mice was inoculated with aliquots of 10^2 B16F10 or B16LS9 cells/1 μL PBS (inoculation day or day 0) using a transconjunctival approach, as previously described (30). There were no

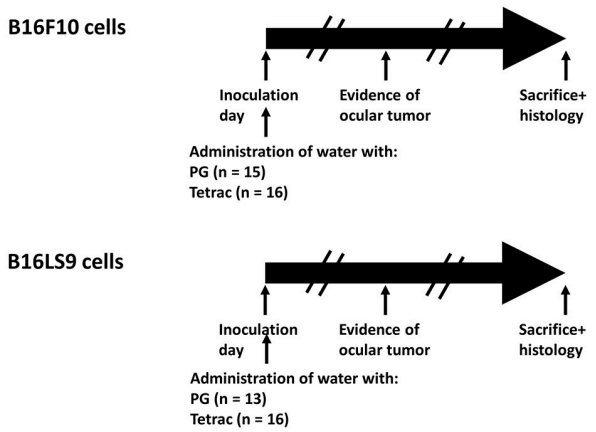

FIGURE 4 | Experimental design. BALB/c mice were inoculated into the right eye subretinal space with aliquots of B16F10 or B16LS9 cells (10^2 cells/1 μL PBS). Each experimental tumor model was divided into mice given tap water with propylene glycol (Control group) or to mice given drinking water containing tetrac (Experimental group). The number of mice in each group is indicated. The mice were monitored on a daily basis and first sign of intraocular tumor growth was recorded. The mice were followed-up and recorded for tumor initiation for 37–40 days.

cases of cell reflux following tumor inoculation and the subconjunctival space remained free of tumor cells. On the same day, each experimental tumor model was divided into mice given tap water (Control group) or drinking water containing tetrac. The mice were monitored on a daily basis and the first sign of intraocular tumor growth was recorded. The experiment design, including the number of mice in each group is indicated in **Figure 4**. The mice were followed-up and recorded for tumor initiation for 37–40 days.

A small proportion of mice from both the B16F10 (**Figure 5A**) and B16LS9 group (**Figure 5B**) were diagnosed with intraocular tumors as early as 2 weeks from inoculation. However, investigating the control groups in both cell lines, tumors were evident in the B16F10 group, as a whole, in a significantly earlier and narrower time frame (15–21 days), as compared to the B16LS9 group (up to 40 days). Results further indicated a delay in tumor onset in the tetrac arm compared to the control group in the B16F10 mice model (Median 24 days to tumor onset versus median of 19 days, respectively). These results reached statistical significance by the logrank test ($p = 0.0195$). In the B16LS9 mice model, both the control and tetrac-treated groups exhibited a similar tumor onset rate.

Mice were sacrificed after 90 days from study initiation, at which point eyes were enucleated and sent for pathological and immunohistochemical processing, including S100 (a melanoma marker) analysis and αvβ3 expression. Results indicate that in both the B16F10 (**Figure 6A**) and the B16LS9 (**Figure 6B**) cell models, the intraocular tumors were positive for S100 immunostaining, confirming the presence of melanoma cells. In accord with the flow-cytometry results, B16F10 (**Figure 6A**), but not B16LS9 (**Figure 6B**), were positively immunostained by the anti-integrin antibody. Lastly, in the B16F10 mice model, tetrac treatment clearly indicated a reduced level of S-100 and integrin staining, suggesting an inhibitory effect on tumor inoculation, growth and integrin expression.

DISCUSSION

The prognosis and survival of patients with ocular melanoma is poor, due to the metastatic nature of the disease and lack of effective therapeutic modalities. Reliable *in vivo* models that can reproduce and mirror human ocular melanoma are essential in order to better understand the disease's characteristics, particularly its metastatic behavior and susceptibility to potential treatment approaches. The significance of results from animal experiments relies on selection of an appropriate animal model. For ocular melanoma, several models exist, consisting of spontaneous, transgenic, and induced models. The latter, compared to the prior two, are easier to handle and are more reproducible (7). The most widely-used induced model in ocular melanoma research is the inoculation model, in which melanoma tumor cells are implanted into the animal's eye, with the mouse being the most widely used species. The small eye size limits the possibility of using routine eye examination. However, due to its cost-effectiveness, rapid reproduction rate, and the fact that 95% of the mouse genome is similar to that of humans, many experiments have been conducted that attempt to replicate in mice the tumor growth and migration behavior of human ocular melanoma. B16 mouse melanoma cell lines are commonly studied and have been successfully inoculated into syngeneic C57BL6 mice eyes (30, 31, 37). A subculture, the B16F10 cell line, which demonstrates high metastatic rate, has also been applied (32, 38–41). In all studies, about 10^5 cells (range: 1–5 $\times$ 10^5 cells) have been injected into the posterior or anterior chambers (31, 32, 34, 38, 42). When such cell numbers were used in our preliminary studies, eyes erupted at about 1 week after inoculation. In addition, tumor cells in these models are highly invasive and the tumor-bearing eye has to be enucleated at 7–14 days post inoculation; mice usually have to be sacrificed shortly after. By optimizing the number of cells required, we have established that an inoculation of 10^2 cells is sufficient to develop an intraocular tumor and to exhibit an extended survival. It has been established that the B16F10 cell line metastasizes predominantly to the lungs (35) and this was confirmed in all of the animals sampled in our study, implying that it was the tumors' systemic spread that eventually killed the mice. In order to improve intraocular tumor visualization, we used Balb/C albino mice which were inoculated with 10^2 B16F10 or B16LS9 melanoma cells. This latter cell line is derived from B16, is liver specific (43) and grows well in the eye (36). It has been previously reported that anterior chamber inoculation of B16LS9 cells in murine eyes results in iris melanoma, but is much less likely to metastasize to the liver than posterior compartment inoculation (44). We have observed that posterior inoculation of 10^2 B16F10 or B16LS9 melanoma cells in the albino mice eye resulted in

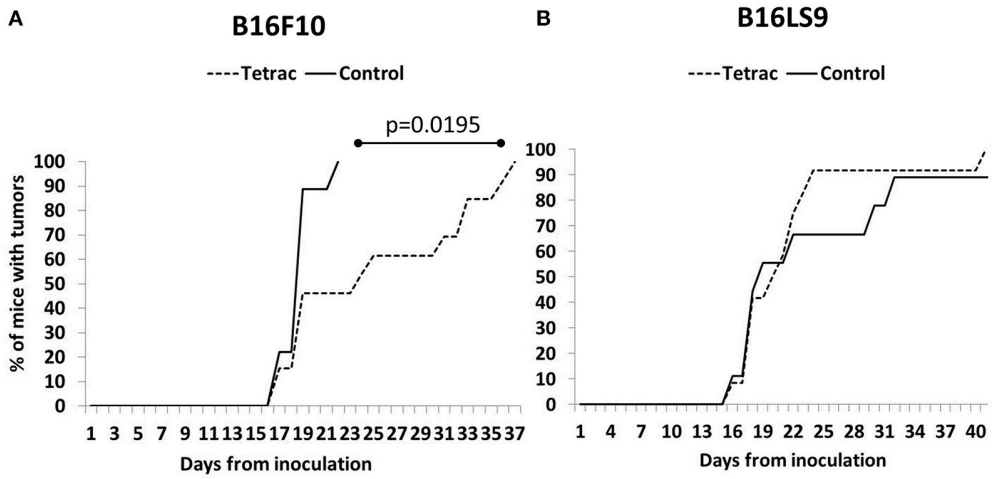

FIGURE 5 | Tetrac delays the onset of ocular melanoma. Mice were inoculated with **(A)** integrin positive cells (B16F10 cells) but not in **(B)** integrin negative model (B16LS9 cells).

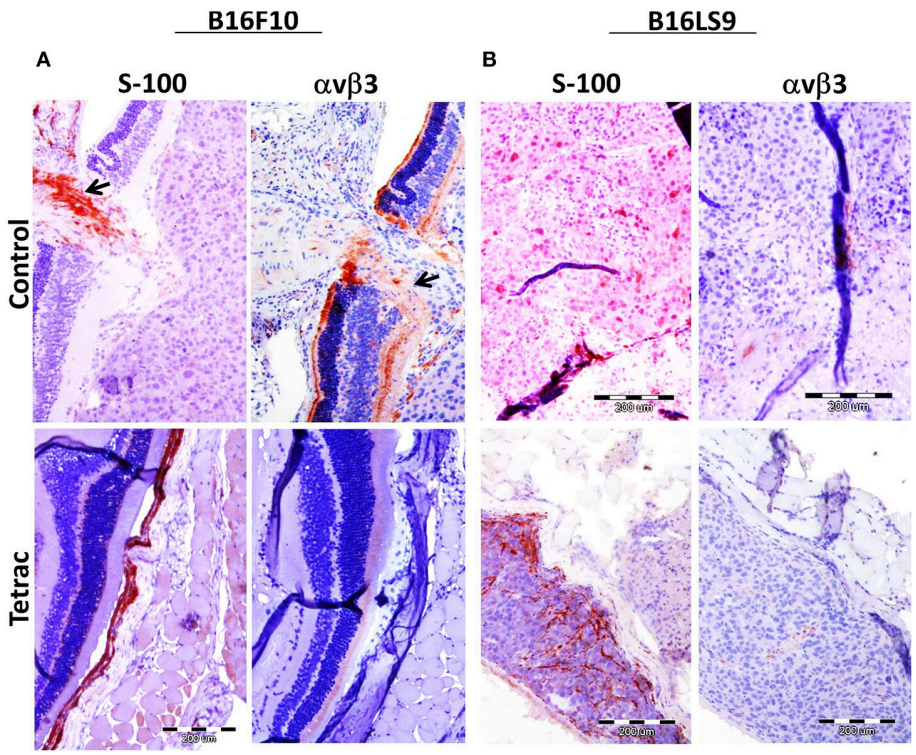

FIGURE 6 | Enucleated murine eye of BALB/c White mice inoculated with **(A)** 100 B16F10 cells and **(B)** 100 B16LS9 cells in control and tetrac treated mice**.** Representative images of the intraocular tumor labeled for S100 (X10) and avb3 integrin (X10) are shown (positive (orange color) stained areas are indicated by arrows).

intraocular tumor behavior which did not metastasize and was associated with extended survival. Additionally, in the B16F10 model group, tumors developed at a significantly earlier stage compared to the time required for the B16LS9-inoculated mice.

The limited efficacy of current treatments in ocular melanoma, highlights the need for more novel treatment approaches (45). Accumulating data suggest that hyperthyroidism may increase the risk of certain non-ocular solid tumors, whereas hypothyroidism may delay disease onset and reduce aggressiveness of cancers (46, 47). We have recently observed a comparable association, both to tumor onset and survival, with states of thyroid function in the ocular melanoma B16F10 mouse

model (24). We have further demonstrated in the same B16F10 melanoma cells as well as in an additional integrin positive human melanoma cell line (Malme-3M), the growth promoting effects of thyroid hormone *in vitro*. These results collectively are attributed to binding of the thyroid hormones to a receptor site on the plasma membrane integrin αvβ3 which may mediate the proliferative action of the hormones on tumor cells (9). The growth-promoting effects by the hormones encouraged us to study the potential of inhibitors of the thyroid hormone-integrin axis in the albino mouse ocular melanoma model. The approach of blocking this specific integrin is highly relevant to ocular melanoma as it is expressed in all tumor subtypes, including spindle, epithelioid, and mixed cell tumors (48). Of note, the B16F10 melanoma cells that were utilized in the present *in vivo* studies, highly express the αvβ3 integrin (24, 49), whereas B16LS9 cells were shown to possess low integrin expression. These findings are important additions for future study design and planning, especially when considering to target the integrin. We have shown that tetrac, a thyroid hormone derivative, clearly delayed tumor onset in mice receiving B16F10 (integrin positive) cells, compared to the untreated group, while in the B16LS9 cells (integrin negative) a similar rate of tumor onset was observed in both groups. Similar pharmacologic targeting of the hormone receptor with a liposomal modified formulation of tetrac was shown in the same B16F10 mice melanoma cells utilized by us (29) as well as in another human melanoma cell line (28). In both cell models tetrac was shown to bind to integrin αvβ3 resulting in reduction in cell proliferation and viability *in vitro* and as well as to reduce tumor growth and metastasis *in vivo*. Delay in tumor growth was observed with tetrac *in vitro* and *in vivo* in an array of tumor types [reviewed in (11)]. A number of laboratories have shown that specific inhibitors of αvβ3 slow growth of these melanoma, cells (50, 51). In contrast to these inhibitors, tetrac acts only at the remarkable thyroid hormone-tetrc receptor on αvβ3 to differentially regulate downstream the expression of a large number of genes related to the cell cycle, apoptosis and other cancer cell survival pathways (52–54). The plasma membrane thyroid hormone-tetrac receptor is exclusively located on integrin αvβ3 and no other alternate receptor was discovered (11). Tetrac binds to the αvβ3 integrin in two orientations and competitively displaces both 3,5,3′-triiodo-L-thyronine (T_3) and T_4, and thus inhibits their tumor-relevant activities (12, 55).

There are several limitations to this study. Although performed in the same manner for all of the experimental groups, the estimation of appearance of tumor after cell inoculation may be subject to error. However, we have used large enough cohorts in order to obtain significant differences between the experimental groups. Another limitation is the use of cutaneous-derived melanoma cells for ocular melanoma studies. However, intraocular inoculated B16 melanoma cells are commonly used to model ocular melanoma, including for evaluation of novel therapeutic approaches (31, 32, 34, 38, 42). For our specific integrin-targeted therapy, the blocking of the αvβ3 integrin, these cells were particularly appropriate, due to a positive high expression of this integrin.

To summarize, we have developed ocular melanoma mice models with an extended therapeutic window that may be exploited for preclinical evaluation of potential drugs. These models enabled us to assess a novel thyroid hormone-αvβ3-integrin targeted therapy, which delayed tumor onset. Tumor biopsies may serve for patient-based therapy following evaluation of the integrin abundance on the tumor cells. This, together with our published results which indicated a beneficial effect of a hypothyroid state on the primary ocular melanoma tumor (24), suggest that this mode of treatment may be administered as soon as the primary tumor is diagnosed.

AUTHOR CONTRIBUTIONS

IDF designed, preformed, analyzed, and interpreted the experimental data. OZ performed the experiments. OA-F designed, analyzed, and interpreted the experimental data. GT performed the ultrasound and doppler measurements. DS performed the statistical analysis. IDF, IF, AH, PD, ME and OA-F wrote the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

IDF was supported by a fellowship from The Dr. Pinchas Borenstein Talpiot Medical Leadership Program 2012.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2018.00775/full#supplementary-material

REFERENCES

1. Oellers P, Karp CL. Management of pigmented conjunctival lesions. *Ocul Surf.* (2012) 10:251–63. doi: 10.1016/j.jtos.2012.08.002
2. Bock F, Maruyama K, Regenfuss B, Hos D, Steven P, Heindl LM, et al. Novel anti (lymph) angiogenic treatment strategies for corneal and ocular surface diseases. *Prog Retin Eye Res.* (2013) 34:89–124. doi: 10.1016/j.preteyeres.2013.01.001
3. Diener-West M, Reynolds S, Agugliaro D, Caldwell R, Cumming K, Earle J, et al. Development of metastatic disease after enrollment in the COMS trials for treatment of choroidal melanoma: collaborative ocular melanoma study group report No. 26. *Arch Ophthalmol.* (2005) 123:1639–43. doi: 10.1001/archopht.123.12.1639
4. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence, treatment, and survival. *Ophthalmology* (2011) 118:1881–5. doi: 10.1016/j.ophtha.2011.01.040
5. Augsburger JJ, Corrêa ZM, Shaikh AH. Effectiveness of treatments for metastatic uveal melanoma. *Am J Ophthalmol.* (2009) 148:119–27. doi: 10.1016/j.ajo.2009.01.023

6. Virgili G, Gatta G, Ciccolallo L, Capocaccia R, Biggeri A, Crocetti E, et al. Survival in patients with uveal melanoma in Europe. *Arch Ophthalmol.* (2008) 126:1413–8. doi: 10.1001/archopht.126.10.1413
7. Cao J, Jager MJ. Animal eye models for uveal melanoma. *Ocul Oncol Pathol.* (2015) 1:141–50. doi: 10.1159/000370152
8. Davis PJ, Lin H-Y, Tang H-Y, Davis FB, Mousa SA. Adjunctive input to the nuclear thyroid hormone receptor from the cell surface receptor for the hormone. *Thyroid* (2013) 23:1503–9. doi: 10.1089/thy.2013.0280
9. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev.* (2010) 31:139–70. doi: 10.1210/er.2009-0007
10. Davis PJ, Davis FB, Mousa SA, Luidens MK, Lin HY. Membrane receptor for thyroid hormone: physiologic and pharmacologic implications. *Annu Rev Pharmacol Toxicol.* (2011) 51:99–115. doi: 10.1146/annurev-pharmtox-010510-100512
11. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol.* (2016) 12:111–21. doi: 10.1038/nrendo.2015.205
12. Bergh JJ, Lin HY, Lansing L, Mohamed SN, Davis FB, Mousa S, et al. Integrin alphaVbeta3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* (2005) 146:2864–71. doi: 10.1210/en.2005-0102
13. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer* (2010) 10:9–22. doi: 10.1038/nrc2748
14. Davis F, Tang H, Shih A, Keating T, Lansing L, Hercbergs A, et al. Acting via a cell surface receptor, thyroid hormone is a growth factor for glioma cells. *Cancer Res.* (2006) 66:7270–5. doi: 10.1158/0008-5472.CAN-05-4365
15. Tang H-Y, Lin H-Y, Zhang S, Davis FB, Davis PJ. Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor. *Endocrinology* (2004) 145:3265–72. doi: 10.1210/en.2004-0308
16. Lin KH, Lin YW, Parkison C, Cheng SY. Stimulation of proliferation by 3,3',5-triiodo-L-thyronine in poorly differentiated human hepatocarcinoma cells overexpressing beta 1 thyroid hormone receptor. *Cancer Lett.* (1994) 85:189–94. doi: 10.1016/0304-3835(94)90274-7
17. Lin H, Tang H, Shih A, Keating T, Cao J, Davis P, et al. Thyroid hormone is a MAPK-dependent growth factor for thyroid cancer cells and is anti-apoptotic. *Steroids* (2007) 72:180–7. doi: 10.1016/j.steroids.2006.11.014
18. Scarlett A, Parsons MP, Hanson PL, Sidhu KK, Milligan TP, Burrin JM. Thyroid hormone stimulation of extracellular signal-regulated kinase and cell proliferation in human osteoblast-like cells is initiated at integrin alphaVbeta3. *J Endocrinol.* (2008) 196:509–17. doi: 10.1677/JOE-07-0344
19. Mousa SA, Davis FB, Mohamed S, Davis PJ, Feng X. Pro-angiogenesis action of thyroid hormone and analogs in a three-dimensional *in vitro* microvascular endothelial sprouting model. *Int Angiol.* (2006) 25:407–13.
20. Cohen K, Ellis M, Khoury S, Davis PJ, Hercbergs A, Ashur-Fabian O. Thyroid hormone is a MAPK-dependent growth factor for human myeloma cells acting via alphavbeta3 integrin. *Mol Cancer Res.* (2011) 9:1385–94. doi: 10.1158/1541-7786.MCR-11-0187
21. Cohen K, Flint N, Shalev S, Erez D, Baharal T, Davis PJ, et al. Thyroid hormone regulates adhesion, migration and matrix metalloproteinase 9 activity via alphavbeta3 integrin in myeloma cells. *Oncotarget* (2014) 5:6312–22. doi: 10.18632/oncotarget.2205
22. Cohen K, Ellis M, Shinderman E, Khoury S, Davis PJ, Hercbergs A, et al. Relevance of the thyroid hormones-alphavbeta3 pathway in primary myeloma bone marrow cells and to bortezomib action. *Leuk Lymphoma* (2015) 56:1107–14. doi: 10.3109/10428194.2014.947612
23. Shinderman-Maman E, Cohen K, Weingarten C, Nabriski D, Twito O, Baraf L, et al. The thyroid hormone-alphavbeta3 integrin axis in ovarian cancer: regulation of gene transcription and MAPK-dependent proliferation. *Oncogene* (2016) 35:1977–87. doi: 10.1038/onc.2015.262
24. Fabian ID, Rosner M, Fabian I, Vishnevskia-Dai V, Zloto O, Shinderman Maman E, et al. Low thyroid hormone levels improve survival in murine model for ocular melanoma. *Oncotarget* (2015) 6:11038–46. doi: 10.18632/oncotarget.3566
25. Halpern J, Hinkle PM. Characterization of thyroid hormone stimulation of uridine uptake by rat pituitary tumor cells. *Endocrinology* (1984) 115:95–101. doi: 10.1210/endo-115-1-95
26. Moreno M, De Lange P, Lombardi A, Silvestri E, Lanni A, Goglia F. Metabolic effects of thyroid hormone derivatives. *Thyroid* (2008) 18:239–53. doi: 10.1089/thy.2007.0248
27. Rutigliano G, Zucchi R. Cardiac actions of thyroid hormone metabolites. *Mol Cell Endocrinol.* (2017) 458:76–81. doi: 10.1016/j.mce.2017.01.003
28. Lee S, Kim J, Shim G, Kim S, Han SE, Kim K, et al. Tetraiodothyroacetic acid-tagged liposomes for enhanced delivery of anticancer drug to tumor tissue via integrin receptor. *J Control Release* (2012) 164:213–20. doi: 10.1016/j.jconrel.2012.05.043
29. Shi S, Zhou M, Li X, Hu M, Li C, Li M, et al. Synergistic active targeting of dually integrin αvβ3/CD44-targeted nanoparticles to B16F10 tumors located at different sites of mouse bodies. *J Control Release* (2016) 235:1–13. doi: 10.1016/j.jconrel.2016.05.050
30. Grossniklaus HE, Barron BC, Wilson MW. Murine model of anterior and posterior ocular melanoma. *Curr Eye Res.* (1995) 14:399–404. doi: 10.3109/02713689508999938
31. Niederkorn JY. Enucleation in consort with immunologic impairment promotes metastasis of intraocular melanomas in mice. *Invest Ophthalmol Vis Sci.* (1984) 25:1080–6. doi: 10.1016/S0161-6420(84)34242-7
32. Yang H, Dithmar S, Grossniklaus HE. Interferon α2b decreases hepatic micrometastasis in a murine model of ocular melanoma by activation of intrinsic hepatic natural killer cells. *Invest Ophthalmol Vis Sci.* (2004) 45:2056–64. doi: 10.1167/iovs.03-1331
33. Ly LV, Baghat A, Versluis M, Jordanova ES, Luyten GP, Van Rooijen N, et al. In aged mice, outgrowth of intraocular melanoma depends on proangiogenic M2-type macrophages. *J Immunol.* (2010) 185:3481–8. doi: 10.4049/jimmunol.0903479
34. De Lange J, Ly L, Lodder K, Verlaan-De Vries M, Teunisse A, Jager M, et al. Synergistic growth inhibition based on small-molecule p53 activation as treatment for intraocular melanoma. *Oncogene* (2012) 31:1105–16. doi: 10.1038/onc.2011.309
35. Harning R, Szalay J. Ocular metastasis of *in vivo* and *in vitro* derived syngeneic murine melanoma. *Invest Ophthalmol Vis Sci.* (1987) 28:1599–604.
36. Diaz CE, Rusciano D, Dithmar S, Grossniklaus HE. B16LS9 melanoma cells spread to the liver from the murine ocular posterior compartment (PC). *Curr Eye Res.* (1999) 18:125–9. doi: 10.1076/ceyr.18.2.125.5380
37. Knisely T, Niederkorn J. Immunologic evaluation of spontaneous regression of an intraocular murine melanoma. *Invest Ophthalmol Vis Sci.* (1990) 31:247–57.
38. Dithmar S, Rusciano D, Grossniklaus H. A new technique for implantation of tissue culture melanoma cells in a murine model of metastatic ocular melanoma. *Melanoma Res.* (2000) 10:2–8. doi: 10.1097/00008390-200002000-00001
39. Yang H, Grossniklaus HE. Combined immunologic and anti-angiogenic therapy reduces hepatic micrometastases in a murine ocular melanoma model. *Curr Eye Res.* (2006) 31:557–62. doi: 10.1080/02713680600718962
40. Yang H, Grossniklaus HE. Constitutive overexpression of pigment epithelium–derived factor inhibition of ocular melanoma growth and metastasis. *Invest Ophthalmol Vis Sci.* (2010) 51:28–34. doi: 10.1167/iovs.09-4138
41. Zhang Q, Yang H, Kang SJ, Wang Y, Wang GD, Coulthard T, et al. *in vivo* high-frequency, contrast-enhanced ultrasonography of uveal melanoma in mice: imaging features and histopathologic correlations. *Invest Ophthalmol Vis Sci.* (2011) 52:2662–8. doi: 10.1167/iovs.10-6794
42. Crosby MB, Yang H, Gao W, Zhang L, Grossniklaus HE. Serum vascular endothelial growth factor (VEGF) levels correlate with number and location of micrometastases in a murine model of uveal melanoma. *Br J Ophthalmol.* (2010) 95:112–7. doi: 10.1136/bjo.2010.182402
43. Rusciano D, Lorenzoni P, Burger M. Murine models of liver metastasis. *Invasion Metastasis* (1994) 14:349–61.
44. Grossniklaus H, Wilson M, Barron B, Lynn M. Anterior versus posterior intraocular melanoma metastatic differences in a murine model. *Arch Ophthalmol.* (1996) 114:1116–20.
45. Buder K, Gesierich A, Gelbrich G, Goebeler M. Systemic treatment of metastatic uveal melanoma: review of literature and future perspectives. *Cancer Med.* (2013) 2:674–86. doi: 10.1002/cam4.133

46. Hercbergs AH, Ashur-Fabian O, Garfield D. Thyroid hormones and cancer: clinical studies of hypothyroidism in oncology. *Curr Opin Endocrinol Diabetes Obes.* (2010) 17:432–6. doi: 10.1097/MED.0b013e32833d9710
47. Moeller LC, Fuhrer D. Thyroid hormone, thyroid hormone receptors, and cancer: a clinical perspective. *Endocr Relat Cancer* (2013) 20:R19–29. doi: 10.1530/ERC-12-0219
48. Elshaw SR, Sisley K, Cross N, Murray AK, Macneil SM, Wagner M, et al. A comparison of ocular melanocyte and uveal melanoma cell invasion and the implication of α1β1, α4β1 and α6β1 integrins. *Br J Ophthalmol.* (2001) 85:732–8. doi: 10.1136/bjo.85.6.732
49. Dahl KDC, Robertson SE, Weaver VM, Simon MC. Hypoxia-inducible factor regulates αvβ3 integrin cell surface expression. *Mol Biol Cell* (2005) 16:1901–12. doi: 10.1091/mbc.e04-12-1082
50. Kim Y-S, Li F, Kong R, Bai Y, Li KC, Fan Y, et al. Multivalency of non-peptide integrin αvβ3 antagonist slows tumor growth. *Mol Pharm.* (2013) 10:3603–11. doi: 10.1021/mp400096z
51. Pickarski M, Gleason A, Bednar B, Duong LT. Orally active αvβ3 integrin inhibitor MK-0429 reduces melanoma metastasis. *Oncol Rep.* (2015) 33:2737–45. doi: 10.3892/or.2015.3910
52. Glinskii AB, Glinsky GV, Lin HY, Tang HY, Sun M, Davis FB, et al. Modification of survival pathway gene expression in human breast cancer cells by tetraiodothyroacetic acid (tetrac). *Cell Cycle* (2009) 8:3554–62. doi: 10.4161/cc.8.21.9963
53. Davis PJ, Glinsky GV, Lin HY, Leith JT, Hercbergs A, Tang HY, et al. Cancer cell gene expression modulated from plasma membrane integrin alphavbeta3 by thyroid hormone and nanoparticulate tetrac. *Front Endocrinol.* (2015) 5:240. doi: 10.3389/fendo.2014.00240
54. Lin HY, Glinsky GV, Mousa SA, Davis PJ. Thyroid hormone and anti-apoptosis in tumor cells. *Oncotarget* (2015) 6:14735–43. doi: 10.18632/oncotarget.4023
55. Freindorf M, Furlani TR, Kong J, Cody V, Davis FB, Davis PJ. Combined QM/MM study of thyroid and steroid hormone analogue interactions with alphavbeta3 integrin. *J Biomed Biotechnol.* (2012) 2012:959057. doi: 10.1155/2012/959057

Conflict of Interest Statement: PD is stockholder and officer in a company developing modified forms of tetrac as anticancer agents.

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

frontiers in Endocrinology

REVIEW
published: 13 February 2019
doi: 10.3389/fendo.2019.00063

Non-genomic Actions of Thyroid Hormones Regulate the Growth and Angiogenesis of T Cell Lymphomas

Florencia Cayrol[1], *Helena A. Sterle*[1], *Maria Celeste Díaz Flaqué*[1], *Maria Laura Barreiro Arcos*[1] *and Graciela A. Cremaschi*[1,2]*

[1] *Instituto de Investigaciones Biomédicas, Consejo Nacional de Investigaciones Científicas y Técnicas, Facultad de Ciencias Médicas, Pontificia Universidad Católica Argentina, Buenos Aires, Argentina,* [2] *Laboratorio de Radioisótopos, Cátedra de Física, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina*

T-cell lymphomas (TCL) are a heterogeneous group of aggressive clinical lymphoproliferative disorders with considerable clinical, morphological, immunophenotypic, and genetic variation, including ∼10–15% of all lymphoid neoplasms. Several evidences indicate an important role of the non-neoplastic microenvironment in promoting both tumor growth and dissemination in T cell malignancies. Thus, dysregulation of integrin expression and activity is associated with TCL survival and proliferation. We found that thyroid hormones acting via the integrin αvβ3 receptor are crucial factors in tumor microenvironment (TME) affecting the pathophysiology of TCL cells. Specifically, TH-activated αvβ3 integrin signaling promoted TCL proliferation and induced and an angiogenic program via the up-regulation of the vascular endothelial growth factor (VEGF). This was observed both on different TCL cell lines representing the different subtypes of human hematological malignancy, and in preclinical models of TCL tumors xenotransplanted in immunodeficient mice as well. Moreover, development of solid tumors by inoculation of murine TCLs in syngeneic hyperthyroid mice, showed increased tumor growth along with increased expression of cell cycle regulators. The genomic or pharmacological inhibition of integrin αvβ3 decreased VEGF production, induced TCL cell death and decreased *in vivo* tumor growth and angiogenesis. Here, we review the non-genomic actions of THs on TCL regulation and their contribution to TCL development and evolution. These actions not only provide novel new insights on the endocrine modulation of TCL, but also provide a potential molecular target for its treatment.

Keywords: VEGF, proliferation, angiogenesis, integrin αvβ3, thyroid hormones, T-cell lymphoma

OPEN ACCESS

Edited by:
Sandra Incerpi,
Università degli Studi Roma Tre, Italy

Reviewed by:
Jean-Yves Scoazec,
Institut Gustave Roussy, France
Dragana Nikitovic,
University of Crete, Greece

***Correspondence:**
Graciela A. Cremaschi
gacremaschi@gmail.com;
graciela_cremaschi@uca.edu.ar

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *15 October 2018*
Accepted: *23 January 2019*
Published: *13 February 2019*

Citation:
Cayrol F, Sterle HA, Díaz Flaqué MC, Barreiro Arcos ML and Cremaschi GA (2019) Non-genomic Actions of Thyroid Hormones Regulate the Growth and Angiogenesis of T Cell Lymphomas. Front. Endocrinol. 10:63. doi: 10.3389/fendo.2019.00063

INTRODUCTION

Thyroid hormones (THs), triiodothyronine (T3), and thyroxine (T4), are involved in different biological processes as cell growth, development, differentiation, and the regulation of metabolism and homeostasis (1). The classical mechanism of action of THs is mediated by the binding of T3 to nuclear receptors (TR) that interact with specific responding elements (TREs) in the promoters of target genes. The binding of T3 to TRs promotes a conformational change that induces the

exchange of corepressors for coactivators, thus leading to gene transcription on responsive genes (2, 3). THs can also trigger their actions by a non-classical mechanism that does not implicate direct gene transcription regulation by nuclear TRs. These non-genomic actions indirectly modulate gene transcription through the activation of intracellular pathways and other transcription factors (3, 4). Despite many of the non-genomic actions have been demonstrated to be initiated by THs through the activation of a membrane receptor (mTR), they can also be initiated at receptors located in the mitochondria or cytoplasm (5).

In the last years, several studies have identified the integrin αvβ3 as the membrane receptor for THs in normal tissues as blood vessels and heart (5); but also in several types of cancer cells (4, 6–9). Integrin αvβ3 is a member of a large group of heterodimeric transmembrane receptors that regulate cell-cell and cell-extracellular matrix (ECM) interactions and enable cells to respond to their environment (10). Several studies related to cancer have implicated the activity of this group of adhesion receptors in the proliferation, migration, and survival of different types of tumor cells (11). Many aspects of the cellular microenvironment, like the composition and structure of the ECM, the signals generated by growth factors or the stimulation of cytokine secretion are regulated by integrins (12, 13). Particularly, integrin αvβ3 mediates the interaction between the cells and the ECM as a result of its binding to plasmatic and ECM ligands that express the peptide sequence RGD (Arginine–Glycine–Aspartate) (14). Interestingly this integrin is highly expressed in proliferating cells, like malignant cancer cells and cells from the endothelial and vascular smooth muscle (14).

It is well-known that the growth, invasiveness, and dissemination of a tumor are highly associated with angiogenesis. In recent studies, our group demonstrated that the interaction of THs with integrin αvβ3 triggers intracellular pathways in T-cell lymphoma (TCL) cells. This further activates transcription factors, thus stimulating gene transcription and the production of angiogenic factors (15). Therefore, the expression of integrin αvβ3 in tumor cells and their vascular network could explain the proangiogenic and proliferative effects of THs on different cancers, including gliomas (9), breast (4), thyroid (6, 8), and renal cancer (7), among others.

In this review, we will focus on the role of integrin αvβ3 as the membrane receptor for THs and how its activation induces the proliferation and survival of different types of cancer cells. Specifically, we will discuss the influence of THs non-genomic actions through integrin αvβ3 activation on TCL malignant phenotype, and the inhibition of this receptor as a potential clinical target.

ROLE OF INTEGRIN αVβ3 IN CANCER AND ANGIOGENESIS

Integrins and Cancer

Despite integrins were initially described as cell adhesion receptors, current studies highlight the idea that these receptors have essential roles in cancer. In fact, one of the well-known mechanisms of cancer is the abnormal function of integrin receptors (16). Cancer is a complex disease and its progression is deeply related with the dynamically evolving extracellular matrix that regulates many aspects of the tumor and tumor-associated cells (16). Integrin bi-directional signaling is essential to sense, modulate, and respond to changes in extracellular stimuli (17). The signal transduction mediated by these receptors usually occurs through direct or indirect interactions between the cytoplasmic domain of the integrin and intracellular effectors, which occasionally can be supported by the interactions with other cell surface proteins that are associated to integrins (14). For example, it has been reported that caveolin is required for the association between Src-family kinases and β1 integrins; moreover the loss of this association results in the loss of FAK phosphorylation induction and the correct development of focal adhesion sites (18). Tetraspanins, on the other hand, are essential for rapid cell migration mediated by α3β1, α6β1, α6β4, and α7β1 integrins, making these integrin partners potential antimetastatic targets (19). In cancer cells, FAK and Src are two of the best-studied integrin-mediated signaling effectors. Different types of solid tumors, including pancreatic, colon, and breast cancers, show high expression and activation of FAK and Src, thus contributing to the progression and the malignant phenotype of these pathologies (20–22). Inhibition of FAK and Src signaling reduces tumorigenic and metastatic potential of breast cancer cells (23). When integrin-mediated cell adhesion occurs, FAK is activated by autophosphorylation, generating a high-affinity binding site for the SH2 domain of Src. These activated FAK/Src complexes are the link between integrins and the downstream signaling effectors such Rac1 GTPase or the MAPKs (24). The interaction of integrins and their ligands, and the consequent activation of these complexes and the intracellular pathways, can influence cancer cells behavior by increasing cell proliferation, survival, and gene expression; therefore contributing to tumor growth and metastasis (24). All these findings point out the mentioned pathways as potential therapeutic targets in different types of cancer (23, 24).

Most solid tumors are originated from epithelial cells that are conferred with the ability to resist apoptosis, migrate, and disseminate through the epithelial-mesenchymal transition (EMT) (25). This process involves the remodeling of the ECM and changes in the interactions of cells with the ECM (26). Many integrins that are expressed by epithelial cells are retained in the tumor, but their levels and physiologic functions may be altered. Integrins α6β4, α6β1, αvβ5, α2β1, and α3β1, regulate the adhesion of epithelial cells to the basement membrane, however, in tumor cells they might involve and contribute to cell migration, proliferation and survival (11). However, during the differentiation into mesenchymal cells some epithelial integrins are downregulated and the expression of other integrins with key roles in EMT progression and tumor invasiveness are activated (24, 26). For example, the expression of α6β4 integrin is down-regulated during EMT in the mammary gland through the epigenetically silencing of the gene encoding β4 integrin (27). Also in mammary epithelial cells, enhanced expression of integrin αvβ3 is required for TGF-β-induced EMT (28). Likewise, α3β1, α5β1, α1β1, and α2β1 integrins are overexpressed

in different stages of EMT (24, 29). Indeed, the expression of many integrin subunits, including α3, α5, α6, αv, β1, β3, and β4 in different types of cancer cells, has been linked to their invasive and metastatic potential (30). The expression of integrins αvβ3, α5β1, and αvβ6 are normally low or undetectable in most adult epithelia but in some tumors their protein levels are overexpressed (11). Elevated αvβ6 integrin levels are associated with fibrosis and cancer in lungs, skin and along the gastrointestinal tract (31). After its activation, α2β1 integrin promotes cell adhesion, proliferation and invasion in liver and lung metastasis (32). In prostate cancer (PCa) integrin α2β1 is overexpressed and its phosphorylation and consequent activation have been associated with the progression of this pathology (33). Also, integrin αvβ3 has been reported to contribute to PCa progression by promoting angiogenesis, survival, and invasion (34, 35). The overexpression of integrin αvβ3 in primary head and neck squamous carcinoma and metastatic lymph nodes was related to lymph node metastasis and worse prognosis (36). In breast cancer, the levels of integrin α6β4 and αvβ3 correlate with tumor size, grade and decreased survival (37, 38). The overexpression of integrin αvβ3 is also involved in the switch from a non-tumorigenic state of melanoma to a tumorigenic and invasive one (10) and increased bone metastasis in prostate cancer (39).

It is well-known that integrins are able to synergistically interact with cytokine receptors and growth factors, thus mediating some features of cancer progression as cell migration, invasion, and survival. In the last years, it has been described that integrin N-glycosylation is essential for integrin heterodimerization and interaction with ligands (16, 40, 41). Currently, several published works indicate that N-glycan alterations on integrin subunits influence their affinity for their ligands, thus contributing to the malignant phenotype. These studies propose the targeting of 1,6-GlcNAc structures, sialic acid, and fucose and their related enzymes, in combination with the inhibition of integrins, represent a promising new therapeutic approach (16).

Mainly two therapeutic strategies based on integrin target were developed in the last decades: inhibition of integrin function and the use of integrin expression patterns for drug delivery (42). The direct inhibition of integrin function with synthetic peptides and humanized antibodies, among others, has so far be the main therapeutic strategy in the clinic and until now is the only form of anti-integrin treatment shown to work in patients (43). The antibodies abituzumab, intetumumab, and the small molecule, cilengitide, are the most advanced molecules studied in clinical trials for the treatment of different types of cancer (44). Despite the promising preclinical results observed, poor efficacy was obtained in late-phase clinical trials (16). The problem in translating the preclinical data of anti-integrin therapies to the clinic, especially in cancer, would be related to the poor knowledge of integrin biology. For example, the profile and distribution of many integrins in normal and pathological tissues from cancer patients is somehow hard to achieve as there is a lack of good antibodies for integrin staining in formalin-fixed-paraffin embedded tissues. The use of integrins as biomarkers could improve the efficacy of anti-integrin cancer treatment (44).

In summary, if we improve the skills for the identification of integrins in patient samples and increases our knowledge on other integrin characteristics, as the internalization and intracellular trafficking response in the oncology process, new effective, and safe therapies would be generated.

Integrins and Tumor Microenvironment

The transformed cells are not capable of generating tumors with metastatic potential by themselves; this process requires a permissive tumor microenvironment (TME) that might be crucial for tumor progression. Recent works have begun to focus more deeply on the study of non-tumor cell components of the stroma and their involvement in the malignant progression (45). The TME include many host cell types, including fibroblasts, endothelial, perivascular, and inflammatory cells, that in some cases can contribute to tumor progression through different processes like angiogenesis, lymphangiogenesis or inflammation. Examples of tumor-associated stromal cells are tumor or cancer-associated fibroblasts (TAFs or CAFs) and tumor-associated macrophages (TAMs) (25, 45, 46). Reciprocal communication between cancer cells and these non-tumoral cells is essential and leads to high proliferation and metastatic capability of the tumor.

Integrins can bidirectionally transduce signals across the cell membrane, (24). The "outside-in" signaling is triggered by chemical or mechanical alterations in the ECM. The interaction of the integrin extracellular head domain with the ECM ligand or divalent cations induces integrin clustering and conformational rearrangements of the cytoplasmic tail that lead to the activation of several signaling pathways that regulate gene transcription and cell shape, survival and migration (47). The "inside-out" signaling, on the other hand, is triggered by a cytoplasmic signal that can alter the integrins' affinity for extracellular ligands (48, 49). These mechanisms are essential for the communication of the cells with their microenvironment and regulate many important biological functions including cell proliferation, survival, and motility. The tumor cells use these same processes to acquire invasive and oncogenic survival properties and to orchestrate changes in the host microenvironment that lead to tumor growth and metastatic dissemination (17).

Additionally to their role in malignant cells, integrins expression on tumor-associated host cells can profoundly influence in the malignant potential of a tumor (17, 50). Integrins are expressed on all the cell types that compose the TME, and modulate functions of both, tumor and stromal cells, that promote the communication between different cell types of the TME, leading to tumor growth and malignant progression (50). For example, integrin α9β1 regulates the signaling that increases tumor growth and lymphatic metastasis via the recruitment of TAFs in breast cancer cells (51). In gastric cancer, C-X-C motif chemokine 12 (CXCL12) derived from CAFs promotes cell invasion by enhancing the clustering of integrin β1 in gastric cancer cells (52). Dr. Cress group demonstrated that the cleavage of integrin α6β1 by the serine protease urokinase plasminogen activator (uPA) induces tumor cell motility, invasion, and metastasis in a xenograft model of PCa cells placed within the living bone matrix (53). The same group described later

that TAMs stimulate the production of uPA inside the tumor, resulting in α6β1 integrin cleavage in PCa cells (54).

The capacity of integrins to regulate cell adhesion and migration alone is enough to drive invasion. Tumor cells must break the ECM barriers to metastasize to a distant organ; this process requires not only the degradation and remodeling of ECM, but it can also involve ECM stiffening. For example, in human breast carcinoma, collagen fibers become bundled and align perpendicularly to the basement membrane, thus converting into tracks for cells to migrate (55). Likewise, in pancreatic ductal adenocarcinoma, increased collagen thickness and matricellular fibrosis in response to elevated β1-integrin mechano-transduction was related to a more aggressive pathology (17). ECM degradation and remodeling is carried out by several proteases. It has been shown that integrins can modulate the expression levels and the activity of those proteases, in particular matrix metalloproteinases (MMPs) and the uPA system (56). The ability to regulate matrix organization and remodeling is a critically important function of integrins (24). For example, the interaction between MMPs and integrin β2 is required for leukocyte migration, and the combined participation of MMPs and other integrins is also necessary for tumor metastasis (56).

The levels of MMPs are always elevated in the presence of tumors (57). The expression of MMP gene can be up-regulated by integrin signaling pathways (58). It has been reported in different studies that integrins αv and β1 are able to increase the levels of several MMPs. It was demonstrated that integrin αvβ6 increases the expression levels of MMPs in oral, ovarian and colon cancers (59–61). In oral squamous cell carcinoma (SCC), the increment of integrin αvβ6 expression activates MMP-3, thus promoting oral SCC cell proliferation and metastasis *in vivo* (61); on the other side, integrin β1 promotes invasion and migration of SCC cells vía MMP7 (62). In ovarian cancer cells, high levels of integrin αvβ6 correlate with an augment of the expression and secretion of pro-MMP-2, pro-MMP-9 and high molecular weight uPA, thus increasing ECM degradation (59).

One of the characteristics that is important to consider is the physical location of MMPs because this dictates their biological functions and is critical for tumor progression. The localization of several MMPs in cell membrane through the interaction with integrins has been demonstrated; one example is the binding of MMP-2 to αvβ3 or MMP-9 to αVβ6 (56, 63). MMP-9 expression levels were found to be increased in colon cancer metastasis to liver, and this metalloproteinases co-localized with integrin αVβ6 at the invading border of the tumor (63). Consequently, integrins have a critical role in TME impact on tumor invasion and spreading.

Integrin αvβ3 and Angiogenesis

Angiogenesis is the formation of new blood vessels from pre-existing ones. Even though it is a fundamental physiological event, in certain situations angiogenesis can also be negative; the formation of new blood vessels contributes to the progression of several pathologies and is crucial in tumor growth and metastasis. Consequently, angiogenesis is essential for the growth, spreading and infiltration of malignant cells within tissues (64). In the beginning, tumors can proliferate and survive by taking advantage of the available vessel of their host and surroundings; nevertheless, malignant cells can become hypoxic if they are too far away from the oxygen and nutrients of those vessels (65). In response to hypoxia tumor cells are able to create new blood vessels to fulfill their metabolic needs.

Tumor angiogenesis depends on ECM disruption, the migratory ability of endothelial cells (ECs) and their adhesion to integrins. As we have already mentioned, integrins are expressed on ECs, lymphatic endothelial cells and pericytes (66) and for this reason, they have been pointed out as important players in cancer angiogenesis (11). They are involved in tumor angiogenesis by interacting with both axis that regulate the maturation and plasticity of the new vessels: the pathway of vascular endothelial growth factor (VEGF) and its receptor (VEGFR) (67) and that of angiopoietins and Tie receptors (ANG-Tie).

Among all integrins, αvβ3 has been thoroughly studied for its localized expression in neovasculature and in aggressive tumors (68). The membrane receptor integrin αvβ3 recognizes ECM proteins expressing the RGD peptide sequence. Despite the expression levels are low in resting endothelial cells and normal organ systems, integrin αvβ3 is highly expressed on activated tumor endothelial cells (11). The latter, makes this integrin an appropriate target for antiangiogenic therapeutics. Moreover, integrin αvβ3 is also express on tumor cells, thus both tumor cells and tumor vasculature can be target by anti-integrin therapy.

It was described that only 20% of integrin αv-null mice survive until birth, and that 100% die within the 1st day of birth (69). These mice develop intracerebral hemorrhage due to the defective interactions between blood vessels and brain parenchymal cells (70). On the other side, the β3 integrin-null mice can survive and apparently develop a normal vascular network (71). Furthermore, no integrin β3 protein levels are detected in quiescent blood vessels, but its expression increases during sprouting angiogenesis (72).

One of the roles of integrin αvβ3 during angiogenesis is to bind and activate MMP-2 on new blood vessels to disrupt ECM and facilitate tumor cell migration and infiltration (64). A cooperative action between activated integrin αvβ3 in tumor cells and platelets, that promotes extravasation and metastasis, has also been reported (73). Integrin αvβ3 also participates in the angiogenic switch. This process is referred the time during tumor progression where the balance between pro- and anti-angiogenic factors tilts toward a pro-angiogenic outcome, resulting in the transition from not vascularized hyperplasia to a vascularized tumor and malignant tumor progression (74). In this sense, it was described that the inhibition of tumor-associated αvβ3 integrin regulates the angiogenic switch in melanoma cells leading to reduced melanoma growth and angiogenesis *in vivo* (74).

In 2004, Davis et al. have shown that THs can induce angiogenesis through a cell surface receptor using a chick chorioallantoic membrane (CAM) model (75). In 2005, Bergh et al. have demonstrated that the membrane receptor for THs is near the RGD binding site of the integrin αvβ3 (76). Additionally, we found that the activation of integrin αvβ3 by THs mediates angiogenesis in malignant T cells (15). A number of *in vitro* and *in vivo* studies have supported a role for THs in the proliferation

of tumor cells (75, 77–79) and as proangiogenic factor in many types of cancer (15, 75, 76, 80). These properties may be relevant to tumor biology and we will discuss them later in this review.

All the mentioned findings highlight integrin αvβ3 as a fundamental tumor angiogenic promotor. Antagonists of αvβ3 integrin were developed and some proved to be very successful antiangiogenic agents both *in vitro* and in preclinical angiogenesis assays *in vivo*. In accordance, integrin αvβ3 antagonists could inhibit tumor growth in several cancer animal models of human breast cancer (81) and glioblastomas (82). Cilengitide, a specific inhibitor of integrin αvβ3, was able to decrease tumor growth in two different angiogenic and invasive glioblastoma models, by decreasing the diameter of tumor vessels thus reducing the infiltration of cells around the tumor center (83). Associated with its function as membrane receptor for THs actions, the effects of the deaminated analog of L-thyroxine, Tetraiodothyroacetic acid (TETRAC) and its nanoparticulate formulation have been reported as antithyroid agents at the integrin (84).

THYROID HORMONE NON-GENOMIC ACTIONS IN T CELL LYMPHOMAS

THs Effects on T Cell Lymphoma Growth and Proliferation

As we have already mentioned, THs are critical for many processes like cell growth, differentiation, metabolism, and homeostasis maintenance (1). The classical effects of THs are initiated when T3 binds to their nuclear receptors (TRs) that interact with specific responding elements (TREs) in the promoters of target genes. The conformational change promoted by the binding of T3 to TRs induces the exchange of corepressors for coactivators, thus leading to gene transcription on responsive genes (2, 3). TRs are encoded by two different genes: the THRA located in chromosome 17, and the THRB located in chromosome 3, codifying for the TRα and TRβ proteins, respectively (2, 3). The expression of these isoforms differs during the embryonic development and in adult tissues (1). Mutations of TRs have been detected in several cancers, such as erythroleukemia and liver, kidney and thyroid cancers (13). These mutations have been suggested to be a selective advantage for malignant transformation (85). Thus, the mutation (86, 87) or aberrant expression (88) of TRs has been demonstrated in several cancer cell lines. Also, biopsies of patients with gastrointestinal tumors showed increased levels of TRα1 that correlate with Wnt pathway activation and tumor proliferation (89).

Several clinical studies show controversial results related to THs status and cancer. On one side, some studies show that hyperthyroidism might be a risk factor for the development and progress of different types of tumors like breast, thyroid and prostate cancers (85, 90, 91), while hypothyroidism could favor the clinical outcome of cancer patients (92, 93). However, hypothyroidism was associated with an increased risk of colorectal cancer and hepatocellular carcinoma, that would be explained by the increased generation of reactive oxidative species associated with lipid peroxidation, that result in chronic inflammation and DNA damage leading to neoplastic transformation (94, 95). The association between THs and cancer is now better understood following the discovery of the αvβ3 integrin plasma membrane receptor for T4 and T3 (see below).

In the last decade several studies reported the proliferative effect that physiological concentrations of T3 and T4 have on different cancer cell lines, such as glioma, papillary, and follicular thyroid carcinoma, lung carcinoma and breast adenocarcinoma, among others (26, 77, 78, 96). These actions induce the activation of intracellular signaling pathways and transcription factors that increase cell proliferation.

In this sense, our group has investigated the effect of genomic and non-genomic actions of THs on normal T lymphocytes (97, 98) and in TCL cell (15, 79, 99–103) proliferation and survival. We found that TH induced cell proliferation of murine TCL cells by triggering a non-genomic intracellular signaling that involves the activity of PKCζ that leads to ERK 1/2 and NF-κB activation and the increase of transcriptional levels of TRs and the inducible nitric oxide synthase (99). We have also found that THs can regulate the balance between proliferation and apoptosis of TCL cells both *in vitro* and in *in vivo* assays (79, 100). Additionally, we studied how the thyroid status modulates the *in vivo* growth of EL4 TCL cells and how the antitumor immune response is affected in euthyroid, hypothyroid, and hyperthyroid mice. The appearance of palpable solid tumors was earlier in hyperthyroid animals, which also developed tumors with a higher growth rate and an increased volume when compared with tumors in euthyroid controls or hypothyroid mice (79). In addition, the larger tumor size in hyperthyroid mice was accompanied by higher expression levels of the proliferating cell nuclear antigen and cell cycle regulators; and with an increase of intratumoral and peritumoral vasculogenesis (79).

Despite TCL tumor growth was not significantly different between hypothyroid and euthyroid mice, hypothyroid animals showed a higher frequency of metastases (102). This was associated to an increased percentage of regulatory T (Treg) cells in their tumor draining lymph nodes, a decrease number and activity of splenic NK cells and a decreased number of splenic myeloid-derived suppressor cells (MDSCs) when compared to control euthyroid tumor-bearing mice (102) (**Figure 1**). Also, tumor-bearing hyperthyroid mice displayed the lowest metastatic dissemination. This was related with an increased systemic antitumor immunity in hyperthyroid mice, reflected by the low number of MDSCs and increased number and activity of both NK and CD8+ cytotoxic T lymphocytes (**Figure 1**), thus strengthening the fact that low levels of circulating THs are related to TCL spreading and metastatic dissemination. These results highlight the importance of monitoring the thyroid status in patients with TCL.

Integrin αvβ3 as the Thyroid Hormone Membrane Receptor in TCL Cells

As we have already mentioned, both T3 and T4, play important roles in regulating the proliferation of several cancer cell types. Their metabolic, developmental and growth effects in normal tissues are mediated primarily by TRs (104), while their surface

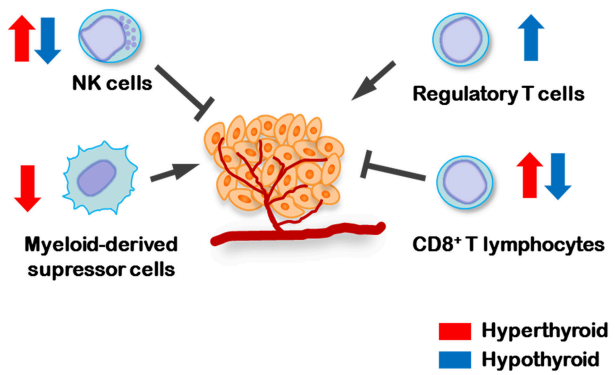

FIGURE 1 | Regulation of antitumor immune cells by circulating levels of THs. In hyperthyroid conditions (blue arrows) increased number and activity of NK and cytotoxic CD8+ lymphocytes, while decreased number of myeloid-derived suppressor cells, were found in the spleens of TCL tumor-bearing mice. However, hypothyroid tumor-bearing animals displayed higher numbers of T regulatory lymphocytes (Treg) in tumor-draining lymph nodes and lower number and activity of splenic NK and CD8+ lymphocytes than control, indicating that the hypothyroid status favors the dissemination of TCL cells.

receptors are involved in the modulation of angiogenesis. Bergh et al. (76) found that physiological concentrations of T4 activate the MAPK pathway in CV-1 cells that lack nuclear TRs, but express the mTR integrin αvβ3. The MAPK-mediated proangiogenic action of T4 was inhibited by TETRAC, RGD peptide, and anti-αvβ3 antibodies (76). These results indicated not only that the surface receptor for THs is on the integrin αvβ3, but also that the binding site for the hormone is either at or near the RGD recognition site. High affinity-binding of radiolabeled hormone to the purified integrin was also demonstrated, and for a complete identification of the mTR, knockdown of integrin αvβ3 by small interfering RNA (siRNA) against both monomers was shown to abrogate the transduction of the THs signaling into MAPK activation (105).

Many laboratories reported the involvement of ERK1/2, Src kinase, and PI3-kinase in the non-genomic mechanisms of THs (106, 107). Studies performed in a glioblastoma cell line showed that not only T3, but also T4 activate the ERK1/2 pathway leading to cell proliferation (26). These results point out a difference between mTR and TRs, the latest is activated with high affinity only by T3, while integrin αvβ3 can bind both T3 and T4.

Studies of the kinetics of thyroid hormone binding performed with crystallographic and mathematical modeling (108, 109) found that THs binding site on integrin αvβ3 has no homology to nuclear TR and contain two binding domains. One domain, namely S1, recognizes exclusively T3 and activates PI3K via Src kinase. The S2 domain regulates MAPK1 and MAPK2 and binds both T4 and T3, however the affinity for T4 is higher than the S1 or S2 sites have for T3 (5). At physiological free hormone concentrations T4 is maximally active at the S2 site on integrin αvβ3, however significantly higher than physiological levels of free T3 are required to induce proliferative activity via this receptor (5).

The identification of αvβ3 integrin as the mTR provides the molecular basis to many actions of TH at cancer cells. THs can influence cell proliferation, survival and angiogenesis in different cancer cells via integrin αvβ3 (110–112). Thus, myeloma cell adhesion to fibronectin is increased by T3 and T4 which induces αvβ3 clustering. In addition, THs induce MMP-9 expression and activation via integrin αvβ3 and MAPK induction, suggesting a role for TH-mediated activation of integrin αvβ3 in myeloma migration and progression (110). THs also promote the proliferation of ovarian cancer cells via integrin αvβ3 that activates extracellular regulated kinase (ERK1/2) (112). In breast cancer cells, THs regulate cell migration via integrin αvβ3 that activates SRC/FAK/PI3-K pathway (111).

Integrin αvβ3 in the Malignant Phenotype of T Cell Lymphomas

T cell lymphomas (TCL) are a broad group of aggressive lymphoproliferative disorders with significant variation clinical, immunophenotypic, and genetic features. This group of hematologic disorders that is characterized by a clonal growth of T cells at different stages of maturation represents ∼10–15% of Non-Hodking lymphomas (113, 114). The last World Health Organization classification has divided this group of hematopoietic malignancies according to its predominant presentation: leukemic (disseminated), nodal, extranodal, or cutaneous (115). The most frequent subtypes include peripheral T cell lymphoma not otherwise specified (PTCL-NOS), anaplastic large cell lymphoma (ALCL) and angioimmunoblastic T cell lymphoma (AITL) (116, 117). Although cutaneous T cell lymphomas (CTCL) are less frequent, is important to note that the skin is the second location in frequency of extranodal primary lymphomas (118). As in other neoplastic disorders, TCL are exposed to a complex environment that comprises among others, growth factors, cytokines, and hormones that are produced by either lymphoma cells or normal cells present in the surrounding or distal tumor microenvironment (119, 120). As we already mentioned, we have demonstrated that one of those factors are THs (15, 79, 99, 100, 103).

Studies of our group demonstrated that both, genomic and non-genomic actions triggered by THs increase cell proliferation of human and murine TCL lines. Moreover, these results described the contribution of the mTR, the integrin αvβ3, in the non-genomic actions of THs in TCL cells (15, 99, 103). The signaling induced by THs through the mTR in murine TCL cells includes the rapid translocation of the ζ isoform of PKC to the cell membrane (99, 103), ERK 1/2 phosphorylation and the activation of the transcription factor NF-κB (15, 99), all molecular processes that are essential for the proliferation and survival of TCL cells.

Recently, we have also demonstrated that integrin αvβ3 is the mTR in human TCL cells. Both T3 and T4 were able to induce *in vitro* the proliferation of tumor, but not normal T lymphocytes (99, 103), being the presence of physiological concentrations of both hormones the most effective to trigger the growth of human TCL cell lines (15). Thus, in a panel of 9 human TCL cell lines, representing the different subtypes of the disease, we showed that the proliferative actions triggered by THs were mediated by the

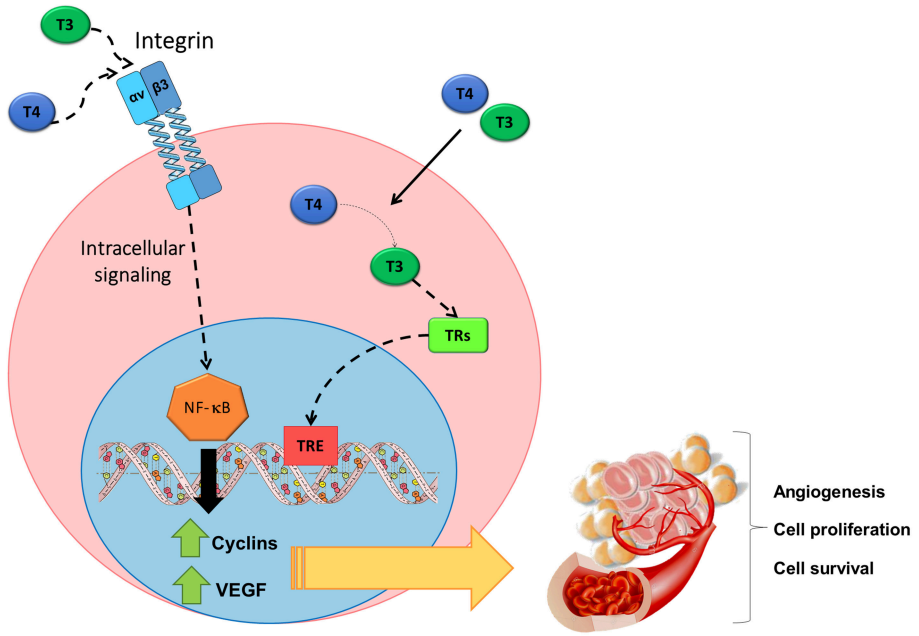

FIGURE 2 | Non-genomic action of THs initiated at the surface receptor of TCL cells on the integrin αvβ3. THs induce signaling pathways triggered after binding to integrin αvβ3 include the activation of NF-κB, thus leading to the production of angiogenic factors such as VEGF and to cell proliferation, cell survival and angiogenesis. Figure adapted from Cremaschi et al. (122) with permission from Elsevier.

activation of integrin αvβ3. This effect was blocked when the mRNA levels of the integrin αv, β3, or both were downregulated using siRNA (15). Additionally, we have evidenced that these effects were accompanied by the regulation of cell cycle markers. According to this, it has been reported in breast cancer cells that TETRAC inhibits the effects of THs on the integrin αvβ3 leading to an increment in the expression of proapoptotic genes, demonstrating that THs non-genomic actions are required for the survival of these cells (4, 121).

We identified the genetic programs activated by THs through their actions on integrin αvβ3 in TCL cells. To this aim we performed RNA sequencing techniques on TCL cells and analyzed results using bioinformatic tools. We found that genes involved in protein translation, lymphocyte proliferation/differentiation, DNA replication and angiogenesis were mobilized by THs through the mTR activation. Remarkably, we found that the intracellular pathways activated by THs through the mTR significantly induced the transcriptional levels VEGFA and VEGFB genes. This induction was abrogated by siRNA against integrin αvβ3 in TCL cells either from immature or mature origins; and dependent on the activation of the transcription factor NF-κB (15). Importantly, when we performed these experiments in the presence of vitronectin, the "natural" ligand of integrin αvβ3, we found that the pathways triggered by THs are different.

It is important to note that it was also evident an association between integrin αvβ3 and VEGF expression in samples from patients with PTCL. By bioinformatic analysis of PTCL tissue microarrays we found a positive correlation between the transcriptional levels of integrin αv or β3 and those of VEGFA or VEGFB. We also verified that the induction of VEGF production in TCL that is regulated by THs functions in a paracrine or autocrine manner. The induction of VEGF production mediated by THs increased the migration of human endothelial cells, and tumor cell proliferation. Moreover, the blocking antibody against VEGF, bevacizumab, abrogated all the mentioned effects. We also found that the proliferative action triggered by THs on TCL cells was impaired by the inhibitor of VEGF receptor, Axitinib, (15, 122). All these findings are resumed in **Figure 2**. In sum, we found that the transcriptional programs initiated by THs, through the activation of integrin αvβ3, stimulate cell proliferation and favor cell survival of TCL, thus, contributing to their malignant phenotype. Furthermore, they also lead to the production and release of angiogenic factors, thus favoring tumor dissemination.

Inhibition of Integrin αvβ3 Receptor for TCL Treatment

As we have already mentioned, integrin αvβ3 is highly expressed on activated tumor endothelial cells, but not on resting endothelial cells and normal organ systems (11). In addition, this membrane receptor is also highly expressed on tumor cells. This characteristic makes integrin αvβ3 an attractive target for both tumor cells and tumor vasculature.

Based on the proliferative and proangiogenic roles of THs mediated by the integrin αvβ3 in TCL cells, we used preclinical models to analyze whether these pathways could be capitalized for the treatment of patients with TCL. We performed xenografts of human TCL in NOD-SCID immunodeficient mice and we evaluated the effect of integrin αvβ3 inhibition on tumor growth.

The negative regulation of the integrins αv or β3 in TCL cells by siRNA reduced the tumor volume and decreased the protein levels of VEGF and the blood vessel area in TCL tumors (15, 122). This suggests a decrease in the angiogenic potential of tumors derived from cells that do not express the integrin αvβ3. We then wondered whether integrin αvβ3 actions on lymphoma cells could be therapeutically capitalized for the treatment of TCL patients; and considering that PTCL-NOS is the most frequent subtype, we developed a xenograft model of human PTCL-NOS cells into SCID mice and evaluated the action of the selective inhibitor of integrin αvβ3 cilengitide. We found that cilengitide treatment reduced tumor volume by decreasing NF-κB pathway activation and the microvascular lumen size, while increasing tumor apoptosis (15). Moreover, similar effects were found in mice bearing ALCL patient-derived tumors (PDX) xenografts (15, 122). It is important to note that in mice treated with cilengitide no toxic effects were observed. These results highlight the importance of these mechanisms for the development of a more effective and less toxic therapy for patients who suffer these pathologies.

Cilengitide was the first integrin antagonist evaluated in clinical phase I and II trials for the treatment of glioblastoma and several other tumor types (123–125). No encouraging results were found in patients with glioblastoma when using cilengitide as a single agent. Some reasons for the unexpected clinical low efficacy in glioblastoma could be related to the fast off-rate of cilengitide from its targets, the rapid plasma clearance, or the poor perfusion of the brain tumor environment (43). However, it is important to note that a beneficial therapeutic action was found when administered in association with standard radiotherapy or chemotherapy (125, 126), and this was also found in other type of tumors (127, 128).

There is not much information on the role of THs and its action on integrin αvβ3 in other hematologic malignancies; however it was shown that this integrin enhance the proliferation of acute myeloid leukemia (AML) cells (129) and it is required for AML cell survival (130). Furthermore, integrin αvβ3 expressed on the worst prognostic AML cells mediates the interaction with stroma cell-derived ligands in the bone marrow niche, thus triggering a signaling cascade that is critical for the proliferation of AML cells (131). Activated integrin αvβ3/β-catenin signaling pathway in tumor microenvironment decreased the sensitivity of AML cells to tyrosine kinase inhibitor sorafenib, as well (132). Thus, inhibition of this integrin signaling pathway would also be of potential therapeutic impact in AML.

CONCLUDING REMARKS

Integrins are crucial mediators for the survival and migration of tumor cells, not only by acting directly on these cells, but also through the influence they exert on the cells of the microenvironment that surround the tumor. Due to the central role that integrins play in tumor angiogenesis and metastasis, they have become promising targets for the treatment of different types of aggressive cancers.

In this sense, integrin αvβ3 has a crucial role in inducing tumor cell migration and metastasis to distant organs. Moreover, being the membrane receptor for thyroid hormone non-genomic actions, integrin αvβ3 triggers intracellular pathways leading to TCL proliferation and survival and to tumor growth and vascularization via the production of angiogenic factors. The selective inhibition of the integrin αvβ3 in different subtypes of TCL results in the decrease of cell proliferation, tumor growth and impaired angiogenesis. The lack or low expression of integrin αvβ3 in non-active endothelial cells and in normal lymphoid cells, important actors in antitumor immune response, offers a rationale and attractive target for TCL treatment.

Moreover, integrin αvβ3 may be an attractive therapeutic tool for other neoplastic diseases. In fact, in patients with advanced solid tumors, as breast, ovary, and pancreas cancers, the benefit of medical induction of euthyroid hypothyroxinemia was demonstrated (133–136). These studies were based on the fact that integrin αvβ3 is overexpressed in these types of tumors, and, by reducing T4 levels, the cancer cell proliferation and survival and the tumor-related angiogenesis can be reduced, without affecting other important metabolic processes that are mainly regulated by T3 levels.

AUTHOR CONTRIBUTIONS

FC: conception and design, acquisition of data, writing/drafting manuscript, revising for important content, final approval of version to be published agreement for accountability of published material; HAS: writing/drafting manuscript, revising for important content, final approval of version to be published; agreement for accountability of published material; MD: revising for important content, final approval of version to be published; agreement for accountability of published material; MB: revising for important content, final approval of version to be published; GC: conception and design, writing/drafting manuscript, revising for important content, final approval of version to be published; agreement for accountability of published material.

FUNDING

This work was supported by the Agencia Nacional para la Promoción Científica y Técnica, PICT 2015/0874, PICT 2015/0876 and Grant for Basic Research Projects from Instituto Nacional del Cáncer, Ministerio de Salud de la República Argentina.

REFERENCES

1. Mendoza A, Hollenberg AN. New insights into thyroid hormone action. *Pharmacol Ther*. (2017) 173:135–45. doi: 10.1016/j.pharmthera.2017.02.012
2. Lazar MA. Thyroid hormone action: a binding contract. *J Clin Invest*. (2003) 112:497–9. doi: 10.1172/JCI19479
3. Brent GA. Mechanisms of thyroid hormone action. *J Clin Invest*. (2012) 122:3035–43. doi: 10.1172/JCI60047

4. De Vito P, Incerpi S, Pedersen JZ, Luly P, Davis FB, Davis PJ. Thyroid hormones as modulators of immune activities at the cellular level. *Thyroid* (2011) 21:879–90. doi: 10.1089/thy.2010.0429
5. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol*. (2016) 12:111–21. doi: 10.1038/nrendo.2015.205
6. Yalcin M, Dyskin E, Lansing L, Bharali DJ, Mousa SS, Bridoux A, et al. Tetraiodothyroacetic acid (tetrac) and nanoparticulate tetrac arrest growth of medullary carcinoma of the thyroid. *J Clin Endocrinol Metab*. (2010) 95:1972–80. doi: 10.1210/jc.2009-1926
7. Yalcin M, Bharali DJ, Lansing L, Dyskin E, Mousa SS, Hercbergs A, et al. Tetraidothyroacetic acid (tetrac) and tetrac nanoparticles inhibit growth of human renal cell carcinoma xenografts. *Anticancer Res*. (2009) 29:3825–31.
8. Yalcin M, Bharali DJ, Dyskin E, Dier E, Lansing L, Mousa SS, et al. Tetraiodothyroacetic acid and tetraiodothyroacetic acid nanoparticle effectively inhibit the growth of human follicular thyroid cell carcinoma. *Thyroid* (2010) 20:281–6. doi: 10.1089/thy.2009.0249
9. Davis FB, Tang HY, Shih A, Keating T, Lansing L, Hercbergs A, et al. Acting via a cell surface receptor, thyroid hormone is a growth factor for glioma cells. *Cancer Res*. (2006) 66:7270–5. doi: 10.1158/0008-5472.CAN-05-4365
10. Huang R, Rofstad EK. Integrins as therapeutic targets in the organ-specific metastasis of human malignant melanoma. *J Exp Clin Cancer Res*. (2018) 37:92. doi: 10.1186/s13046-018-0763-x
11. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer*. (2010) 10:9–22. doi: 10.1038/nrc2748
12. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev*. (2010) 31:139–70. doi: 10.1210/er.2009-0007
13. Chan IH, Privalsky ML. A conserved lysine in the thyroid hormone receptor-alpha1 DNA-binding domain, mutated in hepatocellular carcinoma, serves as a sensor for transcriptional regulation. *Mol Cancer Res*. (2010) 8:15–23. doi: 10.1158/1541-7786.MCR-09-0425
14. Cai W, Chen X. Anti-angiogenic cancer therapy based on integrin αvβ3 antagonism. *Anticancer Agents Med Chem*. (2006) 6:407–28. doi: 10.2174/187152006778226530
15. Cayrol F, Diaz Flaque MC, Fernando T, Yang SN, Sterle HA, Bolontrade M, et al. Integrin αvβ3 acting as membrane receptor for thyroid hormones mediates angiogenesis in malignant T cells. *Blood* (2015) 125:841–51. doi: 10.1182/blood-2014-07-587337
16. Marsico G, Russo L, Quondamatteo F, Pandit A. Glycosylation and integrin regulation in cancer. *Trends Cancer* (2018) 4:537–52. doi: 10.1016/j.trecan.2018.05.009
17. Hamidi H, Pietila M, Ivaska J. The complexity of integrins in cancer and new scopes for therapeutic targeting. *Br J Cancer* (2016) 115:1017–23. doi: 10.1038/bjc.2016.312
18. Chapman HA, Wei Y, Simon DI, Waltz DA. Role of urokinase receptor and caveolin in regulation of integrin signaling. *Thromb Haemost*. (1999) 82:291–7. doi: 10.1055/s-0037-1615845
19. Stipp CS. Laminin-binding integrins and their tetraspanin partners as potential antimetastatic targets. *Expert Rev Mol Med*. (2010) 12:e3. doi: 10.1017/S1462399409001355
20. Ucar DA, Hochwald SN. FAK and interacting proteins as therapeutic targets in pancreatic cancer. *Anticancer Agents Med Chem*. (2010) 10:742–6. doi: 10.2174/187152010794728675
21. Guan JL. Integrin signaling through FAK in the regulation of mammary stem cells and breast cancer. *IUBMB Life* (2010) 62:268–76. doi: 10.1002/iub.303
22. Brunton VG, Frame MC. Src and focal adhesion kinase as therapeutic targets in cancer. *Curr Opin Pharmacol*. (2008) 8:427–32. doi: 10.1016/j.coph.2008.06.012
23. Thakur R, Trivedi R, Rastogi N, Singh M, Mishra DP. Inhibition of STAT3, FAK and Src mediated signaling reduces cancer stem cell load, tumorigenic potential and metastasis in breast cancer. *Sci Rep*. (2015) 5:10194. doi: 10.1038/srep10194
24. Longmate W, DiPersio CM. Beyond adhesion: emerging roles for integrins in control of the tumor microenvironment. *F1000Res.* (2017) 6:1612. doi: 10.12688/f1000research.11877.1
25. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
26. Lin HY, Sun M, Tang HY, Lin C, Luidens MK, Mousa SA, et al. L-Thyroxine vs. 3,5,3'-triiodo-L-thyronine and cell proliferation: activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. *Am J Physiol Cell Physiol*. (2009) 296:C980–91. doi: 10.1152/ajpcell.00305.2008
27. Yang X, Pursell B, Lu S, Chang TK, Mercurio AM. Regulation of beta 4-integrin expression by epigenetic modifications in the mammary gland and during the epithelial-to-mesenchymal transition. *J Cell Sci*. (2009) 122(Pt 14):2473–80. doi: 10.1242/jcs.049148
28. Mori S, Kodaira M, Ito A, Okazaki M, Kawaguchi N, Hamada Y, et al. Enhanced expression of integrin αvβ3 induced by TGF-beta is required for the enhancing effect of Fibroblast Growth Factor 1 (FGF1) in TGF-beta-induced Epithelial-Mesenchymal Transition (EMT) in mammary epithelial cells. *PLoS ONE* (2015) 10:e0137486. doi: 10.1371/journal.pone.0137486
29. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol.* (2014) 15:178–96. doi: 10.1038/nrm3758
30. Carpenter BL, Chen M, Knifley T, Davis KA, Harrison SM, Stewart RL, et al. Integrin α6β4 promotes autocrine Epidermal Growth Factor Receptor (EGFR) signaling to stimulate migration and invasion toward Hepatocyte Growth Factor (HGF). *J Biol Chem*. (2015) 290:27228–38. doi: 10.1074/jbc.M115.686873
31. Koivisto L, Bi J, Hakkinen L, Larjava H. Integrin αvβ6: structure, function and role in health and disease. *Int J Biochem Cell Biol.* (2018) 99:186–96. doi: 10.1016/j.biocel.2018.04.013
32. Casal JI, Bartolome RA. RGD cadherins and α2β1 integrin in cancer metastasis: a dangerous liaison. *Biochim Biophys Acta Rev Cancer* (2018) 1869:321–32. doi: 10.1016/j.bbcan.2018.04.005
33. Juan-Rivera MC, Martinez-Ferrer M. Integrin inhibitors in prostate cancer. *Cancers* (2018) 10:44. doi: 10.3390/cancers10020044
34. Goel HL, Li J, Kogan S, Languino LR. Integrins in prostate cancer progression. *Endocr Relat Cancer* (2008) 15:657–64. doi: 10.1677/ERC-08-0019
35. Ryu S, Park KM, Lee SH. Gleditsia sinensis thorn attenuates the collagen-based migration of PC3 prostate cancer cells through the suppression of α2β1 integrin expression. *Int J Mol Sci.* (2016) 17:328. doi: 10.3390/ijms17030328
36. Li P, Li WL, Qi JX. [Expression of integrin αvβ3, CXC chemokine receptor 4 and CXC chemokine receptor 7 and their relationship with lymph node metastasis in squamous cell carcinoma of head and neck]. *Zhonghua Kou Qiang Yi Xue Za Zhi* (2017) 52:723–9. doi: 10.3760/cma.j.issn.1002-0098.2017.12.003
37. Friedrichs K, Ruiz P, Franke F, Gille I, Terpe HJ, Imhof BA. High expression level of alpha 6 integrin in human breast carcinoma is correlated with reduced survival. *Cancer Res.* (1995) 55:901–6.
38. Tokmakidis SP, Leger LA. Comparison of mathematically determined blood lactate and heart rate "threshold" points and relationship with performance. *Eur J Appl Physiol Occup Physiol.* (1992) 64:309–17. doi: 10.1007/BF00636217
39. McCabe NP, De S, Vasanji A, Brainard J, Byzova TV. Prostate cancer specific integrin αvβ3 modulates bone metastatic growth and tissue remodeling. *Oncogene* (2007) 26:6238–43. doi: 10.1038/sj.onc.1210429
40. Hang Q, Isaji T, Hou S, Wang Y, Fukuda T, Gu J. A key regulator of cell adhesion: identification and characterization of important N-glycosylation sites on integrin alpha5 for cell migration. *Mol Cell Biol.* (2017) 37:e00558-16. doi: 10.1128/MCB.00558-16
41. Cai X, Thinn AMM, Wang Z, Shan H, Zhu J. The importance of N-glycosylation on beta3 integrin ligand binding and conformational regulation. *Sci Rep.* (2017) 7:4656. doi: 10.1038/s41598-017-04844-w
42. Currier NV, Ackerman SE, Kintzing JR, Chen R, Filsinger Interrante M, Steiner A, et al. Targeted drug delivery with an integrin-binding knottin-Fc-MMAF conjugate produced by cell-free protein synthesis. *Mol Cancer Ther.* (2016) 15:1291–300. doi: 10.1158/1535-7163.MCT-15-0881
43. Raab-Westphal S, Marshall JF, Goodman SL. Integrins as therapeutic targets: successes and cancers. *Cancers* (2017) 9:E110. doi: 10.3390/cancers9090110
44. Lasinska I, Mackiewicz J. Integrins as a new target forcancer treatment. *Anticancer Agents Med Chem.* (2018) 15:1291–1300. doi: 10.2174/1871520618666181119103413
45. Yuan Y, Jiang YC, Sun CK, Chen QM. Role of the tumor microenvironment in tumor progression and the clinical applications (Review). *Oncol Rep.* (2016) 35:2499–515. doi: 10.3892/or.2016.4660

46. Marcucci F, Bellone M, Caserta CA, Corti A. Pushing tumor cells towards a malignant phenotype: stimuli from the microenvironment, intercellular communications and alternative roads. *Int J Cancer* (2014) 135:1265–76. doi: 10.1002/ijc.28572
47. Gehler S, Ponik SM, Riching KM, Keely PJ. Bi-directional signaling: extracellular matrix and integrin regulation of breast tumor progression. *Crit Rev Eukaryot Gene Expr.* (2013) 23:139–57. doi: 10.1615/CritRevEukarGeneExpr.2013006647
48. Sun CC, Qu XJ, Gao ZH. Integrins: players in cancer progression and targets in cancer therapy. *Anticancer Drugs* (2014) 25:1107–21. doi: 10.1097/CAD.0000000000000145
49. Vicente-Manzanares M, Sanchez-Madrid F. Targeting the integrin interactome in human disease. *Curr Opin Cell Biol.* (2018) 55:17–23. doi: 10.1016/j.ceb.2018.05.010
50. Larsen M, Artym VV, Green JA, Yamada KM. The matrix reorganized: extracellular matrix remodeling and integrin signaling. *Curr Opin Cell Biol.* (2006) 18:463–71. doi: 10.1016/j.ceb.2006.08.009
51. Ota D, Kanayama M, Matsui Y, Ito K, Maeda N, Kutomi G, et al. Tumor-α9β1 integrin-mediated signaling induces breast cancer growth and lymphatic metastasis via the recruitment of cancer-associated fibroblasts. *J Mol Med.* (2014) 92:1271–81. doi: 10.1007/s00109-014-1183-9
52. Izumi D, Ishimoto T, Miyake K, Sugihara H, Eto K, Sawayama H, et al. CXCL12/CXCR4 activation by cancer-associated fibroblasts promotes integrin beta1 clustering and invasiveness in gastric cancer. *Int J Cancer* (2016) 138:1207–19. doi: 10.1002/ijc.29864
53. King TE, Pawar SC, Majuta L, Sroka IC, Wynn D, Demetriou MC, et al. The role of alpha 6 integrin in prostate cancer migration and bone pain in a novel xenograft model. *PLoS ONE* (2008) 3:e3535. doi: 10.1371/journal.pone.0003535
54. Sroka IC, Sandoval CP, Chopra H, Gard JM, Pawar SC, Cress AE. Macrophage-dependent cleavage of the laminin receptor α6β1 in prostate cancer. *Mol Cancer Res.* (2011) 9: 1319–28. doi: 10.1158/1541-7786.MCR-11-0080
55. Clark AG, Vignjevic DM. Modes of cancer cell invasion and the role of the microenvironment. *Curr Opin Cell Biol.* (2015) 36:13–22. doi: 10.1016/j.ceb.2015.06.004
56. Yue J, Zhang K, Chen J. Role of integrins in regulating proteases to mediate extracellular matrix remodeling. *Cancer Microenviron.* (2012) 5:275–83. doi: 10.1007/s12307-012-0101-3
57. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* (2010) 141:52–67. doi: 10.1016/j.cell.2010.03.015
58. Stamenkovic L. Matrix metalloproteinases in tumor invasion and metastasis. *Semin Cancer Biol.* (2000) 10:415–33. doi: 10.1006/scbi.2000.0379
59. Ahmed N, Pansino F, Clyde R, Murthi P, Quinn MA, Rice GE, et al. Overexpression of αvβ6 integrin in serous epithelial ovarian cancer regulates extracellular matrix degradation via the plasminogen activation cascade. *Carcinogenesis* (2002) 23:237–44. doi: 10.1093/carcin/23.2.237
60. Gu X, Niu J, Dorahy DJ, Scott R, Agrez MV. Integrin αvβ6-associated ERK2 mediates MMP-9 secretion in colon cancer cells. *Br J Cancer* (2002) 87:348–51. doi: 10.1038/sj.bjc.6600480
61. Li X, Yang Y, Hu Y, Dang D, Regezi J, Schmidt BLet al. αvβ6-Fyn signaling promotes oral cancer progression. *J Biol Chem.* (2003) 278:41646–53. doi: 10.1074/jbc.M306274200
62. Takahara T, Kasamatsu A, Yamatoji M, Iyoda M, Kasama H, Saito T, et al. SIPA1 promotes invasion and migration in human oral squamous cell carcinoma by ITGB1 and MMP7. *Exp Cell Res.* (2017) 352:357–63. doi: 10.1016/j.yexcr.2017.02.026
63. Yang GY, Xu KS, Pan ZQ, Zhang ZY, Mi YT, Wang JS, et al. Integrin αvβ6 mediates the potential for colon cancer cells to colonize in and metastasize to the liver. *Cancer Sci.* (2008) 99:879–87. doi: 10.1111/j.1349-7006.2008.00762.x
64. Rocha LA, Learmonth DA, Sousa RA, Salgado AJ. αvβ3 and α5β1 integrin-specific ligands: from tumor angiogenesis inhibitors to vascularization promoters in regenerative medicine? *Biotechnol Adv.* (2018) 36:208–27. doi: 10.1016/j.biotechadv.2017.11.004
65. Nussenbaum F, Herman IM. Tumor angiogenesis: insights and innovations. *J Oncol.* (2010) 2010:132641. doi: 10.1155/2010/132641
66. Silva R, D'Amico G, Hodivala-Dilke KM, Reynolds LE. Integrins: the keys to unlocking angiogenesis. *Arterioscler Thromb Vasc Biol.* (2008) 28:1703–13. doi: 10.1161/ATVBAHA.108.172015
67. Mahabeleshwar GH, Feng W, Reddy K, Plow EF, Byzova: mechanisms of integrin-vascular endothelial growth factor receptor cross-activation in angiogenesis. *Circ Res.* (2007) 101:570–80. doi: 10.1161/CIRCRESAHA.107.155655
68. Borst AJ, James ZM, Zagotta WN, Ginsberg M, Rey FA, DiMaio F, Backovic M, et al. The therapeutic antibody LM609 selectively inhibits ligand binding to human αvβ3 integrin via steric hindrance. *Structure* (2017) 25:1732–39 e5. doi: 10.1016/j.str.2017.09.007
69. Bader BL, Rayburn H, Crowley D, Hynes RO. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. *Cell* (1998) 95:507–19. doi: 10.1016/S0092-8674(00)81618-9
70. McCarty JH, Monahan-Earley RA, Brown LF, Keller M, Gerhardt H, Rubin K, et al. Defective associations between blood vessels and brain parenchyma lead to cerebral hemorrhage in mice lacking alphav integrins. *Mol Cell Biol.* (2002) 22:7667–77. doi: 10.1128/MCB.22.21.7667-7677.2002
71. Reynolds LE, Wyder L, Lively JC, Taverna D, Robinson SD, Huang X, et al. Enhanced pathological angiogenesis in mice lacking beta3 integrin or beta3 and beta5 integrins. *Nat Med.* (2002) 8:27–34. doi: 10.1038/nm0102-27
72. Max R, Gerritsen RR, Nooijen PT, Goodman SL, Sutter A, Keilholz U, Ruiter DJ, et al. Immunohistochemical analysis of integrin alpha vbeta3 expression on tumor-associated vessels of human carcinomas. *Int J Cancer* (1997) 71:320–4.
73. Weber MR, Zuka M, Lorger M, Tschan M, Torbett BE, Zijlstra A, et al. Activated tumor cell integrin αvβ3 cooperates with platelets to promote extravasation and metastasis from the blood stream. *Thromb Res.* (2016) 140 (Suppl. 1):S27–36. doi: 10.1016/S0049-3848(16)30095-0
74. Contois LW, Akalu A, Caron JM, Tweedie E, Cretu A, Henderson T, et al. Inhibition of tumor-associated αvβ3 integrin regulates the angiogenic switch by enhancing expression of IGFBP-4 leading to reduced melanoma growth and angiogenesis *in vivo*. *Angiogenesis* (2015) 18:31–46. doi: 10.1007/s10456-014-9445-2
75. Davis FB, Mousa SA, O'Connor L, Mohamed S, Lin HY, Cao HJ, et al. Proangiogenic action of thyroid hormone is fibroblast growth factor-dependent and is initiated at the cell surface. *Circ Res.* (2004) 94:1500–6. doi: 10.1161/01.RES.0000130784.90237.4a
76. Bergh JJ, Lin HY, Lansing L, Mohamed SN, Davis FB, Mousa S et al. Integrin αvβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* (2005) 146:2864–71. doi: 10.1210/en.2005-0102
77. Lin HY, Tang HY, Shih A, Keating T, Cao G, Davis PJ, et al. Thyroid hormone is a MAPK-dependent growth factor for thyroid cancer cells and is anti-apoptotic. *Steroids* (2007) 72:180–7. doi: 10.1016/j.steroids.2006.11.014
78. Meng R, Tang HY, Westfall J, London D, Cao JH, Mousa SA, et al. Crosstalk between integrin αvβ3 and estrogen receptor-alpha is involved in thyroid hormone-induced proliferation in human lung carcinoma cells. *PLoS ONE* (2011) 6:e27547. doi: 10.1371/journal.pone.0027547
79. Sterle HA, Valli E, Cayrol F, Paulazo MA, Martinel Lamas DJ, Diaz Flaque MC, et al. Thyroid status modulates T lymphoma growth via cell cycle regulatory proteins and angiogenesis. *J Endocrinol.* (2014) 222:243–55. doi: 10.1530/JOE-14-0159
80. Davis PJ, Sudha T, Lin HY, Mousa SA. Thyroid hormone, hormone analogs, and angiogenesis. *Compr Physiol.* (2015) 6:353–62. doi: 10.1002/cphy.c150011
81. Brooks PC, Stromblad S, Klemke R, Visscher D, Sarkar FH, Cheresh DA. Antiintegrin αvβ3 blocks human breast cancer growth and angiogenesis in human skin. *J Clin Invest.* (1995) 96:1815–22. doi: 10.1172/JCI118227
82. Onishi M, Kurozumi K, Ichikawa T, Date I. Mechanisms of tumor development and anti-angiogenic therapy in glioblastoma multiforme. *Neurol Med Chir.* (2013) 53:755–63. doi: 10.2176/nmc.ra2013-0200
83. Le Tourneau C, Faivre S, Raymond E. The role of integrins in colorectal cancer. *Oncology* (2007) 21(Suppl. 3):21–4.

84. Davis PJ, Lin HY, Sudha T, Yalcin M, Tang HY, Hercbergs A, et al. Nanotetrac targets integrin αvβ3 on tumor cells to disorder cell defense pathways and block angiogenesis. *Onco Targets Ther.* (2014) 7:1619–24. doi: 10.2147/OTT.S67393
85. Goemann IM, Romitti M, Meyer ELS, Wajner SM, Maia AL. Role of thyroid hormones in the neoplastic process: an overview. *Endocr Relat Cancer* (2017) 24:R367–85. doi: 10.1530/ERC-17-0192
86. Wang CS, Lin KH, Hsu YC. Alterations of thyroid hormone receptor alpha gene: frequency and association with Nm23 protein expression and metastasis in gastric cancer. *Cancer Lett.* (2002) 175:121–7. doi: 10.1016/S0304-3835(01)00722-4
87. Lin KH, Zhu XG, Hsu HC, Chen SL, Shieh HY, Chen ST, et al. Dominant negative activity of mutant thyroid hormone alpha1 receptors from patients with hepatocellular carcinoma. *Endocrinology* (1997) 138:5308–15. doi: 10.1210/endo.138.12.5625
88. Horkko TT, Tuppurainen K, George SM, Jernvall P, Karttunen TJ, Makinen MJ. Thyroid hormone receptor beta1 in normal colon and colorectal cancer-association with differentiation, polypoid growth type and K-ras mutations. *Int J Cancer* (2006) 118:1653–9. doi: 10.1002/ijc.21556
89. Uchuya-Castillo J, Aznar N, Frau C, Martinez P, Le Neve C, Marisa L, et al. Increased expression of the thyroid hormone nuclear receptor TRalpha1 characterizes intestinal tumors with high Wnt activity. *Oncotarget* (2018) 9:30979–96. doi: 10.18632/oncotarget.25741
90. Hellevik AI, Asvold BO, Bjoro T, Romundstad PR, Nilsen TI, Vatten LJ. Thyroid function and cancer risk: a prospective population study. *Cancer Epidemiol Biomarkers Prev.* (2009) 18:570–4. doi: 10.1158/1055-9965.EPI-08-0911
91. Kitahara CM, Rmendiné Farkas K, Jorgensen JOL, Cronin-Fenton D, Sorensen HT. Benign thyroid diseases and risk of thyroid cancer: a nationwide cohort study. *J Clin Endocrinol Metab.* (2018) 103:2216–24. doi: 10.1210/jc.2017-02599
92. Ashur-Fabian O, Blumenthal DT, Bakon M, Nass D, Davis PJ, Hercbergs A. Long-term response in high-grade optic glioma treated with medically induced hypothyroidism and carboplatin: a case report and review of the literature. *Anticancer Drugs* (2013) 24:315–23. doi: 10.1097/CAD.0b013e32835c7a47
93. Hercbergs AA, Goyal LK, Suh JH, Lee S, Reddy CA, Cohen BH, et al. Propylthiouracil-induced chemical hypothyroidism with high-dose tamoxifen prolongs survival in recurrent high grade glioma: a phase I/II study. *Anticancer Res.* (2003) 23:617–26.
94. Hassan MM, Kaseb A, Li D, Patt YZ, Vauthey JN, Thomas MB, et al. Association between hypothyroidism and hepatocellular carcinoma: a case-control study in the United States. *Hepatology* (2009) 49:1563–70. doi: 10.1002/hep.22793
95. Heemskerk-Gerritsen BA, Seynaeve C, van Asperen CJ, Ausems MG, Collee JM, van Doorn HC, et al. Breast cancer risk after salpingo-oophorectomy in healthy BRCA1/2 mutation carriers: revisiting the evidence for risk reduction. *J Natl Cancer Inst.* (2015) 107:djv033. doi: 10.1093/jnci/djv033
96. Tang HY, Lin HY, Zhang S, Davis FB, Davis PJ. Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor. *Endocrinology* (2004) 145:3265–72. doi: 10.1210/en.2004-0308
97. Klecha AJ, Barreiro Arcos ML, Genaro AM, Gorelik G, Silberman DM, Caro R, et al. Different mitogen-mediated Beta-adrenergic receptor modulation in murine T lymphocytes depending on the thyroid status. *Neuroimmunomodulation* (2005) 12:92–9. doi: 10.1159/000083581
98. Klecha AJ, Genaro AM, Gorelik G, Barreiro Arcos ML, Silberman DM, Schuman M, et al. Integrative study of hypothalamus-pituitary-thyroid-immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signaling pathway. *J Endocrinol.* (2006) 189:45–55. doi: 10.1677/joe.1.06137
99. Barreiro Arcos ML, Sterle HA, Paulazo MAValli E, Klecha AJ, Isse B, et al. Cooperative nongenomic and genomic actions on thyroid hormone mediated-modulation of T cell proliferation involve up-regulation of thyroid hormone receptor and inducible nitric oxide synthase expression. *J Cell Physiol.* (2011) 226:3208–18. doi: 10.1002/jcp.22681
100. Barreiro Arcos ML, Sterle HA, Vercelli C, Valli E, Cayrol MFKlecha AJ, et al. Induction of apoptosis in T lymphoma cells by long-term treatment with thyroxine involves PKCzeta nitration by nitric oxide synthase. *Apoptosis* (2013) 18:1376–90. doi: 10.1007/s10495-013-0869-8
101. MacLeod F, Mate A. Life enrichment for long-stay patients in acute care: an interdisciplinary program. *Perspectives* (1991) 15:2–6.
102. Sterle HA, Barreiro Arcos ML, Valli E, Paulazo MA, Mendez Huergo SP, Blidner AG, et al. The thyroid status reprograms T cell lymphoma growth and modulates immune cell frequencies. *J Mol Med.* (2016) 94:417–29. doi: 10.1007/s00109-015-1363-2
103. Barreiro Arcos ML, Gorelik G, Klecha A, Genaro AM, Cremaschi GA. Thyroid hormones increase inducible nitric oxide synthase gene expression downstream from PKC-zeta in murine tumor T lymphocytes. *Am J Physiol Cell Physiol.* (2006) 291C327–36. doi: 10.1152/ajpcell.00316.2005
104. Lin HY, Chin YT, Yang YC, Lai HY, Wang-Peng J, Liu LF, et al. Thyroid hormone, cancer, and apoptosis. *Compr Physiol.* (2016) 6:1221–37. doi: 10.1002/cphy.c150035
105. Lin HY, Cody V, Davis FB, Hercbergs AA, Luidens MK, Mousa SA, et al. Identification and functions of the plasma membrane receptor for thyroid hormone analogues. *Discov Med.* (2011) 11:337–47.
106. Lei J, Mariash CN, Bhargava M, Wattenberg EV, Ingbar DH. T3 increases Na-K-ATPase activity via a MAPK/ERK1/2-dependent pathway in rat adult alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol.* (2008) 294:L749–54. doi: 10.1152/ajplung.00335.2007
107. Moeller LC, Cao X, Dumitrescu AM, Seo H, Refetoff S. Thyroid hormone mediated changes in gene expression can be initiated by cytosolic action of the thyroid hormone receptor beta through the phosphatidylinositol 3-kinase pathway. *Nucl Recept Signal.* (2006) 4:e020. doi: 10.1621/nrs.04020
108. Cody V, Davis PJ, Davis FB. Molecular modeling of the thyroid hormone interactions with αvβ3 integrin. *Steroids* (2007) 72:165–70. doi: 10.1016/j.steroids.2006.11.008
109. Lin HY, Landersdorfer CB, London D, Meng R, Lim CU, Lin C, et al. Pharmacodynamic modeling of anti-cancer activity of tetraiodothyroacetic acid in a perfused cell culture system. *PLoS Comput Biol.* (2011) 7:e1001073. doi: 10.1371/journal.pcbi.1001073
110. Cohen K, Flint N, Shalev S, Erez D, Baharal T, Davis PJ, et al. Thyroid hormone regulates adhesion, migration and matrix metalloproteinase 9 activity via αvβ3 integrin in myeloma cells. *Oncotarget* (2014) 5:6312–22. doi: 10.18632/oncotarget.2205
111. Flamini MI, Uzair ID, Pennacchio GE, Neira FJ, Mondaca JM, Cuello-Carrion FD, et al. Thyroid hormone controls breast cancer cell movement via integrin alphaV/beta3/SRC/FAK/PI3-Kinases. *Horm Cancer* (2017) 8:16–27. doi: 10.1007/s12672-016-0280-3
112. Hsieh MT, Wang LM, Changou CA, Chin YT, Yang YSH, Lai HY, et al. Crosstalk between integrin αvβ3 and ERalpha contributes to thyroid hormone-induced proliferation of ovarian cancer cells. *Oncotarget* (2017) 8:24237–24249. doi: 10.18632/oncotarget.10757
113. Gru AA. Pathology of T-cell lymphomas: diagnosis and biomarker discovery. *Cancer Treat Res.* (2015) 165:51–95. doi: 10.1007/978-3-319-13150-4_3
114. Ghione P, Moskowitz AJ, De Paola NEK, Horwitz SM, Ruella M. Novel Immunotherapies for T cell lymphoma and leukemia. *Curr Hematol Malig Rep.* (2018) 13:494–506. doi: 10.1007/s11899-018-0480-8
115. Matutes E. The 2017 WHO update on mature T- and natural killer (NK) cell neoplasms. *Int J Lab Hematol.* (2018) 40 (Suppl. 1):97–103. doi: 10.1111/ijlh.12817
116. Boi M, Zucca E, Inghirami G, Bertoni F. Advances in understanding the pathogenesis of systemic anaplastic large cell lymphomas. *Br J Haematol.* (2015) 168:771–83. doi: 10.1111/bjh.13265
117. Vu K, Ai W. Update on the treatment of anaplastic large cell lymphoma. *Curr Hematol Malig Rep.* (2018) 13:135–41. doi: 10.1007/s11899-018-0436-z
118. Wilcox RA. Cutaneous T-cell lymphoma: 2017 update on diagnosis, risk-stratification, and management. *Am J Hematol.* (2017) 92:1085–102. doi: 10.1002/ajh.24876
119. Singh V, Singh SM. Progressive tumor growth-associated altered tumor microenvironment: implications in a tumor stage-dependent modulation in survival of a murine T cell lymphoma. *J Cancer Res Clin Oncol.* (2009) 135:1015–24. doi: 10.1007/s00432-008-0537-5
120. Tse E, Kwong YL. T-cell lymphoma: microenvironment-related biomarkers. *Semin Cancer Biol.* (2015) 34:46–51. doi: 10.1016/j.semcancer.2015.06.001

121. Glinskii AB, Glinsky GV, Lin HY, Tang HY, Sun M, Davis FB, et al. Modification of survival pathway gene expression in human breast cancer cells by tetraiodothyroacetic acid (tetrac). *Cell Cycle* (2009) 8:3562–70. doi: 10.4161/cc.8.21.9963
122. Cremaschi GA, Cayrol F, Sterle HA, Diaz Flaque MC, Barreiro Arcos ML. Thyroid hormones and their membrane receptors as therapeutic targets for T cell lymphomas. *Pharmacol Res.* (2016) 109:55–63. doi: 10.1016/j.phrs.2016.02.001
123. Beekman KW, Colevas AD, Cooney K, Dipaola R, Dunn RL, Gross M, et al. Phase II evaluations of cilengitide in asymptomatic patients with androgen-independent prostate cancer: scientific rationale and study design. *Clin Genitourin Cancer* (2006) 4:299–302. doi: 10.3816/CGC.2006.n.012
124. Friess H, Langrehr JM, Oettle H, Raedle J, Niedergethmann M, Dittrich C, et al. A randomized multi-center phase II trial of the angiogenesis inhibitor Cilengitide (EMD 121974) and gemcitabine compared with gemcitabine alone in advanced unresectable pancreatic cancer. *BMC Cancer* (2006) 6:285. doi: 10.1186/1471-2407-6-285
125. Scaringi C, Minniti G, Caporello P, Enrici RM. Integrin inhibitor cilengitide for the treatment of glioblastoma: a brief overview of current clinical results. *Anticancer Res.* (2012) 32:4213–23.
126. Li M, Song X, Zhu J, Fu A, Li J, Chen T. The interventional effect of new drugs combined with the Stupp protocol on glioblastoma: a network meta-analysis. *Clin Neurol Neurosurg.* (2017) 159:6–12. doi: 10.1016/j.clineuro.2017.05.015
127. Cedra S, Wiegand S, Kolb M, Dietz A, Wichmann G. Reduced cytokine release in *ex vivo* response to cilengitide and cetuximab is a marker for improved survival of head and neck cancer patients. *Cancers* (2017) 9:E117. doi: 10.3390/cancers9090117
128. Massabeau C, Khalifa J, Filleron T, Modesto A, Bigay-Game L, Plat G, et al. Continuous infusion of cilengitide plus chemoradiotherapy for patients with stage III non-small-cell lung cancer: a phase I study. *Clin Lung Cancer* (2018) 19:e277–85. doi: 10.1016/j.cllc.2017.11.002
129. Shah CA, Bei L, Wang H, Altman JK, Platanias LC, Eklund EA. Cooperation between αvβ3 integrin and the fibroblast growth factor receptor enhances proliferation of Hox-overexpressing acute myeloid leukemia cells. *Oncotarget* (2016) 7:54782–94. doi: 10.18632/oncotarget.10189
130. Miller PG, Al-Shahrour F, Hartwell KA, Chu LP, Jaras M, Puram RV, et al. *In vivo* RNAi screening identifies a leukemia-specific dependence on integrin beta 3 signaling. *Cancer Cell* (2013) 24:45–58. doi: 10.1016/j.ccr.2013.05.004
131. Zeisig BB, So CW. Linking MLL leukemia with integrin signaling. *Cancer Cell* (2013) 24:5–7. doi: 10.1016/j.ccr.2013.06.011
132. Yi H, Zeng D, Shen Z, Liao J, Wang X, Liu Y, et al. Integrin αvβ3 enhances beta-catenin signaling in acute myeloid leukemia harboring Fms-like tyrosine kinase-3 internal tandem duplication mutations: implications for microenvironment influence on sorafenib sensitivity. *Oncotarget* (2016) 7:40387–97. doi: 10.18632/oncotarget.9617
133. Hercbergs A, Davis PJ, Lin HY, Mousa SA. Possible contributions of thyroid hormone replacement to specific behaviors of cancer. *Biomed Pharmacother.* (2016) 84:655–9. doi: 10.1016/j.biopha.2016.09.053
134. Hercbergs A, Mousa SA, Leinung M, Lin HY, Davis PJ. Thyroid hormone in the clinic and breast cancer. *Horm Cancer* (2018) 9:139–43. doi: 10.1007/s12672-018-0326-9
135. Mousa SA, Glinsky GV, Lin HY, Ashur-Fabian O, Hercbergs A, Keating KA, et al. Contributions of thyroid hormone to cancer metastasis. *Biomedicines* (2018) 6:E89. doi: 10.3390/biomedicines6030089
136. Hercbergs A, Johnson RE, Ashur-Fabian O, Garfield DH, Davis PJ. Medically induced euthyroid hypothyroxinemia may extend survival in compassionate need cancer patients: an observational study. *Oncologist* (2015) 20:72–6. doi: 10.1634/theoncologist.2014-0308

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

frontiers in Endocrinology

REVIEW
published: 13 February 2019
doi: 10.3389/fendo.2019.00059

Thyroid Hormones and Cancer: A Comprehensive Review of Preclinical and Clinical Studies

Eilon Krashin[1,2†], *Agnieszka Piekiełko-Witkowska*[3†], *Martin Ellis*[1,4,5] *and Osnat Ashur-Fabian*[1,2*]

[1] Translational Hemato-Oncology Laboratory, Meir Medical Center, Kfar-Saba, Israel, [2] Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [3] Department of Biochemistry and Molecular Biology, Centre of Postgraduate Medical Education, Warsaw, Poland, [4] Meir Medical Center, Hematology Institute and Blood Bank, Kfar-Saba, Israel, [5] Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Thyroid hormones take major part in normal growth, development and metabolism. Over a century of research has supported a relationship between thyroid hormones and the pathophysiology of various cancer types. *In vitro* studies as well as research in animal models demonstrated an effect of the thyroid hormones T3 and T4 on cancer proliferation, apoptosis, invasiveness and angiogenesis. Thyroid hormones mediate their effects on the cancer cell through several non-genomic pathways including activation of the plasma membrane receptor integrin αvβ3. Furthermore, cancer development and progression are affected by dysregulation of local bioavailability of thyroid hormones. Case-control and population-based studies provide conflicting results regarding the association between thyroid hormones and cancer. However, a large body of evidence suggests that subclinical and clinical hyperthyroidism increase the risk of several solid malignancies while hypothyroidism may reduce aggressiveness or delay the onset of cancer. Additional support is provided from studies in which dysregulation of the thyroid hormone axis secondary to cancer treatment or thyroid hormone supplementation was shown to affect cancer outcomes. Recent preclinical and clinical studies in various cancer types have further shown promising outcomes following chemical reduction of thyroid hormones or inhibition or their binding to the integrin receptor. This review provides a comprehensive overview of the preclinical and clinical research conducted so far.

Keywords: cancer, thyroid hormone, triiodothyronine, thyroxine, αvβ3 integrin

OPEN ACCESS

Edited by:
Veronica Vella,
Università degli Studi di Catania, Italy

Reviewed by:
Elisabetta Ferretti,
Sapienza Università di Roma, Italy
Rocco Bruno,
ASM Matera, Italy
Alessandro Antonelli,
University of Pisa, Italy
Silvia Martina Ferrari,
University of Pisa, Italy

****Correspondence:***
Osnat Ashur-Fabian
osnataf@gmail.com

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *03 December 2018*
Accepted: *22 January 2019*
Published: *13 February 2019*

Citation:
Krashin E, Piekiełko-Witkowska A, Ellis M and Ashur-Fabian O (2019) Thyroid Hormones and Cancer: A Comprehensive Review of Preclinical and Clinical Studies. Front. Endocrinol. 10:59. doi: 10.3389/fendo.2019.00059

INTRODUCTION

Thyroid hormones (TH) are key regulators of essential cellular processes including proliferation, differentiation, apoptosis, and metabolism. Hypothalamic thyrotropin-releasing hormone (TRH), activates the pituitary gland to synthesize and secret thyroid stimulating hormone (TSH), which in turn acts at the thyroid gland to stimulate TH synthesis and secretion (1). Tetraiodothyronine (T4), the main hormone synthesized in the thyroid gland, is catalyzed to the Triiodothyronine (T3) by specific iodothyronine deiodinases (2). T3 acts as the principal TH mediating metabolic activity, via formation of complexes between T3 and nuclear thyroid hormone receptors alpha (TRα) and beta (TRβ). This nuclear T3-receptor complex binds to thyroid hormone response elements on specific genes, regulating their transcription (3). Diseases associated with excess of TH (hyperthyroidism) and lack of TH (hypothyroidism) are common and present with distinct clinical symptoms.

More than a century ago the association between thyroid hormones and malignancies was first suggested (4). Later, Hercbergs and Leith (5) hypothesized that TH deficiency may affect cancer outcome. This assumption was supported by numerous clinical studies, demonstrating that hypothyroidism inhibits tumor growth, while hyperthyroidism produces an opposite effect (6). A great deal of research has been conducted in recent decades to determine how thyroid hormones exert their growth-promoting effect (7). These mechanisms are now better understood following the discovery of a non-genomic pathway for TH action. αvβ3 is a plasma membrane integrin which acts as a membrane receptor for TH (3). This receptor was shown to contain two distinct binding sites for the hormones, S1 and S2, each translating unique signaling cascades (8). While the S1 site binds solely physiological levels of T3, leading to PI3K activation, the second site, S2, binds T4, and with a lower affinity, T3, activating the ERK1/2 pathway (9). αvβ3 integrin binding facilitates the hormones proliferative action on cancer cells as well as blood vessel cells (3).

In this review, we will provide a summary of studies which examined the link between thyroid hormones and cancer. We will first present the preclinical research on the effects of TH in various cancer models, both *in vitro* and *in vivo*. We will then outline clinical studies examining various aspects of this association including the effect on cancer risk, behavior and outcome.

IN VITRO STUDIES OF THE THYROID-CANCER ASSOCIATION

This section summarizes the *in vitro* studies on thyroid hormone-cancer association, presented in **Table 1**. A comprehensive list of the *in vitro* studies, including cancer cell lines and thyroid hormone concentrations, is presented in **Supplemental Table 1**.

Breast Cancer Cell Models

In vitro, thyroid hormones were shown to induce proliferation of breast cancer cells (10, 11, 14). These growth promoting effects were comparable to that of estrogen (E2) and the proliferative effects of T3 or T4 were blocked by co-administration of an estrogen receptor (ER) antagonist, suggesting a significant cross-talk between the two hormones (11, 12, 14). T3 is able to activate estrogen response elements-mediated gene expression in cancer cells (11). In addition, T3 induces the mRNA expression of the growth factors TGFα and TGFβ in ER positive breast cancer cells, while the ER modulator tamoxifen reverts this effect (13, 101). T3 treatment of ER positive ductal carcinoma cells leads to an increase in P53 and Rb phosphorylation, while an ER antagonist blocks these effects (12). T4 induces serine phosphorylation of ERα, which leads to DNA binding and transcriptional activation by the receptor (14). TH also demonstrates tumor promoting effects irrespective of ER signaling. In aggressive triple negative breast cancer cells, T3 enhances aerobic glycolysis (Warburg effect), a hallmark of transformed cells (22). Via a rapid signaling pathway mediated by the integrin αvβ3, T3 was also shown to regulate actin remodeling and to stimulate breast cancer cell migration and invasion (15). T4 was recently shown to stimulate PD-L1 gene expression and increase PD-L1 protein through activation of ERK1/2, thereby supporting the activity of this defensive checkpoint against immune destruction in breast cancer cells (16). In ER negative breast cancer cells, the αvβ3 inhibitor tetraiodothyroacetic acid (tetrac) hinders thyroid hormone cellular actions initiated via the membrane receptor. Nanoparticulate tetrac induces apoptosis through downregulation of apoptosis inhibitors such as XIAP and MCL1 and upregulation of apoptosis promotors such as CASP2 and BCL2L14. Nanotetrac also increases the expression of angiogenesis inhibitor THBS1 as well as the expression of CBY1, a catenin activity inhibitor, and attenuates Ras-oncogene family members (17). It also reduces the effect of T4 on PDL1 gene and protein expression (16). This further supports the assumption that the growth promoting effects of thyroid hormones in breast cancer are mainly mediated through the membrane receptor αvβ3.

Prostate Cancer Cell Models

Thyroid hormones have shown disparate effects in prostate cancer cells, depending on the thyroid hormone involved (T3 or T4) and cell line investigated. The proliferation of androgen-dependent, but not androgen-independent prostate cancer cells was enhanced by T3. In androgen-dependent cell lines T3 downregulated the expression of the anti-proliferative protein, BTG2 (34). In low invasive prostate cancer cells, but not in highly invasive cancer cells, T4 induced the acquisition of neuroendocrine-like morphology, VEGF secretion and invasive capacity (35). In these cells, while T3 itself had no effect, isoproterenol-stimulated neuroendocrine-like morphology and invasiveness were prevented in the presence of T3. In another study, migration was enhanced and detachment-induced apoptosis was inhibited by T4 in prostate cancer cells, while tetrac, the αvβ3 inhibitor, reversed these effects through diminished activity of the MAPK pathway and inhibited expressions of XIAP, MMP2 and VEGF, suggesting involvement of the integrin in these effects (36).

Lung Cancer Cell Models

T4 at physiologic concentrations and T3 at supraphysiologic concentrations increase abundance of proliferating cell nuclear antigen (PCNA) and ERK1/2 activation, markers of cell proliferation, in small cell and non-small cells lung cancer models (39). Interestingly, thyroid hormones led to phosphorylation of ERα, while an ERα antagonist blocked T4 induced PCNA expression, ERK1/2 activation and ERα phosphorylation. This suggests, as demonstrated in breast cancer cells, that thyroid hormone mitogenic effects mediated via the plasma membrane may involve an ERα dependent pathway. Tetrac, as well as pharmacologic inhibition of the MAPK pathway, blocked lung cancer cell proliferation in response to thyroid hormones (39, 40). Moreover, in human non-small cell lung cancer cells, T4 at physiological concentrations enhanced internalization and nuclear translocation of the integrin αv monomer. αv monomer then binds inside the cell nucleus promoters of central cancer-related genes, such as ERα, cyclooxygenase-2,

TABLE 1 | Preclinical studies on thyroid hormones and cancer.

Cancer type/model	Preclinical studies	Thyroid hormones/Inhibitors effect	Proposed mechanism
Breast	*in vitro*	T3 (10–13) and T4 (14) promoted proliferation T3 stimulated cell migration (15). T4 stimulated PDL1 expression (16). Nanotetrac sensitized to apoptosis and increased angiogenesis inhibitors (17) and reduced PDL1 expression (16)	ER mediated (11–13), TR mediated (10), Membrane receptor (14), Integrin αvβ3 (15–17)
		T3 enhanced apoptosis (18). T3 (19–21) and T4 (19, 21) decreased proliferation. T3 enhanced chemosensitization (22, 23)	SMP30 downregulation (18), c-fos upregulation (19) ,ER expression (20), sensitization of mitochondrial metabolism (22)
	in vivo	T3 treatment increased tumor incidence (24). Hyperthyroidism increased tumor incidence (25, 26) and aggressiveness (26). Hypothyroidism decreased tumor incidence (25–31), increased latency (30), slowed tumor growth (30–32), decreased tumor volume (29) and resulted in disease remission (33)	
		Hypothyroidism enhanced invasiveness and metastases (32)	
Prostate	*in vitro*	T3 induced proliferation (34). T4 induced cell migration (35, 36)	Downregulation of BTG2 (34), integrin αvβ3 (36)
		T3 and T4 inhibited proliferation (19)	c-fos upregulation (19)
	in vivo	Hypothyroidism reduced growth rate (37, 38). T3 reduced tumor growth (35)	
Lung	*in vitro*	T4 and T3 induced proliferation (39, 40). T4 induced HIF-1α expression (41)	Integrin αvβ3 (39–41)
	in vivo	T4 increased tumor growth (42, 43), angiogenesis (43) and metastases (42). Hypothyroidism slowed tumor growth (38). Tetrac suppressed tumor growth (40)	
Ovary	*in vitro*	T3 (44, 45) and T4 (44–46) induced proliferation. T4 induced HIF-1α expression (41). T3 and T4 involved in EMT (47) and induced MAPK and PI3K gene expression (48). Tetrac, Triac, and T1AM inhibited proliferation and induced apoptosis (49)	Akt pathway (45), integrin αvβ3 (41, 44, 46–49)
		T3 and T4 inhibited proliferation (21, 45)	
Cervix	*in vitro*	T4 induced MAPK (50–52)	Membrane receptor (50–52)
Glioma/ glioblastoma	*in vitro*	T3 and T4 induced proliferation (9, 53), and inhibited apoptosis (54)	Integrin αvβ3 (9, 53, 54)
		T3 induced re-differentiation and inhibited proliferation (55)	Akt pathway (55)
	in vivo	Nanotetrac reduced tumor size and decreased vascularity (56)	Integrin αvβ3(56)
Neuroblastoma	*in vitro*	T3 inhibited ras-induced proliferation (57)	TR mediated (57)
Renal	*in vitro*	T3 stimulated proliferation (58)	TR mediated (58)
	in vivo	Tetrac reduced tumor size (59)	Integrin αvβ3 (59)
Gastric	*in vitro*	T3 induces VEGF and HIF1α (60)	Akt pathway (60)
	in vivo	Hyperthyroidism increased cancer incidence (61)	
Pancreas	*in vitro*	T3 increased cell proliferation, migration, and invasion (62)	
		T3 (45, 63) and T4 (45) inhibited proliferation	Cyclin-CDK inhibition(63)
	in vivo	Tetrac inhibited tumor growth and angiogenesis (64)	Integrin αvβ3 (64)
Colon	*in vitro*	T3 promoted cell growth and differentiation (65). T4 promoted cell proliferation (66). T3 and T4 up regulated MDR-1 protein (67). T4 stimulated PDL1 expression (16)	Integrin αvβ3 (16, 66)
	in vitro	T3 reduced cell proliferation and increased differentiation (68)	E-cadherin induction (68)
	in vivo	T4 increased cancer incidence (69)	
Hepatocellular	*in vitro*	T3 increased migration and invasion (70, 71), and activated MAPK and Akt pathway (72) T4 promoted HCC cell self-renewal (73)	TR mediated (70, 71, 73), αvβ3 integrin (72)
		T3 inhibited cell proliferation (74–76) and invasion (77)	TR mediated (74–77)
	in vivo	Hypothyroidism reduced tumor growth (32, 78), number and size of metastases and prolonged survival (78). Hyperthyroidism increased invasion and lung metastases (71)	
		Hyperthyroidism associated with preneoplastic nodule regression (79). Hypothyroidism enhanced invasiveness and metastases (32)	TRβ1 up-regulation (79)
Adrenocortical	*in vitro*	T3 and T4 Induced proliferation (45)	
		T3 and T4 inhibited proliferation (45)	
Thyroid	*in vitro*	T3 and T4 induced proliferation (80)	Integrin αvβ3 (80)

(Continued)

TABLE 1 | Continued

Cancer type/model	Preclinical studies	Thyroid hormones/Inhibitors effect	Proposed mechanism
	in vivo	Hypothyroidism reduced tumor growth (81). Tetrac inhibited tumor growth (82, 83)	Integrin αvβ3 (82, 83)
Melanoma	*in vivo*	Hypothyroidism increased tumor latency and survival (84)	
Basal cell carcinoma	*in vitro*	T3 reduced growth and induced apoptosis (85)	PKA induction (85)
	in vivo	T3 reduced tumor growth (85)	
Ehrlich tumor	*in vivo*	Hyperthyroidism increased tumor size (86) and ascitic volume (87)	
Sarcoma	*in vivo*	Hyperthyroidism increased tumor size and metastases (88)	
Multiple myeloma	*in vitro*	T3 and T4 induced proliferation and viability (89, 90) and increase cell migration and invasion (91)	Integrin αvβ3 (89–91)
	in vitro	Tetrac inhibited proliferation and induced apoptosis (92)	Integrin αvβ3 (92)
Leukemia	*in vitro*	No direct effect of T3 and T4 (93)	
Lymphoma	*in vitro*	T3 and T4 induced proliferation and VEGF expression (94)	Integrin αvβ3 (94)
	in vivo	Hyperthyroidism increased tumor growth (95, 96) and reduced survival (96)	
Angiogenesis	*ex vivo* (CAM model)	T3 (97, 98) and T4 (97–100) induced angiogenesis. Tetrac arrested tumor related angiogenesis (40, 59, 82, 83)	Membrane receptor (98), integrin αvβ3 (40, 59, 82, 83, 97, 99, 100)

hypoxia-inducible factor-1α (HIF1α), and thyroid hormone receptor β1 (41).

Gynecological Cancer Cell Models

In ovarian cancer cells T3 at supra physiological and T4 at physiological concentrations induced cell proliferation, survival and viability and led to αvβ3 mediated ERK up-regulation (44, 46). Genes that constrain cell cycle (p21, p16), promote mitochondrial apoptosis (Nix, PUMA), and tumor suppression (GDF-15, IGFBP-6) were inhibited by TH, while a hypothyroid environment attenuated ovarian cancer growth (44). TH were also shown to be involved in αvβ3 mediated epithelial to mesenchymal (EMT) transition in ovarian cancer cells, inducing mesenchymal markers zeb-1, slug, and vimentin, and inhibiting the epithelial markers, e-cadherin and zo-1 (47). This suggests a possible implication for TH in ovarian cancer metastases. The αvβ3 inhibitor tetrac induced ovarian cell apoptosis as well as upregulation of ATM and PARP-1, proteins that coordinate recognition of DNA damage (49). As demonstrated for lung cancer models, αv monomer internalization and nuclear translocation were induced by T4, activating multiple genes involved in cancer promotion (41). Importantly, and comparable with results from breast cancer, crosstalk between integrin αvβ3 and ERα promoted the proliferation of ovarian cancer cells by TH, mimicking functions of E2. Both T4 and E2 promoted nuclear translocation of the integrin αv monomer as well as the phosphorylation of ERα, while the presence of an antagonist for ERα blocked T4-induced ERK1/2 activation, ERα phosphorylation, PCNA expression and cell proliferation (46).

In cervical cancer cells (HeLa), T4 was demonstrated to rapidly induce phosphorylation and nuclear translocation of MAPK (50) and to potentiate EGF and TGFα-induced MAPK activation (51). These effects could not be mediated through TR, as HeLa cells lack these receptors. These effects were reproduced by T4-agarose and blocked by tetrac, suggesting a membrane receptor involvement (50–52).

Central Nervous System Tumor Cell Models

In glioma cells T4 caused proliferation and upregulation of PCNA and MAPK. This effect was inhibited by tetrac, suggesting mediation by the αvβ3 integrin (53). In another study in glioma cells, T3 and T4, acting on the αvβ3 integrin, induced proliferation and activation of ERK1/2, while only T3 activated Src kinase and its downstream PI3-kinase signaling cascade. These findings suggested that the integrin contains two iodothyronine receptor domains, activating different pathways (9). Resveratrol-induced-apoptosis was inhibited in glioblastoma cells by T4, through interference with nuclear COX-2 and ERK1/2 interaction. This effect was prevented by tetrac (54). Other studies demonstrated conflicting results. In both astrocytoma and glioblastoma cells, T3 promoted re-differentiation. T3 increased cell proliferation and phospho-Akt levels in astrocytoma cells, yet suppressed cell proliferation in glioblastoma cells, suggesting differing effects related to cancer aggressiveness (55). In neuroblastoma cells, T3 inhibited ras-induced proliferation and blocked induction of cyclin D1 expression by the oncogene (57). T3 strongly antagonized the transcriptional response mediated by the Ras/MAPK signaling pathway in neuroblastoma cells expressing TRs.

Renal Cancer Cell Models

Renal cancer is associated with multiple aberrances of thyroid hormone signaling pathway. These include mutations and altered expression of thyroid hormone receptors, decreased intratumoral concentrations of T3, as well lowered expression and disturbed alternative splicing of type 1 iodothyronine deiodinase (102–106). In contrast to normal kidney cells, which decrease proliferation in response to T3, divisions of renal cancer cell

lines are stimulated by TH (58). These different T3 effects are the result of distinct, cell type specific regulation of genes that control cell cycle progression. In renal cancer cells T3 attenuates expressions of E2F4, p107, and p130, while in healthy kidney cells the expression of p107 is stimulated by T3. p107 and p130 are proteins of the retinoblastoma family which enable binding of E2F4 and E2F5 to form CERC (cyclin E repressor complex). During G1 phase, CERC interacts with and negatively affects the activity of promoter of *CCNE1*, encoding cyclin E1, thus repressing proliferation (107). In consequence of these disparate effects on gene expression, T3 accelerates cell cycle progression in renal cancer cells, triggering progression to S phase. In contrast, in normal kidney cells, cell cycle progression is attenuated by T3 (58). These pro-proliferative T3 effects on renal cancer cells were confirmed by an independent study (108).

Gastrointestinal Cancers Cell Models

T4 and T3 differently influence gastrointestinal cancer cells. In contrast to renal tumors, gastric cancers accumulate T3, possibly due to overexpressed transthyretin that mediates cellular T3 import (60). The increased intracellular T3 concentration directly contributes to cancer progression, by inducing the expression of HIF1α, which in turn activates the expression of proangiogenic VEGF. Interestingly, these T3 effects are mediated by accumulation of fumarate, one of the key intermediates of TCA cycle, acting as an inhibitor of HIF1α degradation. These T3 effects are mediated by rapid non-genomic mechanisms, involving PI3K signaling (60). T4, acting on αvβ3 receptors, stimulates colon cancer cell proliferation and activation of PCNA, cyclin D1, and c-Myc (66). These pro-cancerous T4 effects can be prevented by tetrac and nanotetrac. Furthermore, tetrac and nanotetrac potentiate antiproliferative activity of cetuximab, an anti-EGFR antibody, suggesting potential beneficiary effects of these drug combinations in colon cancer patients (66). These pro-mitogenic effects of extracellular T4 are in sharp contrast to mechanisms initiated by T3. Dentice et al. showed that treatment of colon cancer cells with T3 induces their differentiation with concomitant reduced proliferation (68). T3 activated tumor-suppressive E-cadherin, triggering plasma-membrane localization of beta-catenin, thus preventing its nuclear mitogenic activity. These protective T3 actions are prevented by activated beta-catenin which stimulates expression of type 3 deiodinase and downregulates type 2 deiodinase, thereby reducing intracellular T3 pool. In colon cancer cells, T4 mediated the activation of MDR1, suggesting that thyroid hormones may promote drug resistance mechanisms (67, 109).

The effects of T3 in pancreatic tumor cells depend on tumor type. Proliferation of some, but not all, cell lines derived from highly aggressive pancreatic adenocarcinoma was suppressed by T3 (63). Mechanistically, T3 changed expressions of cell cycle regulators, leading to downregulation of cyclins D1 and E, and upregulation of cdk inhibitors, $p21^{cip1}$ and $p27^{kip1}$. Furthermore, T3 attenuated the activity of cyclin-CDK complexes, which resulted in reduced pRb phosphorylation and G1 cell cycle arrest. In contrast, the proliferation, migration, and invasion of pancreatic cancer cells was stimulated by T3 *in vitro* (62). These results fit observations of patients in which hypothyroidism treated with TH supplementation correlated with increased risk of tumor progression and poor prognosis (62). Thyroid hormones were shown to potentiate cytotoxic effects of chemotherapeutics in pancreatic cancer cells (63).

Conflicting *in vitro* results exist regarding the effect of thyroid hormones in hepatocellular carcinoma (HCC). Several studies demonstrated that T3, acting on the TR, leads to inhibition of cancer cell growth. In HCC cells, T3 downregulated oncogenes CDK2, cyclin E and phospho-Rb (74) and up regulated the tumor suppressor p21 and endoglin (74, 75). T3 also induced DKK4, which suppresses cell invasion and metastatic potential via reduction of matrix MMP2 (77) and downregulated ELF2, a transcription factor associated with tumor growth and cell proliferation (76). *In vitro* experiment confirmed that TRβ1 silencing enhanced proliferation and migration of human HCC cells (79). Conversely, T3 action on TR may increase HCC aggressiveness. A high frequency of somatic point mutations of TRα and TRβ were identified in human HCC samples (110, 111). T3 was associated with increased HCC invasiveness through up regulation of furin (70) and lipocalin 2 (71) in a TR dependent manner. Lipocalin 2 and TRα were both overexpressed in HCC patient samples and correlated with cancer grade, stage, and survival (71). T4 action on TRα promoted HCC cells self-renewal, increased cancer stem-like cells and drug resistance and upregulated NF-kB (73). Finally, T3 binding to integrin αvβ3 in HCC cells, induced growth-promoting effects via ERK1/2 and Akt phosphorylation (72).

Hematological Malignancies Cell Models

T4 and T3 stimulate proliferation and viability of multiple myeloma (MM) cells by activating αvβ3 integrin receptor, leading to rapid activation of the MAPK signaling pathway (89, 90). This in turn, results in activation of genes involved in proliferation (PCNA), and reduced expression of genes encoding apoptotic regulators such as apafl, caspase-3, puma, and noxa (90). Remarkably, the integrin-mediated TH actions may contribute to progression of MM by changes in adhesion and remodeling of extracellular matrix. Specifically, T3 and T4 increased adhesion of MM cells to fibronectin and activated expression of MMP-9 via a mechanism involving αvβ3 and MAPK (91). These *in vitro* results are of potential clinical importance, since tetrac inhibited MM cell proliferation and induced apoptosis. Furthermore, tetrac sensitized patient-derived MM cells to bortezomib, providing a potential new therapeutic option (92). Tetrac also blocked TH-mediated induction of MMP-9 (91).

TH affect proliferation of T-cell lymphoma (TCL) cells by simultaneous induction of genomic and non-genomic mechanisms (112, 113). The non-genomic mechanisms involve rapid membrane translocation of PKC ζ isoform and activation of ERK and NF-κB. One of the downstream targets of PKC ζ signaling is inducible nitrix oxide synthase (iNOS), a well-known activator of TLC proliferation. Barreiro Arcos et al. showed that intracellular activity of TH is prerequisite for activation of iNOS expression, along with enhanced expression of TRα (113). Non-genomic TH actions also contributed to survival and progression of TCL. Specifically, binding of TH to αvβ3 receptors, triggered pro-proliferative, and proangiogenic signals

including enhanced expression of cyclins, PCNA, and VEGF. This TH-induced secretion of VEGF stimulated proangiogenic activity of endothelial cells, possibly contributing to TCL progression (94). In another study, *in vitro* treatment of lymphoma cells with T3 and T4 activated proliferation, as indicated by increased expressions of PCNA, as well as cyclins D, A2, and B (95).

In vitro Studies in Other Cancer Models

The cancers originating from thyroid gland are influenced by its own secretory products. Specifically, T4 and T3 promote proliferation of follicular and papillary thyroid cancers *in vitro* (80). These effects are largely mediated by non-genomic signaling involving αvβ3 receptor as indicated by tetrac blockade of the TH-induced proliferation. Furthermore, T4 treatment blocked pro-apoptotic signaling induced by external stimuli such as resveratrol (80). Altogether, this data indicates pro-cancerous and anti-apoptotic role of TH in thyroid cancers. An inhibitory role of TH was reported for skin cancer. T3 inhibited proliferation and induced apoptosis in basal cell carcinoma cells (85). Mechanistically, T3 reduced protein stability and transcriptional activity of Gli2, an oncogenic transcription factor, that promotes G1/S cell cycle phase transition.

Moriggi et al. tested TH influence on six cell lines derived from various types of cancer that differed by the profile of mutations in genes involved in PI3K and beta-catenin signaling pathways. Remarkably, for each cancer type, T3 exerted dual effect, either stimulating or attenuating proliferation. Unfortunately, the presented data did not clarify the cause of this differential T3 effects (45). The results of this study underscore the complexity of mechanisms involved in TH-mediated effects in cancer cells.

Taken together, the results of the *in vitro* studies suggest that effects of thyroid hormones in cancer are mediated by complex genomic and non-genomic signal transduction pathways and are highly dependent on cell type and molecular context. These biological pathways were extensively summarized in a recent review by Goemann et al. (114).

The complex and often contradictory T4 and T3 effects observed *in vitro* underline the importance of *in vivo* studies, which can provide valuable information on the net TH effects in a living organism. On the other hand, *in vitro* experiments provide a unique opportunity to reveal mechanistic details of intra- and extracellular processes initiated by T4 and T3 in cancer cells. Inevitably, both types of studies are required to clarify the role of TH in cancer development and progression.

IN VIVO STUDIES OF THE THYROID-CANCER ASSOCIATION

This section summarizes the *in vivo* studies on the thyroid hormone-cancer association, presented in **Table 1**. A comprehensive list of *in vivo* studies is presented in **Supplemental Table 2**.

Breast Cancer Animal Models

One of the earliest reports analyzing *in vivo* the link between thyroid hormones and breast cancer was published in 1946. Treatment of mice with the thyroid synthesis inhibitor, thiourea, delayed development of spontaneous breast tumors (115). Similar results were achieved when mice were treated with another compound, thiouracil (116). These results were further validated by Vonderhaar et al., who found that thiouracil-induced hypothyroidism delayed development and decreased incidence of spontaneous breast tumors in mice (27). The study suggested that hypothyroidism could contribute to local atrophy of mammary glands, resulting in reduced tumor formation. Contrasting results were obtained on experimental Ehrlich tumors (ET) that arise from mouse mammary adenocarcinoma (86). In that study, hyperthyroidism decreased metabolic activity and proliferation of ET as evidenced by lowers nuclear diameter, mitotic index, and number of nucleolus organizer regions. TH effects were also tested *in vivo* in models of chemically induced breast tumors. Early studies brought inconclusive results, showing that both thyroidectomy and thyroxine supplementation reduced incidence of breast tumors (117). However, a series of later reports clearly demonstrated the protective effect of PTU (propylthiouracil)-induced hypothyroidism. PTU given at a dose that produced severe hypothyroidism in rats, dramatically reduced the incidence of 7,12-dimethylbenz(a)anthracene (DMBA) (28) and N-methyl-N-nitrosourea (MNU) (29) induced breast tumors. A more recent study further demonstrated that PTU-induced hypothyroidism delayed development and reduced incidence of DMBA-induced mammary tumors by activating apoptosis (30). The protective effects of hypothyroidism were also shown in a model of breast tumor xenografts. Treatment with PTU inhibited growth of inoculated mammary adenocarcinomas and improved survival of mice (31). Spectacular effects of PTU treatment were reported by Shoemaker and Dagher who demonstrated complete remission of mammary tumor xenografts in 77% of PTU-treated mice (33). Confounding reports on the influence of thyroidal status in human cancer was partially explained by Martínez-Iglesias et al. (32). Their study revealed that while the growth of breast cancer cells inoculated into hypothyroid hosts was delayed, the tumors were more invasive and metastatic. The tumors grown in hypothyroid animals were more undifferentiated, with reduced expression of epithelial markers (e.g., keratin 8/18, β-catenin) and enhanced expression of mesenchymal markers (vimentin). However, the same study demonstrated that hypothyroidism reduces cancerous proliferation and stimulates necrosis in tumors, resulting in retarded tumor growth (32). In a parallel study, the same group showed that overexpression of thyroid hormone receptor β (TRβ) attenuated growth of breast tumor xenografts in mice, indicating its tumor suppressive activity (118). These studies demonstrated that intracellular and extracellular effects of thyroid hormones can differently contribute to development and progression of breast cancer, affecting both cancer cells and tumor stroma.

Prostate Cancer Animal Models

Consistently with results described above, PTU-induced hypothyroidism attenuated growth of prostate cancer xenografts in athymic mice (37, 38). The latter study clearly demonstrated that PTU did not affect the proliferation of prostate cancer

cells *in vitro*, supporting the conclusion that anticancer effect was the result of hypothyroid state of the animals (38). In contrast to these suppressive effects of hypothyroidism, a more recent study reported that treatment with T3 (2.5 μg/day) inhibited growth of prostate tumors inoculated in nude mice (35).

Lung Cancer Animal Models

One of the early *in vivo* studies on TH effects on lung cancer was performed using a model of Lewis lung carcinoma (3LL), an undifferentiated squamous cell carcinoma that spontaneously developed in the lung of C57/BL6 mouse (42). Hyperthyroidism induced by T4 administration significantly increased growth of tumors inoculated by subcutaneous injections of 3LL cells in mice. In contrast, hypothyroidism triggered by methimazole treatment attenuated tumor growth and increased survival of the animals. Remarkably, T3 and T4 differently affected progression of the disease. The number of pulmonary metastases was reduced by treatments with T3 or methimazole while it was increased by treatment with T4. The reduction of tumor growth by methimazole was probably a result of its direct inhibitory effect on cancer cells, since *in vitro* experiments revealed that methimazole suppressed growth of 3LL cells (42). However, another study showed that hypothyroidism itself can also suppress growth of lung tumors. Treatment with PTU significantly suppressed growth of lung tumors subcutaneously inoculated in mice. This PTU effect was possibly not the result of suppressive effect on cancer cells since in a parallel experiment PTU did not affect growth of prostate cancer cells *in vitro* (38). Interestingly, it was suggested that TH may also affect cancer progression by influencing immune response. In the abovementioned study both T4 and methimazole suppressed the activity of NK cells, while alveolar macrophages were activated by T4 and T3 (42). This data indicates that T4 and T3 have broad effects on lung cancer development and progression, not only via direct effects on cancer cells but also by influencing tumor environment and elements of the immune system. The direct effects of T4 on lung cancer cells are probably the result of non-genomic actions. Interesting data was provided by a large study involving 100 mice with Lewis lung carcinoma tumors in which interactions between thyroid hormone and nitric oxide signaling were analyzed (43). Treatment of mice with T4 resulted in a remarkable increase of tumor weight compared to euthyroid animals. These effects were associated with increased expression of VEGF, suggestive of enhanced vascularization. Furthermore, intraperitoneal injections of tetrac, an antagonist of T4 binding to integrin αvβ3, significantly reduced tumor growth and VEGF expression. These results suggested that pro-tumorous T4 effects in 3LL cells are mediated by αvβ3 integrin receptor (43). Similar data was obtained in non-small cell lung cancer cells in which pro-proliferative T4 actions were blocked by antibody directed against integrin αvβ3 as well as by tetrac (40). These promising therapeutic effects of tetrac were also confirmed in studies involving other tumors (56, 64, 82).

In vivo Studies of Gastrointestinal Cancers

Several lines of evidence indicate that high T4 levels promote gastrointestinal carcinogenesis *in vivo*. In rats, T4 administration increased incidence of chemically induced tumors of colon and stomach (61, 69). TH effects were comprehensively analyzed in models of liver neoplasia. It was shown that hypothyroidism delays progression of experimental Morris hepatoma tumors implanted in female Buffalo Rats (78). Specifically, hypothyroidism induced within 2 weeks from tumor implantation not only attenuated growth of localized tumors but also decreased the number of lung metastases and prolonged survival of the animals. These results were further supported by later studies demonstrating tumor suppressive role of TRβ1 in the progress of hepatocellular carcinoma (HCC). Using rat model of HCC, Frau et al. (79) showed that expressions of TRα1 and TRβ1, along with downregulated expressions of their targets, are decreased in tumors. Notably, downregulation of TRβ1 expression was associated with high proliferative activity of liver cells. TRβ1 expression was also decreased in human HCC tissue samples. In contrast, induction of hyperthyroidism in rats bearing nodules resulted in increased TRβ1 expression and regression of preneoplastic lesions. These results clearly suggest a tumor suppressive role of TRβ1 in HCC. However, contrasting results were published on the role of TRα1 in HCC (71), showing that TRα1 is overexpressed in human HCC and stimulates migration and invasion *in vitro* and *in vivo*. Under hyperthyroid conditions HCC cells expressing TRα1 induced invasion and metastases formation in mice. These effects were mediated via MET/FAK pathways. These results were further confirmed by analysis of human HCC tissue samples in which high expressions of TRα1 were associated with lower patients' survival. Remarkably, no such correlation was observed for TRβ1. The authors suggested that T3/TR could play a dual, oncogenic or tumor suppressive role, depending on the molecular background and stage of the disease. So far, this hypothesis was not supported by experimental data. Curiously, the same research group published contrasting results on the expression of TRs in human HCC tissue samples. In a study published in 2012 they reported decreased expressions of TRs (including TRα1) in HCC specimens and concluded that TRs play a tumor suppressive role (119). Clearly, the role of T3 and TRs in HCC requires further elucidation in independent studies involving both human tissue samples and *in vivo* experiments in mice.

In vivo Studies of Hematological Malignancies

The earliest *in vivo* studies exploring the relations between TH and leukemia brought inconsistent results. One study reported decreased incidence of spontaneous lymphatic leukemia in mice with T4-induced hyperthyroidism when compared with hypothyroid mice treated with PTU (120). The hypo- and hyperthyroid leukemic animals did not differ in their survival rates. However, interpretation of these results is challenging, mainly due to limited information on methods and criteria used for leukemia diagnosis in the animals. In contrast, Morris

et al. showed that PTU-induced hypothyroidism attenuated lymphomatous infiltrations in rats compared with euthyroid animals (121). Furthermore, hypothyroidism prolonged survival of mice and rats with transplanted lymphomas, while T4 treatment of euthyroid animals resulted in the opposite effects. Different results were obtained in a study focused on progression of acute stem-cell leukemia in rats. The animals were rendered hypothyroid by several methods, including thyroidectomy and thyroid gland ablation with radioactive iodine. Apparently, none of these treatments influenced growth of subcutaneously inoculated tumors nor affected survival of the animals (122).

Non-genomic TH effects were reported for T-cell lymphomas (TCL), defined as a group of heterogeneous lymphoproliferative disorders. TH stimulated TCL proliferation and angiogenesis by acting through αvβ3 integrin receptor (94). TH binding to αvβ3 triggered activation of VEGF and NF-κB pathways, resulting in stimulation of angiogenesis and proliferation. This study further showed that selective inhibition of αvβ3 with cilengitide attenuates growth of TCL xenografts in mice. An interesting study providing the link between TH, stress, and cancer was published by Frick et al. (93). They showed that chronic stress led to suppression of TH plasma levels which was associated with attenuation of T-cell proliferation in response to mitogens, suggestive of impaired immune functions. Supplementation with T4 protected T-cells against stress-induced suppression of proliferation. More importantly, treatment with T4 prevented stress-induced growth of lymphoma tumors subcutaneously inoculated in mice. The study also suggested that TH antitumor effects could be mediated by PKC isoforms θ and α. Exposition of the animals to stress diminished activation of these PKC isoforms. In contrast, T4 supplementation counteracted stress-induced attenuation of PKC activation.

Thyroid hormone status can have dual effect on lymphoma growth and metastasis as shown by a study in which hyperthyroidism stimulated local tumor growth while hypothyroidism fostered formation of metastatic lesions in kidneys (95). Interestingly, these dual effects of TH on primary and secondary malignancies seem to be a more generalized mechanism since similar observations were made in the above mentioned mouse model of breast cancer. Lymphoma cells inoculated in hyperthyroid animals grew faster, with enhanced tumoral and peritumoral vasculogenesis, and increased expression of PCNA and caspase 3 (96). These effects were associated with shorter survival of hyperthyroid animals when compared with eu- and hypothyroid mice. Similar to the effects described *in vitro* (95), enhanced expressions of PCNA and cyclins D and E was described in tumors grown in hyperthyroid animals, when compared with eu- and hypothyroid animals. Surprisingly, hyperthyroidism stimulated apoptosis, as demonstrated by activation of caspase 3 and Bax. The enhanced metastasis observed in hypothyroid animals could be the effect of changes in immune responses. Hypothyroidism increased the percentage of $CD4^+CD25^+FoxP3^+$ Treg cells in tumor draining lymph nodes (TDLN). Tregs suppress activation and proliferation of CD4+ and CD8+ T lymphocytes. Likewise, activated $CD8^+$ T cells ($CD8^+CD69^+$ or $CD8^+CD44^{hi}$) were decreased in TDLN of hypothyroid animals. The presence of immunosuppressive Tregs in hypothyroid TLDN possibly contributed to metastatic progression, since depletion of CD8+ cells resulted in enhanced metastasis in mice (95). In contrast, TH could prevent metastasis by activation of apoptosis. Indeed, tumors grown in hyperthyroid animals showed increased presence of apoptotic cells when compared with eu- and hypothyroid mice. Remarkably, no signs of apoptosis were found in highly proliferative regions of tumors, explaining their intense growth. Altogether these results suggest that hypothyroidism creates an immunosuppressive milieu that allows for immune tolerance toward metastasizing tumor cells. Another indication of immune tolerance is reduced accumulation of NK cells in spleens from hypothyroid animals suggestive of reduced ability to remove tumor cells by NK cells (95).

In vivo Studies in Other Cancer Models

Protective effect of hypothyroidism was also shown for uveal melanoma, one of the most common and highly metastatic intraocular malignancies (84). PTU-induced hypothyroidism significantly improved survival of mice with ocular melanoma, in contrast to hyperthyroid animals that demonstrated significantly shorter survival when compared to euthyroid animals. Remarkably, uveal melanoma cells expressed high levels of αv and β3 subunits of the integrin receptor, thus providing the platform for binding of T4 and activation of pro-proliferative intracellular signaling cascades.

Studies on the effects of TH on sarcoma brought conflicting results with early studies demonstrating that thyroid radioablation did not change growth of fibrosarcomas in mice (123), while in another reporting that hyperthyroidism attenuated growth of sarcoma tumors in mice (124). However, these results were later negatively verified by independent studies which showed that T4-induced hyperthyroidism stimulated growth and metastatic progression of sarcoma xenografts in mice, while tumor growth was attenuated in mice rendered hypothyroid by radioablation of the thyroid gland (88).

Antitumor effects of T3 were shown for basal cell carcinoma (BCC), the most common human cancer. Topically applied T3 significantly reduced tumor growth in mouse model of BCC (85). Intracellular T3 levels are regulated by activity of type 3 deiodinase (D3) which degrades T3. Depletion of D3 in skin of BCC bearing mice significantly reduced the occurrence of tumors, suggesting that antitumor T3 actions are mediated via its intracellular activity and not mediated by αvβ3 integrin receptor (125).

In contrast, plasma membrane-initiated TH signaling is well-documented in a mouse model of follicular thyroid carcinoma (FTC). FTC tumors are spontaneously developed by $Thrb^{PV/PV}$ mice in which both alleles of thyroid hormone receptor β bear PV mutation that initially was identified in a patient with thyroid hormone resistance. TRβ with PV mutation are unable to bind T3 and activate transcription. Treatment of $Thrb^{PV/PV}$ mice with PTU reduced the expression of integrin αv subunit, thus leading to attenuation of TH-plasma membrane signaling, including the cascade involving PI3K, AKT, and β-catenin (81). This in turn

resulted in inhibition of cancerous proliferation and reduction of tumor growth.

In conclusion, the *in vivo* studies in animal models indicate that TH have broad effects on cancer development and progression. On one hand, local intracellular changes in TH concentrations contribute to proliferation of cancer cells, stimulating tumor growth. On the other hand, extracellular hypothyroid milieu may support cancer progression by attenuating immune responses. Several lines of evidence strongly indicate that non-genomic T4 actions can trigger cancerous proliferation and that interference with T4-αvβ3 integrin binding can provide efficient therapeutic option for patients.

CLINICAL STUDIES OF THE THYROID-CANCER ASSOCIATION

This section summarizes clinical studies on the thyroid hormone-cancer association, presented in **Table 2**. A detailed list of clinical studies, including study design and number of patients, is presented in **Supplemental Table 3**.

Effect of Thyroid Status on Cancer Risk

Effect on the Overall Risk of Cancer

Hellevik et al. conducted a prospective population based study of 26,691 people without a previously diagnosed thyroid disease (153). Baseline TSH levels were measured and 9 years of follow up of cancer incidence was recorded. Compared to euthyroid reference group, increased cancer risk (HR 1.34) was associated with low TSH levels (<0.5 mU/l), a risk driven by lung cancer (HR 2.34) and prostate cancer (HR 4.99). In another population based cohort study, 17,034 patients with newly diagnosed hyperthyroidism were matched with 34,066 patients without hyperthyroidism. Over a 4 year follow up period, patients with hyperthyroidism were at higher overall risk of cancer (Adjusted HR 1.2, $p < 0.05$) and thyroid cancer (Adjusted HR 6.8, $p < 0.05$), with extended duration of hyperthyroidism associated with greater risk of thyroid cancer (195). The Rotterdam study prospectively included 10,318 patients with baseline measurements for free T4 and TSH, followed for a median of 10.4 years. Higher free T4 levels were associated with higher risk of solid cancers (HR 1.42 per unit increase in free T4), lung cancer (HR 2.33), and breast cancer (HR 1.77), although no association were found for TSH levels (126). Collectively, these prospective studies support a causal association between disorders in thyroid hormones and cancer risk.

Effect on the Risk of Breast Cancer

In a population based case control study including 676 breast cancer patients and 680 controls (127), free T4 levels were associated with a high overall risk of breast cancer (OR 1.4 for free T4 above vs. below the median). This increase was later attributed to a higher incidence rate of less aggressive breast cancer subgroups (128). Another prospective cohort study conducted by the same group included 2,185 women followed for an average of 19.3 years for breast cancer incidence (135). An association was demonstrated between T3 and breast cancer

TABLE 2 | Clinical studies on thyroid function and cancer.

Cancer type	Thyroid function/ Treatment	Clinical outcome
Breast	Hyperthyroidism	Increased risk (126–129), higher mortality (130, 131). Higher T3 (132, 133) and T4 (23, 132–134) in cancer patients. T3 associated with cancer risk (135, 136), large tumors (135), lymph node metastases (135), and cancer death (137, 138)
		No effect on cancer risk (139–141)
	Hypothyroidism	Decreased risk (129, 142, 143), longer progression free survival (144), later diagnosis (142), more localized disease (142), less lymph node involvement (142). Lower mortality (145). Decreased risk of triple+ BC with higher TSH (146)
		Increased risk (147)
		No effect on cancer risk (139–141, 148, 149)
	LT4 treatment	Increased risk (147, 150)
		Lower all-cause mortality (151)
		No effect on cancer risk (152)
Prostate	Hyperthyroidism	Increased risk (153, 154), T3 associated with risk of recurrence (155). Higher T3 in cancer patients (156)
	Hypothyroidism	Lower risk (154, 157)
Lung	Hyperthyroidism	Increased risk (126, 153). Higher T4 and lower TSH in cancer patients (158)
	Hypothyroidism	Longer survival (159, 160) and later diagnosis (159)
	LT4 treatment	Increased risk (161)
Ovary	Hyperthyroidism	Increased risk (162), higher mortality (130, 163). Higher T4 in cancer patients (164)
Uterine	Hypothyroidism	Increased mortality (165), Elevated TSH associated with lower survival (166)
Central nervous system	Hyperthyroidism	Increased risk (167)
	T3 treatment	Prolonged survival (168)
	Induced hypothyroidism	Prolonged survival (169, 170)
Renal	Hypothyroidism	Increased survival (171–178), increased remission (173), tumor regression (179), and response to treatment (180)
		Increased risk (167)
Esophageal cancer	Hyperthyroidism	Higher incidence in cancer (181)
Pancreas	Hyperthyroidism	Increased risk (182), increased mortality (165)
	LT4 treatment	Higher perineural invasion (62), T stage (62), nodal spread (62) and poorer prognostic stage (62)
	Induced hypothyroxinemia	Tumor regression (183)
Colorectal	Hyperthyroidism	Increased risk (184)

(Continued)

TABLE 2 | Continued

Cancer type	Thyroid function/ Treatment	Clinical outcome
	Hypothyroidism	Increased risk in untreated hypothyroidism (184). Higher subclinical hypothyroidism in colorectal cancer (185). Increased risk with higher TSH (186)
	LT4 treatment	Decreased risk (184, 187, 188)
	Free T3/free T4 ratio	Higher ratio associated with increased survival (189)
Hepatocellular	Hyperthyroidism	Low TSH associated with smaller tumors (190). T3 and T4 inversely associated with cancer mortality (191)
		Lower survival with elevated T4 (190)
	Hypothyroidism	Increased risk (192). High TSH associated with larger tumors (190). Increased incidence in HCC patients of unknown etiology (193)
	TSH x free T4	Higher value associated with favorable time to tumor progression and overall survival if chemotherapy provided and unfavorable TTTP and OS if sorafenib administered (194)
Thyroid	Hyperthyroidism	Increased risk (167, 195)
Head and neck	Hypothyroidism	Increased survival (196–198)
Melanoma	Hypothyroidism	No difference in survival (199)
Multiple myeloma	Hyperthyroidism	T3 higher and TSH lower in patients (200)
Leukemia	Hyperthyroidism	T3 and T4 higher and TSH lower in patients (201). Improved outcome in Grave's disease (202)
	Hypothyroidism	Improved outcome in Hashimoto thyroiditis (202)
Myelodysplastic syndrome	Hyperthyroidism	T3 and T4 higher and TSH lower in patients (203)
General	Hyperthyroidism	Increased risk (126, 153, 195), Increased cancer death in hyperthyroidism (165), and toxic nodular goiter (204)
		T3 inversely associated with cancer mortality (191)
		No association to cancer mortality (205, 206)
	Hypothyroidism	Lower mortality (145, 207), Longer survival (208), High response to radiation therapy (209)
		Increased risk (167), Increased cancer mortality (137)
	Induced hypothyroxinemia	Prolonged survival (210)

risk (HR 1.61 of third quartile compared to first). In another large population-based study conducted by Søgaard et al., women with hyper- and hypothyroidism were followed for up to 7.4 years (129). Hyperthyroidism was related to a slight increase in the risk of breast cancer compared to the general population (SIR 1.11), while the opposite was shown for hypothyroidism (SIR 0.94). In a retrospective cohort study of 437 breast cancer patients, elevated levels of TSH were associated with a lower likelihood of triple positive breast cancer (ER+ PR+ Her2/neu+) compared with ER+ PR+ Her2/neu– breast cancer. However, no association was found with tumor grade or stage (146). Interestingly, Brandt et al. were recently able to identify a SNP (rs2235544) in the gene for deiodinase type 1 (DIO1) which was associated with both free T4 level and breast cancer risk (211). A recently published case control study which included 682 breast cancer patients and 731 controls demonstrated an association between higher serum total T4 and breast cancer in both premenopausal (OR 5.98) and post-menopausal women (OR 2.81), whereas a negative association was demonstrated between total T3 and breast cancer (134). Similarly, Huang et al. demonstrated higher free T4 and lower T3 in patients with newly diagnosed breast cancer compared with patients with benign breast lesions (23). However, these findings may demonstrate the effect of malignancy on the thyroid axis, rather than a true demonstration of risk or causality. Specifically, malignancy may be associated with a reduction of T3, resulting the so-called non-thyroidal illness syndrome (NTIS) (212). The association between NTIS and malignancies is later detailed in this review in a designated section. A meta-analysis from 2012, which included 10 case control studies of hyper- and hypothyroidism and breast cancer, failed to find a putative relationship of either disorder. Notably, a high degree of heterogeneity was demonstrated between the six hypothyroidism studies included (139). Another meta- analysis from 2017 included population based studies assessing thyroid dysfunction and the risk of breast cancer. Analysis of 12 studies, including 24,571 cases, also did not find a statistical correlation between hypothyroidism and breast cancer ($p = 0.162$). Similarly, by analyzing 10 studies, which included 21,889 cases, the authors did not demonstrate a statistically significant higher risk of breast cancer in hyperthyroid patients (140).

Effect on the Risk of Prostate Cancer

In a prospective cohort study, sera from 3649 patients were assayed for TSH and free T4 (154). During a 20 year follow up period, 7.8% of males were diagnosed with prostate cancer. Higher TSH was associated with a lower risk of prostate cancer (adjusted HR: 0.7 per 1 mIU/L increase in TSH). Similarly, higher free T4 was associated with increased risk of prostate cancer (adjusted HR: 1.11 per 1 pmol/L increase). In a prospective study of male smokers including 402 prostate cancer patients and 800 controls (157), TSH in the highest quintile was associated with decreased risk of cancer (Q5 vs. Q1–4: OR 0.7). Hypothyroid men (high TSH with normal or low T4) had lower prostate cancer risk compared to euthyroid men (OR 0.48).

Effect on the Risk of Gynecologic Cancers

In a population based case-control study, 767 patients with recent diagnosis of epithelial ovarian cancer were compared with 1,367 community controls (162). Based on data retrieved from interviews, hyperthyroidism history was linked with increased cancer risk (OR 1.8). In another study, Kang et al. evaluated the association of self-reported history of thyroid dysfunction with medical records of confirmed endometrial carcinoma ($n =$ 1,314) and ovarian cancer ($n = 1{,}150$) as part of the Nurses'

Health Study (NHS). In this case, history of hypothyroidism or hyperthyroidism was not associated with cancer risk (213). Lastly, in a retrospective study Brinton et al. assessed the relationship between hospital and outpatient admission for various conditions and subsequent development of uterine and ovarian cancer. A prior diagnosis of thyroid disease was associated with uterine cancer (RR 1.52). However, no specific type of thyroid disease was more strongly linked to risk than others (214).

Effect on the Risk of Gastrointestinal Cancers

In a large population based case control study by Ko et al., 532 patients with pancreatic cancer were matched to 1,701 controls randomly selected from the same population (182). Based on patient self-report, hyperthyroidism history was related with increased cancer risk (OR 2.1).

Unlike pancreatic cancer, data regarding colorectal cancer (CRC) and hepatocellular carcinoma (HCC) produced conflicting results. A single prospective population based study suggested an association between hyperthyroidism and an increased risk of CRC (153), but due to a small cohort, this increase was not statistically significant. In contrast, additional studies demonstrated that hypothyroidism was associated with increased risk of colon cancer. A large case-control study compared 20,990 colorectal cancer (CRC) patients and 82,054 matched controls from a population database, followed for an average of 6.5 years and determined CRC risk in patients with thyroid dysfunction (184). In this study both hyperthyroidism (OR 1.21) and untreated hypothyroidism (OR 1.16) were associated with increased risk of colorectal cancer. Chan et al. conducted a prospective case-control study of 3,836 older men (186). Over a median follow up period of 9 years, 136 men developed colorectal cancer. Following adjustments, higher TSH was related with increased incidence of colorectal cancer (SHR 1.19), an association which was reinforced after eliminating the first year of follow up (SHR 1.23). Free T4 was not associated with cancer incidence in this study.

Similar to CRC, hypothyroidism may also play a role in liver carcinogenesis. Hassan et al. conducted a case-control study including 420 patients with hepatocellular carcinoma and 1,104 healthy controls (192). Hypothyroidism of longer than a decade was associated with significantly higher risk of HCC in women, unrelated to known HCC risk factors (OR 2.9 following regression analysis for risk factors). However, the data on thyroid disorders was based on patient self-report using questionnaires rather than thyroid hormone levels. Reddy et al. (193) demonstrated that in 160 patients with HCC, hypothyroidism was more prevalent in HCC of unknown etiology compared to patients with HCC secondary to HCV or alcoholic liver disease (OR 6.8).

The inverse correlation between thyroid function and cancer risk observed in CRC and HCC may be attributed to the elevation of TSH under hypothyroid state. The overexpression of a functioning thyroid stimulating hormone receptor (TSHR), which was demonstrated in HCC tissues (215), may provide a possible mechanism. TSH elevation in hypothyroidism may lead to HCC progression through direct stimulation of its receptor on cancer cells. However, no documentation for a similar TSHR expression in CRC was reported to date.

Effect on the Risk of Hematologic Malignancies

Dalamaga et al. compared 73 patients with primary multiple myeloma to 73 matched controls admitted for non-neoplastic conditions (200). The prevalence of clinical thyroid disease was higher in multiple myeloma patients compared to controls (adjusted OR 4.03 for thyroid disease, 5.68 for autoimmune thyroid disease). The levels of free T3 was higher (3.5 vs. 2.7 pg/ml, $p = 0.002$) and TSH lower (2.2 vs. 3.1 μIU/ml, $p = 0.001$) in myeloma patients compared to controls, albeit within the normal range. The same group conducted a case control study of 101 patients with histologically and cytogenetically confirmed MDS to 101 matched control. MDS patient had significantly higher serum levels of free T3 and free T4 and lower TSH than controls (203). A small case control study compared thyroid hormone levels between 25 patients with acute leukemia and 25 matched controls. Total T3, free T3, total T4, and free T4 were higher in patients than control, within the normal range, while TSH levels were significantly lower (201). However, since TH levels were not determined prior to the development of disease, an assessment of risk could not be established.

Effect of Thyroid Hormones on Clinical Presentation of Cancer

Cristofallini et al. retrospectively compared 1,136 women with breast cancer with 1,088 controls (142). Prevalence of hypothyroidism was significantly lower in the cancer group compared to the control group (7.0 vs. 14.9%, $p < 0.001$). Hypothyroid breast cancer patients were diagnosed at an older age (58.8 vs. 51.1 years; $p < 0.001$), had higher probability for having a localized disease (95.0 vs. 85.9% clinical T1 or T2 disease, respectively; $p = 0.025$), and were more likely to be lymph node negative (62.8 vs. 54.4%; $p = 0.15$). While these findings suggest that hypothyroidism slows breast cancer progression, the study was limited by its retrospective nature and by the fact that the diagnosis of hypothyroidism was based on information from medical charts rather than hormone values. In the aforementioned study by Tosovic et al. (135), higher T3 level (third quartile compared to first) was associated with large breast tumors (>20 mm) (HR of 3.17) and lymph node metastases (HR 4.53). This association was especially pronounced in post-menopausal women. In a series by Atkins et al. 34 patients with various advanced neoplasms (melanoma, renal cell carcinoma, lymphoma, and colon cancer) had received treatment with interleukin-2 and lymphokine-activated killer (LAK) cells (179). Twenty-one percent of patients had laboratory evidence of hypothyroidism. Patients with hypothyroidism had a higher rate of tumor regression (71 vs. 19%, $p < 0.02$).

Conversely, hypothyroidism was associated with increased aggressiveness of colorectal and liver cancer, comparable with the effect on cancer risk. In a case control study comparing 273 colorectal cancer patients to 819 matched controls, the prevalence of subclinical hypothyroidism was significantly higher in the colorectal neoplasm group (24.5 vs. 15.3%, $p < 0.01$).

Compared with euthyroid subjects, hypothyroid patients had higher likelihood of advanced colonic disease (8.3 vs. 4.4%, $p = 0.028$) (185). In another study by Pinter et al., 667 patients diagnosed with non-surgically treated HCC were retrospectively followed for a mean period of 65.5 months (190). Hypothyroid patients (TSH>3.77 μU/ml) had a higher risk for large lesions (>5 cm), while Hyperthyroid patients (TSH<0.44 μU/ml) had a lower risk.

Effect of Thyroid Status on Cancer Survival

Effect on Overall Cancer Survival

Several population based studies have demonstrated increased cancer mortality in hyperthyroidism, with opposite outcomes in hypothyroidism, supporting the assumption of growth promoting effect of thyroid hormones. Brandt et al. used data of 2,152 patients with Grave's disease and toxic nodular goiter, followed for 11 years (204). Both diseases were associated with increased all-cause mortality compared with non-hyperthyroid controls, and increased cancer mortality was demonstrated for toxic nodular goiter (HR 1.36, $p < 0.05$). In a recent population based study by Journy et al., 75,076 female radiologic technologists who completed medical questionnaires were retrospectively followed for a median of 28 years (130). No association was demonstrated between overall cancer mortality and hyper or hypothyroidism. However, risk of breast cancer mortality after 60 years of age was increased in patients with self-reported hyperthyroidism (HR 2.04, $p < 0.05$). Women with hyperthyroidism treated with radioactive iodine had increased risk of ovarian cancer mortality compared with women without thyroid disease (HR 5.32, $p < 0.05$), based on very few cases. Lechner et al. (216) conducted a retrospective cohort study of 538 patients with various solid malignancies (renal cell carcinoma, GIST, HCC, neuroendocrine, primary central nervous system, other carcinoma, sarcoma) treated with tyrosine kinase inhibitors. Thirteen percent of patients developed subclinical hypothyroidism and 27% developed overt hypothyroidism. Patients with hypothyroidism had significantly longer overall survival (median overall survival 1,005 days in subclinical hypothyroidism and 1,643 in overt hypothyroidism compared with 685 days in euthyroid patients, $p < 0.0001$). In Franklyn et al.'s retrospective cohort of hyperthyroid patients treated with radioiodine (217), mortality from cancers of all sites was reduced following treatment (SMR 0.9, $p = 0.02$). In subgroup analysis lower mortality was significant only for cancer of the bronchus and trachea (SMR 0.78, $p = 0.03$) while for cancers of the small bowel and thyroid, small absolute risk increases in mortality were demonstrated. Opposing results were demonstrated in a large population based study, wherein 115,746 patients were followed for 10 years for evaluation of cancer mortality (137). Following adjustment, patients with biochemically proved subclinical hypothyroidism (1.6%) at study inclusion had higher risk of cancer death (RR 1.51, $p < 0.05$) as well as increased risk of bone, skin and breast cancer (RR 2.79, $p < 0.05$). A prospective study by Zhang et al. (191) was conducted on a cohort of 212,456 middle aged Korean subjects who had undergone thyroid function tests. Following a median follow-up of 4.3 years, an inverse association was demonstrated between free T4 and all-cause mortality (HR = 0.77, $P = 0.01$) as well between free T3 and cancer mortality (HR = 0.62, p for trend = 0.001). TSH was not associated with mortality endpoints. This discrepancy may be at least partially attributed to the large proportion of gastrointestinal tumors in both studies (>45% of cancer deaths) which, as described above, may propagate in hypothyroid conditions.

Effect on Breast Cancer Survival

In 1964, Humphrey and Swerdlow were among the first to demonstrate the effect of thyroid disorders on breast cancer outcomes (218). In their study, the 5-year survival of 14 patients who had undergone a thyroidectomy for non-toxic goiter was significantly longer than nine patients who had undergone thyroidectomy for hyperthyroidism (71 vs. 22%, $p < 0.05$). In another study of 462 cases of breast cancer (131), patients with a history of thyroid disease had significantly lower survival rates at 5 and 10 years compared with controls ($p < 0.005$). In a subgroup analysis, patients with a history of treated hyperthyroidism had significantly shorter 5- and 10-year survival ($p < 0.01$). A population-based prospective cohort study by Tosovic et al. included 2,185 women who had T3 levels measured as part of a preventive health study (138). After a mean follow-up of 24.1 years, 26 women died of breast cancer. T3 levels were correlated with age-adjusted breast cancer related death (HR 2.8, $p = 0.012$), especially in post-menopausal patients (adjusted HR 3.73, $p = 0.001$). Thyroid hormone status may also affect response to breast cancer therapy. A study by Cao et al. included 28 patients with metastatic breast cancer treated with the VEGFR-2 inhibitor famitinib (144). Sixty-four percent of patients had elevated TSH (>4.94 mIU/L) during treatment. Progression free survival (PFS) was longer in these patients compared with patients with normal TSH (107 vs. 53 days, respectively, $p = 0.002$).

Effect on Lung Cancer Survival

Several decades ago, Hercbergs and Leith reported a case of a 69 year old male with metastatic non-small cell lung cancer that resolved spontaneously following resuscitation from myxedema coma, dying of unrelated causes 4 years after the myxedema event (5). In a later retrospective case-controls study (159), Hercbergs et al. compared 85 hypothyroid lung cancer patients to 85 matched euthyroid lung cancer patients. Hypothyroid patients were older at diagnosis (median age 73 vs. 64 years, $p = 0.0006$) and survived longer (stages 1–4: 14.5 vs. 11.1 months, $p = 0.014$; stage 4: 11 vs. 5 months, $p = 0.0018$) compared with controls. In a recent study of 51 non-small-cell lung cancer patients treated with pembrolizumab, a PD-1 inhibitor (160), 21% of patients developed hypothyroidism requiring thyroid hormone replacement, with 80% developing positive antithyroid antibodies. Overall survival (OS) with pembrolizumab was significantly longer in subjects who developed thyroid dysfunction (mean OS 40 vs. 14 months. HR 0.29, $p = 0.04$).

Effect on Ovarian Cancer Survival

Minlikeeva et al. used collective data from 11 studies, including information on thyroid hormone status for a total of 5,822

patients diagnosed with invasive ovarian cancer (163). Increased risk of mortality was demonstrated for patients with a history of hyperthyroidism in the 5 years preceding cancer diagnosis (HR = 1.94; $p = 0.01$). Hypothyroidism was associated with a mildly decreased mortality risk (HR = 1.16; $p = 0.01$). Duration of hypothyroidism or thyroid medications use were not associated with survival.

Effect on Renal Cancer Survival

Much research has been conducted on the possible positive effects of drug-induced hypothyroidism on the outcome of treatment for renal cell carcinoma (RCC). In a small scale study by Weijl et al. (180), patients with metastatic renal cell carcinoma were treated with IL-2 and LAK cells. Forty-seven percent of patients became hypothyroid following treatment. Favorable response to treatment was positively correlated with hypothyroidism ($r = 0.76$, $p = 0.001$).

Over the past decade, several studies have reported on potential favorable outcomes of tyrosine-kinase inhibitors (TKI's)-induced hypothyroidism, specifically by sunitinib. Sunitinib is an oral multitargeted tyrosine kinase inhibitor commonly used in metastatic renal cell carcinoma. Hypothyroidism is a common side effect of this treatment, with up to 85% patients developing abnormality of thyroid function consistent with hypothyroidism, and roughly a third requiring thyroid hormone replacement (219, 220). The mechanism for this effect is not altogether clear, though it may be related to a decreased VEGF binding to normal thyroid cells and/or disruption of thyroid blood flow (219). In a retrospective analysis of metastatic RCC patients who received VEGF receptor tyrosine kinase inhibitors (171), median OS and PFS were significantly longer in patients with a peak TSH >10 mIU/L compared to patients with a peak TSH of ≤10 mIU/L (not reached vs. 21.4 months, $p = 0.005$; 47.7 vs. 9.3 months, $p = 0.009$, respectively). In a series of sunitinib-treated clear cell RCC (172), hypothyroid (TSH >4 mIU/L) patients receiving levothyroxine as thyroid-replacement therapy had prolonged PFS compared with other patients (25.3 vs. 9.0 months; $p = 0.042$). A prospective cohort study by Schmidinger et al. included 87 sunitinib or sorafenib treated patients with metastatic RCC (173). Patients who developed subclinical hypothyroidism had a higher rate of remission compared with euthyroid patients (28.3 vs. 3.3%, $p < 0.001$) and longer median duration of survival (not reached vs. 13.9 months, $p = 0.016$). Other studies have similarly demonstrated prolonged PFS in sunitinib induced hypothyroidism (174, 175). One prospective study did not show such a correlation (221). However, this study was based on only 6 months of follow up. In 2015, Nearchou et al. published a meta-analysis evaluating hypothyroidism as a predictive marker for survival in metastatic RCC patients treated with TKI's (176). Based on six studies, PFS in patients with sunitinib-induced hypothyroidism was not significantly different compared with patients without hypothyroidism. However, in three studies which included patients treated with sunitinib or sorafenib, the difference in PFS was statistically significant in favor of patients with acquired hypothyroidism (HR, 0.59; $p = 0.003$). Moreover, an analysis of four studies indicated a statistically significant improvement in OS in patient who developed sunitinib-induced hypothyroidism compared with patients who did not (HR 0.52, $p = 0.01$).

Effect on Gastrointestinal Cancer Survival

Similar to the effect on cancer risk, CRC and HCC appear to represent cancer subtypes whose association with thyroid status differs from that of other solid tumors. In a recent study by Schirripa et al. (189), a higher baseline free T3/free T4 ratio was associated with increased survival in patients with metastatic colorectal cancer treated with the multikinase inhibitor regorafenib ($p = 0.003$). In Pinter et al.'s study of non-surgically treated HCC patients (190), increased OS was associated with lower TSH (≤1.7 vs. >1.7 μU/ml, median OS 12.3 vs. 7.3 months; $p = 0.003$) and lower free T4 (≤1.66 vs. >1.66 ng/dl, median OS, 10.6 vs. 3.3 months; $p = 0.007$). Similarly, in a study by Zhang et al. (191), both free T3 and free T4 were inversely associated with liver cancer mortality (HR per SD change: 0.64 for free T3, 0.52 for free T4). These findings again support a growth promoting effect of hypothyroidism in HCC, which may be related to activation of the TSH receptor. In a recent case control study (194), the product of TSH and free T4 was calculated for 123 patients with advanced HCC treated with sorafenib or chemotherapy. High TSH x free T4 at baseline (>2.48) was associated with favorable time to tumor progression (TTTP) (HR 0.478, $p = 0.008$) and better OS if chemotherapy was provided (HR 0.44, $p = 0.006$). Conversely, high baseline TSH x free T4 (>2.55) was associated with unfavorable TTTP (HR 2.03, $p = 0.039$) and overall survival (HR 3, $p = 0.007$) if sorafenib was administered. However, the association between this calculated ratio and thyroid status was not fully elucidated.

Effect on Head and Neck Cancer Survival

Head and neck cancer patients commonly undergo involved-field radiation therapy and are prone to iatrogenic hypothyroidism, which can therefore serve as a useful model to study the effect of thyroid dysfunction on cancer outcomes. Nelson et al. conducted a retrospective analysis of 155 patients with advanced head and neck squamous cell carcinoma who were treated with radiation therapy alone or in combination with other treatments (196). Patients who developed new onset hypothyroidism post-treatment had less cancer recurrence ($p = 0.02$), improved survival ($p < 0.001$), and longer recurrence-free survival ($p < 0.001$), compared with patients who did not. In another population based study of patients with head and neck cancer treated with radiotherapy (197), the 10 year incidence of hypothyroidism was 59% and these patients exhibited longer survival (HR 0.42, $p < 0.001$) as well as longer cause-specific survival (HR 0.36, $p < 0.001$). In a phase III trial comparing two cisplatin chemoradiotherapy protocols in 300 patients with locally advances head and neck cancer (222), 38.73% of patient developed hypothyroidism by 2 years of follow up (198). These patients had lower locoregional failure rate (LRFR) (hazard ratio 0.342, $p = 0.043$), and longer overall survival (hazard ratio 0.336, $p = 0.001$). Favorable impact on LRFR, PFS and OS were

associated with hypothyroidism of longer duration and TSH levels up to 40 mIU/L.

Association Between Thyroid Autoimmunity and Cancer

Small scale case control studies have demonstrated an increased prevalence of thyroid autoimmunity in breast cancer (223, 224), gastric cancer (225), pancreatic cancer (226), multiple myeloma (200), and myelodysplastic syndrome (203). In a 2012 meta-analysis, autoimmune thyroiditis, as well as overall thyroid antibody positivity, thyroglobulin antibody positivity and thyroid peroxidase antibody (TPOAb) positivity were associated with increased risk of breast cancer (OR 2.92, 2.02, 2.72, 2.64, respectively), with minimal to moderate heterogeneity (139). Conversely, in a population based case control study by Brandt et al. (128), including 676 breast cancer patients and 680 controls, women with high levels of TPOAb (above 9 kIU/L) were at a lower risk of being diagnosed with breast cancer (OR 0.75), specifically invasive type (OR 0.74).

Thyroid autoimmunity may beneficially affect cancer outcomes. Fiore et al. (227) examined the prognostic value of thyroid autoantibodies in 47 patients with locally metastatic breast cancer referred for mastectomy. Five-year mortality was lower in patients with thyroid autoantibody positivity (6.7 vs. 46.9%, $p = 0.008$). Farahati et al. assessed anti TPOAb in 314 patients with newly diagnosed breast cancer (228). Among 56 patients with TPOAb, no incidences of distant metastasis was documented, whereas in 17 (6.6%) of 258 cases without TPOAb, distant metastases were demonstrated ($p = 0.04$). In Brandt et al., high TPOAb levels were also associated with a lower risk of ductal cancer, large tumors (>20 mm), and ER and PR positive tumors (128). Interestingly, the same group recently identified several TPOAb related SNPs (s11675434, rs3094228, rs1033662, rs301806, and rs207140) which may also be associated with breast cancer risk (211). Another study by Franzke et al. included 329 patients with metastatic RCC treated with systemic IL-2 and IFNα2 (229). Antithyroid autoantibodies were detected in 18% of patients. Thyroid autoantibodies were correlated with increased survival (5-year survival 54 vs. 15%, $p < 0.0001$). Interestingly, HLA-Cw7 expression was more frequent with thyroid autoantibody positivity (69.2 vs. 47.7%, $p = 0.009$), and Cw7 expression was associated with prolonged overall survival, suggesting HLA-dependent thyroid autoimmunity associated with improved cancer outcomes. Thyroid autoantibodies may affect breast cancer behavior irrespective of its effect on the thyroid hormone axis. Thyroid cells and benign and malignant breast tissues share common antigens. The most important of these is the sodium-iodine symporter, which is highly expressed in breast cancer cells (230). Also, lactoperoxidase in breast cancer cells shares a homology with thyroperoxidase (231). T cells directed against thyroid autoantigens could attack breast cancer cells expressing similar antigens (227).

Association of Cancer With Non-thyroidal Illness Syndrome (NTIS)

NTIS, or sick euthyroid syndrome, is characterized by alterations in circulating thyroid hormone levels in euthyroid patients with acute or chronic systemic illnesses. Changes include a decrease in T3 levels, increase in rT3 and inconstant alterations in circulating T4 levels (212). The association of NTIS with cancer was documented in various tumor types including breast cancer (232), gastrointestinal cancers (232), lung cancer (233–235), central nervous system tumors (236), multiple myeloma (237), chronic lymphocytic leukemia (238), and diffuse large cell lymphoma (239).

NTIS may be associated with adverse disease outcomes. In a 1978 study by Ratcliff et al. (233), 6 month mortality was higher among lung cancer patients with low T3 compared with matched lung cancer patients with normal T3 (49 vs. 27%). In a study of 80 patients with newly diagnosed non-small cell lung cancer (234), NTIS was more frequent among stage III (26%) and stage IV (62%) cases, and survival was shorter in patients with NTIS compared with patients without NTIS (mean survival 9.2 vs. 15.2 months, $p = 0.00002$). Similarly, in a cohort of both small cell and non-small cell cancer patients, NTIS was associated with disease stage and served as a poor prognostic factor (235). In a study of 230 patients with primary brain tumors (236), 27% had NTIS syndrome. Glioma patients with NTIS had greater 5-year mortality (HR = 2.197, $p = 0.016$) and shorter OS (249 vs. 352 days; $p = 0.029$). NTIS was also a predictor of poor post-operative outcomes in patients undergoing brain tumor surgery (240). In a study of patients with chronic lymphocytic leukemia (238), NTIS was associated with significantly shorter time to first treatment (2 vs. 11 months, $p < 0.001$) and cancer-specific survival (median survival 51 months vs. not reached) compared to patients with normal T3. In another study by the same group, of 188 patients with diffuse large B cell lymphoma, low T3 was associated with worse PFS (median survival 17 vs. 22 months) and overall survival (median survival 17 vs. 23 months) in the rituximab era (239). However, collectively these results may be a reflection of NTIS as a marker of aggressive disease, rather than a direct effect of this syndrome on cancer outcomes.

The Association Between Thyroid Replacement Therapy and Cancer Incidence and Outcome

In an early study, 5,505 patients referred to a mammography department were interviewed regarding thyroid hormone use (150). Six hundred thirty-five patients used thyroid medications. In patients receiving thyroid supplements, breast cancer incidence was significantly higher than controls without thyroid disease or thyroid medication use (12.13 vs. 6.2%, $p < 0.005$). The difference was especially prominent in patients taking thyroid medication for more than 15 years (19 vs. 6.2%, $p < 0.005$) and nulliparous women taking thyroid medication for more than 5 years (20 vs. 9.2% in nulliparous controls, $p < 0.025$). These findings suggest a relationship between thyroid supplements and cancer associated with the duration of use. In a prospective cohort study, 2,738 post-menopausal women were screened for thyroid hormone parameters and prospectively followed for a period of 9 years (147). New breast cancer was related to previous use of thyroid medication at study inclusion (OR 3.2). However, a meta-analysis from 2017 including six studies evaluating the relationship between thyroid hormone supplementation and risk

of breast cancer found no statistical correlation between the two (140). In a population based study, Cornelli et al. compared the prevalence of breast, colorectal, gastric and lung cancer in women during 2010 with the sales of levothyroxine (LT4) in the previous year in 18 Italian regions (161). Corrected for smoking and age, a significant correlation was demonstrated for lung cancer and levothyroxine sales ($R = 0.485$, $p = 0.04$). Sarosiek et al. performed a retrospective analysis in 504 pancreatic cancer patients who underwent a Whipple procedure or distal pancreatectomy and splenectomy during the course of 7 years (62). 14.1% of patients were hypothyroid. Hypothyroid patients taking exogenous thyroid hormone, in comparison to euthyroid patients, were more likely to have perineural invasion (OR 3.38, $p = 0.012$), have high T stage (T3-T4, OR 2.1, $p = 0.045$), nodal spread (OR 2.05, $p = 0.018$), and have poorer prognostic stage (2B-3, OR 1.89, $p = 0.037$). There was no difference in survival between both groups.

Similar to the apparent protective effect of endogenous thyroid hormones in colorectal cancer, exogenous thyroid hormone supplementation was associated with decreased risk of CRC. In a population based case control study conducted in northern Israel, 2,648 colorectal cancers were matched to 2,566 controls (187). Levothyroxine use for a minimum of 5 years, evaluated by structured interviews and prescription records validation, was associated with a significantly reduced risk of CRC (OR 0.59, $p = 0.001$). However, this study was limited by possible recall bias. Another large population based study compared 20,990 colorectal cancer patient with 82,054 matched control patients (184) and determined CRC risk in patients with thyroid dysfunction, with and without thyroid hormone replacement. Thyroid hormone supplementation use of more than 5 years was related with lower risk for CRC, with a stronger association documented for longer periods since initiation of treatment. The adjusted odds ratio for colorectal cancer associated with thyroid hormone replacement was 0.88 ($p = 0.03$) for treatment initiated 5–10 years before index date and 0.68 ($p < 0.001$) for treatment initiated more than 10 years before index date. In response to this study, Friedman et al. (188) performed a case-control analysis of colon ($n = 12{,}207$) and rectal/rectosigmoid cancers ($n = 4{,}729$), based on the Kaiser permanente cancer registry, and obtained LT4 prescription dispensing records from outpatient pharmacies. Each case patient was matched to up to 50 control subjects. Rectal cancer risk was more than 30% lower in men who used levothyroxine for more than 5 years, compared to non-users (OR 0.66, $p = 0.03$). Although statistically insignificant, colon cancer risk appeared to be somewhat reduced.

The Effect of Induced Hypothyroidism and Hypothyroxinemia on Cancer Outcomes

Few interventional studies exist which examined the effect of chemically induced hypothyroidism and hypothyroxinemia on cancer outcomes. Those are based mainly on case reports and small patient series. A previous report described a patient with inoperable glioblastoma of the optic chiasm who failed standard treatment with radiation and temozolomide (169). The patient underwent induced hypothyroidism with PTU, followed by carboplatin chemotherapy. On two separate occasions, this patient responded clinically and radiographically to treatment with an extended remission period (2.5 years) and prolonged overall survival (4.5 years). In a recent report (183), a patient with triple negative breast cancer and lung metastasis who progressed under chemotherapy was treated with methimazole (45 mg per day) and increasing doses of liothyronine (L-T3). This treatment led to stabilization of the disease and CA-125 levels for several months. A second patient described in this study with metastatic pancreatic adenocarcinoma was treated with a similar protocol. In this case, treatment led to a temporary reduction in CA19-9 and a disappearance of a skin metastasis. In both patients, although L-T3 produced early resistance to treatment, a direct tumor growth inhibition effect was also observed. In a study published in 1976 (168), Yung et al. compared 32 patients with glioblastoma treated with surgery and radiation alone with 18 glioblastoma patients treated with surgery, radiation as well T3 to achieve a hyperthyroid state. Patients in the T3 treatment group had significantly longer median survival (60 vs. 30 weeks, $p = 0.005$). The authors speculated that this may be attributed to radiosensitizing property of triiodothyronine. Hercbergs et al. reported on 22 patients with recurrent glioma who were treated with PTU to induce hypothyroidism, concurrently with tamoxifen (170). Eleven patients became hypothyroid. Median survival was significantly longer in the hypothyroid group (10.1 vs. 3.1 months, $p = 0.03$). Lastly, another study by Hercbergs et al. included 23 patients with end stage cancers of the brain, ovary, lung, pancreas, salivary gland, and breast as wells mesothelioma and soft-tissue sarcoma (210). In euthyroid patients, hypothyroxinemia was reached by using methimazole, with the addition of L-T3 to avoid hypothyroidism side-effects parallel to suppressing endogenous TSH. The survival time of 83% (19 of 23) of patients exceeded the 20% expected 1-year survival for this group based on the SEER database ($p < 0.01$).

THE EFFECTS OF THYROID HORMONES ON ANGIOGENESIS AND ANTICANCER IMMUNE RESPONSES

Generation of dense vasculature and evasion of immune reaction are among the key hallmarks of cancer (241). Recent studies provide evidence that TH affect both these crucial features of tumors, enabling cancer progression.

T4 acting on αvβ3 receptor stimulates formation of new vessels as shown in CAM assay and three-dimensional human microvascular endothelial sprouting model (97, 99, 100) which involves activation of MAPK (97). Inhibition of T4-αvβ3 signaling by tetrac or anti-integrin antibody blocked TH-induced formation of new vessels (97, 99). Importantly, tetrac and its nanoparticulate form also inhibited angiogenesis stimulated by cancer cells, as shown by CAM models implanted with renal cell cancer (59), medullary thyroid cancer (82), follicular thyroid carcinoma (83), and non-small cell lung cancer (40).

T4 may also facilitate cancer progression by interfering with anti-tumor immune responses. Treatment of breast and colon

cancer cells with T4 stimulated the expression of PD-L1, one of the elements of PD-1/PD-L1 immune checkpoint that controls activation of T cells. Cancer cells often overexpress PD-L1 which interacts with PD-1 receptors on the surface of T cells, thus blocking its activation and attenuating immune response directed against tumors (242). In breast cancer cells, T4 activated PD-L1 expression via non-genomic mechanisms involving αvβ3 receptor. Remarkably, NDAT (nanotetrac) blocked T4-mediated activation of PD-L1, providing a possibility for restoring immune defense against cancer cells. One of the key drawbacks of cancer therapies directed against PD-1/PDL-1 checkpoint, aimed at activation of immune responses, is the risk of autoimmune disease in treated patients. Since αvβ3 is specifically overexpressed in cancer cells, and rarely functions on the surface of healthy cells, treatment of patients with NDAT would possibly not lead to autoimmune responses directed against non-cancer cells (16). Clearly, the results of these promising *in vitro* studies require further validation *in vivo* on a larger group of cancer types.

THYROID HORMONES AND RESPONSE TO THERAPY

The results of several *in vitro* studies suggest that thyroid hormones may influence responses to chemotherapy. Through promotion of cancer cell proliferation, mitochondrial activity, and cell cycle progression from G0-G1 to S, T3 enhances the sensitivity of breast cancer cells to various chemotherapies (22, 23). In pancreatic cancer cells T3 treatment potentiated cytotoxic activities of chemotherapeutics such as cisplatin or gemcitabine (63). Contrasting observations were made for cell lines derived from colonic tumors. In colon cancer cells T3 increases the expression of P-gp (MDR1), one of the key mediators of xenobiotic efflux (67, 109). In contrast to the above mentioned prevalent non-genomic TH effects stimulating cancer progression, T3 activates P-gp expression by TR binding to the direct repeat elements located upstream of the transcription start site of the P-gp gene (243). These results suggest that T3 may possibly interfere with drug treatments of colon cancer; however this hypothesis requires experimental verification. T4 and T3 also interfered with the activity of bortezomib, a key drug in treatment of MM patients (90).

CONCLUSION

The thyroid hormones are increasingly acknowledged for their tumor-promoting effects. We aimed in this review to shed light on this association in order to clarify which types of cancer are thyroid-hormone sensitive and therefore are expected to favorably respond to manipulation of the thyroid hormone axis. This is highly relevant, specifically in the context of the discovery of the T4 receptor site upon the αvβ3 integrin, which is overexpressed in many tumor cells and vasculature and may serve as an attractive target for inhibition. However, currently few T4-αvβ3 inhibitors which demonstrated efficacy in several cancer types are under preclinical studies. Moreover, TH status is currently not yet considered as a risk factor or a prognostic factor in the clinical practice of cancer management, partly due to the conflicting data reported over the years. Future prospective studies to evaluate this link, with strict inclusion/exclusion criteria and predefined cut-off values, are therefore merited. Convincing results may promote the inclusion of thyroid status in the assessment of cancer patients and interventional studies may lead to novel treatment modalities that are desperately lacking in many aggressive cancers.

AUTHOR CONTRIBUTIONS

EK, AP-W, ME, and OA-F designed the review, collected the data, wrote, and approved the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2019.00059/full#supplementary-material

REFERENCES

1. Ortiga-Carvalho TM, Chiamolera MI, Pazos-Moura CC, Wondisford FE. Hypothalamus-pituitary-thyroid axis. *Compr Physiol.* (2016) 6:1387–428. doi: 10.1002/cphy.c150027
2. Piekiełko-Witkowska A, Nauman A. Iodothyronine deiodinases and cancer. *J Endocrinol Invest.* (2011) 34:716-28. doi: 10.3275/7754
3. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol.* (2016) 12:111-21. doi: 10.1038/nrendo.2015.205
4. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases. *Trans Med Chir Soc Edinb.* (1896) 15:153–79.
5. Hercbergs A, Leith JT. Spontaneous remission of metastatic lung cancer following myxedema coma–an apoptosis-related phenomenon? *J Natl Cancer Inst.* (1993) 85:1342–3. doi: 10.1093/jnci/85.16.1342
6. Hercbergs AH, Ashur-Fabian O, Garfield D. Thyroid hormones and cancer: clinical studies of hypothyroidism in oncology. *Curr Opin Endocrinol Diabetes Obes.* (2010) 17:432-6. doi: 10.1097/MED.0b013e32833d9710
7. Moeller LC, Führer D. Thyroid hormone, thyroid hormone receptors, and cancer: a clinical perspective. *Endocr Relat Cancer* (2013) 20:R19–29. doi: 10.1530/ERC-12-0219
8. Freindorf M, Furlani TR, Kong J, Cody V, Davis FB, Davis PJ. Combined QM/MM study of thyroid and steroid hormone analogue interactions with αvβ3 integrin. *J Biomed Biotechnol.* (2012) 2012:959057. doi: 10.1155/2012/959057
9. Lin HY, Sun M, Tang HY, Lin C, Luidens MK, Mousa SA, et al. L-Thyroxine vs. 3,5,3'-triiodo-L-thyronine and cell proliferation: activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. *Am J Physiol Cell Physiol.* (2009) 296:C980–91. doi: 10.1152/ajpcell.00305.2008
10. Burke RE, McGuire WL. Nuclear thyroid hormone receptors in a human breast cancer cell line. *Cancer Res.* (1978) 38(11 Pt 1):3769–73.
11. Hall LC, Salazar EP, Kane SR, Liu N. Effects of thyroid hormones on human breast cancer cell proliferation. *J Steroid Biochem Mol Biol.* (2008) 109:57–66. doi: 10.1016/j.jsbmb.2007.12.008
12. Dinda S, Sanchez A, Moudgil V. Estrogen-like effects of thyroid hormone on the regulation of tumor suppressor proteins, p53 and retinoblastoma,

in breast cancer cells. *Oncogene* (2002) 21:761–8. doi: 10.1038/sj.onc.1205136
13. Nogueira CR, Brentani MM. Triiodothyronine mimics the effects of estrogen in breast cancer cell lines. *J Steroid Biochem Mol Biol.* (1996) 59:271–9. doi: 10.1016/S0960-0760(96)00117-3
14. Tang HY, Lin HY, Zhang S, Davis FB, Davis PJ. Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor. *Endocrinology* (2004) 145:3265–72. doi: 10.1210/en.2004-0308
15. Flamini MI, Uzair ID, Pennacchio GE, Neira FJ, Mondaca JM, Cuello-Carrión FD, et al. Thyroid hormone controls breast cancer cell movement via integrin αV/β3/SRC/FAK/PI3-kinases. *Horm Cancer* (2017) 8:16–27. doi: 10.1007/s12672-016-0280-3
16. Lin HY, Chin YT, Nana AW, Shih YJ, Lai HY, Tang HY, et al. Actions of l-thyroxine and Nano-diamino-tetrac (Nanotetrac) on PD-L1 in cancer cells. *Steroids* (2016) 114:59–67. doi: 10.1016/j.steroids.2016.05.006
17. Glinskii AB, Glinsky GV, Lin HY, Tang HY, Sun M, Davis FB, et al. Modification of survival pathway gene expression in human breast cancer cells by tetraiodothyroacetic acid (tetrac). *Cell Cycle* (2009) 8:3562–70. doi: 10.4161/cc.8.21.9963
18. Sar P, Peter R, Rath B, Das Mohapatra A, Mishra SK. 3, 3'5 Triiodo L thyronine induces apoptosis in human breast cancer MCF-7 cells, repressing SMP30 expression through negative thyroid response elements. *PLoS ONE* (2011) 6:e20861. doi: 10.1371/journal.pone.0020861
19. Martinez MB, Ruan M, Fitzpatrick LA. Altered response to thyroid hormones by prostate and breast cancer cells. *Cancer Chemother Pharmacol.* (2000) 45:93–102. doi: 10.1007/s002800050016
20. Cestari SH, Figueiredo NB, Conde SJ, Clara S, Katayama ML, Padovani CR, et al. Influence of estradiol and triiodothyronine on breast cancer cell lines proliferation and expression of estrogen and thyroid hormone receptors. *Arq Bras Endocrinol Metabol.* (2009) 53:859–64. doi: 10.1590/S0004-27302009000700010
21. Martinez MB, Ruan M, Fitzpatrick LA. Altered response to thyroid hormones by breast and ovarian cancer cells. *Anticancer Res.* (2000) 20:4141–6.
22. Suhane S, Ramanujan VK. Thyroid hormone differentially modulates Warburg phenotype in breast cancer cells. *Biochem Biophys Res Commun.* (2011) 414:73–8. doi: 10.1016/j.bbrc.2011.09.024
23. Huang J, Jin L, Ji G, Xing L, Xu C, Xiong X, et al. Implication from thyroid function decreasing during chemotherapy in breast cancer patients: chemosensitization role of triiodothyronine. *BMC Cancer* (2013) 13:334. doi: 10.1186/1471-2407-13-334
24. Sellitti DF, Tseng YC, Latham KR. Effect of 3,5,3'-triiodo-L-thyronine on the incidence and growth kinetics of spontaneous mammary tumors in C3H/HeN mice. *Cancer Res.* (1981) 41(Pt. 1):5015–9.
25. Vermey ML, Marks GT, Baldridge MG. Effect of thyroid function on MNU-induced mammary carcinogenesis. *Zool Sci.* (2015) 32:272–7. doi: 10.2108/zs140124
26. Glushakov RI, Proshin SN, Tapil'skaya NI. The incidence of breast tumor during experimental hyperthyroidism. *Bull Exp Biol Med.* (2013) 156:245–7. doi: 10.1007/s10517-013-2322-y
27. Vonderhaar BK, Greco AE. Effect of thyroid status on development of spontaneous mammary tumors in primiparous C3H mice. *Cancer Res.* (1982) 42:4553–61.
28. Goodman AD, Hoekstra SJ, Marsh PS. Effects of hypothyroidism on the induction and growth of mammary cancer induced by 7,12-dimethylbenz(a)anthracene in the rat. *Cancer Res.* (1980) 40:2336–42.
29. Macejova D, Radikova Z, Macho L, Liska J, Brtko J. MNU-induced carcinogenesis of rat mammary gland: effect of thyroid hormone on expression of retinoic acid receptors in tumours of mammary gland. *Mol Cell Endocrinol.* (2005) 244:47–56. doi: 10.1016/j.mce.2005.01.014
30. López-Fontana CM, Sasso CV, Maselli ME, Santiano FE, Semino SN, Cuello Carrión FD, et al. Experimental hypothyroidism increases apoptosis in dimethylbenzanthracene-induced mammary tumors. *Oncol Rep.* (2013) 30:1651–60. doi: 10.3892/or.2013.2648
31. Shoemaker JP, Bradley RL, Hoffman RV. Increased Survival and Inhibition of Mammary Tumors in Hypothyroid Mice. *J Surg Res.* (1976) 21:151–4. doi: 10.1016/0022-4804(76)90151-7
32. Martínez-Iglesias O, García-Silva S, Regadera J, Aranda A. Hypothyroidism enhances tumor invasiveness and metastasis development. *PLoS ONE* (2009) 4:e6428. doi: 10.1371/journal.pone.0006428
33. Shoemaker JP, Dagher RK. Remissions of mammary adenocarcinoma in hypothyroid mice given 5-fluorouracil and chloroquine phosphate. *J Natl Cancer Inst.* (1979) 62:1575–8.
34. Tsui KH, Hsieh WC, Lin MH, Chang PL, Juang HH. Triiodothyronine modulates cell proliferation of human prostatic carcinoma cells by downregulation of the B-cell translocation gene 2. *Prostate* (2008) 68:610–9. doi: 10.1002/pros.20725
35. Delgado-González E, Sánchez-Tusie AA, Morales G, Aceves C, Anguiano B. Triiodothyronine attenuates prostate cancer progression mediated by β-adrenergic stimulation. *Mol Med.* (2016) 22:1–11. doi: 10.2119/molmed.2015.00047
36. Zhang P, Chen L, Song Y, Li X, Sun Y, Xiao Y, et al. Tetraiodothyroacetic acid and transthyretin silencing inhibit pro-metastatic effect of L-thyroxin in anoikis-resistant prostate cancer cells through regulation of MAPK/ERK pathway. *Exp Cell Res.* (2016) 347:350–9. doi: 10.1016/j.yexcr.2016.08.019
37. Theodossiou C, Schwarzenberger P. Propylthiouracil reduces xenograft tumor growth in an athymic nude mouse prostate cancer model. *Am J Med Sci.* (2000) 319:96–9. doi: 10.1016/S0002-9629(15)40695-0
38. Theodossiou C, Skrepnik N, Robert EG, Prasad C, Axelrad TW, Schapira DV, et al. Propylthiouracil-induced hypothyroidism reduces xenograft tumor growth in athymic nude mice. *Cancer* (1999) 86:1596–601. doi: 10.1002/(SICI)1097-0142(19991015)86:8<1596::AID-CNCR30>3.0.CO;2-C
39. Meng R, Tang HY, Westfall J, London D, Cao JH, Mousa SA, et al. Crosstalk between integrin αvβ3 and estrogen receptor-α is involved in thyroid hormone-induced proliferation in human lung carcinoma cells. *PLoS ONE* (2011) 6:e27547. doi: 10.1371/journal.pone.0027547
40. Mousa SA, Yalcin M, Bharali DJ, Meng R, Tang HY, Lin HY, et al. Tetraiodothyroacetic acid and its nanoformulation inhibit thyroid hormone stimulation of non-small cell lung cancer cells in vitro and its growth in xenografts. *Lung Cancer* (2012) 76:39–45. doi: 10.1016/j.lungcan.2011.10.003
41. Lin HY, Su YF, Hsieh MT, Lin S, Meng R, London D, et al. Nuclear monomeric integrin αv in cancer cells is a coactivator regulated by thyroid hormone. *FASEB J.* (2013) 27:3209–16. doi: 10.1096/fj.12-227132
42. Kinoshita S, Sone S, Yamashita T, Tsubura E, Ogura T. Effects of experimental hyper- and hypothyroidism on natural defense activities against Lewis lung carcinoma and its spontaneous pulmonary metastases in C57BL/6 mice. *Tokushima J Exp Med.* (1991) 38:25–35.
43. Carmona-Cortés J, Rodríguez-Gómez I, Wangensteen R, Banegas I, García-Lora Á, Quesada A, et al. Effect of thyroid hormone-nitric oxide interaction on tumor growth, angiogenesis, and aminopeptidase activity in mice. *Tumour Biol.* (2014) 35:5519–26. doi: 10.1007/s13277-014-1726-2
44. Shinderman-Maman E, Cohen K, Weingarten C, Nabriski D, Twito O, Baraf L, et al. The thyroid hormone-αvβ3 integrin axis in ovarian cancer: regulation of gene transcription and MAPK-dependent proliferation. *Oncogene* (2016) 35:1977–87. doi: 10.1038/onc.2015.262
45. Moriggi G, Verga Falzacappa C, Mangialardo C, Michienzi S, Stigliano A, Brunetti E, et al. Thyroid hormones (T3 and T4): dual effect on human cancer cell proliferation. *Anticancer Res.* (2011) 31:89–96.
46. Hsieh MT, Wang LM, Changou CA, Chin YT, Yang YSH, Lai HY, et al. Crosstalk between integrin αvβ3 and ERα contributes to thyroid hormone-induced proliferation of ovarian cancer cells. *Oncotarget* (2017) 8:24237–49. doi: 10.18632/oncotarget.10757
47. Weingarten C, Jenudi Y, Tshuva RY, Moskovich D, Alfandari A, Hercbergs A, et al. The interplay between Epithelial-Mesenchymal Transition (EMT) and the thyroid hormones-αvβ3 axis in ovarian cancer. *Horm Cancer* (2018) 9:22–32. doi: 10.1007/s12672-017-0316-3
48. Shinderman-Maman E, Weingarten C, Moskovich D, Werner H, Hercbergs A, Davis PJ, et al. Molecular insights into the transcriptional regulatory role of thyroid hormones in ovarian cancer. *Mol Carcinog.* (2018) 57:97–105. doi: 10.1002/mc.22735
49. Shinderman-Maman E, Cohen K, Moskovich D, Hercbergs A, Werner H, Davis PJ, et al. Thyroid hormones derivatives reduce proliferation and induce cell death and DNA damage in ovarian cancer. *Sci Rep.* (2017) 7:16475. doi: 10.1038/s41598-017-16593-x

50. Lin HY, Davis FB, Gordinier JK, Martino LJ, Davis PJ. Thyroid hormone induces activation of mitogen-activated protein kinase in cultured cells. *Am J Physiol.* (1999) 276(5 Pt 1):C1014–24. doi: 10.1152/ajpcell.1999.276.5.C1014
51. Lin HY, Shih A, Davis FB, Davis PJ. Thyroid hormone promotes the phosphorylation of STAT3 and potentiates the action of epidermal growth factor in cultured cells. *Biochem J.* (1999) 338(Pt 2):427–32.
52. Shih A, Zhang S, Cao HJ, Tang HY, Davis FB, Davis PJ, et al. Disparate effects of thyroid hormone on actions of epidermal growth factor and transforming growth factor-alpha are mediated by 3',5'-cyclic adenosine 5'-monophosphate-dependent protein kinase II. *Endocrinology* (2004) 145:1708–17. doi: 10.1210/en.2003-0742
53. Davis FB, Tang HY, Shih A, Keating T, Lansing L, Hercbergs A, et al. Acting via a cell surface receptor, thyroid hormone is a growth factor for glioma cells. *Cancer Res.* (2006) 66:7270–5. doi: 10.1158/0008-5472.CAN-05-4365
54. Lin HY, Tang HY, Keating T, Wu YH, Shih A, Hammond D, et al. Resveratrol is pro-apoptotic and thyroid hormone is anti-apoptotic in glioma cells: both actions are integrin and ERK mediated. *Carcinogenesis* (2008) 29:62–9. doi: 10.1093/carcin/bgm239
55. Liappas A, Alexandros L, Mourouzis I, Iordanis M, Zisakis A, Athanasios Z, et al. Cell-type-dependent thyroid hormone effects on glioma tumor cell lines. *J Thyroid Res.* (2011) 2011:856050. doi: 10.4061/2011/856050
56. Sudha T, Bharali DJ, Sell S, Darwish NHE, Davis PJ, Mousa SA. Nanoparticulate tetrac inhibits growth and vascularity of glioblastoma xenografts. *Horm Cancer* (2017) 8:157–65. doi: 10.1007/s12672-017-0293-6
57. García-Silva S, Aranda A. The thyroid hormone receptor is a suppressor of ras-mediated transcription, proliferation, and transformation. *Mol Cell Biol.* (2004) 24:7514–23. doi: 10.1128/MCB.24.17.7514-7523.2004
58. Poplawski P, Nauman A. Thyroid hormone - triiodothyronine - has contrary effect on proliferation of human proximal tubules cell line (HK2) and renal cancer cell lines (Caki-2, Caki-1) - role of E2F4, E2F5 and p107, p130. *Thyroid Res.* (2008) 1:5. doi: 10.1186/1756-6614-1-5
59. Yalcin M, Bharali DJ, Lansing L, Dyskin E, Mousa SS, Hercbergs A, et al. Tetraidothyroacetic acid (tetrac) and tetrac nanoparticles inhibit growth of human renal cell carcinoma xenografts. *Anticancer Res.* (2009) 29:3825–31.
60. Liu R, Li Z, Bai S, Zhang H, Tang M, Lei Y, et al. Mechanism of cancer cell adaptation to metabolic stress: proteomics identification of a novel thyroid hormone-mediated gastric carcinogenic signaling pathway. *Mol Cell Proteomics* (2009) 8:70–85. doi: 10.1074/mcp.M800195-MCP200
61. Iishi H, Tatsuta M, Baba M, Yamamoto R, Taniguchi H. Enhancement by thyroxine of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. *Br J Cancer* (1993) 68:515–8. doi: 10.1038/bjc.1993.378
62. Sarosiek K, Gandhi AV, Saxena S, Kang CY, Chipitsyna GI, Yeo CJ, et al. Hypothyroidism in pancreatic cancer: role of exogenous thyroid hormone in tumor invasion-preliminary observations. *J Thyroid Res.* (2016) 2016:2454989. doi: 10.1155/2016/2454989
63. Michienzi S, Bucci B, Verga Falzacappa C, Patriarca V, Stigliano A, Panacchia L, et al. 3,3',5-Triiodo-L-thyronine inhibits ductal pancreatic adenocarcinoma proliferation improving the cytotoxic effect of chemotherapy. *J Endocrinol.* (2007) 193:209–23. doi: 10.1677/joe.1.07065
64. Yalcin M, Lin HY, Sudha T, Bharali DJ, Meng R, Tang HY, et al. Response of human pancreatic cancer cell xenografts to tetraiodothyroacetic acid nanoparticles. *Horm Cancer* (2013) 4:176–85. doi: 10.1007/s12672-013-0137-y
65. Rognoni JB, Pichard V, Honore S, Rigot V, Lehmann M, Roccabianca M, et al. Convergent effects of growth factors, hormones, and fibronectin are necessary for the enterocyte differentiation of a colon adenocarcinoma cell line (HT29-D4). *Differentiation* (1998) 63:305–17. doi: 10.1046/j.1432-0436.1998.6350305.x
66. Lee YS, Chin YT, Yang YSH, Wei PL, Wu HC, Shih A, et al. The combination of tetraiodothyroacetic acid and cetuximab inhibits cell proliferation in colorectal cancers with different K-ras status. *Steroids* (2016) 111:63–70. doi: 10.1016/j.steroids.2016.03.006
67. Mitin T, Von Moltke LL, Court MH, Greenblatt DJ. Levothyroxine up-regulates P-glycoprotein independent of the pregnane X receptor. *Drug Metab Dispos.* (2004) 32:779–82. doi: 10.1124/dmd.32.8.779
68. Dentice M, Luongo C, Ambrosio R, Sibilio A, Casillo A, Iaccarino A, et al. beta-Catenin regulates deiodinase levels and thyroid hormone signaling in colon cancer cells. *Gastroenterology* (2012) 143:1037–47. doi: 10.1053/j.gastro.2012.06.042
69. Iishi H, Tatsuta M, Baba M, Okuda S, Taniguchi H. Enhancement by thyroxine of experimental carcinogenesis induced in rat colon by azoxymethane. *Int J Cancer* (1992) 50:974–6. doi: 10.1002/ijc.2910500625
70. Chen RN, Huang YH, Lin YC, Yeh CT, Liang Y, Chen SL, et al. Thyroid hormone promotes cell invasion through activation of furin expression in human hepatoma cell lines. *Endocrinology* (2008) 149:3817–31. doi: 10.1210/en.2007-0989
71. Chung IH, Chen CY, Lin YH, Chi HC, Huang YH, Tai PJ, et al. Thyroid hormone-mediated regulation of lipocalin 2 through the Met/FAK pathway in liver cancer. *Oncotarget* (2015) 6:15050–64. doi: 10.18632/oncotarget.3670
72. Gnoni GV, Rochira A, Leone A, Damiano F, Marsigliante S, Siculella L. 3,5,3'triiodo-L-thyronine induces SREBP-1 expression by non-genomic actions in human HEP G2 cells. *J Cell Physiol.* (2012) 227:2388–97. doi: 10.1002/jcp.22974
73. Wang T, Xia L, Ma S, Qi X, Li Q, Xia Y, et al. Hepatocellular carcinoma: thyroid hormone promotes tumorigenicity through inducing cancer stem-like cell self-renewal. *Sci Rep.* (2016) 6:25183. doi: 10.1038/srep25183
74. Yen CC, Huang YH, Liao CY, Liao CJ, Cheng WL, Chen WJ, et al. Mediation of the inhibitory effect of thyroid hormone on proliferation of hepatoma cells by transforming growth factor-beta. *J Mol Endocrinol.* (2006) 36:9–21. doi: 10.1677/jme.1.01911
75. Lin YH, Huang YH, Wu MH, Wu SM, Chi HC, Liao CJ, et al. Thyroid hormone suppresses cell proliferation through endoglin-mediated promotion of p21 stability. *Oncogene* (2013) 32:3904–14. doi: 10.1038/onc.2013.5
76. Chung IH, Liu H, Lin YH, Chi HC, Huang YH, Yang CC, et al. ChIP-on-chip analysis of thyroid hormone-regulated genes and their physiological significance. *Oncotarget* (2016) 7:22448–59. doi: 10.18632/oncotarget.7988
77. Chi HC, Liao CH, Huang YH, Wu SM, Tsai CY, Liao CJ, et al. Thyroid hormone receptor inhibits hepatoma cell migration through transcriptional activation of Dickkopf 4. *Biochem Biophys Res Commun.* (2013) 439:60–5. doi: 10.1016/j.bbrc.2013.08.028
78. Mishkin SY, Pollack R, Yalovsky MA, Morris HP, Mishkin S. Inhibition of local and metastatic hepatoma growth and prolongation of survival after induction of hypothyroidism. *Cancer Res* (1981) 41:3040–5.
79. Frau C, Loi R, Petrelli A, Perra A, Menegon S, Kowalik MA, et al. Local hypothyroidism favors the progression of preneoplastic lesions to hepatocellular carcinoma in rats. *Hepatology* (2015) 61:249–59. doi: 10.1002/hep.27399
80. Lin HY, Tang HY, Shih A, Keating T, Cao G, Davis PJ, et al. Thyroid hormone is a MAPK-dependent growth factor for thyroid cancer cells and is anti-apoptotic. *Steroids* (2007) 72:180–7. doi: 10.1016/j.steroids.2006.11.014
81. Lu C, Zhu X, Willingham MC, Cheng SY. Activation of tumor cell proliferation by thyroid hormone in a mouse model of follicular thyroid carcinoma. *Oncogene* (2012) 31:2007–16. doi: 10.1038/onc.2011.390
82. Yalcin M, Dyskin E, Lansing L, Bharali DJ, Mousa SS, Bridoux A, et al. Tetraiodothyroacetic acid (tetrac) and nanoparticulate tetrac arrest growth of medullary carcinoma of the thyroid. *J Clin Endocrinol Metab.* (2010) 95:1972–80. doi: 10.1210/jc.2009-1926
83. Yalcin M, Bharali DJ, Dyskin E, Dier E, Lansing L, Mousa SS, et al. Tetraiodothyroacetic acid and tetraiodothyroacetic acid nanoparticle effectively inhibit the growth of human follicular thyroid cell carcinoma. *Thyroid* (2010) 20:281–6. doi: 10.1089/thy.2009.0249
84. Fabian ID, Rosner M, Fabian I, Vishnevskia-Dai V, Zloto O, Shinderman Maman E, et al. Low thyroid hormone levels improve survival in murine model for ocular melanoma. *Oncotarget* (2015) 6:11038–46. doi: 10.18632/oncotarget.3566
85. Luongo C, Ambrosio R, Salzano S, Dlugosz AA, Missero C, Dentice M. The sonic hedgehog-induced type 3 deiodinase facilitates tumorigenesis of basal cell carcinoma by reducing Gli2 inactivation. *Endocrinology* (2014) 155:2077–88. doi: 10.1210/en.2013-2108
86. Ferreira E, da Silva AE, Serakides R, Gomes MG, Cassali GD. Ehrlich tumor as model to study artificial hyperthyroidism influence on breast cancer. *Pathol Res Pract.* (2007) 203:39–44. doi: 10.1016/j.prp.2006.09.005
87. Silva AE, Ferreira E, Ocarino NM, Moraes JR, Cassali GD, Serakides R. Effect of hypothyroidism on growth, viability and characteristics of ascitic Ehrlich

tumor in ovariectomized and non-ovariectomized mice. *J Appl Res.* (2007) 7:158–66.
88. Kumar MS, Chiang T, Deodhar SD. Enhancing effect of thyroxine on tumor growth and metastases in syngeneic mouse tumor systems. *Cancer Res.* (1979) 39:3515–8.
89. Cohen K, Ellis M, Khoury S, Davis PJ, Hercbergs A, Ashur-Fabian O. Thyroid hormone is a MAPK-dependent growth factor for human myeloma cells acting via αvβ3 integrin. *Mol Cancer Res.* (2011) 9:1385–94. doi: 10.1158/1541-7786.MCR-11-0187
90. Cohen K, Ellis M, Shinderman E, Khoury S, Davis PJ, Hercbergs A, et al. Relevance of the thyroid hormones-αvβ3 pathway in primary myeloma bone marrow cells and to bortezomib action. *Leuk Lymphoma* (2015) 56:1107–14. doi: 10.3109/10428194.2014.947612
91. Cohen K, Flint N, Shalev S, Erez D, Baharal T, Davis PJ, et al. Thyroid hormone regulates adhesion, migration and matrix metalloproteinase 9 activity via αvβ3 integrin in myeloma cells. *Oncotarget* (2014) 5:6312–22. doi: 10.18632/oncotarget.2205
92. Cohen K, Abadi U, Hercbergs A, Davis PJ, Ellis M, Ashur-Fabian O. The induction of myeloma cell death and DNA damage by tetrac, a thyroid hormone derivative. *Endocr Relat Cancer* (2018) 25:21–34. doi: 10.1530/ERC-17-0246
93. Frick LR, Rapanelli M, Bussmann UA, Klecha AJ, Arcos ML, Genaro AM, et al. Involvement of thyroid hormones in the alterations of T-cell immunity and tumor progression induced by chronic stress. *Biol Psychiatry* (2009) 65:935–42. doi: 10.1016/j.biopsych.2008.12.013
94. Cayrol F, Díaz Flaqué MC, Fernando T, Yang SN, Sterle HA, Bolontrade M, et al. Integrin αvβ3 acting as membrane receptor for thyroid hormones mediates angiogenesis in malignant T cells. *Blood* (2015) 125:841–51. doi: 10.1182/blood-2014-07-587337
95. Sterle HA, Barreiro Arcos ML, Valli E, Paulazo MA, Méndez Huergo SP, Blidner AG, et al. The thyroid status reprograms T cell lymphoma growth and modulates immune cell frequencies. *J Mol Med.* (2016) 94:417–29. doi: 10.1007/s00109-015-1363-2
96. Sterle HA, Valli E, Cayrol F, Paulazo MA, Martinel Lamas DJ, Diaz Flaqué MC, et al. Thyroid status modulates T lymphoma growth via cell cycle regulatory proteins and angiogenesis. *J Endocrinol.* (2014) 222:243–55.
97. Mousa SA, Davis FB, Mohamed S, Davis PJ, Feng X. Pro-angiogenesis action of thyroid hormone and analogs in a three-dimensional *in vitro* microvascular endothelial sprouting model. *Int Angiol.* (2006) 25:407–13.
98. Davis FB, Mousa SA, O'Connor L, Mohamed S, Lin HY, Cao HJ, et al. Proangiogenic action of thyroid hormone is fibroblast growth factor-dependent and is initiated at the cell surface. *Circ Res.* (2004) 94:1500–6. doi: 10.1161/01.RES.0000130784.90237.4a
99. Bergh JJ, Lin HY, Lansing L, Mohamed SN, Davis FB, Mousa S, et al. Integrin alphaVbeta3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* (2005) 146:2864–71. doi: 10.1210/en.2005-0102
100. Mousa SA, O'Connor LJ, Bergh JJ, Davis FB, Scanlan TS, Davis PJ. The proangiogenic action of thyroid hormone analogue GC-1 is initiated at an integrin. *J Cardiovasc Pharmacol.* (2005) 46:356–60. doi: 10.1097/01.fjc.0000175438.94906.a0
101. Conde SJ, Luvizotto RA, Síbio MT, Katayama ML, Brentani MM, Nogueira CR. Tamoxifen inhibits transforming growth factor-alpha gene expression in human breast carcinoma samples treated with triiodothyronine. *J Endocrinol Invest.* (2008) 31:1047–51. doi: 10.1007/BF03345650
102. Pachucki J, Ambroziak M, Tanski Z, Luczak J, Nauman J, Nauman A. Type I 5'-iodothyronine deiodinase activity and mRNA are remarkably reduced in renal clear cell carcinoma. *J Endocrinol Invest.* (2001) 24:253–61. doi: 10.1007/BF03343855
103. Puzianowska-Kuznicka M, Nauman A, Madej A, Tanski Z, Cheng S, Nauman J. Expression of thyroid hormone receptors is disturbed in human renal clear cell carcinoma. *Cancer Lett.* (2000) 155:145–52. doi: 10.1016/S0304-3835(00)00416-X
104. Kamiya Y, Puzianowska-Kuznicka M, McPhie P, Nauman J, Cheng SY, Nauman A. Expression of mutant thyroid hormone nuclear receptors is associated with human renal clear cell carcinoma. *Carcinogenesis* (2002) 23:25–33. doi: 10.1093/carcin/23.1.25
105. Piekielko-Witkowska A, Master A, Wojcicka A, Boguslawska J, Brozda I, Tanski Z, et al. Disturbed expression of type 1 iodothyronine deiodinase splice variants in human renal cancer. *Thyroid* (2009) 19:1105–13. doi: 10.1089/thy.2008.0284
106. Master A, Wójcicka A, Piekiełko-Witkowska A, Bogusławska J, Popławski P, Tanski Z, et al. Untranslated regions of thyroid hormone receptor beta 1 mRNA are impaired in human clear cell renal cell carcinoma. *Biochim Biophys Acta* (2010) 1802:995–1005. doi: 10.1016/j.bbadis.2010.07.025
107. Le Cam L, Polanowska J, Fabbrizio E, Olivier M, Philips A, Ng Eaton E, et al. Timing of cyclin E gene expression depends on the regulated association of a bipartite repressor element with a novel E2F complex. *EMBO J.* (1999) 18:1878–90. doi: 10.1093/emboj/18.7.1878
108. Czarnecka AM, Matak D, Szymanski L, Czarnecka KH, Lewicki S, Zdanowski R, et al. Triiodothyronine regulates cell growth and survival in renal cell cancer. *Int J Oncol.* (2016) 49:1666–78. doi: 10.3892/ijo.2016.3668
109. Nishio N, Katsura T, Inui K. Thyroid hormone regulates the expression and function of P-glycoprotein in Caco-2 cells. *Pharm Res.* (2008) 25:1037–42. doi: 10.1007/s11095-007-9495-x
110. Wu SM, Cheng WL, Lin CD, Lin KH. Thyroid hormone actions in liver cancer. *Cell Mol Life Sci.* (2013) 70:1915–36. doi: 10.1007/s00018-012-1146-7
111. Chan IH, Privalsky ML. Thyroid hormone receptor mutants implicated in human hepatocellular carcinoma display an altered target gene repertoire. *Oncogene* (2009) 28:4162–74. doi: 10.1038/onc.2009.265
112. Barreiro Arcos ML, Gorelik G, Klecha A, Genaro AM, Cremaschi GA. Thyroid hormones increase inducible nitric oxide synthase gene expression downstream from PKC-zeta in murine tumor T lymphocytes. *Am J Physiol Cell Physiol.* (2006) 291:C327–36. doi: 10.1152/ajpcell.00316.2005
113. Barreiro Arcos ML, Sterle HA, Paulazo MA, Valli E, Klecha AJ, Isse B, et al. Cooperative nongenomic and genomic actions on thyroid hormone mediated-modulation of T cell proliferation involve up-regulation of thyroid hormone receptor and inducible nitric oxide synthase expression. *J Cell Physiol.* (2011) 226:3208–18. doi: 10.1002/jcp.22681
114. Goemann IM, Romitti M, Meyer ELS, Wajner SM, Maia AL. Role of thyroid hormones in the neoplastic process: an overview. *Endocr Relat Cancer* (2017) 24:R367–85. doi: 10.1530/ERC-17-0192
115. Morris HP, Dubnik CS, Dalton AJ. Effect of prolonged ingestion of thiourea on mammary glands and the appearance of mammary tumors in adult C3H mice. *J Natl Cancer Inst.* (1946) 7:159–69.
116. Dubnik CS, Morris HP, Dalton AJ. Inhibition of mammary-gland development and mammary-tumor formation in female C3H mice following ingestion of thiouracil. *J Natl Cancer Inst.* (1950) 10:815–41.
117. Jull JW, Huggins C. Influence of hyperthyroidism and of thyroidectomy on induced mammary cancer. *Nature* (1960) 188:73. doi: 10.1038/188073a0
118. Martínez-Iglesias O, Garcia-Silva S, Tenbaum SP, Regadera J, Larcher F, Paramio JM, et al. Thyroid hormone receptor beta1 acts as a potent suppressor of tumor invasiveness and metastasis. *Cancer Res.* (2009) 69:501–9. doi: 10.1158/0008-5472.CAN-08-2198
119. Liao CH, Yeh CT, Huang YH, Wu SM, Chi HC, Tsai MM, et al. Dickkopf 4 positively regulated by the thyroid hormone receptor suppresses cell invasion in human hepatoma cells. *Hepatology* (2012) 55:910–20. doi: 10.1002/hep.24740
120. Grad B. The influence of hyper- and hypothyroidism on the incidence of lymphatic leukemia of AKR mice. *Cancer Res.* (1957) 17:266–71.
121. Morris DM, Wolff FF, Upton AC. The influence of the thyroid gland on the survival of rats and mice bearing transplanted lymphoid leukemia. *Cancer Res.* (1957) 17:325–8.
122. Morris DM. The effect of thyroidectomy and thyroid-stimulating hormone on a transplanted acute leukemia in the Fischer rat. *Cancer Res.* (1960) 20:373–6.
123. Sloviter HA. The effect of complete ablation of thyroid tissue by radioactive iodine on the survival of tumor-bearing mice. *Cancer Res.* (1951) 11:447–9.
124. Williams MW, Williams CS. Depression of growth of sarcoma 180 by hyperthyroidism. *Cancer Chemother Rep.* (1965) 45:1–4.
125. Di Girolamo D, Ambrosio R, De Stefano MA, Mancino G, Porcelli T, Luongo C, et al. Reciprocal interplay between thyroid hormone and microRNA-21 regulates hedgehog pathway-driven skin tumorigenesis. *J Clin Invest.* (2016) 126:2308–20. doi: 10.1172/JCI84465

126. Khan SR, Chaker L, Ruiter R, Aerts JG, Hofman A, Dehghan A, et al. Thyroid function and cancer risk: the rotterdam study. *J Clin Endocrinol Metab.* (2016) 101:5030–6. doi: 10.1210/jc.2016-2104
127. Tosovic A, Becker C, Bondeson AG, Bondeson L, Ericsson UB, Malm J, et al. Prospectively measured thyroid hormones and thyroid peroxidase antibodies in relation to breast cancer risk. *Int J Cancer* (2012) 131:2126–33. doi: 10.1002/ijc.27470
128. Brandt J, Borgquist S, Manjer J. Prospectively measured thyroid hormones and thyroid peroxidase antibodies in relation to risk of different breast cancer subgroups: a Malmö Diet and Cancer Study. *Cancer Causes Control.* (2015) 26:1093–104. doi: 10.1007/s10552-015-0602-8
129. Søgaard M, Farkas DK, Ehrenstein V, Jørgensen JO, Dekkers OM, Sørensen HT. Hypothyroidism and hyperthyroidism and breast cancer risk: a nationwide cohort study. *Eur J Endocrinol.* (2016) 174:409–14. doi: 10.1530/EJE-15-0989
130. Journy NMY, Bernier MO, Doody MM, Alexander BH, Linet MS, Kitahara CM. Hyperthyroidism, hypothyroidism, and cause-specific mortality in a large cohort of women. *Thyroid* (2017) 27:1001–10. doi: 10.1089/thy.2017.0063
131. Moossa AR, Evans DA, Brewer AC. Thyroid status and breast cancer. Reappraisal of an old relationship. *Ann R Coll Surg Engl.* (1973) 53:178–88.
132. Ditsch N, Liebhardt S, Von Koch F, Lenhard M, Vogeser M, Spitzweg C, et al. Thyroid function in breast cancer patients. *Anticancer Res.* (2010) 30:1713–7.
133. Saraiva PP, Figueiredo NB, Padovani CR, Brentani MM, Nogueira CR. Profile of thyroid hormones in breast cancer patients. *Braz J Med Biol Res.* (2005) 38:761–5. doi: 10.1590/S0100-879X2005000500014
134. Ortega-Olvera C, Ulloa-Aguirre A, Ángeles-Llerenas A, Mainero-Ratchelous FE, González-Acevedo CE, Hernández-Blanco ML, et al. Thyroid hormones and breast cancer association according to menopausal status and body mass index. *Breast Cancer Res.* (2018) 20:94. doi: 10.1186/s13058-018-1017-8
135. Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Manjer J. T3 levels in relation to prognostic factors in breast cancer: a population-based prospective cohort study. *BMC Cancer* (2014) 14:536. doi: 10.1186/1471-2407-14-536
136. Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Malm J, Manjer J. Prospectively measured triiodothyronine levels are positively associated with breast cancer risk in postmenopausal women. *Breast Cancer Res.* (2010) 12:R33. doi: 10.1186/bcr2587
137. Tseng FY, Lin WY, Li CI, Li TC, Lin CC, Huang KC. Subclinical hypothyroidism is associated with increased risk for cancer mortality in adult Taiwanese-a 10 years population-based cohort. *PLoS ONE* (2015) 10:e0122955. doi: 10.1371/journal.pone.0122955
138. Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Manjer J. Triiodothyronine levels in relation to mortality from breast cancer and all causes: a population-based prospective cohort study. *Eur J Endocrinol.* (2013) 168:483–90. doi: 10.1530/EJE-12-0564
139. Hardefeldt PJ, Eslick GD, Edirimanne S. Benign thyroid disease is associated with breast cancer: a meta-analysis. *Breast Cancer Res Treat.* (2012) 133:1169–77. doi: 10.1007/s10549-012-2019-3
140. Fang Y, Yao L, Sun J, Yang R, Chen Y, Tian J, et al. Does thyroid dysfunction increase the risk of breast cancer? A systematic review and meta-analysis. *J Endocrinol Invest.* (2017) 40:1035–47. doi: 10.1007/s40618-017-0679-x
141. Simon MS, Tang MT, Bernstein L, Norman SA, Weiss L, Burkman RT, et al. Do thyroid disorders increase the risk of breast cancer? *Cancer Epidemiol Biomarkers Prev.* (2002) 11:1574–8.
142. Cristofanilli M, Yamamura Y, Kau SW, Bevers T, Strom S, Patangan M, et al. Thyroid hormone and breast carcinoma. Primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma. *Cancer* (2005) 103:1122–8. doi: 10.1002/cncr.20881
143. Brinton LA, Hoffman DA, Hoover R, Fraumeni JF. Relationship of thyroid disease and use of thyroid supplements to breast cancer risk. *J Chronic Dis.* (1984) 37:877–93. doi: 10.1016/0021-9681(84)90062-6
144. Cao J, Zhang J, Wang Z, Wang B, Lv F, Wang L, et al. Hypothyroidism as a potential biomarker of efficacy of famitinib, a novel VEGFR-2 inhibitor in metastatic breast cancer. *Cancer Chemother Pharmacol.* (2014) 74:389–98. doi: 10.1007/s00280-014-2505-x
145. Goldman MB, Monson RR, Maloof F. Benign thyroid diseases and the risk of death from breast cancer. *Oncology* (1992) 49:461–6. doi: 10.1159/000227093
146. Villa NM, Li N, Yeh MW, Hurvitz SA, Dawson NA, Leung AM. Serum thyrotropin concentrations are not predictive of aggressive breast cancer biology in euthyroid individuals. *Endocr Pract.* (2015) 21:1040–5. doi: 10.4158/EP15733.OR
147. Kuijpens JL, Nyklíctek I, Louwman MW, Weetman TA, Pop VJ, Coebergh JW. Hypothyroidism might be related to breast cancer in post-menopausal women. *Thyroid* (2005) 15:1253–9. doi: 10.1089/thy.2005.15.1253
148. Franceschi S, la Vecchia C, Negri E, Parazzini F, Boyle P. Breast cancer risk and history of selected medical conditions linked with female hormones. *Eur J Cancer* (1990) 26:781–5. doi: 10.1016/0277-5379(90)90151-I
149. Angelousi AG, Anagnostou VK, Stamatakos MK, Georgiopoulos GA, Kontzoglou KC. Mechanisms in endocrinology: primary HT and risk for breast cancer: a systematic review and meta-analysis. *Eur J Endocrinol.* (2012) 166:373–81. doi: 10.1530/EJE-11-0838
150. Kapdi CC, Wolfe JN. Breast cancer. Relationship to thyroid supplements for hypothyroidism. *JAMA* (1976) 236:1124–7. doi: 10.1001/jama.1976.03270110022020
151. Sandhu MK, Brezden-Masley C, Lipscombe LL, Zagorski B, Booth GL. Autoimmune hypothyroidism and breast cancer in the elderly. *Breast Cancer Res Treat.* (2009) 115:635–41. doi: 10.1007/s10549-008-0104-4
152. Hoffman DA, McConahey WM, Brinton LA, Fraumeni JF Jr. Breast cancer in hypothyroid women using thyroid supplements. *JAMA* (1984) 251:616–9. doi: 10.1001/jama.1984.03340290030015
153. Hellevik AI, Asvold BO, Bjøro T, Romundstad PR, Nilsen TI, Vatten LJ. Thyroid function and cancer risk: a prospective population study. *Cancer Epidemiol Biomarkers Prev.* (2009) 18:570–4. doi: 10.1158/1055-9965.EPI-08-0911
154. Chan YX, Knuiman MW, Divitini ML, Brown SJ, Walsh J, Yeap BB. Lower TSH and higher free thyroxine predict incidence of prostate but not breast, colorectal or lung cancer. *Eur J Endocrinol.* (2017) 177:297–308. doi: 10.1530/EJE-17-0197
155. Lehrer S, Diamond EJ, Bajwa AM, Kornreich R, Stagger S, Stone NN, et al. Association between serum triiodothyronine (t3) level and risk of disease recurrence in men with localized prostate cancer. *Prostate Cancer Prostatic Dis.* (2001) 4:232–4. doi: 10.1038/sj.pcan.4500542
156. Lehrer S, Diamond EJ, Stone NN, Droller MJ, Stock RG. Serum triiodothyronine is increased in men with prostate cancer and benign prostatic hyperplasia. *J Urol.* (2002) 168:2431–3. doi: 10.1016/S0022-5347(05)64161-4
157. Mondul AM, Weinstein SJ, Bosworth T, Remaley AT, Virtamo J, Albanes D. Circulating thyroxine, thyroid-stimulating hormone, and hypothyroid status and the risk of prostate cancer. *PLoS ONE* (2012) 7:e47730. doi: 10.1371/journal.pone.0047730
158. Mazzoccoli G, Pazienza V, Piepoli A, Muscarella LA, Giuliani F, Sothern RB. Alteration of hypothalamic-pituitary-thyroid axis function in non-small-cell lung cancer patients. *Integr Cancer Ther.* (2012) 11:327–36. doi: 10.1177/1534735411413269
159. Hercbergs A, Mason J, Reddy C, Elson P. Thyroid hormones and lung cancer: primary hypothyroidism is prognostically significant for survival in lung cancer. *Cancer Res.* (2004). 1024.
160. Osorio JC, Ni A, Chaft JE, Pollina R, Kasler MK, Stephens D, et al. Antibody-mediated thyroid dysfunction during T-cell checkpoint blockade in patients with non-small-cell lung cancer. *Ann Oncol.* (2017) 28:583–9. doi: 10.1093/annonc/mdw640
161. Cornelli U, Belcaro G, Recchia M, Finco A. Levothyroxine and lung cancer in females: the importance of oxidative stress. *Reprod Biol Endocrinol.* (2013) 11:75. doi: 10.1186/1477-7827-11-75
162. Ness RB, Grisso JA, Cottreau C, Klapper J, Vergona R, Wheeler JE, et al. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. *Epidemiology* (2000) 11:111–7. doi: 10.1097/00001648-200003000-00006
163. Minlikeeva AN, Freudenheim JL, Cannioto RA, Eng KH, Szender JB, Mayor P, et al. History of thyroid disease and survival of ovarian cancer patients: results from the Ovarian Cancer Association Consortium, a brief report. *Br J Cancer* (2017) 117:1063–9. doi: 10.1038/bjc.2017.267

164. Rasool M, Naseer MI, Zaigham K, Malik A, Riaz N, Alam R, et al. Comparative study of alterations in Tri-iodothyronine (T3) and thyroxine (T4) hormone levels in breast and ovarian cancer. *Pak J Med Sci.* (2014) 30:1356–60. doi: 10.12669/pjms.306.5294
165. Goldman MB, Monson RR, Maloof F. Cancer mortality in women with thyroid disease. *Cancer Res.* (1990) 50:2283–9.
166. Seebacher V, Hofstetter G, Polterauer S, Reinthaller A, Grimm C, Schwameis R, et al. Does thyroid-stimulating hormone influence the prognosis of patients with endometrial cancer? A multicentre trial. *Br J Cancer* (2013) 109:215–8. doi: 10.1038/bjc.2013.282
167. Mellemgaard A, From G, Jørgensen T, Johansen C, Olsen JH, Perrild H. Cancer risk in individuals with benign thyroid disorders. *Thyroid* (1998) 8:751–4. doi: 10.1089/thy.1998.8.751
168. Yung WK, Steward W, Marks JE, Griem ML, Mullan JF. Glioblastoma multiforme: treatment with radiation and triiodothyronine. *Int J Radiat Oncol Biol Phys.* (1976) 1:645–50. doi: 10.1016/0360-3016(76)90146-2
169. Ashur-Fabian O, Blumenthal DT, Bakon M, Nass D, Davis PJ, Hercbergs A. Long-term response in high-grade optic glioma treated with medically induced hypothyroidism and carboplatin: a case report and review of the literature. *Anticancer Drugs* (2013) 24:315–23. doi: 10.1097/CAD.0b013e32835c7a47
170. Hercbergs AA, Goyal LK, Suh JH, Lee S, Reddy CA, Cohen BH, et al. Propylthiouracil-induced chemical hypothyroidism with high-dose tamoxifen prolongs survival in recurrent high grade glioma: a phase I/II study. *Anticancer Res.* (2003) 23:617–26.
171. Bailey EB, Tantravahi SK, Poole A, Agarwal AM, Straubhar AM, Batten JA, et al. Correlation of degree of hypothyroidism with survival outcomes in patients with metastatic renal cell carcinoma receiving vascular endothelial growth factor receptor tyrosine kinase inhibitors. *Clin Genitourin Cancer* (2015) 13:e131–7. doi: 10.1016/j.clgc.2014.11.002
172. Kust D, Prpić M, Murgić J, Jazvić M, Jakšić B, Krilić D, et al. Hypothyroidism as a predictive clinical marker of better treatment response to sunitinib therapy. *Anticancer Res.* (2014) 34:3177–84.
173. Schmidinger M, Vogl UM, Bojic M, Lamm W, Heinzl H, Haitel A, et al. Hypothyroidism in patients with renal cell carcinoma: blessing or curse? *Cancer* (2011) 117:534–44. doi: 10.1002/cncr.25422
174. Riesenbeck LM, Bierer S, Hoffmeister I, Köpke T, Papavassilis P, Hertle L, et al. Hypothyroidism correlates with a better prognosis in metastatic renal cancer patients treated with sorafenib or sunitinib. *World J Urol.* (2011) 29:807–13. doi: 10.1007/s00345-010-0627-2
175. Baldazzi V, Tassi R, Lapini A, Santomaggio C, Carini M, Mazzanti R. The impact of sunitinib-induced hypothyroidism on progression-free survival of metastatic renal cancer patients: a prospective single-center study. *Urol Oncol.* (2012) 30:704–10. doi: 10.1016/j.urolonc.2010.07.015
176. Nearchou A, Valachis A, Lind P, Akre O, Sandström P. Acquired hypothyroidism as a predictive marker of outcome in patients with metastatic renal cell carcinoma treated with tyrosine kinase inhibitors: a literature-based meta-analysis. *Clin Genitourin Cancer* (2015) 13:280–6. doi: 10.1016/j.clgc.2014.10.002
177. Sella A, Hercbergs AH, Hanovich E, Kovel S. Does sunitinib-induced hypothyroidism play a role in the activity of sunitinib in metastatic renal cell carcinoma? *Chemotherapy* (2012) 58:200–5. doi: 10.1159/000337079
178. Shinohara N, Takahashi M, Kamishima T, Ikushima H, Otsuka N, Ishizu A, et al. The incidence and mechanism of sunitinib-induced thyroid atrophy in patients with metastatic renal cell carcinoma. *Br J Cancer* (2011) 104:241–7. doi: 10.1038/sj.bjc.6606029
179. Atkins MB, Mier JW, Parkinson DR, Gould JA, Berkman EM, Kaplan MM. Hypothyroidism after treatment with interleukin-2 and lymphokine-activated killer cells. *N Engl J Med.* (1988) 318:1557–63. doi: 10.1056/NEJM198806163182401
180. Weijl NI, Van der Harst D, Brand A, Kooy Y, Van Luxemburg S, Schroder J, et al. Hypothyroidism during immunotherapy with interleukin-2 is associated with antithyroid antibodies and response to treatment. *J Clin Oncol.* (1993) 11:1376–83. doi: 10.1200/JCO.1993.11.7.1376
181. Turkyilmaz A, Eroglu A, Aydin Y, Yilmaz O, Karaoglanoglu N. A new risk factor in oesophageal cancer aetiology: hyperthyroidism. *Acta Chir Belg.* (2010) 110:533–6. doi: 10.1080/00015458.2010.11680671
182. Ko AH, Wang F, Holly EA. Pancreatic cancer and medical history in a population-based case-control study in the San Francisco Bay Area, California. *Cancer Causes Control* (2007) 18:809–19. doi: 10.1007/s10552-007-9024-6
183. Rodríguez-Molinero A, Hercbergs A, Sarrias M, Yuste A. Plasma 3,3',5-Triiodo-L-thyronine [T3] level mirrors changes in tumor markers in two cases of metastatic cancer of the breast and pancreas treated with exogenous L-T3. *Cancer Biomark.* (2018) 21:433–8. doi: 10.3233/CBM-170668
184. Boursi B, Haynes K, Mamtani R, Yang YX. Thyroid dysfunction, thyroid hormone replacement and colorectal cancer risk. *J Natl Cancer Inst.* (2015) 107:djv084. doi: 10.1093/jnci/djv084
185. Mu G, Mu X, Xing H, Xu R, Sun G, Dong C, et al. Subclinical hypothyroidism as an independent risk factor for colorectal neoplasm. *Clin Res Hepatol Gastroenterol.* (2015) 39:261–6. doi: 10.1016/j.clinre.2014.08.002
186. Chan YX, Alfonso H, Chubb SA, Fegan PG, Hankey GJ, Golledge J, et al. Higher thyrotropin concentration is associated with increased incidence of colorectal cancer in older men. *Clin Endocrinol.* (2017) 86:278–85. doi: 10.1111/cen.13271
187. Rennert G, Rennert HS, Pinchev M, Gruber SB. A case-control study of levothyroxine and the risk of colorectal cancer. *J Natl Cancer Inst.* (2010) 102:568–72. doi: 10.1093/jnci/djq042
188. Friedman GD, Schwalbe JS, Habel LA. Re: a case-control study of levothyroxine and the risk of colorectal cancer. *J Natl Cancer Inst.* (2011) 103:1637–9. doi: 10.1093/jnci/djr374
189. Schirripa M, Pasqualetti G, Giampieri R, Scartozzi M, Lonardi S, Rumanò L, et al. Prognostic value of thyroid hormone ratios in patients with advanced metastatic colorectal cancer treated with regorafenib: the TOREADOR study. *Clin Colorectal Cancer* (2018) 17:e601–15. doi: 10.1016/j.clcc.2018.05.013
190. Pinter M, Haupt L, Hucke F, Bota S, Bucsics T, Trauner M, et al. The impact of thyroid hormones on patients with hepatocellular carcinoma. *PLoS ONE* (2017) 12:e0181878. doi: 10.1371/journal.pone.0181878
191. Zhang Y, Chang Y, Ryu S, Cho J, Lee WY, Rhee EJ, et al. Thyroid hormones and mortality risk in euthyroid individuals: the Kangbuk Samsung health study. *J Clin Endocrinol Metab.* (2014) 99:2467–76. doi: 10.1210/jc.2013-3832
192. Hassan MM, Kaseb A, Li D, Patt YZ, Vauthey JN, Thomas MB, et al. Association between hypothyroidism and hepatocellular carcinoma: a case-control study in the United States. *Hepatology* (2009) 49:1563–70. doi: 10.1002/hep.22793
193. Reddy A, Dash C, Leerapun A, Mettler TA, Stadheim LM, Lazaridis KN, et al. Hypothyroidism: a possible risk factor for liver cancer in patients with no known underlying cause of liver disease. *Clin Gastroenterol Hepatol.* (2007) 5:118–23. doi: 10.1016/j.cgh.2006.07.011
194. Chu YD, Lin KH, Huang YH, Lin CC, Hung CF, Yeh TS, et al. A novel thyroid function index associated with opposite therapeutic outcomes in advanced hepatocellular carcinoma patients receiving chemotherapy or sorafenib. *Asia Pac J Clin Oncol.* (2018). doi: 10.1111/ajco.12983
195. Yeh NC, Chou CW, Weng SF, Yang CY, Yen FC, Lee SY, et al. Hyperthyroidism and thyroid cancer risk: a population-based cohort study. *Exp Clin Endocrinol Diabetes* (2013) 121:402–6. doi: 10.1055/s-0033-1341474
196. Nelson M, Hercbergs A, Rybicki L, Strome M. Association between development of hypothyroidism and improved survival in patients with head and neck cancer. *Arch Otolaryngol Head Neck Surg.* (2006) 132:1041–6. doi: 10.1001/archotol.132.10.1041
197. Smith GL, Smith BD, Garden AS, Rosenthal DI, Sherman SI, Morrison WH, et al. Hypothyroidism in older patients with head and neck cancer after treatment with radiation: a population-based study. *Head Neck* (2009) 31:1031–8. doi: 10.1002/hed.21066
198. Patil VM, Noronha V, Joshi A, Bhattacharjee A, Goel A, Talreja V, et al. Influence of hypothyroidism after chemoradiation on outcomes in head and neck cancer. *Clin Oncol.* (2018) 30:675. doi: 10.1016/j.clon.2018.07.002
199. Ellerhorst JA, Cooksley CD, Grimm EA. Autoimmunity and hypothyroidism in patients with uveal melanoma. *Melanoma Res.* (2001) 11:633–7. doi: 10.1097/00008390-200112000-00010
200. Dalamaga M, Karmaniolas K, Papadavid E, Pelecanos N, Migdalis I. Association of thyroid disease and thyroid autoimmunity with multiple myeloma risk: a case-control study. *Leuk Lymphoma* (2008) 49:1545–52. doi: 10.1080/10428190802165946

201. Ghalaut VS, Yadav S, Ghalaut PS, Yadav A, Sachdeva A, Yadav R, et al. Association of insulin like growth factor-1 (IGF-1) and thyroid hormones in patients of acute leukemia. *Clin Lab.* (2012) 58:227–31.
202. Moskowitz C, Dutcher JP, Wiernik PH. Association of thyroid disease with acute leukemia. *Am J Hematol.* (1992) 39:102–7. doi: 10.1002/ajh.2830390206
203. Dalamaga M, Lekka A, Karmaniolas K, Stathopoulou E, Dionyssiou-Asteriou A. Is thyroid autoimmunity a risk factor for developing primary myelodysplastic syndrome? *Cancer Causes Control* (2008) 19:371–8. doi: 10.1007/s10552-007-9096-3
204. Brandt F, Thvilum M, Almind D, Christensen K, Green A, Hegedüs L, et al. Graves' disease and toxic nodular goiter are both associated with increased mortality but differ with respect to the cause of death: a Danish population-based register study. *Thyroid* (2013) 23:408–13. doi: 10.1089/thy.2012.0500
205. Ittermann T, Haring R, Sauer S, Wallaschofski H, Dörr M, Nauck M, et al. Decreased serum TSH levels are not associated with mortality in the adult northeast German population. *Eur J Endocrinol.* (2010) 162:579–85. doi: 10.1530/EJE-09-0566
206. Vadiveloo T, Donnan PT, Cochrane L, Leese GP. The Thyroid Epidemiology, Audit, and Research Study (TEARS): morbidity in patients with endogenous subclinical hyperthyroidism. *J Clin Endocrinol Metab.* (2011) 96:1344–51. doi: 10.1210/jc.2010-2693
207. Metso S, Jaatinen P, Huhtala H, Auvinen A, Oksala H, Salmi J. Increased cardiovascular and cancer mortality after radioiodine treatment for hyperthyroidism. *J Clin Endocrinol Metab.* (2007) 92:2190–6. doi: 10.1210/jc.2006-2321
208. Lechner MG, Vyas CM, Hamnvik OR, Alexander EK, Larsen PR, Choueiri TK, et al. Hypothyroidism during tyrosine kinase inhibitor therapy is associated with longer survival in patients with advanced nonthyroidal cancers. *Thyroid* (2018) 28:445–53. doi: 10.1089/thy.2017.0587
209. Hercbergs A. High tumor response rate to radiation therapy in biochemically hypothyroid patients. *Proc Am Assoc Cancer Res.* (1997) 248:1667.
210. Hercbergs A, Johnson RE, Ashur-Fabian O, Garfield DH, Davis PJ. Medically induced euthyroid hypothyroxinemia may extend survival in compassionate need cancer patients: an observational study. *Oncologist* (2015) 20:72–6. doi: 10.1634/theoncologist.2014-0308
211. Brandt J, Borgquist S, Almgren P, Försti A, Huss L, Melander O, et al. Thyroid-associated genetic polymorphisms in relation to breast cancer risk in the Malmö Diet and Cancer Study. *Int J Cancer* (2018) 142:1309–21. doi: 10.1002/ijc.31156
212. Hercbergs A, Mousa SA, Davis PJ. Nonthyroidal Illness Syndrome and Thyroid Hormone Actions at Integrin αvβ3. *J Clin Endocrinol Metab* (2018) 103:1291–5. doi: 10.1210/jc.2017-01939
213. Kang JH, Kueck AS, Stevens R, Curhan G, De Vivo I, Rosner B, et al. A large cohort study of hypothyroidism and hyperthyroidism in relation to gynecologic cancers. *Obstet Gynecol Int.* (2013) 2013:743721. doi: 10.1155/2013/743721
214. Brinton LA, Sakoda LC, Frederiksen K, Sherman ME, Kjaer SK, Graubard BI, et al. Relationships of uterine and ovarian tumors to pre-existing chronic conditions. *Gynecol Oncol.* (2007) 107:487–94. doi: 10.1016/j.ygyno.2007.08.002
215. Shih YL, Huang YH, Lin KH, Chu YD, Yeh CT. Identification of functional thyroid stimulating hormone receptor and TSHR gene mutations in hepatocellular carcinoma. *Anticancer Res.* (2018) 38:2793–802. doi: 10.21873/anticanres.12523
216. Lechner MG, Vyas CM, Hamnvik OR, Alexander EK, Larsen PR, Choueiri TK, et al. Risk factors for new hypothyroidism during tyrosine kinase inhibitor therapy in advanced nonthyroidal cancer patients. *Thyroid* (2018) 28:437–44. doi: 10.1089/thy.2017.0579
217. Franklyn JA, Maisonneuve P, Sheppard M, Betteridge J, Boyle P. Cancer incidence and mortality after radioiodine treatment for hyperthyroidism: a population-based cohort study. *Lancet* (1999) 353:2111–5. doi: 10.1016/S0140-6736(98)12295-X
218. Humphrey LJ, Swedlow M. The relationship of breast disease to thyroid disease. *Cancer* (1964) 17:1170–3. doi: 10.1002/1097-0142(196409)17:9<1170::AID-CNCR2820170912>3.0.CO;2-R
219. Rini BI, Tamaskar I, Shaheen P, Salas R, Garcia J, Wood L, et al. Hypothyroidism in patients with metastatic renal cell carcinoma treated with sunitinib. *J Natl Cancer Inst.* (2007) 99:81–3. doi: 10.1093/jnci/djk008
220. Wolter P, Stefan C, Decallonne B, Dumez H, Bex M, Carmeliet P, et al. The clinical implications of sunitinib-induced hypothyroidism: a prospective evaluation. *Br J Cancer* (2008) 99:448–54. doi: 10.1038/sj.bjc.6604497
221. Sabatier R, Eymard JC, Walz J, Deville JL, Narbonne H, Boher JM, et al. Could thyroid dysfunction influence outcome in sunitinib-treated metastatic renal cell carcinoma? *Ann Oncol.* (2012) 23:714–21. doi: 10.1093/annonc/mdr275
222. Noronha V, Joshi A, Patil VM, Agarwal J, Ghosh-Laskar S, Budrukkar A, et al. Once-a-week versus once-every-3-weeks cisplatin chemoradiation for locally advanced head and neck cancer: a phase III randomized noninferiority trial. *J Clin Oncol.* (2018) 36:1064–72. doi: 10.1200/JCO.2017.74.9457
223. Jiskra J, Límanová Z, Barkmanová J, Smutek D, Friedmannová Z. Autoimmune thyroid diseases in women with breast cancer and colorectal cancer. *Physiol Res.* (2004) 53:693–702.
224. Giustarini E, Pinchera A, Fierabracci P, Roncella M, Fustaino L, Mammoli C, et al. Thyroid autoimmunity in patients with malignant and benign breast diseases before surgery. *Eur J Endocrinol.* (2006) 154:645–9. doi: 10.1530/eje.1.02108
225. Syrigos KN, Konstantoulakis MM, Constantoulakis M, Marafelia P, Koutras D, Golematis BC. Thyroid autoantibodies and thyroid function in patients with gastric cancer. *Acta Oncol.* (1994) 33:905–7. doi: 10.3109/02841869409098454
226. Fyssas I, Syrigos KN, Konstantoulakis MM, Tsibloulis V, Manouras A, Peveretos P, et al. Thyroid autoantibodies and thyroid function in patients with pancreatic adenocarcinoma. *Acta Oncol.* (1997) 36:65–8. doi: 10.3109/02841869709100735
227. Fiore E, Giustarini E, Mammoli C, Fragomeni F, Campani D, Muller I, et al. Favorable predictive value of thyroid autoimmunity in high aggressive breast cancer. *J Endocrinol Invest.* (2007) 30:734–8. doi: 10.1007/BF03350810
228. Farahati J, Roggenbuck D, Gilman E, Schütte M, Jagminaite E, Seyed Zakavi R, et al. Anti-thyroid peroxidase antibodies are associated with the absence of distant metastases in patients with newly diagnosed breast cancer. *Clin Chem Lab Med.* (2012) 50:709–14. doi: 10.1515/CCLM.2011.819
229. Franzke A, Peest D, Probst-Kepper M, Buer J, Kirchner GI, Brabant G, et al. Autoimmunity resulting from cytokine treatment predicts long-term survival in patients with metastatic renal cell cancer. *J Clin Oncol.* (1999) 17:529–33. doi: 10.1200/JCO.1999.17.2.529
230. Tazebay UH, Wapnir IL, Levy O, Dohan O, Zuckier LS, Zhao QH, et al. The mammary gland iodide transporter is expressed during lactation and in breast cancer. *Nat Med.* (2000) 6:871–8. doi: 10.1038/78630
231. Ruf J, Toubert ME, Czarnocka B, Durand-Gorde JM, Ferrand M, Carayon P. Relationship between immunological structure and biochemical properties of human thyroid peroxidase. *Endocrinology* (1989) 125:1211–8. doi: 10.1210/endo-125-3-1211
232. Dişel U, Beşen A, Karadeniz C, Mertsoylu H, Sezer A, Köse F, et al. Prevalence of thyroid dysfunction in untreated cancer patients: a cross-sectional study. *Med Oncol.* (2012) 29:3608–13. doi: 10.1007/s12032-012-0254-4
233. Ratcliffe JG, Stack BH, Burt RW, Radcliffe WA, Spilg WG, Cuthbert J, et al. Thyroid function in lung cancer. *Br Med J.* (1978) 1:210–2. doi: 10.1136/bmj.1.6107.210
234. Cengiz SE, Cetinkaya E, Altin S, Gunluoglu Z, Demir A, Gunluoglu G, et al. Nutritional and prognostic significance of sick euthyroid syndrome in non-small cell lung cancer patients. *Intern Med.* (2008) 47:211–6. doi: 10.2169/internalmedicine.47.0423
235. Yasar ZA, Kirakli C, Yilmaz U, Ucar ZZ, Talay F. Can non-thyroid illness syndrome predict mortality in lung cancer patients? A prospective cohort study. *Horm Cancer* (2014) 5:240–6. doi: 10.1007/s12672-014-0183-0
236. Bunevicius A, Deltuva VP, Tamasauskas S, Smith T, Laws ER, Bunevicius R, et al. Preoperative low tri-iodothyronine concentration is associated with worse health status and shorter five year survival of primary brain tumor patients. *Oncotarget* (2017) 8:8648–56. doi: 10.18632/oncotarget.14376
237. Hrycek A, Gruszka A. Thyroid hormone and insulin-like growth factor-I in patients with multiple myeloma treated with melphalan and prednisone. *Arch Med Res.* (2006) 37:74–8. doi: 10.1016/j.arcmed.2005.04.015

238. Gao R, Chen RZ, Xia Y, Liang JH, Wang L, Zhu HY, et al. Low T3 syndrome as a predictor of poor prognosis in chronic lymphocytic leukemia. *Int J Cancer* (2018) 143:466–77. doi: 10.1002/ijc.31327
239. Gao R, Liang JH, Wang L, Zhu HY, Wu W, Wu JZ, et al. Low T3 syndrome is a strong prognostic predictor in diffuse large B cell lymphoma. *Br J Haematol.* (2017) 177:95–105. doi: 10.1111/bjh.14528
240. Bunevicius A, Deltuva V, Tamasauskas S, Tamasauskas A, Laws ER, Bunevicius R. Low triiodothyronine syndrome as a predictor of poor outcomes in patients undergoing brain tumor surgery: a pilot study: clinical article. *J Neurosurg.* (2013) 118:1279–87. doi: 10.3171/2013.1.JNS121696
241. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
242. Hamanishi J, Mandai M, Matsumura N, Abiko K, Baba T, Konishi I. PD-1/PD-L1 blockade in cancer treatment: perspectives and issues. *Int J Clin Oncol.* (2016) 21:462–73. doi: 10.1007/s10147-016-0959-z
243. Kurose K, Saeki M, Tohkin M, Hasegawa R. Thyroid hormone receptor mediates human MDR1 gene expression-Identification of the response region essential for gene expression. *Arch Biochem Biophys.* (2008) 474:82–90. doi: 10.1016/j.abb.2008.03.020

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

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 07 March 2019
doi: 10.3389/fendo.2019.00139

Molecular Actions of Thyroid Hormone on Breast Cancer Cell Migration and Invasion via Cortactin/N-WASP

Ivonne Denise Uzair[1], *Jeremias Conte Grand*[1], *Marina Ines Flamini*[1,2] *and Angel Matias Sanchez*[1]*

[1] *Laboratory of Signal Transduction and Cell Movement, Institute of Medicine and Experimental Biology of Cuyo, National Scientific and Technical Research Council (CONICET), Mendoza, Argentina,* [2] *Tumor Biology Laboratory, Institute of Medicine and Experimental Biology of Cuyo, National Scientific and Technical Research Council (CONICET), Mendoza, Argentina*

The thyroid hormone triiodothyronine (T3) plays a fundamental role in growth regulation, differentiation, metabolism and cellular movement. These processes are particularly important considering that deregulation of T3 levels could promote abnormal responsiveness of mammary epithelial cells, which may lead to the development and progression of breast cancer (BC). Once cells migrate and invade different tissues, BC metastasis is the main cause of cancer-related death because it is particularly difficult to revert this multistep process. Cell migration integrates several steps that induce changes in cell structure and morphology to promote BC cell invasion. These sequential steps include actin cytoskeleton remodeling, focal adhesion complex formation and, finally, the turnover of branched actin filament networks. In this article, we demonstrate that T3 has the ability to modify the Epithelial-Mesenchymal Transition process. In addition, we show that T3 induces actin cytoskeleton reorganization, triggers focal adhesion formation and, as a consequence, promotes actin nucleation via non-genomic pathway. These events are specifically modulated by T3 via integrin αvβ3 to FAK/paxillin/cortactin/N-WASP/Arp2/3 complex signaling pathway, increasing cell adhesion, migration and invasion of T-47D BC cells. We suggest that T3 influences the progression of tumor metastasis by controlling signaling pathways that converge in cell motility. This knowledge is crucial for the development of novel therapeutic strategies for BC treatment.

Keywords: triiodothyronine, cortactin, N-WASP, cell motility and invasion, breast cancer

OPEN ACCESS

Edited by:
Paul Davis,
Albany Medical College, United States

Reviewed by:
Osnat Ashur-Fabian,
Tel Aviv University, Israel
Eva Surmacz,
Temple University, United States
Dragana Nikitovic,
University of Crete, Greece

***Correspondence:**
Angel Matias Sanchez
amsanchez@mendoza-conicet.gov.ar

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *16 October 2018*
Accepted: *13 February 2019*
Published: *07 March 2019*

Citation:
Uzair ID, Conte Grand J, Flamini MI and Sanchez AM (2019) Molecular Actions of Thyroid Hormone on Breast Cancer Cell Migration and Invasion via Cortactin/N-WASP. Front. Endocrinol. 10:139. doi: 10.3389/fendo.2019.00139

INTRODUCTION

Breast cancer (BC) is one of the most common cancer types in women worldwide. Nearly 300.000 new cases of invasive BC are registered every year, and more than 10% of the affected patients die due to this disease (1). Because metastasis is the main cause of death in BC patients, the study of molecular mechanisms that drive cells into an invasive phenotype could offer new perspectives for the development of targeted therapies (2). In order to invade distant tissues, BC cells need to adhere and migrate from the primary tumor, for which actin cytoskeleton reorganization is crucial.

Deregulation of the signaling pathways that control actin dynamics marks the onset of cancer progression and metastasis process (3).

Metastasis consists of several steps that include migration of cells away from the primary tumor, their invasion of different tissues and, finally, the generation of a new tumor (4). To achieve these steps, carcinoma cells change their shape, losing their apical-basal polarity to acquire a front-rear polarity that allows cell migration. This process is called Epithelial-Mesenchymal Transition (EMT) (5). This transition involves a downregulation of epithelial proteins expression (E-cadherin) and an upregulation of mesenchymal proteins (vimentin), driving modifications in cell morphology through the reorganization of cytoskeletal architecture (5). Recent studies have demonstrated that the thyroid hormones triiodo-L-thyronine (T3) and tetraiodo-L-thyronine (T4) enhance EMT activity and the metastasis process via integrin αvβ3, reducing E-cadherin and increasing vimentin expression in ovarian cancer cells (6), but the action of T3 on EMT in BC cells remains to be determined.

The thyroid hormones (TH), T3 and T4 regulate cell development, differentiation, metabolism, membrane transport and morphology (7). TH also support BC development by acting as proliferative factors and enhancers of cell migration and invasion (8). T4 has been involved in the promotion of cell proliferation through the ER by a MAPK-dependent pathway in MCF-7 BC cells (9). The effects of TH are generally mediated by genomic mechanisms via nuclear TH receptors that finally regulate specific gene expression. However, in the last decade, several studies have supported the existence of non-genomic or rapid mechanisms triggered by TH, independent of nuclear receptors (10). These rapid actions can be mediated by different plasma membrane-receptors, among them integrin αvβ3 (8). We have recently shown that T3 binds to the integrin αvβ3 receptor, which is highly expressed in several cancer cell lines (8, 10, 11), and it regulates actin cytoskeleton proteins such as Src, FAK, and PI3K, increasing BC cell adhesion and migration (11). Similarly, Lin et al. (12) demonstrated that T3, but not T4, increases Src/PI3K phosphorylation through integrin αvβ3 in human glioma cells. These kinases are over-expressed in different BC cell lines. They activate proteins that are crucial for actinic nucleation, the final step to trigger BC cell motility (13).

One of these proteins is paxillin, a scaffold protein with several key domains for the binding of adhesion molecules and the recruitment of signaling components for actin nucleation (14).

Actinic nucleation, a fundamental mechanism for the branching of actin filaments, is responsible for modifying the cell's cytoskeletal architecture by generating new filaments from pre-existing ones at the cell's leading edge. This crucial step provides the force necessary to trigger cell movement (15, 16). The Arp2/3 complex is one of the main modulators of this process; it is activated by nucleation promoter factors, such as cortactin and N-WASP. Cortactin is able to bind and activate the Arp2/3 complex by its N-terminal region (17). On the other hand, N-WASP belongs to the family of the Wiskott–Aldrich Syndrome Proteins (WASP) and also regulates actinic nucleation by activating the Arp2/3 complex synergistically with cortactin (18). Despite the fact that deregulation of these proteins promotes BC progression, it remains to be studied whether T3 could modulate cell adhesion, migration and invasion, via integrin αvβ3, thus controlling cortactin, N-WASP and Arp2/3 complex activity.

The aim of the present study was therefore to continue elucidating the molecular signal pathway triggered by T3, via integrin αvβ3, on cell morphology and motility in BC cells. In particular, we pretended to deepen our understanding of the rapid actions of T3 on the generation of dynamic structural modifications of the cytoskeleton through key proteins involved in actinic nucleation, representing the starting platform for BC cell migration, invasion, and metastasis.

MATERIALS AND METHODS

Cell Culture and Treatments

The T-47D human breast carcinoma cell line was obtained from the American Type Culture Collection. T-47D cells were grown in RPMI 1640 supplemented with L-glutamine (2 mM), 10% fetal bovine serum (FBS), penicillin and streptomycin under 5% CO_2 atmosphere at 37°C. Before long treatments, BC cells were kept 24 h in medium containing steroid-deprived FBS. Before experiments investigating non-transcriptional effects, BC were kept in medium containing no FBS for 8 h. T3 was obtained from Sigma-Aldrich, 4-amino-5-(4-chlorophenyl)-7-(t-butyl)-pyrazolo-(3,4-d) pyrimidine (PP2, 10 μM) was from Calbiochem (La Jolla, CA); and Tetraidothyroacetic acid (Tetrac, 10 μM), FAK inhibitor (FAKi, 1 μM), Wiskostatin (10 μM) and CK-666 (4 μM) were from Santa Cruz Biotechnology (Santa Cruz, CA). Whenever an inhibitor was used, the compound was added 45–60 min before starting the active treatments. PP2, FAKi, Wiskostatin and CK-666 were dissolved in DMSO, Tetrac was dissolved in acetone, and Triiodothyronine (T3) was dissolved in RPMI 1640 Medium.

Immunoblottings

Cell lysates were separated by SDS-PAGE in 8–10% gels and transferred into PVDF membranes. Antibodies used were: p-FAK^{Y397} (611807), FAK (610088) (BD Transduction Laboratories, Lexington, KY); p-FAK (Tyr^{397}) (sc-11765-R), cortactin (H-191), p-cortactin (Tyr^{466}), paxillin (T-16), p-paxillin (Tyr^{118}), E-cadherin (G-10), vimentin (E-5), actin (C-11) (Santa Cruz Biotechnology); α-Tubulin (T9026) (Sigma Aldrich); N-WASP (30D10) (Cell Signaling Technology); p-N-WASP (Ser$^{484/485}$) (Chemicon International); p-Arp2 (Thr237) (Biorbyt). Primary and secondary antibodies were incubated with the membranes using standard techniques. Immunodetection was accomplished using enhanced chemiluminescence and recorded with a quantitative digital imaging system (Chemidoc XRS with Image Lab, Bio-Rad, USA).

Cell Immunofluorescence

T-47D cells were grown on coverslips. Cells were fixed with 4% paraformaldehyde for 30 min and permeabilized with 0.1% Triton for 5 min. Blocking was performed with PBS containing 3% bovine serum albumin for 30 min at room temperature.

Cells were incubated with antibodies against p-paxillin^{Y118}; vimentin (E-5) (Santa Cruz Biotechnology); E-cadherin (24E10) (Cell Signaling Technology); p-N-WASP$^{S484/485}$ (Chemicon International); and p-Arp2^{T237} (Biorbyt) overnight at 4 °C, followed by incubation with DyLight^{488}/ DyLight^{594} and/or fluorescein-conjugated secondary antibody (FITC 1:150; Vector Laboratories, Burlingame, CA). Cells were then incubated with Texas Red-phalloidin (Sigma-Aldrich, Saint-Louis, MO) for 30 min. After washing, the nuclei were counterstained with or 4'-6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, Saint-Louis, MO) and mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA). Immunofluorescence was visualized using a Nikon Eclipse E200 microscope and recorded with a high-resolution DP70 Olympus digital camera.

Gene Silencing With RNA Interference

Synthetic small interfering RNAs targeting paxillin (siRNA paxillin) and control siRNAs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The siRNAs were used at the final concentration of 50 nM. T-47D BC cells were treated 48 h after siRNAs transfection. Efficacy of gene silencing was checked with Western analysis and found to be optimal at 48 h.

Transfection Experiments

Dominant negative constructs for cortactin (*cortactin3YF*, non-phosphorylatable mutant of cortactin) was generously provided by Ph.D John Cooper (Washington University School of Medicine, USA). The inserts were cloned in pcDNA 2AB Flag-cortactin 3YF (19). The plasmids (10 μg) were transfected into T-47D cells using Lipofectamine 2000 (Invitrogen, USA). BC cells were treated 24–48 h after the transfection. Efficacy of transfection was checked with Western analysis and found to be optimal at 36 h.

Cell Migration Assay

Cell migration was assayed with razor scrape assays. Briefly, a razor blade was pressed through the confluent T-47D BC cell monolayer into the plastic plate to mark the starting line. T-47D cells were swept away on one side of that line. Cells were washed, and 2.0 mL of RPMI 1640 containing steroid-deprived FBS and gelatin (1 mg/mL) were added. Cytosine β-D-arabinofuranoside hydrochloride (Sigma) (10 μM), a selective inhibitor of DNA synthesis that does not inhibit RNA synthesis, was used 1 h before the test substance was added to prevent cell proliferation. Immunofluorescence protocol was performed as previously described in *Immunoflourescence assay*. Only the nuclei were counterstained with or 4'-6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, Saint-Louis, MO) and mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA). Migration was monitored for 48 h. Cells were digitally imaged and the migration distance was measured by using Nikon Eclipse E200 microscope and recorded with a high-resolution DP70 Olympus digital camera.

Cell Adhesion Assays

Five hundred thousand cells per well were seeded into 6-well plates on coverslips previously coated with 1% sterile gelatin and exposed to different treatments. The cells were incubated at 37°C for 2 h. Non-adherent T-47D cells were then removed by gently washing with PBS. The attached cells were fixed with 4% formaldehyde and stained with 10% ethanol/crystal violet for 20 min. Cells of attached images were captured and counted in 10 randomly chosen fields per well using a Nikon Eclipse E200 microscope coupled to a high-resolution CCD digital camera, as previously described (20).

Cell Invasion Assay

Cell invasion was assayed using the BD BioCoatTM Growth Factor Reduced (GFR) MatrigelTM Invasion Chamber (BD Bioscience, USA). In brief, after rehydrating the GFR Matrigel inserts, the test substance was added to the wells. An equal number of Control Inserts (no GFR Matrigel coating) were prepared as control. 0.5 mL of T-47D cell suspension (2.5×10^4 cells/mL) was added to the inside of the inserts. Cytosine β-D-arabinofuranoside hydrochloride (Sigma) (10 μM), a selective inhibitor of DNA synthesis that does not inhibit RNA synthesis, was used 1 h before the test substance was added to prevent cell proliferation. The chambers were incubated for 48 h at 37°C, 5% CO2 atmosphere. After incubation, the non-invading cells were removed from the upper surface of the membrane using cotton-tipped swabs. The cells on the lower surface of the membrane were then stained with Diff-Quick stain. The invading cells were observed and photographed under the microscope at 100 X magnification. Cells were counted in the central field of triplicate membranes.

Statistical Analysis

All values are expressed as (mean ± SD) of three independent experiments. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by Barlett's multiple comparisons test using GraphPad Prism 5 software. $P < 0.05$ was considered as statistically significant.

RESULTS

T3 Enhances EMT in Breast Cancer Cells

Epithelial cells have an inherent plasticity that allows them to partially or fully transition into mesenchymal cells by downregulating epithelial and upregulating mesenchymal characteristics in response to an external signal (5). As TH are able to rapidly induce EMT in ovarian cancer cell lines (6), as a first approach we decided to investigate the action of T3 on E-cadherin and vimentin expression, two important markers of epithelial and mesenchymal cells, respectively. After treatment with T3 (10 nM) during different periods (30 min, 1, 6, 12, and 24 h), we observed that T3 induced a progressive decrease in E-cadherin levels starting at 30 min, which became statistically significant at 1 and 6 h and then returned to basal levels at 12 and 24 h (**Figures 1A,B**). We observed an opposite pattern when we analyzed the action of T3 on vimentin expression. T3 increased vimentin levels starting at 30 min, which became significant at 1 and 6 h and returned to basal levels at 12 and 24 h (**Figures 1A,B**).

In parallel, we examined the cellular localization of E-cadherin and vimentin with immunofluorescence analysis after 1 h of T3 treatment. In control cells, we observed that E-cadherin was intensely localized in the plasma membrane, whereas vimentin showed a weak cytosplasmatic stain (**Figure 1C**). After T3 exposure for 1 h, E-cadherin reduced its membrane intensity level whereas vimentin filaments showed an intense cytoplasmatic stain (**Figure 1C**).

To determine whether T3 initiates its signaling pathway via integrin αvβ3, we treated the BC cells with T3 in the presence of the integrin αvβ3 receptor antagonist tetraiodothyroacetic acid (Tetrac). Tetrac impaired the expression and redistribution of both EMT markers (**Figures 1C,D**). By western blot analysis we demonstrated that T3 for 1 h induces E-cadherin downregulation and vimentin upregulation, and this effect was impared by Tetrac (**Figure 1E**), suggesting that T3 promotes EMT activity via integrin αvβ3 in T-47D BC cell.

Thyroid Hormone T3 Induces Rapid Cytoskeletal and Cell Membrane Remodeling in BC Cells

To determine the effects of T3 on BC cell morphology, we analyzed actin cytoskeleton remodeling by means of an immunofluorescence assay. T3 enhanced actin membrane reorganization, which was evidenced by a remodeling of the cytoskeleton toward the plasmatic membrane. The latter led to a thickening of the membrane and, the formation of specialized cell membrane structures involved in the generation of cellular locomotive force, such as lamellipodia, filopodia, and membrane ruffles (**Figure 2A**, 20 min, red arrows). This event was time-dependent; a maximal remodeling was observed after 20 min of T3 exposure, which returned to basal levels after 60 min (**Figure 2A**).

Cortactin and N-WASP are two important actin nucleation regulators that are modulated by specific phosphorylation. We evaluated the phosphorylation of cortactin and N-WASP after increasing doses of T3 for 20 min. We observed that the phosphorylation of both proteins was maximal with 10 nM (**Figures 2B,C**) and therefore used this concentration for the rest of the experiments.

In addition, T3 rapidly induced $Ser^{484/485}$ N-WASP phosphorylation in a time-dependent manner, with a maximal effect at 20 min and a return to basal levels after 60 min (**Figures 2D,E**).

T3 Signal to FAK/paxillin Phosphorylation via Integrin αvβ3 Increasing T-47D Cell Migration

We evaluated the role of diverse protein kinases in the cascades involved in the signaling to cortactin and N-WASP in BC

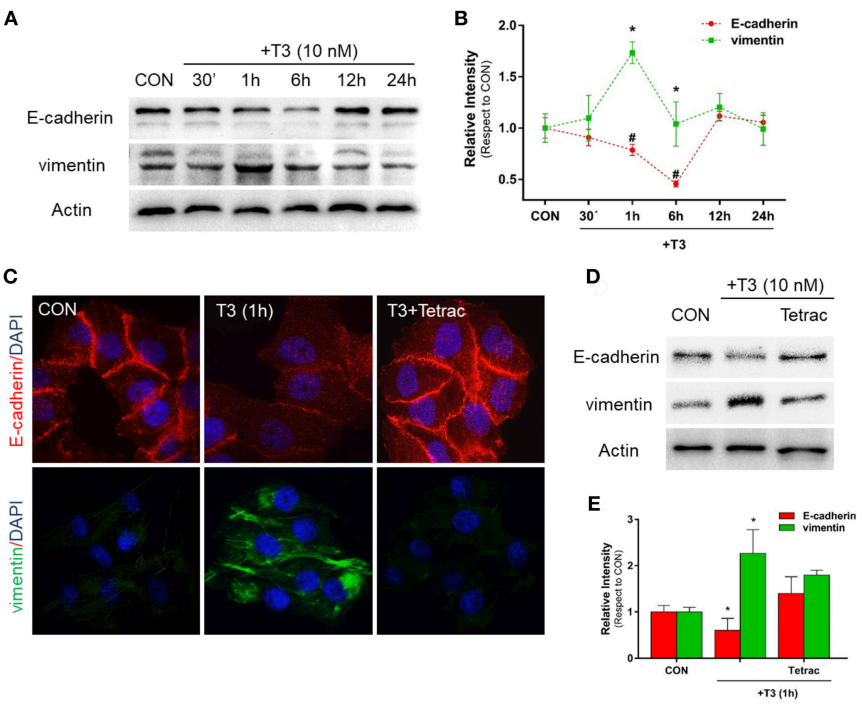

FIGURE 1 | T3 modulates EMT via E-cadherin and vimentin expression**. (A)** T-47D BC cells were treated with T3 for different times (30 min, 1, 6, 12, and 24 h) and Western blot expression patterns for E-cadherin and vimentin were performed. **(B)** E-cadherin and vimentin densitometry values were adjusted to actin intensity, then normalized to the control sample. Results are expressed as mean ± S.D. $^{*}P < 0.05$ vs. control. **(C,D)** An immunofluorescence assay and Western blot analysis were performed to determine E-cadherin and vimentin expression and localization in BC cells. Cells were treated with T3 for 1 h, in the presence or absence of Tetrac. Cells were stained with E-cadherin linked to $DyLight^{594}$ and vimentin linked to $DyLight^{488}$; nuclei were counterstained with DAPI. CON, Control. **(E)** Each EMT marker densitometry values were adjusted to actin intensity, then normalized to the control sample. Results are expressed as the mean ± S.D. $^{*}P < 0.05$ vs. control. $^{\#}P < 0.05$ vs. control. The experiments were performed in triplicate; representative images are shown.

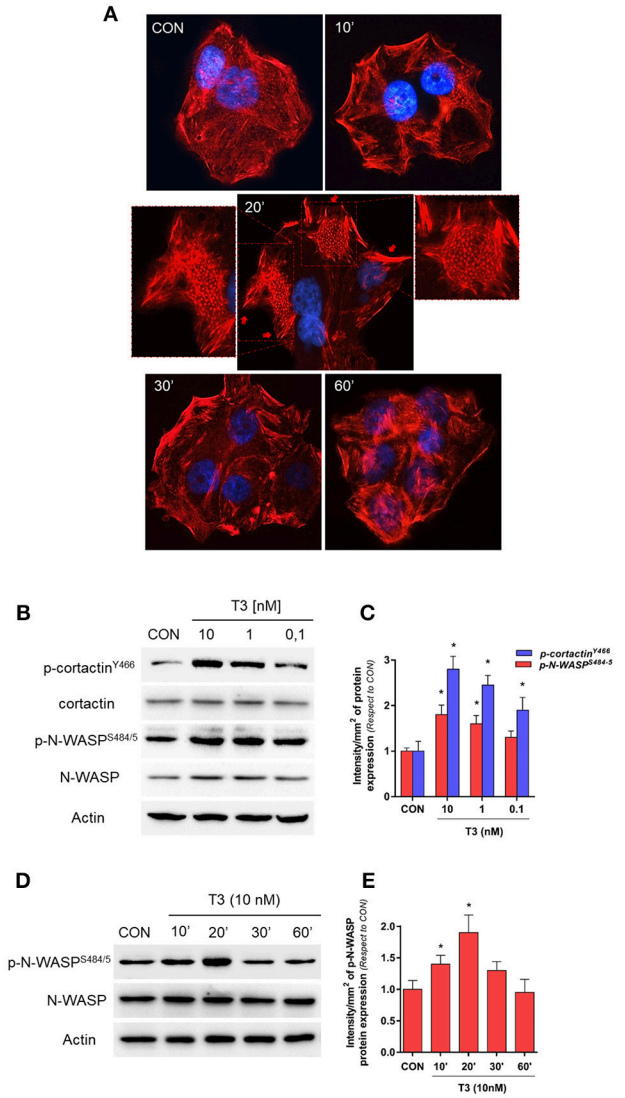

FIGURE 2 | T3 induces dynamic actin remodeling in BC cells. **(A)** T-47D cells were treated for different times with T3 (10 nM). Actin fibers were stained with phalloidin linked to Texas Red (red labeling) and nuclei were counterstained with DAPI (blue labeling). The inserts show the generation of dynamic structural modifications of the cytoskeleton, with the development of protrusive membrane structures, such as lamellipodia and filopodia at higher magnification. **(B,C)** Dose-dependent and **(D,E)** time-dependent cortactin and N-WASP phosphorylation of T-47D cells after T3 treatment. Total cell amount of wild-type cortactin and phosphorylated cortactin (p-cortactin^{Y466}) or N-WASP and phosphorylated N-WASP (p-N-WASP$^{S484/5}$) are shown by Western blot analysis. Phospho-cortactin and p-N-WASP densitometry values were adjusted to cortactin and N-WASP intensity, respectively, then normalized to the control sample. *$P < 0.05$ vs. corresponding control. All experiments were performed in triplicate with consistent results; representative images are shown.

cells. We had previously determined that T3 induces rapid phosphorylation of Src, FAK and PI3K, via integrin αvβ3, and promotes BC cell motility (11). We thus analyzed two central proteins involved in focal adhesion complex formation, FAK and paxillin phosphorylation/activation, after T3 stimulation in T-47D cells. We observed that a rapid pulse of 10 nM T3 (20 min) increased FAK^{Tyr397} and paxillin^{Tyr118} phosphorylation and that this effect was prevented by the integrin αvβ3 receptor antagonist Tetrac (**Figures 3A,B**). Src and FAK are the main kinases involved in focal adhesion signaling; they are responsible for the recruitment and phosphorylation of paxillin. We therefore used pharmacological inhibitors of Src (PP2) and FAK (FAKi) in cells exposed to T3 and observed that the increase of phospho-FAK^{Tyr397} and phospho-paxillin^{Tyr118} was impaired by PP2 and FAKi, suggesting that T3 induces paxillin phosphorylation via Src and FAK (**Figures 3C,D**).

To determine the role of paxillin in the formation of focal adhesion complex, we next examined its subcellular localization in the presence of T3 by means of immunofluorescence. Breast cancer cells treated with T3 triggered a significant increase of phospho-paxillin^{Tyr118} at the cell membrane periphery where cortical actin complexes were formed, and this was impaired by the blockade of Src (PP2), FAK (FAKi), and paxillin (siRNAs) (**Figures 3E,F**).

Finally, we evaluated BC cell migration through a wound-healing assay during exposure to T3 during 48 h. We observed that T3 significantly enhanced BC cell migration; this effect was prevented by the use of PP2, FAKi and the silencing of paxillin with specific siRNAs (**Figures 3G,H**). Altogether, these results suggest that T3 signals to paxillin through a Src/FAK cascade. When paxillin is phosphorylated, it consequently translocates to the cell periphery, promoting BC cell migration.

Intracellular Events Linking Activation of Paxillin to Cortactin, N-WASP and Arp2/3 Complex

In order to continue elucidating the signaling by which T3 enhances BC cell movement, we evaluated the role of paxillin toward three fundamental components of actin nucleation: cortactin, N-WASP and Arp2/3 complex. We observed that the rapid treatment with 10 nM of T3 (20 min) significantly increased the phosphorylation levels of paxillin, cortactin, N-WASP and the subunit Arp2 (Arp2/3 complex) (**Figures 4A,B**). Paxillin phosphorylation was only prevented by the use of the specific siRNA vs. paxillin, whereas phospho-cortactin was inhibited by the siRNA vs. paxillin and its dominant-negative cortactin^{3YF} construct (cortactin^{3YF}), but not with the specific inhibitor of N-WASP, Wiskostatin. Finally, N-WASP and Arp2 phosphorylation were markedly reduced by the silencing of paxillin, blockade of cortactin^{3YF} and the inhibition of N-WASP (**Figures 4A,B**), suggesting that integrin αvβ3 signals to Arp2/3 complex via FAK, paxillin, cortactin, and N-WASP.

The efficacy of transfections was assayed by Western blot. The siRNA of paxillin significantly reduced its expression (**Figures 4C,D**), whereas the expression of cortactin^{3YF} construct significantly increased the amount of cortactin present in these BC cells (**Figures 4E,F**).

We next evaluated the subcellular localization of phosphorylated N-WASP$^{S484/485}$ and Arp2$^{T237/238}$. In control cells, phospho-N-WASP and phospho-Arp2 were weakly distributed throughout the cytoplasm (**Figures 4G,H**). T3

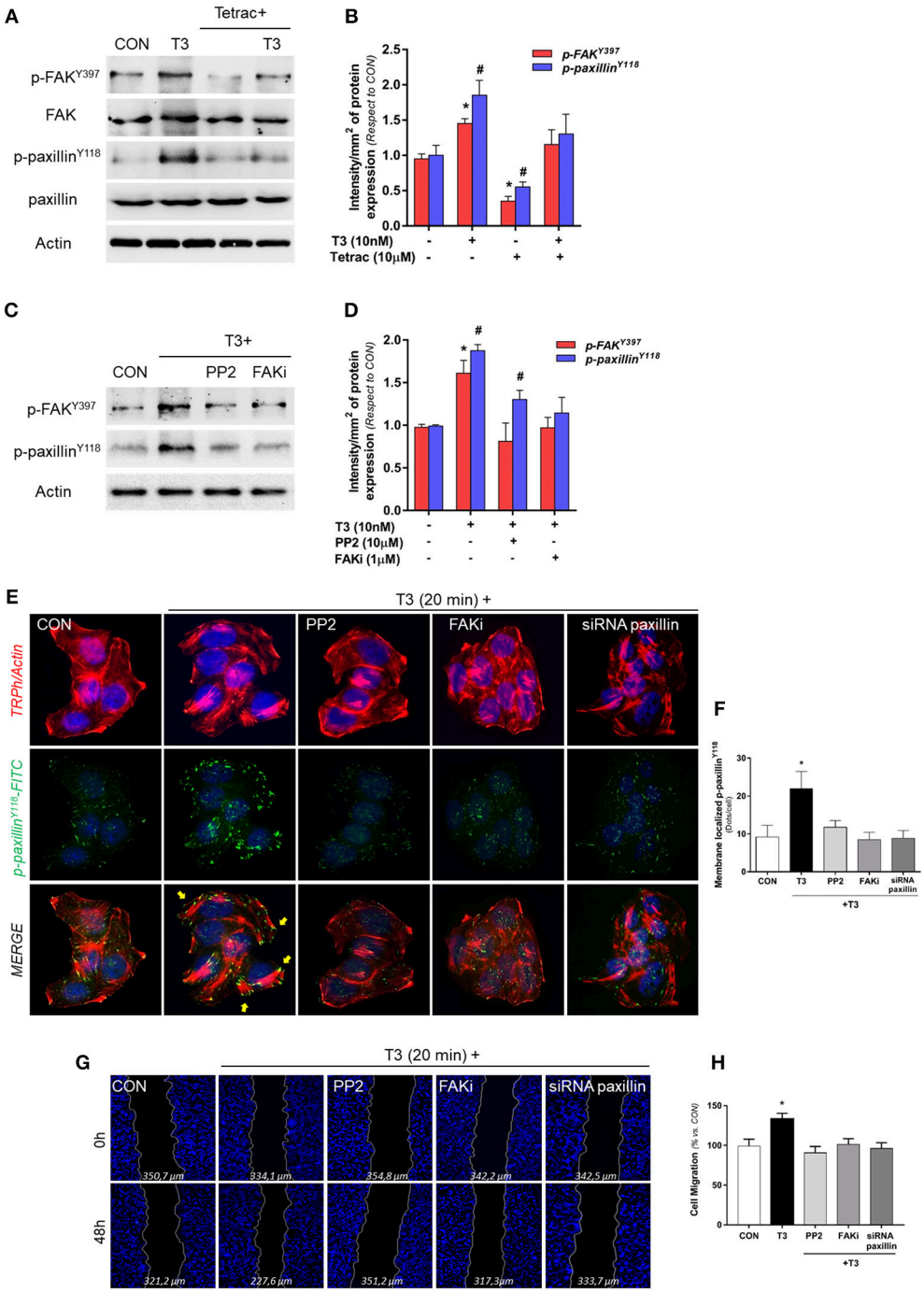

FIGURE 3 | T3 promotes FAK and paxillin phosphorylation through integrin αvβ3**. (A)** T-47D cells were treated with T3 (10 nM) for 20 min in the presence or absence of Tetrac. Total cell amount of wild-type FAK and paxillin, or phospho-FAK and p-paxillin, are shown with Western blot. **(B)** Phospho-FAK and phospho-paxillin densitometry values were adjusted to FAK and paxillin intensity, respectively, then normalized to the control sample. Results are expressed as the mean ± S.D. $^*P <$ 0.05 vs. control. $^{\#}P < 0.05$ vs. control. **(C)** Cells were exposed to T3 (10 nM) for 20 min in the presence or absence of PP2 (10 μM) and FAK (1 μM), and FAK and paxillin phosphorylation were analyzed through Western blot assay. **(D)** Phospho-FAK and p-paxillin densitometry values were adjusted to FAK and paxillin and/or actin intensity and normalized to the control. Results are expressed as the mean ± S.D. $^*P < 0.05$ vs. control. $^{\#}P < 0.05$ vs. control. **(E)** BC cells were stained with anti-phospho-paxillinTyr^{118} linked to FITC, filamentous actin was stained with phalloidin linked to Texas Red and nuclei were counterstained with DAPI. CON, Control. Yellow arrows indicate membrane-localized paxillinTyr^{118}-. **(F)** Quantification of the membrane-localized p-paxillin in the different conditions. Results are expressed as Dots/cells (mean ± SD). $^*P < 0.05$ vs. control. Membrane-localized p-paxillin was counted in 40 different cells. The experiments were repeated three times with consistent results. **(G)** Cells were treated with T3 (10 nM) for 48 h in the presence or absence of PP2, FAKi and siRNA paxillin. Representative images are shown. Migration assay was monitored at 48 h by taking photographs. DAPI was used to stain nucleus and Gap closure was quantified with the use of NIH image J software. $^*P < 0.05$ vs. control. **(H)** Cell migration distances were measured; values are presented as % of control. $^*P < 0.05$ vs. control. The experiments were performed in triplicate with consistent results; representative images are shown.

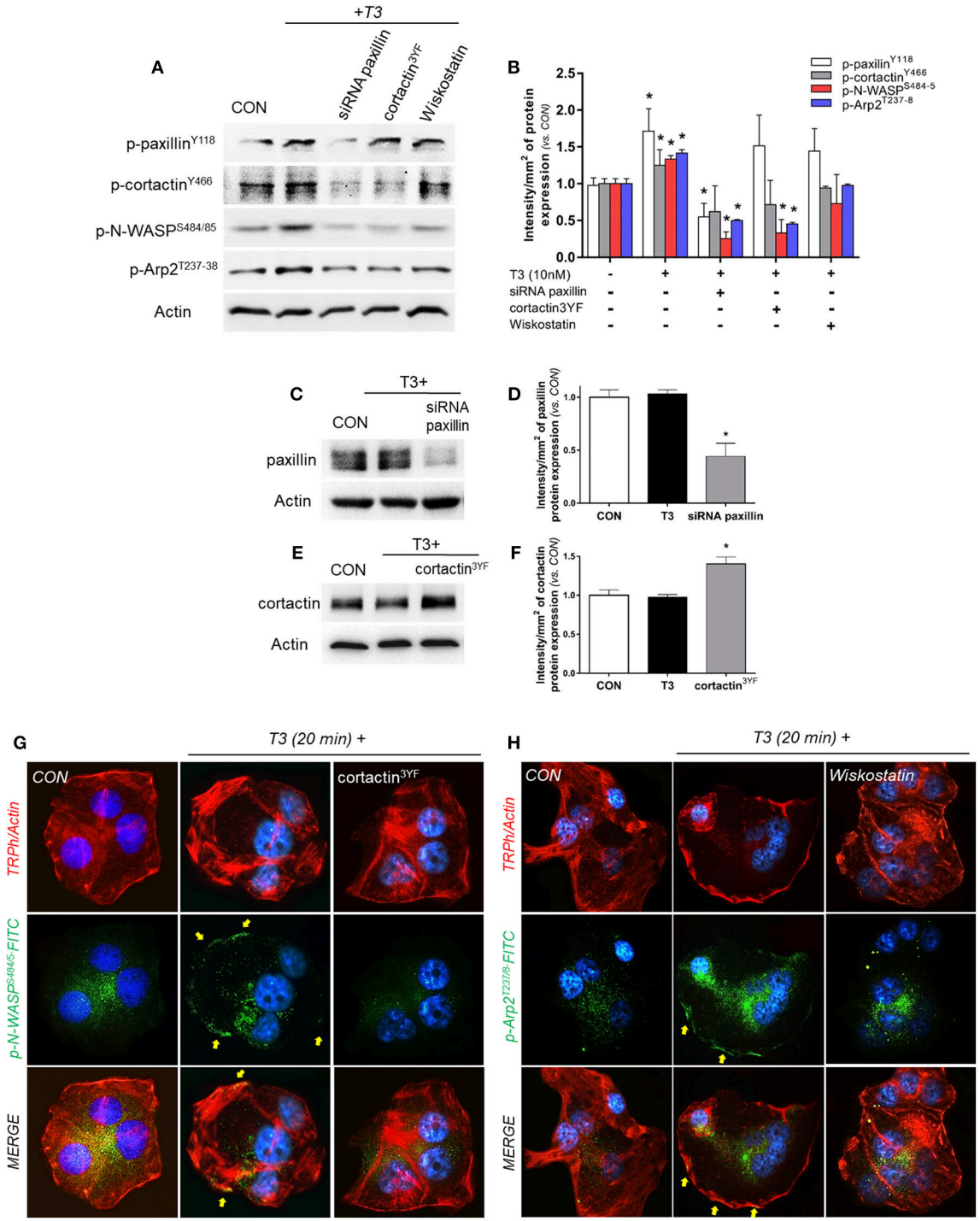

FIGURE 4 | T3 signals to paxillin, cortactin, N-WASP and Arp2/3 complex. **(A,B)** T-47D cells were incubated in the presence of 10 nM T3 for 20 min with or without silencing of paxillin with specific siRNAs and/or inhibition of cortactin and N-WASP. Actin, phospho-paxillin^{Y118}, p-cortactin^{Y466}, p-N-WASP$^{S484/485}$ and p-Arp2^{Y237} were assayed in cell extracts. The densitometry values were adjusted to actin intensity, then normalized to the control sample. **(C–F)** T-47D cells were transfected, with paxillin-targeted siRNAs or the dominant negative constructs of cortactin (*cortactin3YF)* and incubated with T3 (10 nM) for 20 min. Paxillin protein expression was detected by Western blot, and actin intensity was used as loading control. Paxillin and cortactin densitometry values were adjusted to actin intensity, then normalized to the control sample. *$P < 0.05$ vs. corresponding control. **(G)** Cells were stained with anti-phospho-N-WASP$^{S484/485}$ linked to FITC (green) and **(H)** phospho-Arp2$^{T237/8}$ linked to FITC (green), filamentous actin was stained with phalloidin linked to Texas Red and nuclei were counterstained with DAPI. CON, Control. Yellow arrows indicate membrane-localized p-N-WASP$^{S484/485}$ and p-Arp2$^{T237/8}$. All experiments were performed in triplicate with consistent results; representative images are shown.

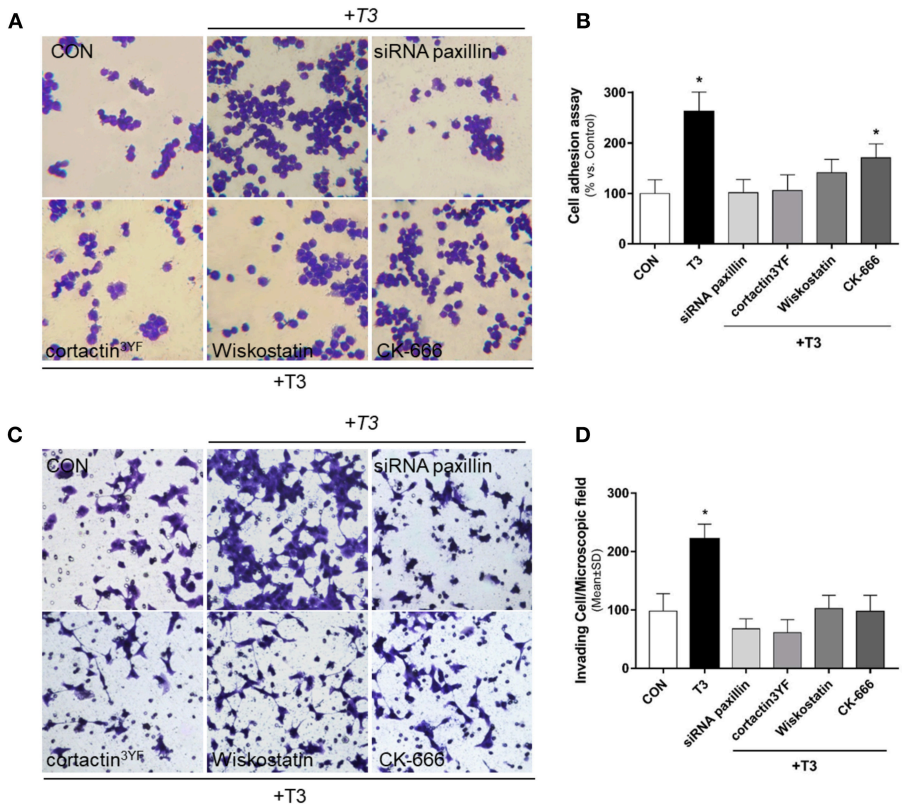

FIGURE 5 | BC cell adhesion and invasion is triggered by T3 via paxillin/cortactin-N-WASP/ Arp 2/3 Complex pathway**.** T-47D cells were treated with T3 (10 nM) for 2 h **(A)** and 48 h **(C)** in the presence or absence of Wiskostatin or CK-666, and transfected with siRNAs vs. paxillin or mutant constructs for cortactin (*cortactin3YF*). **(A)** After the treatment, cells were placed on coverslips previously covered with gelatin and a cell adhesion assay was performed. Representative images of adhered cells are shown. **(B)** Percentage of attached cells vs. CON, Control cells. Experiments were performed in triplicate; *$P < 0.05$ vs. CON. **(C)** Breast cancer cell invasion through matrigel was assayed with invasion chambers. Representative images in chambers with matrigel are shown. **(D)** Invading cells were counted in the central field of triplicate membranes. *$P < 0.05$ vs. CON.

exposure for 20 min increased N-WASP$^{S484/485}$ and Arp2$^{T237/238}$ phosphorylation and translocation to the plasmatic membrane (**Figures 4G,H**). This relocalization was prevented by the use of cortactin3YF (**Figure 4G**) and Wiskostatin (**Figure 4H**).

T3 Enhances Cell Adhesion and Invasion via Paxillin/Cortactin/N-WASP/Arp2/3 Complex

We performed an adhesion and three-dimensional invasion assay using Matrigel to determine the ability of cancer cells to adhere and invade the surrounding environment. Treatment with T3 (10 nM) enhanced the capacity of BC cells to adhere (**Figures 5A,B**, yellow arrows) and invade ECM (**Figures 5C,D**, yellow arrows). This ability was drastically diminished in treatments where specific inhibitors were used, such as siRNA paxillin, cortactin^{3YF}, Wiskostatin and the specific inhibitor of the Arp2/3 complex (CK-666) (**Figures 5A,D**). These results support the concept that paxillin, cortactin, N-WASP and Arp2/3 Complex are involved in adhesion and cellular invasion processes triggered by T3.

DISCUSSION

In the last years, several studies have evidenced a link between thyroid hormones (TH) and cancer development (21). Because TH regulate growth, differentiation, development and metabolism, altered levels of these hormones could play a significant role in the development and progression of diverse types of cancer, including breast cancer (BC). Published works about the relationship between hyper- and hypothyroidism and the incidence of BC are controversial. Some authors consider hypothyroidism an important enhancer factor in invasion and metastasis (22), other studies suggest that hypothyroidism does not affect, or, rather, reduces the risk of developing the disease (23), whereas other not found association between hypothyroidism or hyperthyroidism and BC (24). In addition, an important association between hyperthyroidism and the risk of developing BC has been established (25, 26). Søgaard et al. (27) described an increased risk of BC in women with hyperthyroidism and a slightly reduced risk in women with hypothyroidism, suggesting that a correlation exists between TH levels and BC risk.

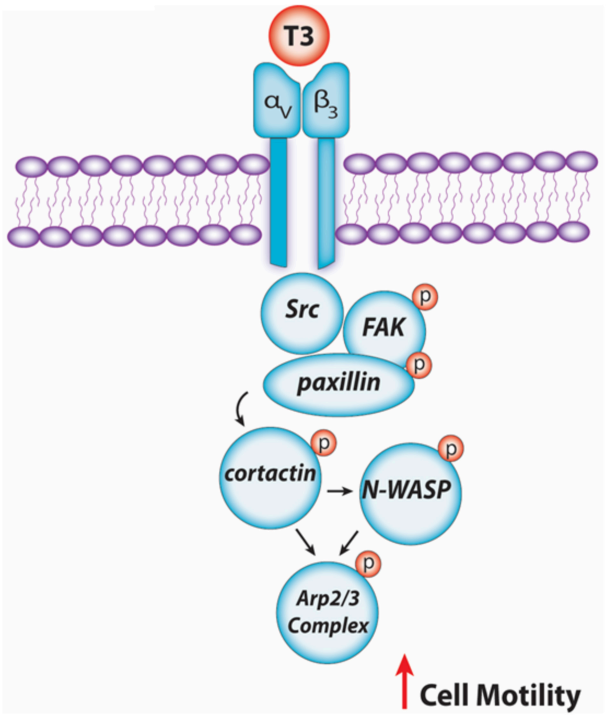

FIGURE 6 | Schematic signaling cascade triggered by T3 promoting BC cell migration and invasion. Binding of T3 to the specific membrane integrin αvβ3 receptor induces phosphorylation of the Src/FAK/paxillin/cortactin/N-WASP/Arp2/3 Complex cascade, promoting BC cell motility.

Furthermore, a significant positive associations between higher prediagnostic T3 levels, larger tumors and occurrence of lymph node metastases have been demonstrated, suggesting that this association, can be related to both a higher incidence and more aggressive forms of BC, increasing BC mortality (28).

For this reason, in this work we evaluated the effect of T3 in a supraphysiological concentration (10 nM) taking into account that altered levels of this hormone could be related with BC.

We have previously shown that reducing T3 levels downregulates the expression of key cell motility regulators, such as Src, FAK, and PI3K kinase, in T-47D BC cells (11). In the present work we observed that treatment with T3 (10 nM) results in a maximal phosphorylation of cortactin and N-WASP (**Figures 2B,C**), which suggests that altered levels of T3 affect BC cells' ability to induce the adhesion, migration and invasion processes.

As a first approach, we studied the involvement of T3 in the epithelial-mesenchymal transition (EMT), a key step for the early development of metastasis. We observed a progressive reduction of E-cadherin expression and an increase in vimentin expression in a time-dependent manner, being maximal at 1 h and returning to basal levels at 12 and 24 h. We also showed, by means of immunofluorescence experiments, that these changes were accompanied by a reduction of E-cadherin at the plasma membrane and an increase in the intensity of vimentin filaments in the cytoplasm. Lamouille et al. (5) have described that during the EMT initiation, E-cadherin expression is reduced because it is cleaved at the plasma membrane and degraded in the cytoplasm. This could explain the protein level reduction observed in this work. Furthermore, an increased expression of intermediate filaments, such as vimentin, is necessary to determine the beginning of the transition (29), by promoting the directional cell migration by regulating microtubule polarity and focal adhesion dynamics (30). Previous works have also reported this altered expression of E-cadherin/vimentin in response to TH. Weingarten et al. (6) have determined that 1-4 h of T3 and T4 treatment drastically increases the expression of vimentin and reduces E-cadherin mRNA level to half of its basal level in OVCAR-3 and SKOV-3 ovarian cancer cell lines. They demonstrated that the modifications in EMT proteins were mediated by integrin αvβ3 membrane receptor, which is consistent with our results (6). The EMT is an orchestrated sequential steps process in which cell-cell and cell-extracellular matrix (ECM) interactions are modified to release epithelial cells from the surrounding tissue, along with actin cytoskeleton rearrangements to confer cells the ability to migrate through a three-dimensional ECM. In fact, TH are capable of modify the expression of several and crucial components of ECM. In mouse mammary epithelial cells, T3 increases the expression of the proteases stromelysin 1 and 2 and stimulates their activity leading to a gelatinolytic activity of type IV collagenase (31). In human hepatoma cells and fibroblast, T3 induces fibronectin expression by activating hypoxia-inducible factor-1 (HIF-1) (32). Furthermore, in astrocytes TH regulates integrin interactions with ECM proteins like laminin, being essential for their migration during brain development (33, 34). These studies indicate that TH may exert an integrated regulation of tumor progression by modifying the ECM, the EMT and the signaling pathways implicated in cell migration and invasion. Our study, specifically reveals that T3 enhance the EMT and this leads to an important cell cytoskeleton reorganization, increasing cellular motility and enabling cells to develop an invasive phenotype driven by T3.

Another finding of this work is that T3 stimulates morphological changes that depend on the generation of dynamic structural modifications of the actin cytoskeleton reorganization, via actin polymerization/depolymerization, in association with specialized membrane structures, such as lamellipodia, filopodia and membrane ruffles. These structures are fundamental to promote cell adhesion, migration and invasion processes.

Cell adhesion is carried out by different integrins including αvβ3 that recruits Src and FAK kinases, which are critical for the formation of focal adhesion (FAs) complexes (16, 34, 35). Cohen et al. (36) have shown that T3 and T4 regulate adhesion, migration and matrix metalloproteinase activity via integrin α_v/β_3 in myeloma cells. We have previously reported that T3, via integrin αvβ3, represents the starting platform to activate Src, FAK, and PI3K, which leads to increased BC cell motility (11). We have not evaluated the use of Tetrac to block cell adhesion, migration and invasion, but Weingarten et al. (6) are currently developing a nano-particle antagonist of thyroid-integrin binding that could represent a novel agent that may limit the metastasic potential of cancer cells. For this reason,

we continued evaluating the non-genomic effects of T3 on several kinases and scaffold proteins related to cell movement, such as paxillin. Paxillin has many binding partners and acts as a pivot molecule between the formation of focal adhesion complex and the actin nucleation, key steps in the regulation of cell motility (3, 37). Our results show that T3 induces paxillin phosphorylation whereas the specific integrin αvβ3 receptor antagonist Tetrac inhibits this action. Once paxillin is activated by T3, it translocates to sites where FAs are formed. This effect is dependent on integrin αvβ3, Src and FAK proteins. This finding is in agreement with our previous work, in which we have shown that paxillin is recruited to FAs by Src and FAK in response to rapid treatments of BC cells with estradiol (37). Similarly, Deramaudt et al. (38) have shown that a FAK mutant construct deficient in binding paxillin disrupts FA formation and drastically reduces cell adhesion, migration and invasion of mouse fibroblasts. When paxillin is phosphorylated and recruited to FAs, it becomes a docking site for many downstream signaling molecules, among them the actin nucleation regulators cortactin and N-WASP (37, 39).

Actin nucleation is crucial for directional cell motility through the actin polymerization process. The latter involves the generation and turnover of actin filaments, which form subcellular structures (lamellipodia and filopodia) that are key for cell movement. In this context, many signaling pathways drive actin nucleation by regulating actin-binding protein activity. In order to be functional, Arp2/3 complex needs to be activated by proteins called nucleation promoting factors or NPFs. In this work we evaluated the role of T3 on two main NPFs, cortactin and N-WASP. We identified the recruitment of the FAK/paxillin/cortactin by T3, which is a step required for N-WASP and Arp2/3 complex phosphorylation and translocation to the plasma membrane. Blocking this event by using specific inhibitors or mutant constructs drastically affects cell adhesion and invasion, which highlights the importance of actin nucleation proteins in tumor progression. These findings emphasize the relevance of the cortactin/N-WASP/Arp2/3 complex phosphorylation and regulation for cancer metastasis. The discovery that cortactin/N-WASP controls the Arp2/3 complex via thyroid hormones may thus offer novel insights to better understand the action of these hormones on BC metastasis, although there is considerable evidence of the role of TH in enhancing metastasis in several types of cancer (40). The use of specific NPF inhibitors could thus be an attractive alternative to counteract the ability of BC cells to metastasize. Although no drug is available to target cortactin, Dasatinib is currently used to disrupt the Src/cortactin signaling pathway for blocking BC metastasis (41). A recent study demonstrated that using nanobodies to target the VCA domain of N-WASP results in a diminished invasiveness of breast, prostate, and head and neck squamous cancer cells by disrupting N-WASP/Arp2/3 complex interaction (42).

Additionally, we also studied the phosphorylation of Arp2 subunit, which is fundamental to the fully functioning of Arp2/3 complex (43). Our results suggest that paxillin, cortactin, and N-WASP relay signals from integrin αvβ3 to the Arp2/3 complex.

One limitation of this study is the use of only one BC cell line. Also, it would be interesting to validate these findings in other models, mainly in primary cell cultures because integrin α_v/β_3 is universally expressed in cancer cells.

In conclusion, our findings provide new insights about the non-genomic action of T3 in BC cell progression via integrin αvβ3/FAK/paxillin/cortactin/N-WASP/Arp2/3 complex (**Figure 6**). We observed how T3 induces rapid alterations in the plasma membrane, leading to a rearrangement of the actin cytoskeleton and consequent formation of structures related with cell motility, increasing cell adhesion, migration, and invasion in T-47D BC cells. These findings could be helpful to develop new drugs that interfere with the ability of breast tumors to diffuse locally or at distant sites in patients with thyroid disorders and breast cancer, counteracting BC cell progression by controlling circulating T3 levels.

AUTHOR CONTRIBUTIONS

IU carried out different experiments, cell culture and treatments. JC performed immunofluorescence and migration assays. MF was instrumental in funding the study and participated to the writing of the manuscript. AS planned and funded the project, supervised the experiments, wrote the paper.

FUNDING

This work has been supported by the National Agency for Scientific and Technological Promotion (The Ministry of Science, Technology and Productive Innovation) through grants PICT-2015-0864 to MF and PICT-2015-2175 to AS.

ACKNOWLEDGMENTS

We thank Ph.D. Mariella Superina for correcting this manuscript.

REFERENCES

1. DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A. Breast cancer statistics, 2017, racial disparity in mortality by state. *CA Cancer J Clin.* (2017) 67:439–48. doi: 10.3322/caac.21412
2. Scully OJ, Bay BH, Yip G, Yu Y. Breast cancer metastasis. *Cancer Genomics Proteomics*. (2012) 9:311–20.
3. Lin HY, Cody V, Davis FB, Hercbergs AA, Luidens MK, Mousa SA, et al. Identification and functions of the plasma membrane receptor for thyroid hormone analogues. *Discov Med.* (2011) 11:337–47.
4. Jacquemet G, Hamidi H, Ivaska J. Filopodia in cell adhesion, 3D migration and cancer cell invasion. *Curr Opin Cell Biol.* (2015) 36:23–31. doi: 10.1016/j.ceb.2015.06.007
5. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol.* (2014) 15:178–96. doi: 10.1038/nrm3758
6. Weingarten C, Jenudi Y, Tshuva RY, Moskovich D, Alfandari A, Hercbergs A, et al. The interplay between epithelial-mesenchymal transition (EMT) and the thyroid hormones-alphavbeta3 axis in ovarian cancer. *Horm Cancer.* (2018) 9:22–32. doi: 10.1007/s12672-017-0316-3

7. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev.* (2010) 31:139–70. doi: 10.1210/er.2009-0007
8. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol.* (2016) 12:111–21. doi: 10.1038/nrendo.2015.205
9. Franson KL, Buring SM, Davis PJ, Krueger KP, Umland EM, Woodward JM, et al. Report of the 2014-2015 academic affairs standing committee: addressing affordability, accessibility, and accountability. *Am J Pharm Educ.* (2015) 79:S13. doi: 10.5688/ajpe798S13
10. Senese R, Cioffi F, de Lange P, Goglia F, Lanni A. Thyroid: biological actions of 'nonclassical' thyroid hormones. *J Endocrinol.* (2014) 221:R1–12. doi: 10.1530/JOE-13-0573
11. Flamini MI, Uzair ID, Pennacchio GE, Neira FJ, Mondaca JM, Cuello-Carrión FD, et al. Thyroid hormone controls breast cancer cell movement via integrin alphaV/beta3/SRC/FAK/PI3-kinases. *Horm Cancer.* (2017) 8:16–27. doi: 10.1007/s12672-016-0280-3
12. Lin HY, Sun M, Tang HY, Lin C, Luidens MK, Mousa SA, et al. L-Thyroxine vs. 3,5,3'-triiodo-L-thyronine and cell proliferation: activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. *Am J Physiol Cell Physiol.* (2009) 296:C980–91. doi: 10.1152/ajpcell.00305.2008
13. Luo M, Guan JL. Focal adhesion kinase: a prominent determinant in breast cancer initiation, progression and metastasis. *Cancer Lett.* (2010) 289:127–39. doi: 10.1016/j.canlet.2009.07.005
14. Maziveyi M, Alahari SK. Cell matrix adhesions in cancer: the proteins that form the glue. *Oncotarget.* (2017) 8:48471–87. doi: 10.18632/oncotarget.17265
15. LeClaire LL, Baumgartner M, Iwasa JH, Mullins RD, Barber DL. Phosphorylation of the Arp2/3 complex is necessary to nucleate actin filaments. *J Cell Biol.* (2008) 182:647–54. doi: 10.1083/jcb.200802145
16. Davis PJ, Davis FB, Mousa SA, Luidens MK, Lin HY. Membrane receptor for thyroid hormone: physiologic and pharmacologic implications. *Annu Rev Pharmacol Toxicol.* (2011) 51:99–115. doi: 10.1146/annurev-pharmtox-010510-100512
17. Yin M, Ma W, An L. Cortactin in cancer cell migration and invasion. *Oncotarget.* (2017) 8:88232–43. doi: 10.18632/oncotarget.21088
18. Helgeson LA, Prendergast JG, Wagner AR, Rodnick-Smith M, Nolen BJ. Interactions with actin monomers, actin filaments, and Arp2/3 complex define the roles of WASP family proteins and cortactin in coordinately regulating branched actin networks. *J Biol Chem.* (2014) 289:28856–69. doi: 10.1074/jbc.M114.587527
19. Tehrani S, Tomasevic N, Weed S, Sakowicz R, Cooper JA. Src phosphorylation of cortactin enhances actin assembly. *Proc Natl Acad Sci USA.* (2007) 104:11933–8. doi: 10.1073/pnas.0701077104
20. Flamini MI, Gauna GV, Sottile ML, Nadin BS, Sanchez AM, Vargas-Roig LM. Retinoic acid reduces migration of human breast cancer cells: role of retinoic acid receptor beta. *J Cell Mol Med.* (2014) 18:1113–23. doi: 10.1111/jcmm.12256
21. Hercbergs A, Mousa SA, Leinung M, Lin HY, Davis PJ. Thyroid hormone in the clinic and breast cancer. *Horm Cancer.* (2018) 9:139–43. doi: 10.1007/s12672-018-0326-9
22. Martínez-Iglesias O, García-Silva S, Regadera J, Aranda A. Hypothyroidism enhances tumor invasiveness and metastasis development. *PLoS ONE.* (2009) 4:e6428. doi: 10.1371/journal.pone.0006428
23. Smyth PP. The thyroid, iodine and breast cancer. *Breast Cancer Res.* (2003) 5:235–8. doi: 10.1186/bcr638
24. Ellis M, Cohen K, Maman ES, Hercbergs A, Davis PJ, Ashur-Fabian O, et al. [The Involvement of Thyroid Hormones in Cancer]. *Harefuah.* (2015) 154:512–5.
25. Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Malm J, Manjer J. Prospectively measured triiodothyronine levels are positively associated with breast cancer risk in postmenopausal women. *Breast Cancer Res.* (2010) 12:R33. doi: 10.1186/bcr2587
26. Saraiva PP, Figueiredo NB, Padovani CR, Brentani MM, Nogueira CR. Profile of thyroid hormones in breast cancer patients. *Braz J Med Biol Res.* (2005) 38:761–5. doi: 10.1590/S0100-879X2005000500014
27. Søgaard M, Farkas DK, Ehrenstein V, Jørgensen JO, Dekkers OM, Sørensen HT. Hypothyroidism and hyperthyroidism and breast cancer risk: a nationwide cohort study. *Eur J Endocrinol.* (2016) 174:409–14. doi: 10.1530/EJE-15-0989
28. Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Manjer J. T3 levels in relation to prognostic factors in breast cancer: a population-based prospective cohort study. *BMC Cancer.* (2014) 14:536. doi: 10.1186/1471-2407-14-536
29. Mendez MG, Kojima S, Goldman RD. Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. *FASEB J.* (2010) 24:1838–51. doi: 10.1096/fj.09-151639
30. Liu CY, Lin HH, Tang MJ, Wang YK. Vimentin contributes to epithelial-mesenchymal transition cancer cell mechanics by mediating cytoskeletal organization and focal adhesion maturation. *Oncotarget.* (2015) 6:15966–83. doi: 10.18632/oncotarget.3862
31. López-Barahona M, Fialka I, González-Sancho JM, Asunción M, González M, Iglesias T, et al. Thyroid hormone regulates stromelysin expression, protease secretion and the morphogenetic potential of normal polarized mammary epithelial cells. *EMBO J.* (1995) 14:1145–55. doi: 10.1002/j.1460-2075.1995.tb07098.x
32. Farwell AP, Tranter MP, Leonard JL. Thyroxine-dependent regulation of integrin-laminin interactions in astrocytes. *Endocrinology.* (1995) 136:3909–15. doi: 10.1210/endo.136.9.7649099
33. Leonard JL. Non-genomic actions of thyroid hormone in brain development. *Steroids.* (2008) 73(9–10):1008–12. doi: 10.1016/j.steroids.2007.12.016
34. Mitra SK, Schlaepfer DD. Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr Opin Cell Biol.* (2006) 18:516–23. doi: 10.1016/j.ceb.2006.08.011
35. Davis PJ, Sudha T, Lin HY, Mousa SA. Thyroid hormone, hormone analogs, and angiogenesis. *Compr Physiol.* (2015) 6:353–62. doi: 10.1002/cphy.c150011
36. Cohen K, Flint N, Shalev S, Erez D, Baharal T, Davis PJ, et al. Thyroid hormone regulates adhesion, migration and matrix metalloproteinase 9 activity via alphavbeta3 integrin in myeloma cells. *Oncotarget.* (2014) 5:6312–22. doi: 10.18632/oncotarget.2205
37. Shortrede JE, Uzair ID, Neira FJ, Flamini MI, Sanchez AM. Paxillin, a novel controller in the signaling of estrogen to FAK/N-WASP/Arp2/3 complex in breast cancer cells. *Mol Cell Endocrinol.* (2016) 430:56–67. doi: 10.1016/j.mce.2016.04.007
38. Deramaudt TB, Dujardin D, Noulet F, Martin S, Vauchelles R, Takeda K, et al. Altering FAK-paxillin interactions reduces adhesion, migration and invasion processes. *PLoS ONE.* (2014) 9:e92059. doi: 10.1371/journal.pone.0092059
39. Mezi S, Todi L, Orsi E, Angeloni A, Mancini P. Involvement of the Src-cortactin pathway in migration induced by IGF-1 and EGF in human breast cancer cells. *Int J Oncol.* (2012) 41:2128–38. doi: 10.3892/ijo.2012.1642
40. Mousa SA, Glinsky GV, Lin HY, Ashur-Fabian O, Hercbergs A, Keating KA, et al. Contributions of thyroid hormone to cancer metastasis. *Biomedicines.* (2018) 6:e89. doi: 10.3390/biomedicines6030089
41. Meirson T, Gil-Henn H. Targeting invadopodia for blocking breast cancer metastasis. *Drug Resist Updat.* (2018) 39:1–17. doi: 10.1016/j.drup.2018.05.002
42. Hebbrecht T, Van Audenhove I, Zwaenepoel O, Verhelle A, Gettemans J. VCA nanobodies target N-WASp to reduce invadopodium formation and functioning. *PLoS ONE.* (2017) 12:e0185076. doi: 10.1371/journal.pone.0185076
43. Choi CH, Thomason PA, Zaki M, Insall RH, Barber DL. Phosphorylation of actin-related protein 2 (Arp2) is required for normal development and cAMP chemotaxis in Dictyostelium. *J Biol Chem.* (2013) 288:2464–74. doi: 10.1074/jbc.M112.435313

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

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 12 March 2019
doi: 10.3389/fendo.2019.00130

Tetrac and NDAT Induce Anti-proliferation via Integrin αvβ3 in Colorectal Cancers With Different *K-RAS* Status

Yu-Tang Chin[1,2†], Zong-Rong He[3,4†], Chi-Long Chen[5†], Hsiao-Ching Chu[6], Yih Ho[1,7], Po-Yu Su[8], Yu-Chen S. H. Yang[9], Kuan Wang[1,8], Ya-Jung Shih[1,8], Yi-Ru Chen[1,8], Jens Z. Pedersen[10], Sandra Incerpi[11], André Wendindondé Nana[12], Heng-Yuan Tang[13], Hung-Yun Lin[1,12,13,14,15], Shaker A. Mousa[13], Paul J. Davis[13,16] and Jacqueline Whang-Peng[1,2,12*]*

[1] Taipei Cancer Center, Taipei Medical University, Taipei, Taiwan, [2] Cancer Center, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan, [3] Department of Pediatrics, E-Da Hospital, Kaohsiung, Taiwan, [4] School of Medicine, I-Shou University, Kaohsiung, Taiwan, [5] School of Medicine, Taipei Medical University, Taipei, Taiwan, [6] Division of Medical Imaging, E-Da Cancer Hospital, I-Shou University, Kaohsiung, Taiwan, [7] School of Pharmacy, Taipei Medical University, Taipei, Taiwan, [8] Graduate Institute of Nanomedicine and Medical Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan, [9] Joint Biobank, Office of Human Research, Taipei Medical University, Taipei, Taiwan, [10] Department of Biology, University of Rome Tor Vergata, Rome, Italy, [11] Department of Sciences, Roma Tre University, Rome, Italy, [12] Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan, [13] Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, United States, [14] TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei, Taiwan, [15] Traditional Herbal Medicine Research Center of Taipei Medical University Hospital, Taipei Medical University, Taipei, Taiwan, [16] Department of Medicine, Albany Medical College, Albany, NY, United States

Colorectal cancer is a serious medical problem in Taiwan. New, effective therapeutic approaches are needed. The selection of promising anticancer drugs and the transition from pre-clinical investigations to clinical trials are often challenging. The deaminated thyroid hormone analog (tetraiodothyroacetic acid, tetrac) and its nanoparticulate analog (NDAT) have been shown to have anti-proliferative activity *in vitro* and in xenograft model of different neoplasms, including colorectal cancers. However, mechanisms involved in tetrac- and NDAT-induced anti-proliferation in colorectal cancers are incompletely understood. We have investigated possible mechanisms of tetrac and NDAT action in colorectal cancer cells, using a perfusion bellows cell culture system that allows efficient, large-scale screening for mechanisms of drug actions on tumor cells. Although integrin αvβ3 in *K-RAS* wild type colorectal cancer HT-29 cells was far less than that in *K-RAS* mutant HCT116 cells, HT-29 was more sensitive to both tetrac and NDAT. Results also indicate that both tetrac and NDAT bind to tumor cell surface integrin αvβ3, and the agents may have different mechanisms of anti-proliferation in colorectal cancer cells. *K-RAS* status appears to play an important role in drug resistance that may be encountered in treatment with this drug combination.

Keywords: perfusion bellows cell culture system, colorectal cancer cells, anticancer, phosphoERK1/2, NDAT, tetrac, integrin αvβ3

OPEN ACCESS

Edited by:
Gabriella Castoria,
Second University of Naples, Italy

Reviewed by:
Marzia Di Donato,
Università degli Studi Della Campania Luigi Vanvitelli Caserta, Italy
Mario Rotondi,
University of Pavia, Italy

****Correspondence:***
Hung-Yun Lin
linhy@tmu.edu.tw
Jacqueline Whang-Peng
jqwpeng@nhri.org.tw

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *16 November 2018*
Accepted: *12 February 2019*
Published: *12 March 2019*

Citation:
Chin Y-T, He Z-R, Chen C-L, Chu H-C, Ho Y, Su P-Y, Yang Y-CSH, Wang K, Shih Y-J, Chen Y-R, Pedersen JZ, Incerpi S, Nana AW, Tang H-Y, Lin H-Y, Mousa SA, Davis PJ and Whang-Peng J (2019) Tetrac and NDAT Induce Anti-proliferation via Integrin αvβ3 in Colorectal Cancers With Different K-RAS Status. Front. Endocrinol. 10:130. doi: 10.3389/fendo.2019.00130

INTRODUCTION

The well-demonstrated receptor of thyroid hormone on the extracellular domain of plasma membrane integrin αvβ3 is mainly expressed on tumor cells and dividing blood vessel cells. Steroid hormones (1–3) and thyroid hormones (L-thyroxine, T_4, and 3,5,3′-triiodo-L-thyronine, T_3) are able to bind to integrin αvβ3 on cells (4–11). The interaction between thyroid hormone and integrin αvβ3 (11) has been demonstrated to promote cancer cell proliferation in various types of cancer cells, including breast cancer (12, 13), lung cancer (10, 14), glioma cells (15, 16), myeloma cells (17), pancreatic cancer (18), and colorectal cancer cells (6, 7, 13, 19). Crosstalk between epidermal growth factor (EGFR) and the integrin has been reported to be involved in regulation of cancer cell proliferation (7, 13). Signal transduction mechanisms mediate the promotion of cancer cell proliferation by thyroid hormone and such mechanisms can be blocked by tetraiodothyroacetic acid (tetrac) (7, 13), a deaminated analog of thyroid hormone.

Tetrac has been shown to compete with thyroid hormone, e.g., L-thyroxine (T_4) for the iodothyronine receptor on integrin αvβ3. Tetrac inhibits binding of thyroid hormones, thus inhibiting the downstream signal transduction pathways of nongenomically initiated effects of thyroid hormone (4, 7, 19–21). Tetrac also has actions at the receptor that are independent of the functions of thyroid hormone, for example, modulation of angiogenesis by multiple mechanisms and regulation of tumor cell metabolism (22). Its interaction with integrin αvβ3 permits tetrac to modify differentially regulated gene expression that is related to cancer cell survival pathways. Tetrac up-regulates expression of pro-apoptotic *BcL-x* short form (12), anti-angiogenic thrombospondin 1 (*THBS1*) and other pro-apoptotic genes (4). In addition, tetrac downregulates transcription of several families of anti-apoptotic genes. A nano-formulation of tetrac, nano-diamino-tetrac (NDAT), has been shown to act primarily at the cell surface and does not enter the nucleus when it does enter the cell.

Recently we have studied the anti-proliferative effects of tetrac, NDAT, and their combinations with other anticancer drugs on colorectal cancer cells (4, 7, 13, 19–21). In combination with resveratrol, NDAT downregulated resveratrol-induced ribonucleotide reductase regulatory subunit M2 (*RRM2*) gene expression *in vivo* and potentiated the anticancer effect of the stilbene (20). In addition, tetrac enhanced nuclear abundance of chibby family member 1 (CBY1), a nuclear β-catenin antagonist, which is a protein that may compromise nuclear β-catenin-dependent gene expression and proliferation (6). Gefitinib-induced anti-proliferation in gefitinib-resistant colorectal cancer cells is restored by NDAT; the mechanism involves inhibition of beta-galactoside alpha-2, 6-sialyltransferase 1 (ST6Gal1) activity and PI3K activation (7). These observations indicate that added or enhanced effects are obtained with combinations of tetrac or NDAT and other chemotherapeutic agents.

In the current report, we investigated mechanisms by which tetrac- and NDAT induced anti-proliferation in colorectal cancer cells. In addition, we report studies conducted to define the different gene profiles induced by tetrac and NDAT in colorectal cancer cell lines. Finally, using a novel perfusion bellows cell culture system, we have distinguished the mechanisms by which tetrac or NDAT work on human colorectal cancer cells with different *K-RAS* status.

MATERIALS AND METHODS

Cell Cultures

Human colorectal cancer cell lines HT-29 (ATCC® HTB-38™) and HCT 116 (ATCC® CCL-247™) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were maintained in RPMI-1640 medium (Life Technologies Corp. Carlsbad, CA, USA) supplemented with 10% FBS and grown under 5% CO_2/95% air at 37°C routinely. Prior to treatments, cells were washed with phosphate buffered saline (PBS) and then serum-free medium was added for starvation for 48 h. Then, the serum-free medium was replaced by 5% stripped FBS containing medium at the initiations of studies.

Pharmacodynamics

Anti-proliferative effects of tetrac and NDAT were defined in a well-established perfusion bellows cell culture system (13, 23). At the outset, 5×10^7 cells were seeded in perfusion bellows cell culture system and incubated at 37°C overnight. Then polymer flakes were harvested, trypsinized, and cells were collected and counted. The number of original cells attached to flakes was 0.5×10^7 cells/bottle. Cell cultures were refreshed with 1% stripped FBS-containing medium. Tetrac or NDAT was added in a medium bottle to the final concentrations indicated in the Results section. Specific concentration of tetrac and NDAT were chosen according to the physiological concentration of T_4 (10^{-7} M) as described previously (24–26). The samples of cell-bearing flakes were then treated as indicated, and cells were harvested at timeframe indicated, trypsinized, and collected for counting. The cell cultures were refreshed with 10% hormone-stripped FBS containing medium.

Quantitative Real-Time PCR (QPCR)

Total RNA was extracted and genomic DNA was eliminated with the Illustra RNAspin Mini RNA Isolation Kit (GE Healthcare Life Sciences, Buckinghamshire, UK). One microgram of DNase I-treated total RNA was reverse-transcribed with a RevertAid H Minus First Strand cDNA Synthesis Kit (Life Technologies Corp.) into cDNA and used as the template for real-time PCR reactions and analysis. The real-time PCR reactions were performed using QuantiNovaTM SYBR® Green PCR Kit (QIAGEN, Valencia, CA, USA) on a CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). This involved an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturing at 95°C for 5 s and combined annealing/extension at 60°C for 10 s, as described in the manufacturer's instructions. The primer sequences were: *Homo sapiens* integrin, alpha v (*ITG αv*), forward 5′-TCC GATTCCAAACTGGGAGC-3′ and reverse 5′-AAGGCCACTG AAGATGGAGC-3′ (Accession No.: NM_002210.4); *Homo sapiens* integrin, beta 3 (*ITG β3*), forward 5′-CTGGTGT TTACCACTGATGCCAAG-3′ and reverse 5′-TGTTGAGG

CAGGTGGCATTGAAGG-3′ (Accession No.: NM_000212.2); *Homo sapiens* caspase 2, apoptosis-related cysteine peptidase (*CASP2*), forward 5′-GCATGTACTCCC ACCGTTGA-3′ and reverse 5′-GACAGGCGGAGCTTCTTGTA-3′ (Accession No.: NM_032982.3); *Homo sapiens* v-myc avian myelocytomatosis viral oncogene homolog, (*c-Myc*), forward 5′-TTCGGGTAG TGGAAAACCAG-3′ and reverse 5′-CAGCAGCTCGAATT TCTTCC-3′ (Accession No.: NM_002467); *Homo sapiens* p53-inducible gene 3 (*PIG3*) / tumor protein p53 inducible protein 3 (*TP53I3*), forward 5′-TTGAGGCATCTGGACAT GTG-3′ and reverse 5′-GGGTCAATCCCTCTGGGATAG-3′ (Accession No.: NM_004881.4); *Homo sapiens* tumor protein p53 (*p53*), forward 5′-AAGTCTAGAGCCACCGTCCA-3′ and reverse 5′-CAGTCTGGCTGCCAATCCA-3′ (Accession No.: NM_000546.5); *Homo sapiens* cyclin-dependent kinase inhibitor 1A (*p21*), forward 5′- CTGGGGATGTCCGTCAGAAC-3′ and reverse 5′-CATTAGCGCATCACAGTCGC-3′ (Accession No.: BT006719.1); *Homo sapiens* BCL2-associated agonist of cell death (*BAD*), forward 5′- CTTTAAGAAGGGACTTCCTCGCC-3′ and reverse 5′-AAGTTCCGATCCCACCAGGA-3′ (Accession No.: NM_032989.2); *Homo sapiens* vascular endothelial growth factor A (*VEGF-A*), forward 5′-TACCTCCACCATGCCAA GTG-3′ and reverse 5′-GATGATTCTGCCCTCCTCCTT-3′ (Accession No.: NM_001204384.1); *Homo sapiens* programmed death ligand 1 (*PD-L1*) (*CD274*), forward 5′-GTTGAAG GACCAGCTCTCCC-3′ and reverse 5′-ACCCCTGCA TCC TGCAATTT-3′ (Accession No. NM_014143.3); *Homo sapiens* thrombospondin 1, (*THBS1*), forward 5′-ATCCTGGACTCGC TGTAGGT-3′ and reverse 5′-GTCATCGTCCCTTTCGGTGT-3′ (accession no.: BC136470.1); and *Homo sapiens* 18S ribosomal RNA (*18S*), forward 5′-GTAACCCGTTGAACCCCATT-3′ and reverse 5′-CCATCCAATCGGTAGTAGCG-3′ (accession no. NR_003286). The relative gene expression normalized to the internal control 18S rRNA was calculated based on the ΔΔCT method and the fidelity of the PCR reactions was determined with melting temperature analysis.

Western Blotting

Western blotting analyses were conducted followed routine protocol described previously (6, 7, 19–21, 23). Consistent amount of protein samples were resolved on a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). A 20 μg quantity of protein was loaded in each well with 5x sample buffer, and the protein samples were resolved with electrophoresis at 100 V for 2 h. The resolved proteins were transferred from the polyacrylamide gel to Millipore Immobilon-PSQ Transfer PVDF membranes (Millipore, Billerica, MA, USA) with the Mini Trans-Blot® Cell (Bio-Rad Laboratories). The membranes were blocked with a solution of 2% FBS in Tris-buffered saline and incubated with primary antibodies to pERK1/2 (GeneTex, Inc., Hsinchu, Taiwan), Cyclin D1 (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), ERK1/2 (Santa Cruz Biotechnology), and α-Tubulin (Novus Biologicals, Littleton, CO, USA) at 4°C overnight and washed; the proteins were detected with HRP-conjugated secondary antibodies and Immobilon™ Western HRP Substrate Luminol Reagent (Millipore). Images of the western blots were visualized and recorded with an Amersham Imager 600 (GE Healthcare, Chicago, IL, USA). The blots were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Flow Cytometry Analysis

HCT116 cells and HT-29 cells were harvested from polymer flakes by trypsinization, washed with PBS, and resuspended in 1 mL PBS ($1 \times 10^6 - 5 \times 10^6$ cells). To quantify the expression of integrin αvβ3 on the cell membrane, cells were fixed with 70% ethanol for 30 min at 4°C. Cells were washed in PBS three times and then resuspended in 100 μL PBS with 1% bovine serum albumin (BSA) at a concentration of 5×10^6 cells per mL. Cells were incubated with mouse monoclonal anti-integrin αvβ3 antibody (1:50; Santa Cruze Biotechnology Inc.) at room temperature for 1 h. Cells were further incubated with an Alexa-647-labeled goat anti-rabbit antibody (1:200, GeneTex International Corporation, Hsinchu City, Taiwan) at room temperature for 30 min in the dark. Flow cytometry was carried out on a FACSCalibur™ (Becton Dickinson) instrument, using CellQuest software to determine the expression of integrin αvβ3. Fluorescence-activated cell sorting (FACS) analysis used integrin αvβ3-Alexa-647. Relative percentages of integrin αvβ3-positive cells were calculated from FL-3 histograms using ModFit LT software.

Data Analysis and Statistics

Data are presented as the mean ± S.E. The data were analyzed using IBM SPSS Statistics software version 19.0 (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) with Duncan's *post-hoc* test was used to analyze the differences between experimental groups followed by a paired Student's *t*-test. Student's *t*-tests for paired data were also used in some cases as indicated. $p < 0.05$ was considered statistically significant.

RESULTS

Different Expression Levels of Integrin αvβ3 Are Present on the Cell Surface of Colorectal Cancer Cells

To evaluate the expression of integrin αvβ3 in the colorectal cancer cell lines HCT116 and HT-29, studies of QPCR and flow cytometry analysis of integrin αvβ3 were conducted. The expressions of *ITGαV* and *ITGβ3* in HCT116 cells were significantly higher than that in HT-29 cells (2.7 fold in *ITGαV* and 9.4 fold in *ITGβ3*) (**Figure 1A,** left panel). The expression of integrin αvβ3 on the cell surface of HCT116 cells (35.6%) was also notably greater than that on HT-29 cells (12.6%) (**Figure 1A**, right panel).

Both Tetrac and NDAT Bind to Cell Surface Integrin αvβ3 in Colorectal Cancer Cells

To determine whether the signal transduction pathways activated by tetrac and NDAT are integrin αvβ3-dependent, colorectal

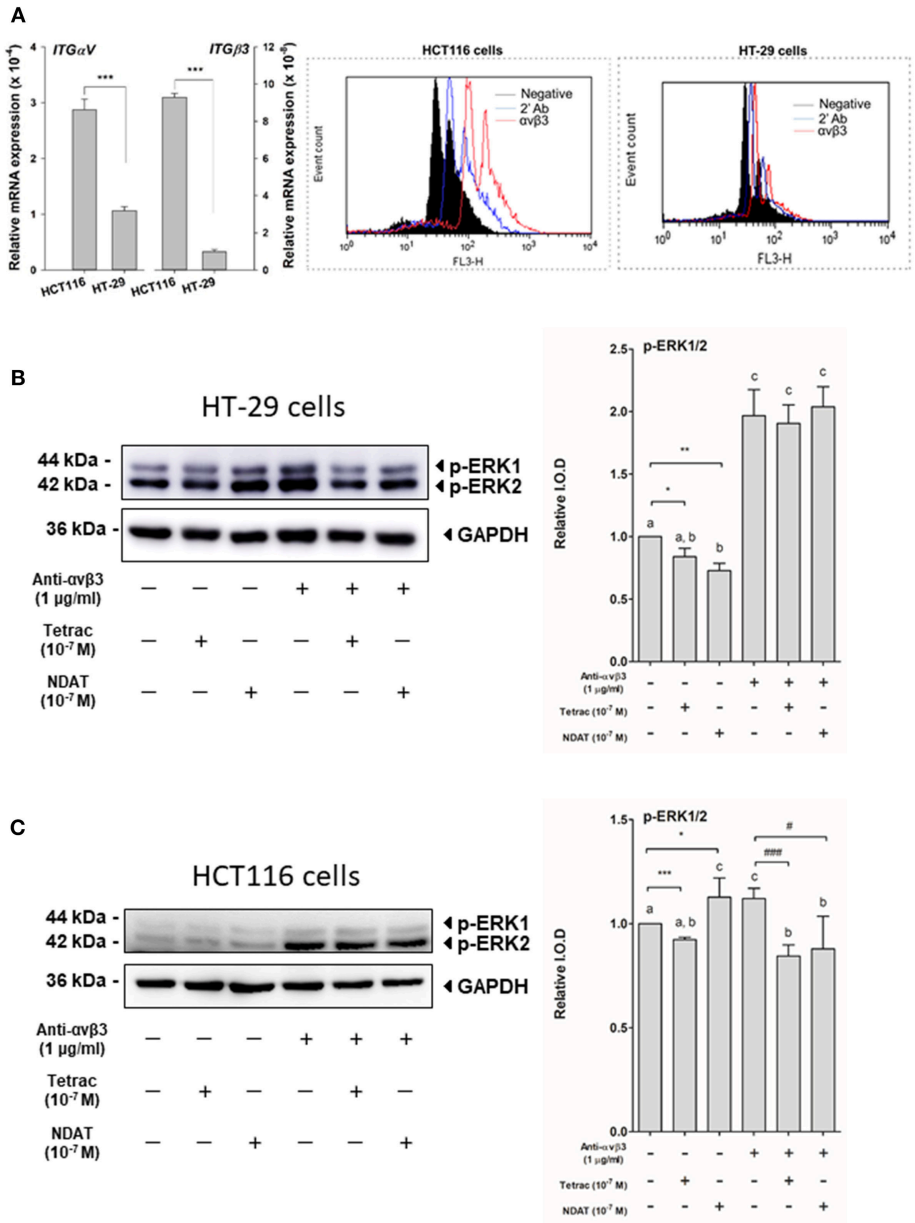

FIGURE 1 | Cell surface integrin αvβ3 is the binding site of tetrac and NDAT in colorectal cancer cells. **(A)** Eighty-five percent confluent colorectal cancer cells, HT-29 cells, and HCT116 cells, grown in 10-cm Petri dishes were harvested for studies of QPCR and flow cytometry analysis of integrin αvβ3. For studies of ERK1/2 activation, HT-29 cells and HCT116 cells seeded in 10-cm Petri dishes were pretreated with 1 μg/mL of anti-integrin αvβ3 antibody for 30 min and then treated with either 10^{-7} M tetrac or 10^{-7} M NDAT for 30 min. Total proteins were extracted, then Western blot analyses were conducted. **(B)** Activation of ERK1/2 was induced by NDAT but not tetrac in HT-29 cells. **(C)**. Activation of ERK1/2 was inhibited by tetrac and NDAT in HCT116 cells. Pretreatment of anti-integrin αvβ3 antibody reversed their effects. Number of independent experiments. $N = 3$. (Data are expressed as mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with untreated control; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$, compared with anti-integrin αvβ3 antibody treatment. a-c: the subsets after *post hoc* analysis after the significant differences were obtained using one-way ANOVA).

cancer *K-RAS*-mutant HCT116 cells and *K-RAS*-wild type HT-29 cells were set in 10 mL Petri dishes and starved with serum-free media for 2 days. Prior to starting each experiment, cells were re-fed with fresh medium. Cells were treated with 1 μg/mL anti-integrin αvβ3 30 min before treatment of either 10^{-7} M tetrac or NDAT for 30 min. Total proteins were extracted and Western blot analyses were conducted for pERK1/2 study. Tetrac inhibited activation of ERK1/2, but NDAT increased activated ERK1/2 in HT-29 cells (**Figure 1B**). On the other hand, tetrac and NDAT inhibited constitutively

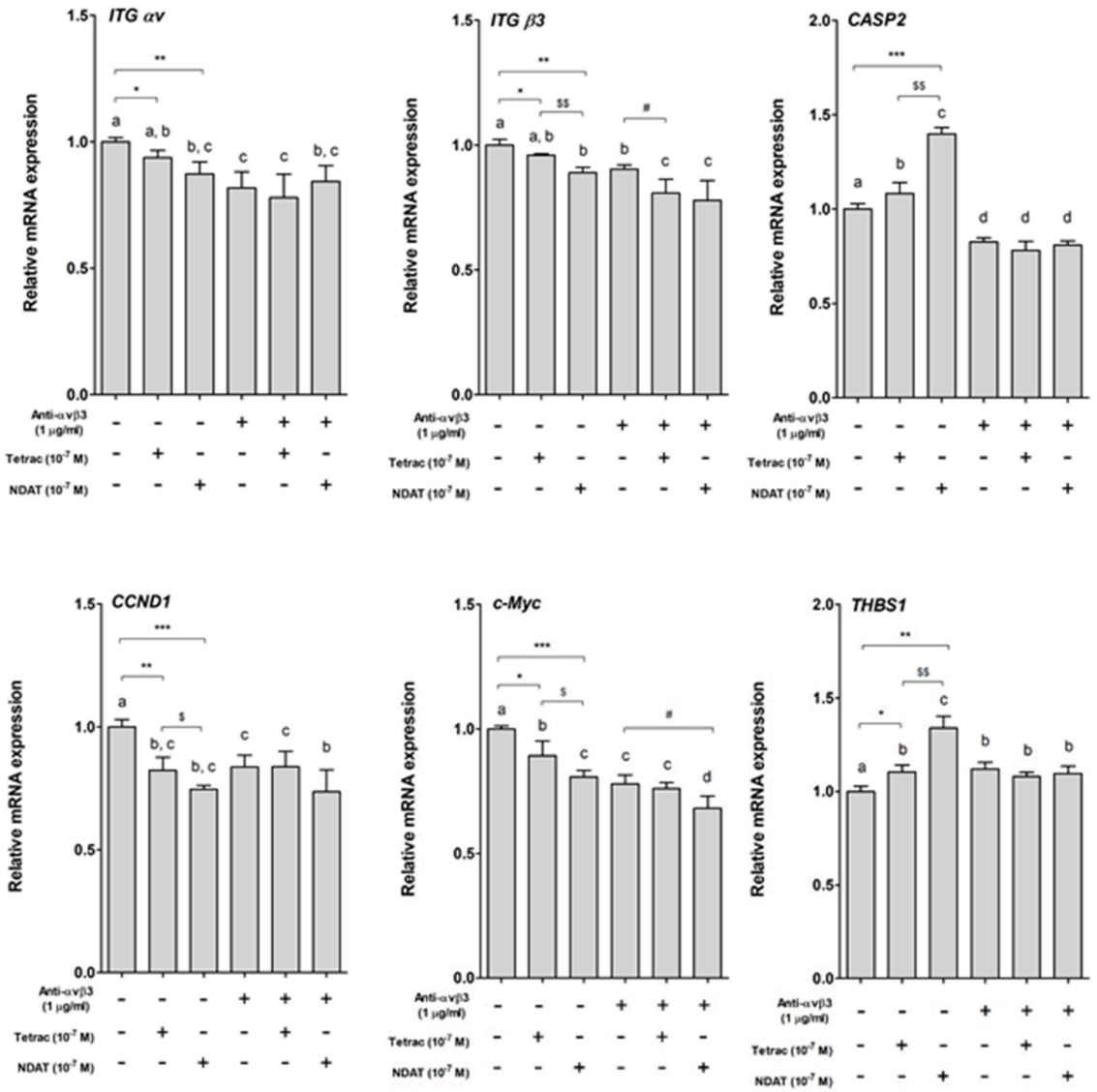

FIGURE 2 | Tetrac and NDAT via cell surface integrin αvβ3 regulate gene expression in colorectal cancer cells. HCT116 cells seeded in 6-well plates were pretreated with 1 μg/mL of anti-integrin αvβ3 antibody for 30 min and then treated with either 10^{-7} M tetrac or 10^{-7} M NDAT for 24 h. Total RNA was extracted and QPCR was conducted. $N = 3$. (Data are expressed as mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with untreated control; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, compared with tetrac group; $\#p < 0.05$, compared with anti-integrin αvβ3 antibody treatment. a–d: the subsets after *post hoc* analysis after the significant differences were obtained using one-way ANOVA).

activated ERK1/2 in HCT116 cells (**Figure 1C**). However, this effect was partially removed by anti-integrin αvβ3 antibody pretreatment (**Figures 1B,C**).

Parallel studies were conducted to define the expression of specific genes controlled by tetrac or NDAT. Colorectal cancer HCT116 cells were set in 6-well trays and starved with serum-free media for 2 days. Prior to starting each experiment, cells were re-fed with fresh medium. Cells were treated with 1 μg/mL anti-integrin αvβ3 for 30 min prior to treatment with 10^{-7} M tetrac or NDAT for 24 h. Total RNA was extracted and qPCR was conducted for integrin αv (*ITG αv*), integrin β3 (*ITG β3*), *CASP2*, *CCND1*, *c-Myc*, and *THBS1*. Results shown in **Figure 2** indicate that both tetrac and NDAT inhibited expression of *CCND1* and *c-Myc*, but promoted expression of *CASP2* and *THBS1*. The effect of NDAT was much greater than that of tetrac. However, pretreatment of cells with anti-integrin αvβ3 blocked the effect of tetrac and NDAT (**Figure 2**). These results suggest that both tetrac and NDAT bind to integrin αvβ3 to induce downstream signals that play an important role in tetrac/NDAT-dependent cellular activities.

Tetrac and NDAT Induce Anti-proliferation in Colorectal Cancer Cells With Different *K-RAS* Status

Two colorectal cancer cell lines were seeded in the perfusion bellows cell culture system. Different concentrations of tetrac (10^{-8}, 10^{-7}, and 10^{-6} M) or NDAT (10^{-9}, 10^{-8}, and 10^{-7} M) were added to medium bottles. Tetrac of various concentrations (10^{-8}, 10^{-7}, and 10^{-6} M) significantly reduced cell number of *K-RAS*-wild type HT-29 cells after 4 days of treatment, as did NDAT (10^{-8} and 10^{-7} M). NDAT (10^{-8} M) reduced 37% of cell number after 4 days of treatment, but tetrac (10^{-7} M) only reduced 28.4% of cell number compared to untreated control (**Figure 3**).

After 6 days of treatment, tetrac (10^{-8} and 10^{-7} M) significantly reduced cell number of *K-RAS*-mutant HCT116 cells (**Figure 4**). On the other hand, different concentrations of NDAT (10^{-9}, 10^{-8}, and 10^{-7} M) inhibited cancer cell proliferation significantly after 2 days of treatment. NDAT (10^{-9} M) reduced 52.5% of cell number at day 2, but tetrac (10^{-8} M) reduced 11.5% of cell number at day 5 compared to untreated control (**Figure 4**).

Cells treated with tetrac and NDAT were also prepared for flow cytometry studies. Results shown in **Figure 5** indicate that tetrac induced cancer cell arrested in G2/M stage. However, low concentration 10^{-8} M NDAT induced G2/M arrest and 10^{-7} M NDAT shut down DNA synthesis.

NDAT and Tetrac Modulate Expression of Different Genes in Colorectal Cancer Cells

We observed previously that treatment with tetrac or NDAT results in different gene expression profiles in MDA-MB-231 cells and in medullary thyroid carcinoma cells (24). In order to define the gene transcription profiles induced by tetrac and by NDAT in colorectal cancer cells with different *K-RAS* status, HT-29 cells and HCT116 cells were treated with 10^{-8} and 10^{-7} M tetrac or NDAT, respectively for 24 h. RNA was extracted from the harvested cells at the end of treatment for qPCR studies. Treatment with NDAT (10^{-8} M) for 24 h increased expression of *p53* and p53-responsible genes such as *p21*, *PIG3 (TP53I3)*, *BAD*, and *CASP2* in HT-29 cells significantly (**Figure 5**). In addition, NDAT increased expression of *THBS1*. Protein TSP1 is an endogenous suppressor of angiogenesis and is invariably suppressed in cancer cells. Although tetrac also increased expression of those genes, the effect was 10-fold less (**Figure 5**). In contrast, tetrac (10^{-8} and 10^{-7} M) significantly increased expression of *p21*, *p53*, *PIG3*, *BAD*, and *THBS1* genes in HCT116 cells (**Figure 6**). However, 10^{-8} M tetrac did not significantly inhibit expression of *VEGF-A*, which plays an important role in HCT116 cells metastasis (**Figure 6**). On the other hand, NDAT also increased expression of the mentioned genes and significantly decreased expression of *VEGF* at 10^{-8} M.

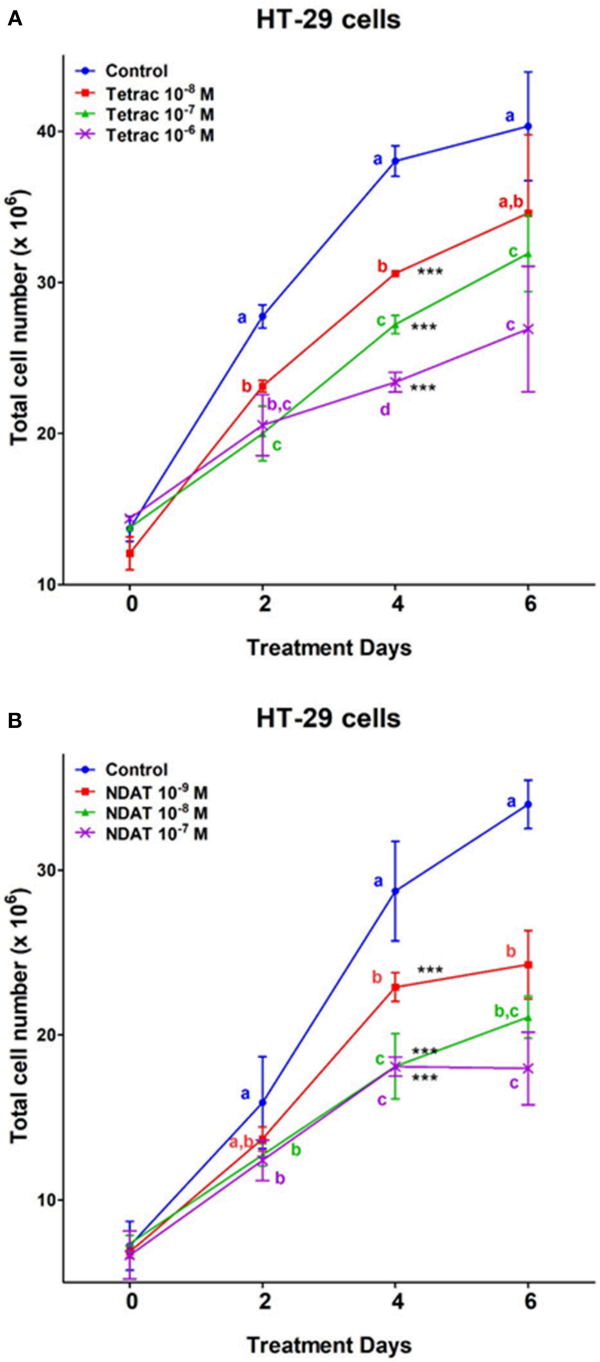

FIGURE 3 | Tetrac **(A)** and NDAT **(B)** induce anti-proliferation of *K-RAS* wild-type colorectal cancer HT-29 cells. HT-29 colorectal cancer cells were grown in the perfusion bellows system and treated with tetrac (10^{-8} to 10^{-6} M) or NDAT (10^{-9} to 10^{-7} M) for 6 days. After incubated in the treatment, cells were isolated and subjected to analysis by cell count, $N = 3$. Data are expressed as mean ± SD; ***$p < 0.001$, compared with untreated control. a–c: the subsets after *post hoc* analysis after the significant differences were obtained using one-way ANOVA.

DISCUSSION

The perfusion bellows cell culture studies we describe here provide useful pharmacodynamic information of the application of new drugs or combinations of various agents *in vitro* to human cancer cell lines (13). Pharmacodynamic modeling based

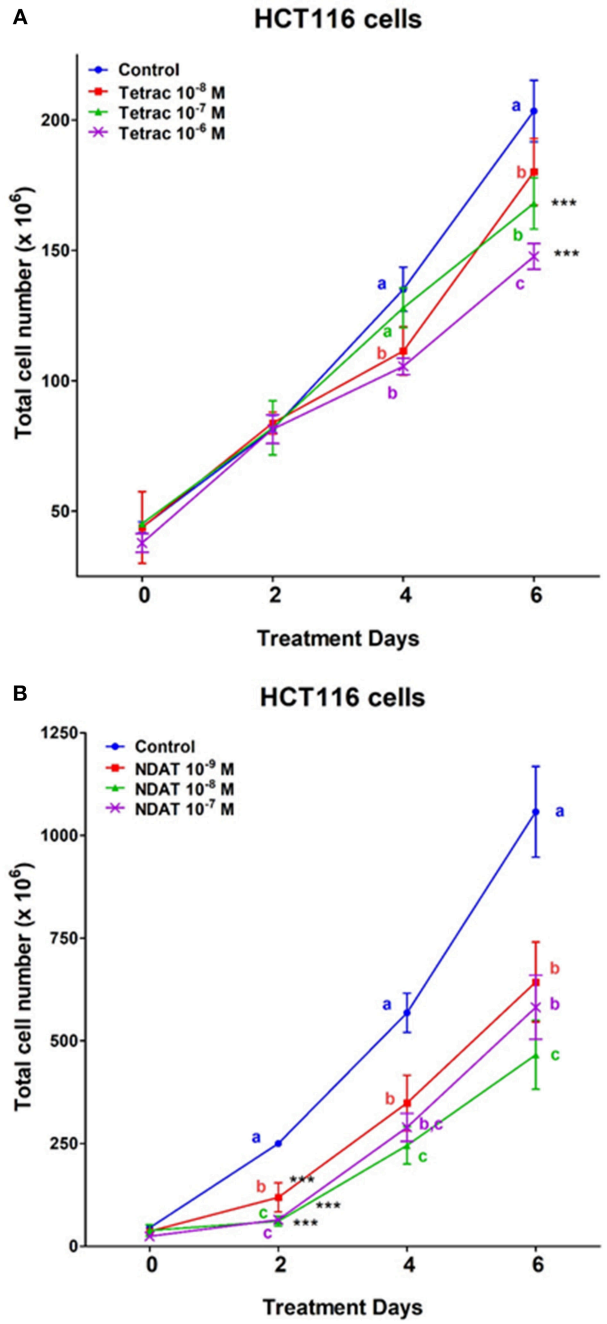

FIGURE 4 | Tetrac **(A)** and NDAT **(B)** induce anti-proliferation of *K-RAS*-mutant colorectal cancer HCT116 cells. HCT116 colorectal cancer cells were grown in the perfusion bellows system and treated with tetrac (10^{-8} to 10^{-6} M) or NDAT (10^{-9} to 10^{-7} M) for 6 days. After incubated in the treatment, cells were isolated and subjected to analysis by cell count. $N = 3$. Data are expressed as mean ± SD; $^{***}p < 0.001$, compared with untreated control. a–c: the subsets after *post hoc* analysis after the significant differences were obtained using one-way ANOVA.

on the expected pharmacokinetics of a drug permits—in the perfusion bellows cell culture system– the understanding of dose-response relationship of antineoplastic agents over a very wide range of concentration in *vitro* and can support translation from *in vitro* models to animal models and ultimately human clinical trials.

Via binding to the thyroid hormone receptor on plasma membrane integrin αvβ3 (27, 28), NDAT induces signal transduction and biological activities similar to those of unmodified tetrac (**Figures 1**, **2**), but with desirable effects on cell survival pathway genes that differ from the parent thyroid hormone analog (**Figures 2**, **5**, **6**) (5, 24). Studies indicated that NDAT but not tetrac activated ERK1/2 in *K-RAS*-wild type colorectal cancer HT-29 cells (**Figure 1A**), which confirms previous results that NDAT activates ERK1/2 in HT-29 cells (24). On the other hand, both tetrac and NDAT inhibited ERK1/2 activation in *K-RAS*-mutant HCT-116 cells (**Figure 1B**). Interestingly, NDAT is more sensitive to the inhibitory effect of pretreatment of cells with of anti-integrin αvβ3 (**Figure 2**). Both tetrac and NDAT induced anti-proliferation in colorectal cancer *K-RAS*-wild type HT-29 cells and in *K-RAS*-mutant HCT116 cells (**Figures 3**, **4**). The growth-inhibitory effects of tetrac and NDAT on HCT116 colon cancer cell xenografts are evident in 2–4 d after the onset of treatment (6, 7, 20)**.** The anti-proliferative effect of tetrac and NDAT on cancer cells in the perfusion bellows cell culture system was seen at 3 d after the start of treatment (**Figures 3**, **4**). Pharmacodynamics of anti-proliferative activities *in vitro* of tetrac and NDAT in colorectal cancer cells were compared in the perfusion bellows cell culture system. Results revealed that NDAT had a higher potency than tetrac as an anti-proliferative agent (**Figure 3**). We have previously shown that the anticancer effects of tetrac and NDAT in colorectal cancer HCT116 cell xenografts are well-established within 3 d after onset of drug administration (6, 7). Although inhibitory effects of tetrac and NDAT in the perfusion bellows cell culture system are limited to suppression of cell proliferation, these results in the perfusion system reproduce findings obtained earlier in the same cells in xenografts. Tetrac-/NDAT-induced anti-tumor effects in xenografts have been shown to involve both primary effects on tumor cell proliferation and anti-angiogenesis effect (6, 7). Results in these sets of studies similarly demonstrate that the anticancer effects of tetrac/NDAT occur first as anticancer cell proliferation.

The inhibition of HT-29 cell growth by NDAT and tetrac was comparable at a higher drug concentration (10^{-7} M), but there was increased sensitivity to NDAT at a lower drug concentration of the used agents (**Figure 3**). In addition to the anti-proliferative effects initiated at the cell surface integrin receptor, tetrac may penetrate into cells to exert low-grade thyromimetic (proliferative) effects in the nucleus of treated colorectal cancer cells. Therefore, the net anti-proliferative effect of tetrac decreases. On the other hand, NDAT does not gain access to the cell nucleus and shows a more robust anti-proliferative effect.

In addition to their anti-proliferative effects on cancer cells, tetrac and NDAT have been shown to enhance anti-cancer growth by other anticancer drugs. Cetuximab (Erbitux®) inhibits *K-RAS* wild-type but not *K-RAS* mutant colorectal cancer cell growth (19). The combination of tetrac and cetuximab significantly reduced cell proliferation compared to cetuximab

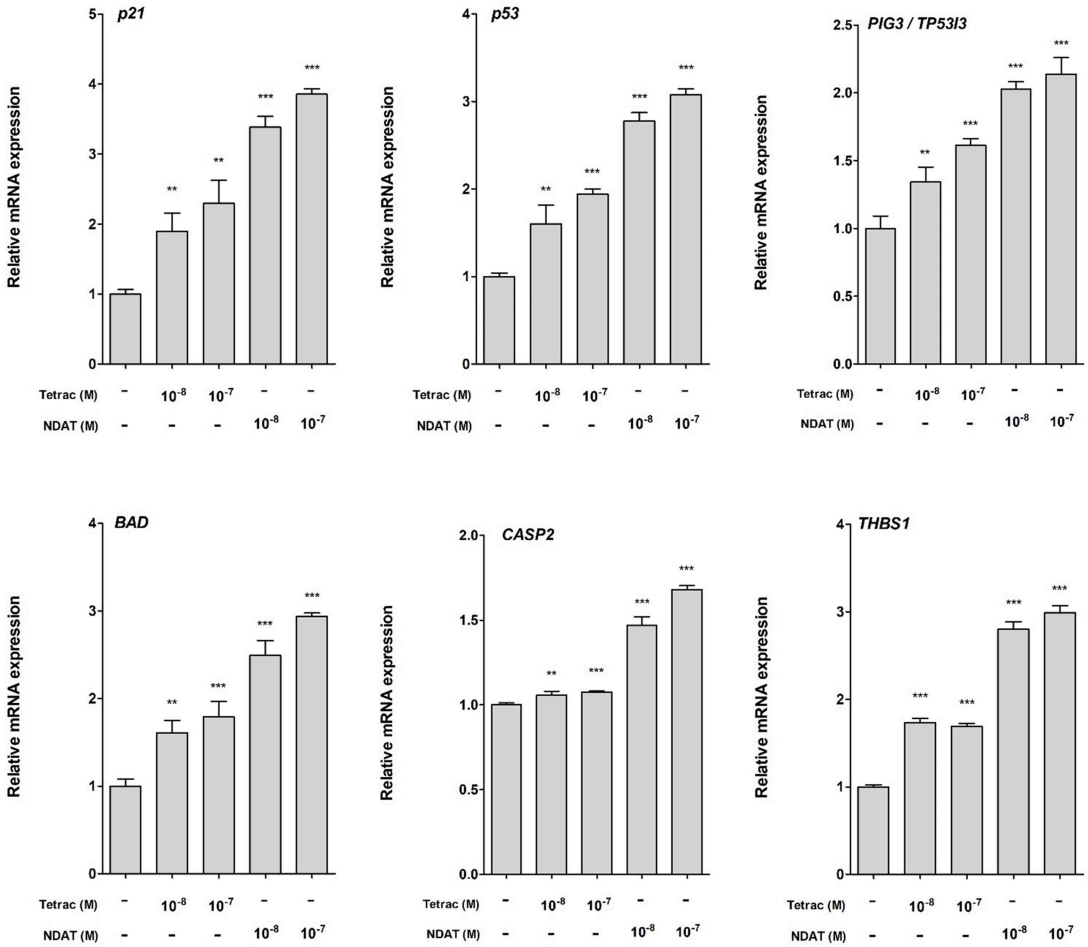

FIGURE 5 | Tetrac and NDAT suppress expression of proliferative and metastasis-related genes in HT-29 cancer cells. HT-29 cells were seeded in 6-well plates and treated with different concentrations of 10^{-8} and 10^{-7} M tetrac or NDAT for 24 h. Cells were harvested and total RNA was extracted. qPCR experiments were conducted to examine expression of anti-proliferative genes (*p21, p53,* and *PIG3*), apoptotic genes (*BAD* and *CASP2*) and metastasis-related genes (*THBS1*). $N = 6$. (Data are expressed as mean. ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with untreated control).

alone in *K-RAS* mutant HCT 116 cells, but not in *K-RAS* wild type COLO 205 cells (19). NDAT also rescued the anti-proliferative effect of cetuximab in both colorectal cancer cell lines (19). These results suggest that tetrac and NDAT may be used in the clinic in the future to treat *K-RAS*-mutant colorectal cancer patient. However, present studies indicated that NDAT was much more effective than tetrac (**Figures 5**, **6**). Furthermore, NDAT reversed *K-RAS* mutant-dependent cetuximab resistance. In addition, xenograft weights in NDAT-treated alone animals are not significantly decreased compared to those in untreated control [result not shown (6, 7)]. Therefore, NDAT alone or combination with low dosage of cetuximab may be a new chemotherapeutic approach in the future.

Studies have shown that tetrac affects only XIAP gene expression (12) in breast cancer cells. On the other hand, NDAT downregulates expression of apoptosis inhibitors *XIAP*, myeloid cell leukemia sequence 1 (*MCL1*), and apoptosis-promoting genes such as *CASP2* and *BCL2L14* (12). Expression of the angiogenesis inhibitor thrombospondin 1 (*THBS1*) gene is increased by both unmodified tetrac and NDAT, as is the expression of *CBY1*, a nuclear inhibitor of catenin activity (12). The majority of differentially regulated *K-RAS*-oncogene family members and the epidermal growth factor receptor gene are downregulated by NDAT, but not by tetrac (12). β-Catenin belongs to a class of catenins that have been shown to play roles in cell-cell adhesion. It also has transcription functions. Various cancers such as those of the colorectum,

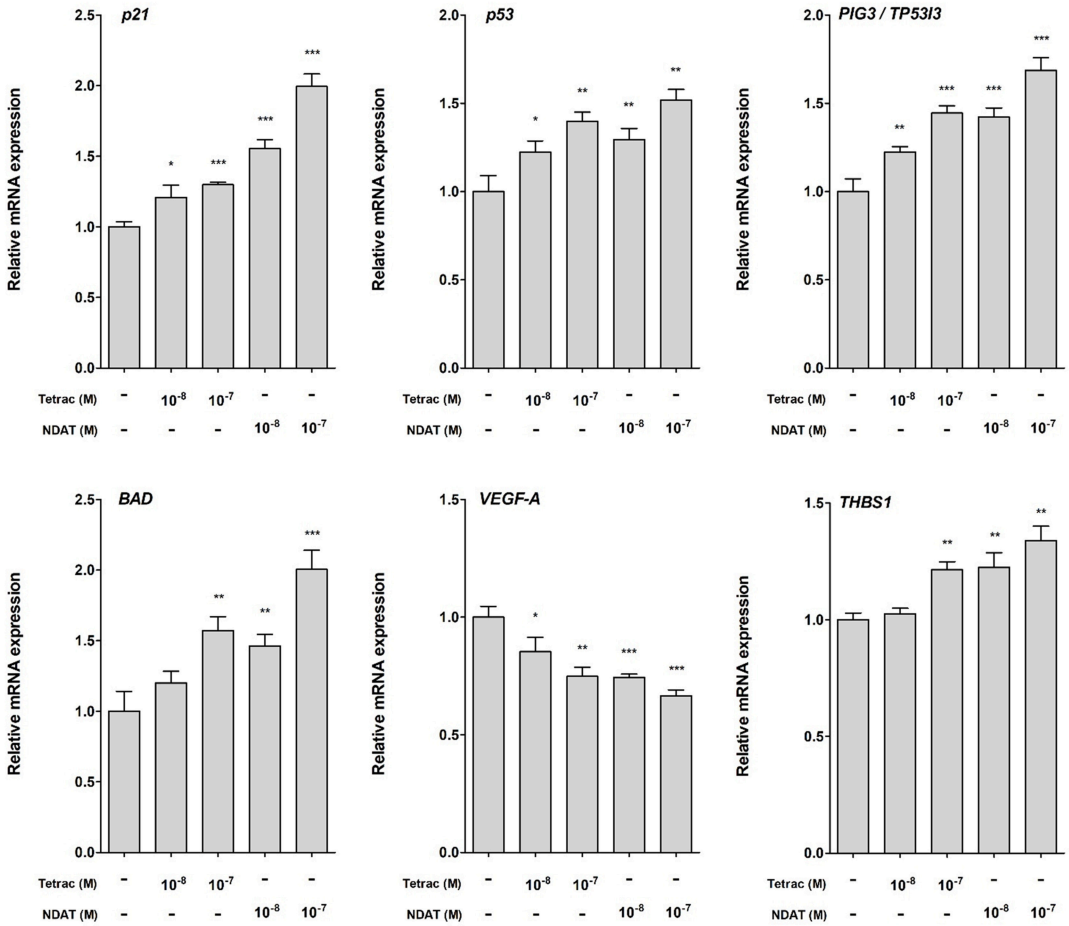

FIGURE 6 | Tetrac and NDAT suppress expression of proliferative and metastasis-related genes in HCT116 cancer cells. HCT116 cells were seeded in 6-well plates and treated with different concentrations of 10^{-8} and 10^{-7} M tetrac or NDAT for 24 h. Cells were harvested and total RNA was extracted. Studies of qPCR were conducted to examine expression of anti-proliferative genes (*p21, p53,* and *PIG3*), apoptotic gene (*BAD*), and metastasis-related genes (*VEGF-A* and *THBS1*). $N = 6$. (Data are expressed as mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with untreated control).

breast, and ovary have been shown to contain mutant and overexpressed *β-catenin* genes. CBY1, an inhibitor of nuclear functions of β-catenin, is upregulated by NDAT. Like β-catenins, integrin αvβ3 participates in cellular adhesion complexes. NDAT downregulates expression of the gene for integrin β3 monomer. Since integrin αvβ3 has been shown to involve metastasis and migration, this may be a desirable action of NDAT at αvβ3 in cancer cells.

NDAT also downregulates expression of the genes for *α-catenins*, *CTNNA1* and *CTNNA2* (8). Mutation of CTNNA2 is associated with tumor invasiveness and thus inhibition of transcription of the gene is desirable. The nonmutated gene product of CTNNA1 can function as a tumor invasion suppressor, but mutation is associated with gastrointestinal tract and other cancers.

Thyroid hormone induces expression of MMP-2 and MMP-9 in myeloma cells and colorectal cancer cells (7). This action is inhibited by tetrac and NDAT; this implies that αvβ3 can be involved in the contribution of matrix metalloproteinases to cancer-relevant angiogenesis and to tumor invasiveness. Tetrac and NDAT were able to inhibit constitutive expression of *VEGF-A* (**Figure 6**). Tetrac and NDAT also increased transcription of thrombospondin 1 (*THBS1*, *TSP1*), which suppresses angiogenesis.

In summary, tetrac and NDAT via integrin αvβ3 regulate thyroid hormone-dependence of various independent cancer cell activities. NDAT is much more potent than tetrac and has a

broader range of transcriptional actions in cancer cells that may be clinically desirable.

AUTHOR CONTRIBUTIONS

Y-TC, Z-RH, C-LC, KW, H-YL, and JW-P: conceptualizing research idea. H-CC, YH, P-YS, Y-CY, and KW: developing methodologies. P-YS, Y-CY, KW, Y-JS, Y-RC, AWN, and H-YT: performing experiments. JZP, SI, Y-JS, Y-RC, AWN, and H-YT: collecting and analyzing data. JZP and SI: Interpreting data. H-YL and PJD: writing manuscript. SAM, PJD, and JZP: proofreading and editing.

FUNDING

This work was supported in part by grant from Wan-Fan Hospital Collaborating Program (JW-P, 98TMU-WFH-02-1), by Chair Professor Research Fund, to KW, by Chair Professor Research Fund to JW-P, by the TMU Research Center of Cancer Translational Medicine from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan, general grant of Ministry of Science and Technology Taiwan (H-YL, MOST 107-2314-B-038-017), a gift from PJD to Albany College of Pharmacy and Health Sciences and by E-DaHospital, EDAHP107018 to Z-RH.

REFERENCES

1. Davis PJ, Sudha T, Lin HY, Mousa SA. Thyroid hormone, hormone analogs, and angiogenesis. *Compr Physiol.* (2015) 6:353–62. doi: 10.1002/cphy.c150011
2. Lin HY, Hsieh MT, Cheng GY, Lai HY, Chin YT, Shih YJ, et al. Mechanisms of action of nonpeptide hormones on resveratrol-induced antiproliferation of cancer cells. *Ann NY Acad Sci.* (2017) 1403:92–100. doi: 10.1111/nyas.13423
3. Chin YT, Wei PL, Ho Y, Nana AW, Changou CA, Chen YR, et al. Thyroxine inhibits resveratrol-caused apoptosis by PD-L1 in ovarian cancer cells. *Endocr Relat Cancer*. (2018) 25:533–45. doi: 10.1530/ERC-17-0376
4. Lin HY, Chin YT, Yang YC, Lai HY, Wang-Peng J, Liu LF, et al. Thyroid hormone, cancer, and apoptosis. *Compr Physiol.* (2016) 6:1221–37. doi: 10.1002/cphy.c150035
5. Lee YS, Chin YT, Shih YJ, Nana AW, Chen YR, Wu HC, et al. Thyroid hormone promotes beta-catenin activation and cell proliferation in colorectal cancer. *Horm Cancer*. (2018) 9:156–65. doi: 10.1007/s12672-018-0324-y
6. Nana AW, Chin YT, Lin CY, Ho Y, Bennett JA, Shih YJ, et al. Tetrac downregulates beta-catenin and HMGA2 to promote the effect of resveratrol in colon cancer. *Endocr Relat Cancer*. (2018) 25:279–93. doi: 10.1530/ERC-17-0450
7. Chang TC, Chin YT, Nana AW, Wang SH, Liao YM, Chen YR, et al. Enhancement by nano-diamino-tetrac of antiproliferative action of gefitinib on colorectal cancer cells: mediation by EGFR sialylation and PI3K activation. *Horm Cancer*. (2018) 9:420–32. doi: 10.1007/s12672-018-0341-x
8. Davis PJ, Glinsky GV, Lin HY, Leith JT, Hercbergs A, Tang HY, et al. Cancer cell gene expression modulated from plasma membrane integrin alphavbeta3 by thyroid hormone and nanoparticulate tetrac. *Front Endocrinol.* (2014) 5:240. doi: 10.3389/fendo.2014.00240
9. Incerpi S, Hsieh MT, Lin HY, Cheng GY, De Vito P, Fiore AM, et al. Thyroid hormone inhibition in L6 myoblasts of IGF-I-mediated glucose uptake and proliferation: new roles for integrin alphavbeta3. *Am J Physiol Cell Physiol.* (2014) 307:C150–61. doi: 10.1152/ajpcell.00308.2013
10. Meng R, Tang HY, Westfall J, London D, Cao JH, Mousa SA, et al. Crosstalk between integrin alphavbeta3 and estrogen receptor-alpha is involved in thyroid hormone-induced proliferation in human lung carcinoma cells. *PLoS ONE*. (2011) 6:e27547. doi: 10.1371/journal.pone.0027547
11. Davis PJ, Mousa SA, Lin HY. Tetraiodothyroacetic acid (tetrac), integrin alphavbeta3 and disabling of immune checkpoint defense. *Future Med Chem.* (2018) 10:1637–9. doi: 10.4155/fmc-2018-0123
12. Glinskii AB, Glinsky GV, Lin HY, Tang HY, Sun M, Davis FB, et al. Modification of survival pathway gene expression in human breast cancer cells by tetraiodothyroacetic acid (tetrac). *Cell Cycle*. (2009) 8:3562–70. doi: 10.4161/cc.8.21.9963
13. Lin HY, Landersdorfer CB, London D, Meng R, Lim CU, Lin C, et al. Pharmacodynamic modeling of anti-cancer activity of tetraiodothyroacetic acid in a perfused cell culture system. *PLoS Comput Biol.* (2011) 7:e1001073. doi: 10.1371/journal.pcbi.1001073
14. Mousa SA, Yalcin M, Bharali DJ, Meng R, Tang HY, Lin HY, et al. Tetraiodothyroacetic acid and its nanoformulation inhibit thyroid hormone stimulation of non-small cell lung cancer cells in vitro and its growth in xenografts. *Lung Cancer*. (2012) 76:39–45. doi: 10.1016/j.lungcan.2011.10.003
15. Hercbergs AH, Lin HY, Davis FB, Davis PJ, Leith JT. Radiosensitization and production of DNA double-strand breaks in U87MG brain tumor cells induced by tetraiodothyroacetic acid (tetrac). *Cell Cycle*. (2011) 10:352–7. doi: 10.4161/cc.10.2.14641
16. Lin HY, Glinsky GV, Mousa SA, Davis PJ. Thyroid hormone and anti-apoptosis in tumor cells. *Oncotarget*. (2015) 6:14735–43. doi: 10.18632/oncotarget.4023
17. Cohen K, Flint N, Shalev S, Erez D, Baharal T, Davis PJ, et al. Thyroid hormone regulates adhesion, migration and matrix metalloproteinase 9 activity via alphavbeta3 integrin in myeloma cells. *Oncotarget*. (2014) 5:6312–22. doi: 10.18632/oncotarget.2205
18. Yalcin M, Lin HY, Sudha T, Bharali DJ, Meng R, Tang HY, et al. Response of human pancreatic cancer cell xenografts to tetraiodothyroacetic acid nanoparticles. *Horm Cancer*. (2013) 4:176–85. doi: 10.1007/s12672-013-0137-y
19. Lee YS, Chin YT, Yang YSH, Wei PL, Wu HC, Shih A, et al. The combination of tetraiodothyroacetic acid and cetuximab inhibits cell proliferation in colorectal cancers with different K-ras status. *Steroids*. (2016) 111:63–70. doi: 10.1016/j.steroids.2016.03.006
20. Nana AW, Wu SY, Yang YS, Chin YT, Cheng TM, Ho Y, et al. Nano-diamino-tetrac (NDAT) enhances resveratrol-induced antiproliferation by action on the RRM2 pathway in colorectal cancers. *Horm Cancer*. (2018) 9:349–60. doi: 10.1007/s12672-018-0334-9
21. Lin SJ, Chin YT, Ho Y, Chou SY, Sh Yang YC, Nana AW, et al. Nano-diamino-tetrac (NDAT) inhibits PD-L1 expression which is essential for proliferation in oral cancer cells. *Food Chem Toxicol.* (2018) 120:1–11. doi: 10.1016/j.fct.2018.06.058
22. Davis PJ, Lin HY, Sudha T, Yalcin M, Tang HY, Hercbergs A, et al. Nanotetrac targets integrin alphavbeta3 on tumor cells to disorder cell defense pathways and block angiogenesis. *Onco Target Ther*. (2014) 7:1619–24. doi: 10.2147/OTT.S67393
23. Chin YT, Hsieh MT, Yang SH, Tsai PW, Wang SH, Wang CC, et al. Anti-proliferative and gene expression actions of resveratrol in breast cancer cells *in vitro*. *Oncotarget*. (2014) 5:12891–907. doi: 10.18632/oncotarget.2632
24. Lin HY, Chin YT, Nana AW, Shih YJ, Lai HY, Tang HY, et al. Actions of L-thyroxine and Nano-diamino-tetrac (Nanotetrac) on PD-L1 in cancer cells. *Steroids*. (2016) 114:59–67. doi: 10.1016/j.steroids.2016.05.006
25. Lin HY, Su YF, Hsieh MT, Lin S, Meng R, London D, et al. Nuclear monomeric integrin alphav in cancer cells is a coactivator regulated

by thyroid hormone. *FASEB J.* (2013) 27:3209–16. doi: 10.1096/fj.12-227132

26. Lin HY, Shih A, Davis FB, Davis PJ. Thyroid hormone promotes the phosphorylation of STAT3 and potentiates the action of epidermal growth factor in cultured cells. *Biochem J.* (1999) 338 (Pt. 2):427–32. doi: 10.1042/bj3380427
27. Sudha T, Bharali DJ, Yalcin M, Darwish NH, Debreli Coskun M, Keating KA, et al. Targeted delivery of paclitaxel and doxorubicin to cancer xenografts via the nanoparticle of nano-diamino-tetrac. *Int J Nanomed.* (2017) 12:1305–15. doi: 10.2147/IJN.S123742
28. Sudha T, Bharali DJ, Yalcin M, Darwish NH, Coskun MD, Keating KA, et al. Targeted delivery of cisplatin to tumor xenografts via the nanoparticle component of nano-diamino-tetrac. *Nanomedicine*. (2017) 12:195–205. doi: 10.2217/nnm-2016-0315

Conflict of Interest Statement: Co-authors SAM and PJD are co-inventors of NDAT and stockholders in a company that is commercializing the agent.

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

frontiers in Endocrinology

CORRECTION
published: 09 April 2019
doi: 10.3389/fendo.2019.00241

Corrigendum: Tetrac and NDAT Induce Anti-proliferation via Integrin αvβ3 in Colorectal Cancers With Different *K-RAS* Status

Yu-Tang Chin[1,2†], Zong-Rong He[3,4†], Chi-Long Chen[5†], Hsiao-Ching Chu[6], Yih Ho[1,7], Po-Yu Su[8], Yu-Chen S. H. Yang[9], Kuan Wang[1,8], Ya-Jung Shih[1,8], Yi-Ru Chen[1,8], Jens Z. Pedersen[10], Sandra Incerpi[11], André Wendindondé Nana[12], Heng-Yuan Tang[13], Hung-Yun Lin[1,12,13,14,15], Shaker A. Mousa[13], Paul J. Davis[13,16] and Jacqueline Whang-Peng[1,2,12*]*

[1] Taipei Cancer Center, Taipei Medical University, Taipei, Taiwan, [2] Cancer Center, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan, [3] Department of Pediatrics, E-Da Hospital, Kaohsiung, Taiwan, [4] School of Medicine, I-Shou University, Kaohsiung, Taiwan, [5] School of Medicine, Taipei Medical University, Taipei, Taiwan, [6] Division of Medical Imaging, E-Da Cancer Hospital, I-Shou University, Kaohsiung, Taiwan, [7] School of Pharmacy, Taipei Medical University, Taipei, Taiwan, [8] Graduate Institute of Nanomedicine and Medical Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan, [9] Joint Biobank, Office of Human Research, Taipei Medical University, Taipei, Taiwan, [10] Department of Biology, University of Rome Tor Vergata, Rome, Italy, [11] Department of Sciences, Roma Tre University, Rome, Italy, [12] Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan, [13] Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, United States, [14] TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei, Taiwan, [15] Traditional Herbal Medicine Research Center of Taipei Medical University Hospital, Taipei Medical University, Taipei, Taiwan, [16] Department of Medicine, Albany Medical College, Albany, NY, United States

Keywords: perfusion bellows cell culture system, colorectal cancer cells, anticancer, phosphoERK1/2, NDAT, tetrac, integrin αvβ3

OPEN ACCESS

Approved by:
Frontiers in Endocrinology Editorial Office, Frontiers Media SA, Switzerland

***Correspondence:**
Hung-Yun Lin
linhy@tmu.edu.tw
Jacqueline Whang-Peng
jqwpeng@nhri.org.tw

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *26 March 2019*
Accepted: *27 March 2019*
Published: *09 April 2019*

Citation:
Chin Y-T, He Z-R, Chen C-L, Chu H-C, Ho Y, Su P-Y, Yang Y-CSH, Wang K, Shih Y-J, Chen Y-R, Pedersen JZ, Incerpi S, Nana AW, Tang H-Y, Lin H-Y, Mousa SA, Davis PJ and Whang-Peng J (2019) Corrigendum: Tetrac and NDAT Induce Anti-proliferation via Integrin αvβ3 in Colorectal Cancers With Different K-RAS Status. Front. Endocrinol. 10:241. doi: 10.3389/fendo.2019.00241

A Corrigendum on

Tetrac and NDAT Induce Anti-proliferation via Integrin αvβ3 in Colorectal Cancers With Different *K-RAS* Status

by Chin, Y.-T., He, Z.-R., Chen, C.-L., Chu, H.-C., Ho, Y., Su, P.-Y, et al. (2019). Front. Endocrinol. 10:130. doi: 10.3389/fendo.2019.00130

In the original article, we neglected to include the funder "E-Da Hospital, EDAHP107018" to Z-RH.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

frontiers in Endocrinology

MINI REVIEW
published: 23 August 2019
doi: 10.3389/fendo.2019.00565

Clinical Implications and Impact of Discovery of the Thyroid Hormone Receptor on Integrin αvβ3–A Review

*Aleck Hercbergs**

Department of Radiation Oncology, Cleveland Clinic, Cleveland, OH, United States

Hypothyroidism has been reported to improve survival in cancer patients but only recently has the putative mechanism been identified as a receptor for thyroxine and tri-iodothyronine on integrin αvβ3. Recognition of divergence of action of the pro-oncogenic L-thyroxine (T4) from pro-metabolic 3,5,3′-triiodo-L-thyronine (T3) has enabled clinical implementation whereby exogenous T3 may replace exogenous (or endogenous) T4 to maintain clinical euthyroid hypothyroxinemia that results in significantly better survival in advanced cancer patients without the morbidity of clinical hypothyroidism.

Keywords: cancer, euthyroid hypothyroxinemia, hypothyroidism, integrin αvβ3, L-thyroxine, thyroid hormone receptor

OPEN ACCESS

Edited by:
Sandra Incerpi,
Roma Tre University, Italy

Reviewed by:
Roberto De Luca,
Beth Israel Deaconess Medical Center and Harvard Medical School, United States
Hynda K. Kleinman,
George Washington University, United States

***Correspondence:**
Aleck Hercbergs
hercbergs@gmail.com

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *13 May 2019*
Accepted: *02 August 2019*
Published: *23 August 2019*

Citation:
Hercbergs A (2019) Clinical Implications and Impact of Discovery of the Thyroid Hormone Receptor on Integrin αvβ3–A Review. Front. Endocrinol. 10:565. doi: 10.3389/fendo.2019.00565

INTRODUCTION

In recent years there have been reports that blood thyroxine depletion in individuals with advanced solid cancers–e.g., glioblastoma, high grade soft tissue sarcoma, was associated with regression of advanced tumors. Exogenous 3,5,3′-triiodo-L-thyronine (L-T3) was used to maintain metabolic euthyroidism (1, 2).

How did this approach come about?

The 1993 report of spontaneous remission (3) and 5-year survival of a patient with metastatic non-small cell lung cancer inspired this novel line of research, which has evolved from early experimental studies to the clinic, wherein proactive medically induced thyroid suppression was investigated in the treatment of failed high grade brain tumors (glioblastoma multiforme) (4). Significant prolongation of life (from 4 to 10 months median survival) was associated with a drop of >40% of circulating free thyroxine (T4) levels. Tumor regression was also observed in patients. There was short survival in all of the 50% of patients who did not experience free thyroxine depletion. The delay in thyroid hormone decrease remained an obstacle in extended implementation of this approach until the discovery by Davis and Mousa et al. of a newly identified cell surface receptor for thyroid hormone (both analogs T4 and T3) on integrin αvβ3 expressed on cancer cell membranes and actively dividing vascular endothelium (5).

This contribution is not intended as a comprehensive review of the literature of thyroid hormones and cancer but is focused on relevance of thyroxine impact on cancer growth and biology, now understood to be mediated through the cell surface integrin αvβ3 receptor.

THYROID HORMONE IN PHYSIOLOGY AND CANCER BIOLOGY

T4 and T3–A Pivotal Difference and Roles

The use of exogenous T3 as a substitute for T4 as thyroid hormone replacement has enabled withdrawal of the potent pro-oncogenic hormone T4 in cancer patients without resulting in clinical hypothyroidism (1).

In the absence of T4, cancer cell mitogenesis and proliferation does not occur. Absence of T3 impacts severely mitogenesis and metabolism. The pivotal role of thyroid hormones thyroxine T4 and T3 in cancer growth and biology and the impact on emerging clinical practice has become understood following identification of integrin αvβ3 and the interaction with and binding differences between T3 and T4 (1). T4 is the principal secretory product of the thyroid gland. It serves as a prohormone for T3 in the latter's intracellular functions (1). The reported literature refers solely to blood thyroid hormone levels individually, e.g., FT4 or FT3 and/or TSH or under the "hypothyroid" label.

T3-directed gene expression in cells requires primary interactions between T3 and its nuclear receptor proteins (TRs) (1, 2). T3 also regulates mitochondrial respiration (3). Because its biological half-life is significantly longer than that of T3 (6), T4 is the most commonly prescribed form of thyroid hormone replacement for clinical hypothyroidism and for suppression of endogenous pituitary thyrotropin (TSH) (1).

It is becoming accepted that cancer depends on both analogs to exist, survive, proliferate, and grow into tumors. Thyroxine is the sole endogenous and pleiotropic pro-oncogenic hormone that abets cancer, acting as a ligand for the membrane expressed receptor on integrin αvβ3 (7).

Thyroid hormone is a pivotal crucial pro-growth pro-oncogenic hormone for most if not all malignant tumors and the crucial interaction of T4 is with the integrin αvβ3. Thyroid hormone drives and is pro-proliferation and pro-angiogenic for cancer (7–9).

It will become evident how this discovery has led to a new and novel paradigm in understanding and management of solid cancers.

Thyroxine is the more potent pro-oncogenic thyroid hormone analog and is the pro-hormone for T3, which is the dominant pro-metabolic thyroid hormone (10, 11). T3 and T4 bind to the plasma membrane protein integrin αvβ3, which mediates the signal across the membrane (5). T4 is active at this receptor (7, 11). *In vitro* evidence indicates that T3, bound with a lower affinity by αvβ3 (7), is of low activity at physiological levels at the receptor.

Integrin αvβ3 contains two thyroid hormone binding sites, S1 and S2, which activate different downstream pathways (12): S1 binds T3 and activates the PI3K/AKT-pathway whereas S2 binds T4 and, *with lower affinity*, T3, and activates PI3K/AKT-pathway and MAPK-pathway. At physiological concentrations, T4 (and not T3) is the principal ligand at S2. Thyroid hormone action at αvβ3 is inhibited by the deaminated and decarboxylated T4 derivative tetrac (3,3,5′,5′-tetraiodothyroacetic acid) (5). Blocking the thyroid hormone receptor on the integrin (equivalent to total thyroxine depletion) downregulates multiple pro-oncogenic genes and upregulates pro-apoptotic genes (12). The consequences of tetrac on human xenograft growth and a triple negative breast cancer gene expression are profoundly anti-oncogenic (12, 13).

Thyroid hormone is therefore a high priority molecule promoting interaction with tumor cell membranes and is not tumor specific. The pursuit of strategies to implement "precision" medicine is clearly irrelevant here.

Genomic vs. Non-genomic Actions of Thyroid Hormone

Classical effects of thyroid hormones are initiated when T3 binds to its TRs that interact with specific responding elements (TREs). Thyroid hormones can also elicit their actions by a non-classical mechanism without direct gene transcription regulation by nuclear TRs (**Figure 1**). These non-genomic actions indirectly modulate gene transcription by activating intracellular pathways and other transcription factors (10, 14). Many of the non-genomic actions are via a receptor expressed on a membrane integrin for thyroxine and T3. The availability of T4 is the crucial step for activation of the integrin and consequent trans-membrane signaling into the cell (**Figure 2**). Total withdrawal or depletion of T4 will arrest this entire process

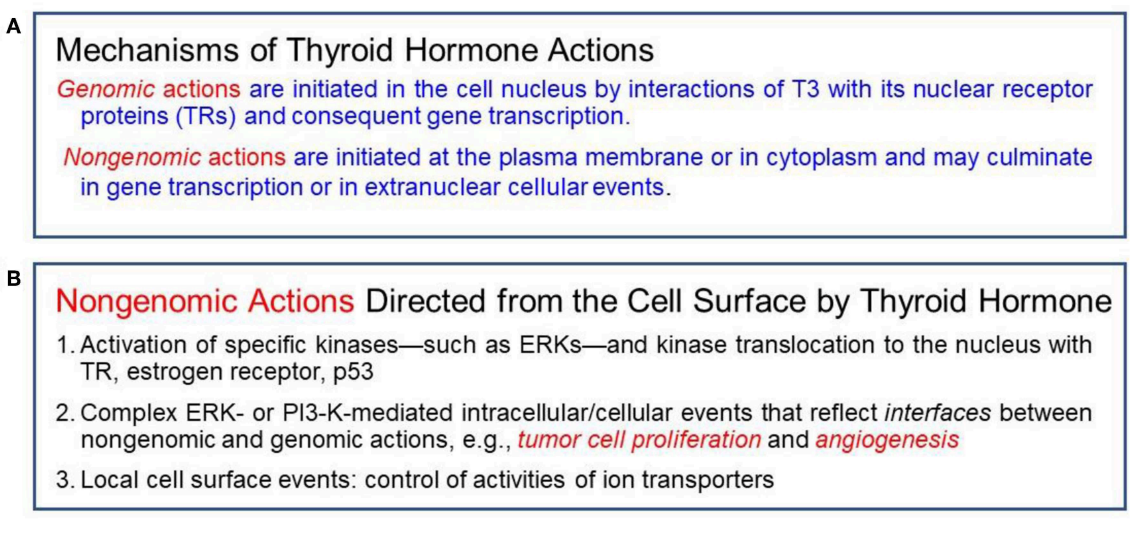

FIGURE 1 | (A) Actions of thyroid hormone are genomic or non-genomic in mechanism. **(B)** Specific non-genomic actions.

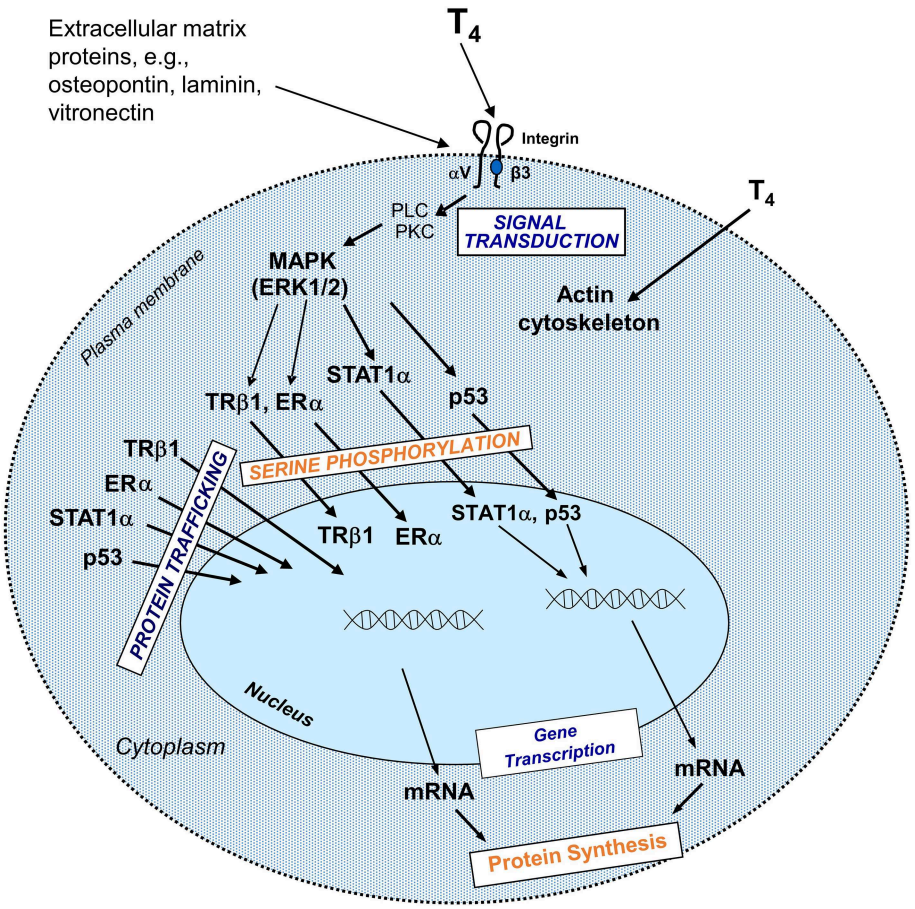

FIGURE 2 | Non-genomic actions of the hormone that begin at integrin αvβ3 include regulation of intracellular trafficking of specific proteins to the nucleus and serine phosphorylation of some of these proteins in the course of nuclear entry. Directed to the nucleus from the cytoplasm, some of these proteins might be involved in modulation of transcription of specific genes and in cell proliferation. These pathways depend on activation of phospholipase C (PLC), protein kinase C (PKC), mitogen activated protein kinase (MAPK)1, and MAPK2. T4 non-genomically rapidly activates actin polymerization in hypothyroid astocytes and osteoblastic cells (15). Reprinted with permission from Hercbergs et al. (16).

with consequences for the viability of the tumor and/or vascular cell (11).

THYROID HORMONE, CANCER, AND THE INTEGRIN αvβ3-EXPRESSED THYROID HORMONE RECEPTOR

T4 as a Hormone

Observations of thyroid hormone's impact on cancer prognosis predate the discovery of the integrin αvβ3 and thyroid hormone receptor on cancer cells and membranes and vascular endothelium. These studies are summarized in **Table 1**.

The discovery of the receptor and activation by thyroxine and significantly less by T3 has led to successful clinical translation and implementation in the treatment of compassionate care cancer patients by simple substitution of exogenous T3 for exogenous L-thyroxine in hypothyroxinemia. Discovery of the thyroid hormone receptor on integrin αvβ3 advances the understanding of the interaction and relationship to cancer with ambient thyroid hormone levels (1).

T4 and Cancer Biology

T4 in physiological free hormone concentrations stimulates proliferation of cancer cells *in vitro* and in xenografts (13, 39–45). Preclinical studies of tetrac, which blocks thyroid hormone action at integrin αvβ3, have shown arrested tumor growth (7, 11) in a variety of tumor xenografts including xenografts of renal cell carcinoma (13), non-small cell lung carcinoma (46), medullary carcinoma of the thyroid (41), pancreatic carcinoma (43), and multi-drug resistant breast cancer (47).

CLINICAL TRANSLATIONAL STUDY TO INDUCE EUTHYROID HYPOTHYROXINEMIA

Euthyroid Hypothyroxinemia and Divergence of Action Between T4 and T3

This is a eumetabolic state maintained in the total absence of blood thyroxine by providing exogenous T3. The individual can therefore live and perform all normal daily activities and

TABLE 1 | Cancer outcomes across a spectrum of thyroid functions.

Thyroid function	Type of research	No. of cases	Cancer type/disease	Clinical outcome	References
Spontaneous hyperthyroid	Prospective population study	29,691	Several malignancies	Significantly higher hazard ratios for lung and prostate cancer vs. significantly lower for HT	(17)
	Case-control	532	Pancreas	Increased risk with prior hypothyroidism	(18)
	Case-control	26, 22 matched controls	Breast	Subclinical hyperthyroidism associated with more frequent cancers	(19)
Spontaneous hypothyroid	Case report	1	NSCLC, metastatic	'Spontaneous' CR following myxedema coma	(20)
	Series	28	Various solid tumors	100% response (CR and PR) rate to radiation therapy in chemically HT pts	(21)
Primary hypothyroidism-Thyroid hormone supplemented	Population-based	1,136, 1,088 controls	Breast, primary	Less aggressive disease in HT group, fewer metastases, 7 years older age at onset, smaller tumors	(22)
	Comparative study	280	Breast, all stages	5 years older for HT	(23)
	Comparative study	68, 91 matched controls	Breast, all stages	6 years older, smaller tumors, lower stage, lower S phase for HT	(24)
	Comparative study	85, 85 matched controls	Lung, all stages	4.3 years older, longer survival for HT	(25)
	Comparative study	247, 234 matched controls	RCC, all stages	Greater use of TH in RCC pts	(26)
	Case report	1	Breast	Apparent tumor stimulation with TH	(27)
	Case report/review	1	NSCLC	Apparent tumor stimulation with TH	(20)
	Case report	1	Anaplastic thyroid	Apparent tumor stimulation with TH, CR while clinically HT, 10-year survival	(28)
	Series	5	Pancreas, CRC	Long-term survival while on lower dose; TH/TH discontinued	(29)
	Series	176	Breast	Pts taking TH before diagnosis had greater relapse rate, larger tumors	(30)
Hypothyroid –[iatrogenic] 2° to XRT/CHEMORX/ SURG/Biologics	Retrospective	54	RCC treated with sunitinib	Pts becoming HT with sunitinib and treated with TH seemed to have worse outcome	(31)
	Retrospective	155, with 59 developing HT	HNSCC	Pts developing HT seemed to have better survival	(32)
	Population-based	5,916 (age >65)	HN (excluding thyroid, larynx, prior HT)	Longer survival in those developing HT	(33)
	Phase II, subset analysis	34	RCC, melanoma treated with IL-2/LAK cells	Higher responses with development of HT	(34)
	Phase II, subset analysis	16	RCC, metastatic, treated with IL-2/LAK cells	Development of HT correlated with better response rate	(35)
Interventional hypothyroxinemia	Phase I-II	36	Recurrent, high-grade gliomas made HT with PTU	Early-onset HT associated with improved survival	(4, 36)
	Phase II	20	Recurrent, high-grade gliomas made HT with PTU	HT associated with improved survival	(37)
Recurrent disease following [re-] initiation of L-thyroxine in HT pts	Case reports	4	Breast	7/9 women given TH after mastectomy developed recurrence, 4 of which were late	(30)
	Case report	1	Breast	Rapid progression, death after re-starting TH, 3+ years after being in CR	(27)

CR, complete response; CRC, colorectal cancer; HN, head and neck; HNSCC, head and neck squamous cell carcinoma; HT, hypothyroidism; IL-2, interleukin 2; LAK, lymphokine-activated killer; NSCLC, non-small cell lung cancer; PR, partial response; pts, patients; PTU, propylthiouracil; RCC, renal cell carcinoma; TH, thyroid hormone; XRT, radiation therapy. Reprinted with permission from Hercbergs et al. (38).

functions as prior to removal of the source of T4 or post total thyroidectomy. Preclinical evidence (12) indicates that T3, bound with a lower affinity by αvβ3 (9), is of low activity at physiological levels at the receptor (5, 39). The clinical ramifications of these effects on cancer cells are supported by the results of induction of the state of euthyroid hypothyroxinemia in patients with advanced cancers and with normal thyroid function (1).

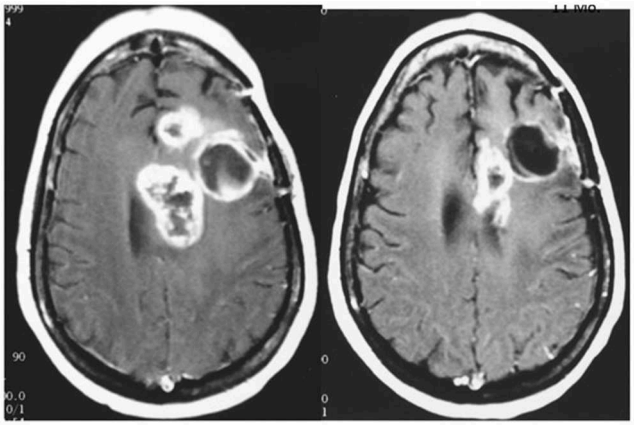

FIGURE 3 | MRI of brain of a 42 year old female with recurrent glioblastoma showing significant mass reduction with free thyroxine depletion at 4 months. Left, pre-thyroxine depletion; right, 4 months later. The patient survived for 3 years. Reprinted with permission from Hercbergs et al. (4).

In a compassionate-need study of terminal patients with a variety of incurable solid tumors, extended survival was observed in a majority of patients using exogenous T3 to induce and maintain hypothyroxinemia. T3 administration prevented symptomatic hypothyroidism. Low odds of survival were surmounted in 19 of 23 patients (83%) who exceeded the expected median survival of literature-reported series used as controls (1). An additional approach is to terminate exogenous T4 supplementation to allow the T4 level to decline and supplement with T3 titrated individually to the patient's functional needs. Examples of the outcomes of this strategy on advanced disease (glioblastoma and sarcoma) are shown in **Figures 3–5**.

CLINICAL STUDIES

Clinical studies have shown that survival is significantly prolonged (almost 3-fold) in failed glioblastoma patients treated with propylthiouracil to inhibit thyroxine synthesis.

More information available about T4 action on lung cancer is limited, but euthyroid hypothyroxinemia appears to slow the course of and extend survival of non-small cell, small cell lung, pancreas, mesothelioma, glioblastoma, and soft tissue sarcoma (1, 2).

Going forward it is suggested and clinically pivotal that the term hypothyroxinemia be employed and not the clinically imprecise term hypothyroidism, which is misleading in the emerging era as shown in this paper.

DISCUSSION

The identification of the pro-oncogenic exogenous thyroxine and its replacement by the metabolically dominant T3 has had a significant impact on the palliation and survival of advanced cancer patients, who live longer. This discovery followed identification of the integrin αvβ3 thyroid hormone receptor and lower binding of T3 than T4. There is also divergence of signaling pathways between the pro-metabolic T3 and the pro-oncogenic T4 (8).

Tetrac is a specific molecular blocker of the thyroid hormone receptor on integrin αvβ3 and is anti-oncogenic. The effects of tetrac occlusion of the receptor reveal the pleiotropic pro-oncogenic unopposed T4 effect on mitogenesis, angiogenesis, and apoptosis (48).

Both T3 and T4 ligand have a receptor on the cancer cell plasma membrane and on dividing vascular endothelial cells. This receptor on integrin αvβ3 is activated to transduce mitogenic signaling to the interior of the cell. Similarly, blocking of the receptor with tetrac effectively blocks all (most) T4 and T3 signal transduction as occurs following T4 and T3 depletion. This results in regression of established tumors, inhibits angiogenesis, and potentiates ionizing radiation-induced cell death (2). Separation and divergence of the effects and functions of T4 from T3 on integrin αvβ3-expressed receptors occurs as a result of binding to the receptor. T4 is significantly more potent than T3 (48) on cancer and blood vessel cells, as noted above.

T4 depletion with use of an antithyroid drug such as methimazole eliminates circulating T4 and T3, whether the latter is derived from T4 in the peripheral circulation or directly released by the thyroid gland. This approach has been utilized in cancer patients with considerable efficacy, resulting in significant prolongation of survival and tumor regression in some patients with large tumor masses, e.g., GBM and soft tissue sarcoma (1).

A rare spontaneous regression of metastatic lung cancer has occurred whereby T4 and T3 became depleted to life threatening levels with myxedema coma (3).

It is of note that there is a declining continuum of risk for free thyroxine levels from high supraphysiological (hyperthyroidism) to frank hypothyroxinemia and to blocking of the integrin αvβ3 thyroid hormone receptor, which would equate to a zero ambient free T4.

FUTURE DIRECTIONS

The capacity/ability to impact on cancer progression in the occult or preclinical stage with only a molecular diagnosis and identification by altering endogenous thyroid hormone (thyroxine) levels might become a minimally morbid, but effective, treatment approach to pre-emptively treat solid tumor types prior to their emergence as clinically evident disease.

SUMMARY

Discovery of integrin αvβ3 receptor and binding to the thyroid hormone ligand have led to the strategy of rapid T4 depletion of the pro-oncogenic stimulus of T4 both in the euthyroid and exogenous T4 supplemented individuals. A significantly faster release from T4 driven tumor growth has resulted in rapid tumor regression, with palliation in life threatening situations and better survival. The discovery of tetrac binding to the receptor promises even greater therapeutic gains. The wide range of tumor types responding to T4 depletion suggests there is potential for globally impacting on cancer from the very early

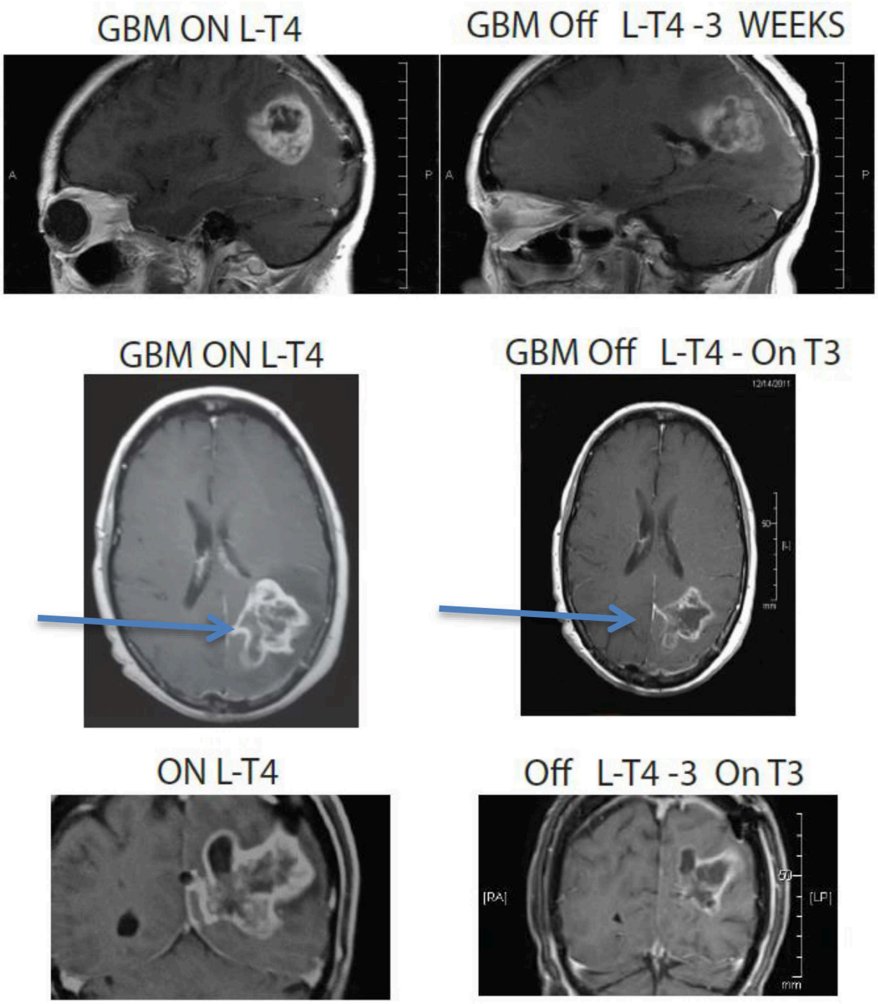

FIGURE 4 | MRI images of a 67 year old female patient who was deteriorating neurologically with hemiplegia unresponsive to high dose dexamethasone. Discontinuation of L-T4 was followed within 1 week by significant clinical improvement and tumor regression. Arrows point to tumor mass, showing reduction in size in all dimensions following cessation of exogenous L-T4.

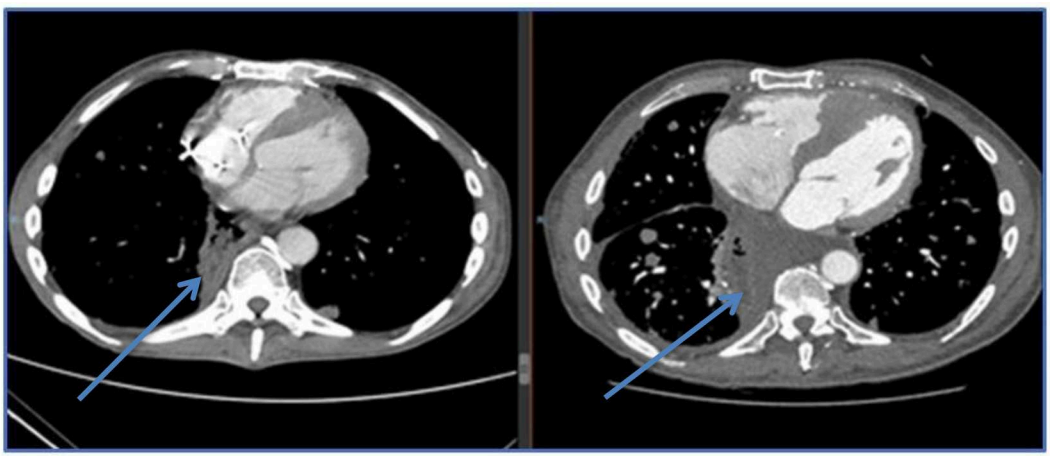

FIGURE 5 | CT images of esophageal sarcoma metastatic with cardiac infiltration and cardiac failure, right image is on exogenous L-T4, left image is post L-T4 discontinuation and oral cyclophosphamide. Patient improved clinically and was discharged from intensive care.

to late stages. It is rational to consider application of this approach to much earlier states of cancer and thereby prolong survival significantly.

CONCLUSION

Identification of the integrin αvβ3 receptor pathway promises a paradigm changing approach to manage and treat cancer more effectively than currently available modalities.

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENTS

The author thanks Kelly A. Keating of the Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY for her editing of the manuscript.

REFERENCES

1. Hercbergs A, Johnson RE, Ashur-Fabian O, Garfield DH, Davis PJ. Medically induced euthyroid hypothyroxinemia may extend survival in compassionate need cancer patients: an observational study. *Oncologist.* (2015) 20:72–6. doi: 10.1634/theoncologist.2014-0308
2. Hercbergs A, Davis PJ, Lin H-Y, Keating KA, Mousa SA. Thyroid Hormone Replacement Therapy in Patients with Various Types of Cancer. in *Hormone Therapy and Replacement in Cancer and Aging-related Diseases*. InTechOpen (2019). Available online at: https://cdn.intechopen.com/pdfs/67201.pdf
3. Hercbergs A, Leith JT. Spontaneous remission of metastatic lung cancer following myxedema coma–an apoptosis-related phenomenon? *J Natl Cancer Inst.* (1993) 85:1342–3. doi: 10.1093/jnci/85.16.1342
4. Hercbergs AA, Goyal LK, Suh JH, Lee S, Reddy CA, Cohen BH, et al. Propylthiouracil-induced chemical hypothyroidism with high-dose tamoxifen prolongs survival in recurrent high grade glioma: a phase I/II study. *Anticancer Res.* (2003) 23:617–26.
5. Bergh JJ, Lin HY, Lansing L, Mohamed SN, Davis FB, Mousa S, et al. Integrin αVβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology.* (2005) 146:2864–71. doi: 10.1210/en.2005-0102
6. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, et al. Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association Task Force on thyroid hormone replacement. *Thyroid.* (2014) 24:1670–751. doi: 10.1089/thy.2014.0028
7. Davis PJ, Davis FB, Mousa SA, Luidens MK, Lin HY. Membrane receptor for thyroid hormone: physiologic and pharmacologic implications. *Annu Rev Pharmacol Toxicol.* (2011) 51:99–115. doi: 10.1146/annurev-pharmtox-010510-100512
8. Lin HY, Sun M, Tang HY, Lin C, Luidens MK, Mousa SA, et al. L-Thyroxine vs. 3,5,3'-triiodo-L-thyronine and cell proliferation: activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. *Am. J. Physiol. Cell Physiol.* (2009) 296:C980–91. doi: 10.1152/ajpcell.00305.2008
9. Davis PJ, Glinsky GV, Lin HY, Leith JT, Hercbergs A, Tang HY, et al. Cancer cell gene expression modulated from plasma membrane integrin αvβ3 by thyroid hormone and nanoparticulate tetrac. *Front Endocrinol.* (2014) 5:240. doi: 10.3389/fendo.2014.00240
10. Brent GA. Mechanisms of thyroid hormone action. *J Clin Invest.* (2012) 122:3035–43. doi: 10.1172/JCI60047
11. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol.* (2016) 12:111–21. doi: 10.1038/nrendo.2015.205
12. Glinskii AB, Glinsky GV, Lin HY, Tang HY, Sun M, Davis FB, et al. Modification of survival pathway gene expression in human breast cancer cells by tetraiodothyroacetic acid (tetrac). *Cell Cycle.* (2009) 8:3562–70. doi: 10.4161/cc.8.21.9963
13. Yalcin M, Bharali DJ, Lansing L, Dyskin E, Mousa SS, Hercbergs A, et al. Tetraidothyroacetic acid (tetrac) and tetrac nanoparticles inhibit growth of human renal cell carcinoma xenografts. *Anticancer Res.* (2009) 29:3825–31.
14. Cayrol F, Sterle HA, Diaz Flaque MC, Barreiro Arcos ML, Cremaschi GA. Non-genomic actions of thyroid hormones regulate the growth and angiogenesis of T cell lymphomas. *Front Endocrinol.* (2019) 10:63. doi: 10.3389/fendo.2019.00063
15. Silva FG, Giannocco G, Santos MF, Nunes MT. Thyroid hormone induction of actin polymerization in somatotrophs of hypothyroid rats: potential repercussions in growth hormone synthesis and secretion. *Endocrinology.* (2006) 147:5777–85. doi: 10.1210/en.2006-0110
16. Hercbergs A, Davis PJ, Lin HY, Mousa SA. Possible contributions of thyroid hormone replacement to specific behaviors of cancer. *Biomed Pharmacother.* (2016) 84:655–9. doi: 10.1016/j.biopha.2016.09.053
17. Hellevik AI, Asvold BO, Bjoro T, Romundstad PR, Nilsen TI, Vatten LJ. Thyroid function and cancer risk: a prospective population study. *Cancer Epidemiol Biomarkers Prev.* (2009) 18:570–4. doi: 10.1158/1055-9965.EPI-08-0911
18. Ko AH, Wang F, Holly EA. Pancreatic cancer and medical history in a population-based case-control study in the San Francisco Bay Area, California. *Cancer Causes Control.* (2007) 18:809–19. doi: 10.1007/s10552-007-9024-6
19. Saraiva PP, Figueiredo NB, Padovani CR, Brentani MM, Nogueira CR. Profile of thyroid hormones in breast cancer patients. *Braz J Med Biol Res.* (2005) 38:761–5. doi: 10.1590/S0100-879X2005000500014
20. Hercbergs A. Spontaneous remission of cancer–a thyroid hormone dependent phenomenon? *Anticancer Res.* (1999) 19:4839–44.
21. Hercbergs A. High tumor response rate to radiation therapy in biochemically hypothyroid patients (Abstract #167). In: *Proceedings of AACR*. (San Diego, CA) 248.
22. Cristofanilli M, Yamamura Y, Kau SW, Bevers T, Strom S, Patangan M, et al. Thyroid hormone and breast carcinoma - primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma. *Cancer.* (2005) 103:1122–8. doi: 10.1002/cncr.20881
23. Backwinkel K, Jackson AS. Some features of breast cancer + thyroid deficiency - report of 280 cases. *Cancer.* (1964) 17:1174. doi: 10.1002/1097-0142(196409)17:9<1174::AID-CNCR2820170913>3.0.CO;2-C
24. Hercbergs A, Daw H, Thakur S. Primary hypothyroidism and breast cancer: clinical and pathological risk reduction correlates from a case control study (Abstract # 178). In: *Proceedings of the 80th Annual Meeting of the American Thyroid Association*. (Palm Beach, FL) (2009).
25. Hercbergs A, Mason J, Reddy C. Thyroid hormones and lung cancer: primary hypothyroidism is prognostically significant for survival in lung cancer (abstract #4440). In: *95th Annual Meeting*, AACR. (Orlando, FL) (2004).
26. Rosenberg AG, Dexeus F, Swanson DA, Von Eschenbach AC. Relationship of thyroid disease to renal cell carcinoma. *An Epidemiol Study Urol.* (1990) 35:492–8. doi: 10.1016/0090-4295(90)80101-R
27. Hercbergs A. The thyroid gland as an intrinsic biologic response-modifier in advanced neoplasia - a novel paradigm. *In Vivo.* (1996) 10:245–7.
28. Hercbergs A, Setrakian S, Abdelmannan D. Anaplastic thyroid CA: 10-year survival in a man with uncorrected primary hypothyroidism. *Thyroid.* (2007) 17:S—93.
29. Hercbergs A, Daw H, Ayoub W. Possible association of hypothyroidism with longevity in advanced colorectal and pancreatic cancer: a case study. In: *79th Annual Meeting of American Thyroid Association*. (Chicago, IL: Thyroid) (2008).

30. Burt J, Schapira D. Thyroid hormone: a modifier of the natural history of breast cancer (Abstract # 738). In: *Annual Meeting of the American Association for Cancer Research*. (San Diego, CA) (1983).
31. Sabatier RGG, Deville, J. Hypothyroidism and survival during sunitinib therapy in metastatic renal cell cancer: a prospective observational analysis (Abstract # 317). In: *ASCO Genitourinary Cancers Symposium*. (Orlando, FL) (2009).
32. Nelson M, Hercbergs A, Rybicki L, Strome M. Association between development of hypothyroidism and improved survival in patients with head and neck cancer. *Arch Otolaryngol-Head Neck Surgery*. (2006) 132:1041–6. doi: 10.1001/archotol.132.10.1041
33. Smith GL, Smith BD, Garden AS, Rosenthal DI, Sherman SI, Morrison WH, et al. Hypothyroidism in older patients with head and neck cancer after treatment with radiation: a population-based study. *Head Neck*. (2009) 31:1031–8. doi: 10.1002/hed.21066
34. Atkins MB, Mier JW, Parkinson DR, Gould JA, Berkman EM, Kaplan MM. Hypothyroidism after treatment with interleukin-2 and lymphokine-activated killer cells. *N Engl J Med*. (1988) 318:1557–63. doi: 10.1056/NEJM198806163182401
35. Weijl NI, Vanderharst D, Brand A, Kooy Y, Vanluxemburg S, Schroder J, et al. Hypothyroidism during immunotherapy with interleukin-2 Is associated with antithyroid antibodies and response to treatment. *J Clin Oncol*. (1993) 11:1376–83. doi: 10.1200/JCO.1993.11.7.1376
36. Hercbergs ASJ, Reddy C, Goyal L. Early onset propylthiouracil-induced hypothyroidism is associated with improved survival in recurrent high grade glioma (Abstract # 1211). In: *99th Annual Meeting AACR*. San Diego, CA (2008).
37. Linetsky E, Hercbergs A, Dotan S. Time to tumor progression (TTP) and quality of life (QOL) following propylthiouracil induction of chemical hypothyroidism in failed malignant gliomas (Abstract # 144). In: *World Federation of Neuro-Oncology – Second Quadrennial Meeting*. (Edinburgh) (2005).
38. Hercbergs AH, Ashur-Fabian O, Garfield D. Thyroid hormones and cancer: clinical studies of hypothyroidism in oncology. *Curr Opin Endocrinol Diabetes Obes*. (2010) 17:432–6. doi: 10.1097/MED.0b013e32833d9710
39. Davis FB, Tang HY, Shih A, Keating T, Lansing L, Hercbergs A, et al. Acting via a cell surface receptor, thyroid hormone is a growth factor for glioma cells. *Cancer Res*. (2006) 66:7270–5. doi: 10.1158/0008-5472.CAN-05-4365
40. Lin HY, Tang HY, Shih A, Keating T, Cao G, Davis PJ, et al. Thyroid hormone is a MAPK-dependent growth factor for thyroid cancer cells and is anti-apoptotic. *Steroids*. (2007) 72:180–7. doi: 10.1016/j.steroids.2006.11.014
41. Yalcin M, Dyskin E, Lansing L, Bharali DJ, Mousa SS, Bridoux A, et al. Tetraiodothyroacetic acid (tetrac) and nanoparticulate tetrac arrest growth of medullary carcinoma of the thyroid. *J Clin Endocrinol Metab*. (2010) 95:1972–80. doi: 10.1210/jc.2009-1926
42. Meng R, Tang HY, Westfall J, London D, Cao JH, Mousa SA, et al. Crosstalk between integrin αvβ3 and estrogen receptor-α is involved in thyroid hormone-induced proliferation in human lung carcinoma cells. *PLoS ONE*. (2011) 6:e27547. doi: 10.1371/journal.pone.0027547
43. Yalcin M, Lin HY, Sudha T, Bharali DJ, Meng R, Tang HY, et al. Response of human pancreatic cancer cell xenografts to tetraiodothyroacetic acid nanoparticles. *Horm Cancer*. (2013) 4:176–85. doi: 10.1007/s12672-013-0137-y
44. Hsieh M-T, Wang L-M, Changou CA, Chin Y-T, Yang Y-CSH, Lai H-Y, et al. Crosstalk between integrin αvβ3 and ERα contributes to thyroid hormone-induced proliferation of ovarian cancer cells. *Oncotarget*. (2016) 8:24237–49. doi: 10.18632/oncotarget.10757
45. Lin HY, Chin YT, Yang YC, Lai HY, Wang-Peng J, Liu LF, et al. Thyroid hormone, cancer, and apoptosis. *Compr Physiol*. (2016) 6:1221–37. doi: 10.1002/cphy.c150035
46. Mousa SA, Yalcin M, Bharali DJ, Meng R, Tang HY, Lin HY, et al. Tetraiodothyroacetic acid and its nanoformulation inhibit thyroid hormone stimulation of non-small cell lung cancer cells in vitro and its growth in xenografts. *Lung Cancer*. (2012) 76:39–45. doi: 10.1016/j.lungcan.2011.10.003
47. Bharali DJ, Yalcin M, Davis PJ, Mousa SA. Tetraiodothyroacetic acid-conjugated PLGA nanoparticles: a nanomedicine approach to treat drug-resistant breast cancer. *Nanomedicine*. (2013) 8:1943–54. doi: 10.2217/nnm.12.200
48. Lin HY, Cody V, Davis FB, Hercbergs AA, Luidens MK, Mousa SA, et al. Identification and functions of the plasma membrane receptor for thyroid hormone analogues. *Discov Med*. (2011) 11:337–47.

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

The handling editor declared a past co-authorship with the author.

frontiers in Endocrinology

REVIEW
published: 30 August 2019
doi: 10.3389/fendo.2019.00532

The Role of Thyroid Hormones in Hepatocyte Proliferation and Liver Cancer

*Fabio Gionfra[1], Paolo De Vito[2], Valentina Pallottini[1], Hung-Yun Lin[3,4,5,6,7], Paul J. Davis[5,7], Jens Z. Pedersen[2] and Sandra Incerpi[1]**

[1] Department of Sciences, University Roma Tre, Rome, Italy, [2] Department of Biology, University of Rome Tor Vergata, Rome, Italy, [3] Ph.D. Program for Cancer Molecular Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan, [4] Taipei Cancer Center, Taipei Medical University, Taipei, Taiwan, [5] Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, United States, [6] Traditional Herbal Medicine Research Center of Taipei Medical University Hospital, Taipei Medical University, Taipei, Taiwan, [7] Department of Medicine, Albany Medical College, Albany, NY, United States

OPEN ACCESS

Edited by:
Gabriella Castoria,
Second University of Naples, Italy

Reviewed by:
Marzia Di Donato,
University of Campania Luigi Vanvitelli, Italy
Jiayi Wang,
Shanghai Jiaotong University, China

***Correspondence:**
Sandra Incerpi
sandra.incerpi@gmail.com

Specialty section:
This article was submitted to Cancer Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 25 March 2019
Accepted: 17 July 2019
Published: 30 August 2019

Citation:
Gionfra F, De Vito P, Pallottini V, Lin H-Y, Davis PJ, Pedersen JZ and Incerpi S (2019) The Role of Thyroid Hormones in Hepatocyte Proliferation and Liver Cancer. Front. Endocrinol. 10:532. doi: 10.3389/fendo.2019.00532

Thyroid hormones T3 and T4 (thyroxine) control a wide variety of effects related to development, differentiation, growth and metabolism, through their interaction with nuclear receptors. But thyroid hormones also produce non-genomic effects that typically start at the plasma membrane and are mediated mainly by integrin αvβ3, although other receptors such as TRα and TRβ are also able to elicit non-genomic responses. In the liver, the effects of thyroid hormones appear to be particularly important. The liver is able to regenerate, but it is subject to pathologies that may lead to cancer, such as fibrosis, cirrhosis, and non-alcoholic fatty liver disease. In addition, cancer cells undergo a reprogramming of their metabolism, resulting in drastic changes such as aerobic glycolysis instead of oxidative phosphorylation. As a consequence, the pyruvate kinase isoform M2, the rate-limiting enzyme of glycolysis, is dysregulated, and this is considered an important factor in tumorigenesis. Redox equilibrium is also important, in fact cancer cells give rise to the production of more reactive oxygen species (ROS) than normal cells. This increase may favor the survival and propagation of cancer cells. We evaluate the possible mechanisms involving the plasma membrane receptor integrin αvβ3 that may lead to cancer progression. Studying diseases that affect the liver and their experimental models may help to unravel the cellular pathways mediated by integrin αvβ3 that can lead to liver cancer. Inhibitors of integrin αvβ3 might represent a future therapeutic tool against liver cancer. We also include information on the possible role of exosomes in liver cancer, as well as on recent strategies such as organoids and spheroids, which may provide a new tool for research, drug discovery, and personalized medicine.

Keywords: integrin αvβ3, deiodinase, hypothyroidism, tetrac, celiac disease, exosomes, organoids, spheroids

INTRODUCTION

Thyroid hormones 3,5,3′-triiodothyronine (T3) and 3,5,3′,5′-tetraiodothyronine (T4) play an essential role in the regulation of cell function during growth, development and metabolism, through two different mechanisms: genomic and non-genomic. The genomic action takes place through the classical nuclear receptors TRα and TRβ, together with modulatory factors such as coactivators and corepressors to regulate gene expression and protein synthesis (1). TRα can stimulate both proliferation and differentiation through β-catenin, while TRβ shows antiproliferative effects in cancer cells and is a differentiation factor. Loss of TRβ is followed by oncogenic transformation. Thyroid hormone receptors and estrogen receptors can cross-talk to modulate physio-pathological responses (2, 3). But thyroid hormones may also give rise to non-genomic effects mediated by integrin αvβ3. These non-genomic effects mainly occur at the plasma membrane level and involve membrane transport systems such as the transporters for glucose and amino acids, the Na^+/H^+ exchanger, Na^+/K^+-ATPase activity, and kinase activities such as Mitogen-Activated Protein Kinase (MAPK) and Phosphatidyl Inositol 3-Kinase (PI3-K) (4–6), thus increasing angiogenesis and tumor cells proliferation. Integrins are plasma membrane integral proteins that bind extracellular matrix (ECM) proteins such as vitronectin, fibronectin and osteopontin, and regulate cell-cell adhesion (7). Both hormones bind to integrin αvβ3, but T3 binds to the S1 receptor site, activating PI3-K through src, whereas both T3 and T4 bind to a second site S2, leading to the activation of the MAPK pathway and cell proliferation (8). Integrin αvβ3 can also bind other small molecules, such as resveratrol and the steroid hormones (estrogens and androgens) and cancer growth may be modulated by this type of interaction [(9); **Figure 1**].

THE LIVER

The liver can be considered privileged from an immunological point of view because it receives 75% of its blood from the portal vein coming from splanchnic districts and about 25% from hepatic artery blood. The major fraction from the portal vein is blood coming from the intestine, stomach, spleen, pancreas, and other organs. Therefore, the portal blood contains components from intestinal uptake of nutrients without the lipid components that go to the lymphatic vessels (10). This special feature of the liver cells makes human hepatocellular carcinoma (HCC) the second leading cause of death in the world.

The liver is composed mainly of hepatocytes, but there is also a small fraction of cells that are important both in physiology and pathology, such as Kupffer cells of the immune system with phagocyte activity, endothelial cells, and Hepatic Stellate Cells. The liver, unlike other tissues in the body, is capable of renewing itself in a very efficient way, and many papers have been published on liver regeneration after partial hepatectomy. The pathways followed in the regenerative process have turned out to be interesting because it is possible in this way to obtain knowledge on cell proliferation, differentiation and tumor growth (11). The first phase of regeneration, called the "priming phase," prepares the cells to respond to growth factors. The second phase is initiated by the activation of growth factor receptors, and among these the most important appears to be the Epidermal Growth Factor Receptor (EGFR) and c-MET or Hepatocyte Growth Factor Receptor (11). They act in concert until the end of the regenerative process, and then proliferation stops. Important inhibitors of liver regeneration are transforming growth factor-β (TGF-β) and the integrins, which allow the communication between ECM proteins and the cells. Thus, thyroid hormones are important modulators of the regeneration process, because they are able to cross-talk with growth factors such as EGF, TGF-β, and IGF-1 as well as with the integrins, essential players in the mechanism of thyroid hormones (12).

The aim of the present paper is an evaluation of our current knowledge of thyroid hormones in the liver and of the mechanisms related to cell growth and metabolism that may lead to liver cancer. We consider some particular features of liver cells, such as regeneration and the capability to give rise to several metabolic pathologies (fibrosis, cirrhosis, non-alcoholic fatty liver disease). We also consider the possibility that exosomes might modulate thyroid hormone responses in the context of liver cancer, and we provide some information on the frontiers of biotechnology concerning organoids and spheroids.

THYROID HORMONES AND LIVER DISEASE

The liver represents a major target for thyroid hormones, which are involved in the regulation of body weight, lipogenesis, lipid metabolism, and insulin resistance. Therefore, they may have a key role in the pathogenesis of several diseases that affect the liver, such as Alcoholic Liver Disease and non-alcoholic steatohepatitis (NASH), which may evolve into cirrhosis and HCC. Among the thyroid hormone receptors, TRβ is the one mainly expressed in the liver, while TRα is more common in the cardiovascular system and in bone (13). The role of TRβ in mice was demonstrated by the group of Cheng, studying a dominant negative mutation in TRβ ($Thr\beta^{PV/PV}$). These mice develop hepatic steatosis within a few months and have significantly larger livers (14). The mutated mice show increased activation of Peroxisome Proliferator–Activated Receptor-γ signaling and decreased fatty acid β-oxidation, leading to lipid accumulation and increased hepatic triglyceride content (14). At variance with this, mice with a mutation in TRα ($Thr\alpha^{PV/PV}$) showed decreased weight and less hepatic lipid accumulation and also decreased lipogenesis.

Thyroid hormones increase the levels of free fatty acids by stimulating lipolysis from dietary fats, although they also stimulate the uptake of free fatty acids by the fatty acid binding protein and fatty acid translocase. The conversion of glucose to fatty acids and *de novo* lipogenesis is stimulated by other hormones and by the diet. Thyroid hormones also regulate the expression and activities of many transcription factors involved in lipogenesis, such as the Sterol Regulatory Element-Binding Protein (SREBP)-1C, liver X receptors and Carbohydrate-Responsive Element-Binding Protein (15). Despite the role of

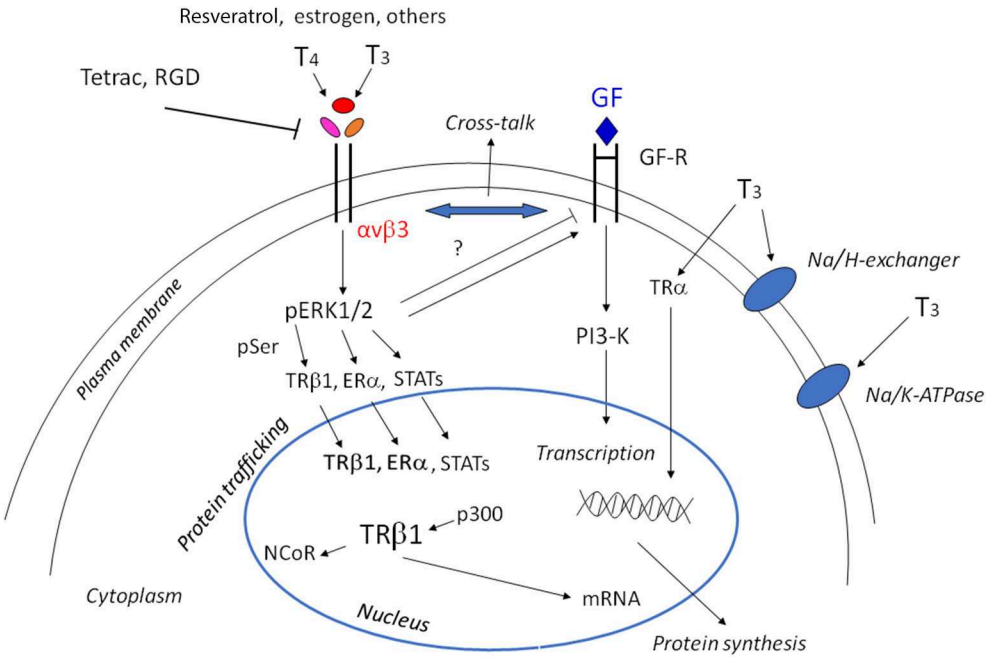

FIGURE 1 | Scheme of non-genomic and genomic actions of thyroid hormones. Non-genomic actions start at the integrin αvβ3, through MAPK/ERK1/2 they may go to the cytoplasm and nucleus to modulate gene expression. The shuttling of the αv monomer to the nucleus is not shown for the sake of simplicity. The cross-talk of thyroid hormones with growth factors is indicated by the double arrow and can be modulated by ERK1/2 activation. The modulation of the membrane Na/K-ATPase pump, either in activation or inhibition depending on the cell type and context, and activation of the Na/H exchanger is also indicated. In the nucleus, interaction of T3 gives rise to the shedding of the corepressor and the interaction with coactivators. Downstream of the activation of TRβ1, Erα, STATS there is the activation of tumor cell proliferation, angiogenesis, and growth factors (GF), but also cytokines through JAK1/2.

thyroid hormones in *de novo* lipogenesis, they do not increase triglyceride levels but reduce the apolipoprotein B100 and also Very Low-Density Lipoproteins (VLDL) and Low-Density Lipoproteins (LDL) (16). Thyroid hormones also maintain constant sterol levels by modulating all possible pathways of synthesis, export, import, and the conversion to bile acids. In particular, thyroid hormones induce the expression of the limiting enzyme of the cholesterol synthesis, HMG-CoA reductase (17).

Liver fibrosis and cirrhosis are characterized by chronic damage to liver tissue, leading to chronic inflammation, and to altered matrix tissue generation and vascularization. Therefore, the liver progressively looses its functions and this may give rise to the development of cancer. An important role in liver tissue regulation and dysregulation is provided by the ECM proteins that convey information from cell to cell and also from the extracellular to the intracellular compartments. These proteins, which include integrins and collagen, may be important for tissue remodeling and also in the progression of fibrosis, cirrhosis, and cancer (18, 19).

Alcoholic fatty liver disease and non-alcoholic fatty liver disease (NAFLD) represent a major public problem all over the world. Alcohol abuse is the primary cause of several diseases such as fatty liver, alcoholic hepatitis, and cirrhosis (20, 21). Alcohol is metabolized by the liver, which is the primary site of damage. Alcoholic steatohepatitis follows Alcoholic Liver Disease and is characterized by hepatic fat accumulation, infiltration of inflammatory cells, and injury to liver tissue. The process of infiltration by macrophages and neutrophils is mediated by osteopontin produced by the liver. The effects of this protein can be mediated by integrins (22), and osteopontin also appears to be involved in NAFLD/NASH diseases. This cytokine is increased in model systems of these pathologies. Compared to wild type animals, osteopontin-knock-out mice showed decreased liver injury and fibrosis (22). Osteopontin levels are instead increased in some models of liver injury, such as treatment with CCl_4, although the mechanisms are not clear at present (23).

Epidemiological and clinical reports show an association between NAFLD/NASH and thyroid dysfunction in the form of established or subclinical hypothyroidism. The percentage of hypothyroidism was 15–36% among patients with NAFLD/NASH (20). It has been suggested that NAFLD/NASH are hepatic markers of insulin resistance and metabolic syndrome (24, 25), and insulin resistance can in part be prevented by treating hypothyroidism (20). Among the possible mechanisms proposed is a role of adipocytokines in NAFLD in the presence of hypothyroidism (26). An increased level of leptin has also been reported for hypothyroid patients; this may be responsible for the development of NAFLD/NASH. Leptin is an adipocytokine; it is increased in obesity and may give rise to insulin resistance (27). NAFLD patients

show abnormal lipid profiles, with high levels of cholesterol, LDL, and triglycerides. Thyroid hormones acting through the β receptor may cause a reduction of body weight and fat and a decrease in cholesterol and triglyceride levels in hepatocytes (28, 29).

In the context of liver cancer and liver pathologies, the possible effects of oxidative stress, mitochondrial dysfunction, and reactive oxygen species (ROS) production should also be mentioned. Oxidative stress alters the activity of deiodinases, as discussed in the paragraphs that follow, and thyroid hormones can modulate cell function through oxidative stress (30, 31). The role of ROS in thyroid hormone signaling is well-known from the cross-talk between thyroid hormones and the immune system (32, 33).

Liver fibrosis begins with some damage to liver cells that can be of different nature: physical injury, infection by virus or bacteria (lipopolysaccharides), alcohol, etc. This gives rise to mitochondrial dysfunction and an increase in free fatty acids and ROS, leading to lipid peroxidation, activation of Kupffer cells and Hepatic Stellate Cells. In the case of hepatic injury, expression of the nuclear thyroid hormone receptor in Hepatic Stellate Cells is inhibited, and the dominant hormone receptor becomes TRα, which participates in the fibrogenic response, producing a stronger wound-healing response and higher contractility (34). The levels of inflammatory cytokines increase, causing a further increase in ROS formation, impairment of deiodinase activity, an increase in cell proliferation, and ultimately fibrosis leading to cancer, as will be described in the following sections [(20, 35); **Figure 2**].

Oxidative markers and inflammation actually appear very early in younger populations as well. A recent paper showed that Ox-LDL and the serum level of Triggering Receptor Expressed on Myeloid cells-1 are associated with cardiovascular risk and other health risks (36). In humans the association of oxidative and inflammatory markers with cholesterol levels, reported for a young healthy population, indicates that it could be very important to start early with an evaluation of these markers, in order to prevent future cardiac pathologies (36). The paper cited does not deal with liver diseases, but it draws attention to the condition of a young human population and their lifestyle. Prevention of some diseases such as liver diseases should start as soon as possible.

In line with the previous topic, we want to recall another pathology affecting more and more children and adolescents: Celiac disease. This is an inflammatory disease of the gut that may develop when persons are exposed to a gluten-containing diet. The intestine as well as the liver are the organs mainly involved, particularly in the young population affected by NAFLD, the most common liver disease in school-age individuals. Very often the diagnosis of Celiac disease precedes that of liver disease (37). Celiac disease is often present in hypothyroid patients, who are more prone to develop cancer and in particular liver cancer (38, 39). Therefore, the development of liver disease and hypothyroidism is becoming more complicated for younger generations.

THYROID HORMONE PATHWAYS LEADING TO PHYSIO-PATHOLOGICAL RESPONSES IN LIVER DISEASES UP TO CANCER

Normal hepatocytes replicate by entering the cell cycle, and in the presence of an injury the process is the same, but it may become dysregulated following conspicuous tissue damage with associated oxidative stress. In any case a regenerative response takes place. In the period of injury-activated regeneration, genes that normally are quiescent become activated through a processes recalling fetal development. Among these processes is the activation of deiodinases, seleno-dependent enzymes that are able to both activate and inactivate thyroid hormone formation in the peripheral tissues (40–42). In particular the levels of deiodinase 3, which hydrolyzes and inactivates both T3 and T4, become upregulated following liver damage and oxidative stress, and the result is a decrease in active T3 levels and increased formation of reverse T3 (rT3) and increased cell proliferation (43–47). Elevated levels of deiodinase 3, mediated by HIF-1, are also reported in both fetal and cancer development (48–50). In liver injury, hepatocytes show a decreased expression of deiodinase 1 and increased levels of deiodinase 3; these variations are regulated by Hedgehog ligands (51). Deiodinase 3 is also more expressed in non-differentiated tissues, such as the developing embryo and cancer (45, 51). Tumor growth or HCC give similar responses to development and injury, and hypothyroidism is associated with a 2- to 3-fold increased risk of cancer development in women. A similar association has not been reported for men (52–54).

Deiodinase 2 is not highly expressed in the liver tissue of an adult, although it is briefly expressed in mouse hepatocytes around birth. This brief appearance seems to be important for the future sensitivity to diet-induced lipid asset for the posttranslational modifications involving DNA methylation and leading to hepatic steatosis, hyperlipidemia and obesity (55). This has been demonstrated by the development of D2-KO mice (ALB-D2KO) with a selective inactivation of deiodinase 2, the resulting phenotype shows resistance to steatosis, hyperlipidemia and obesity. The same researchers also studied the molecular mechanisms involved in this mouse phenotype; the results show that this decreased vulnerability to liver steatosis and diet-induced obesity in the ALB-D2KO mice is due to a reduction in the hepatocyte expression of liver zinc-finger protein-125 (zfp125), a FoxO1-inducible transcriptional repressor responsible for lipid accumulation through a reduced secretion of VLDL. The situation is complicated from both metabolic and hormonal points of view because Forkhead box O1 (FoxO1) is known to be inhibited by insulin, which normally decreases the lipidemia (56).

T3 acts as a mitogen via Protein Kinase A (PKA)/β-catenin activation, leading to activation of cyclin D1 in normal hepatocytes (50). Thyroid hormones have a very complex interaction with their receptors and deiodinases. T3/TR interaction leads to inhibition of the Wnt/β-catenin pathway via Dickkopf Wnt signaling inhibitor 4 (DKK4), resulting in the inhibition of hepatoma cell proliferation

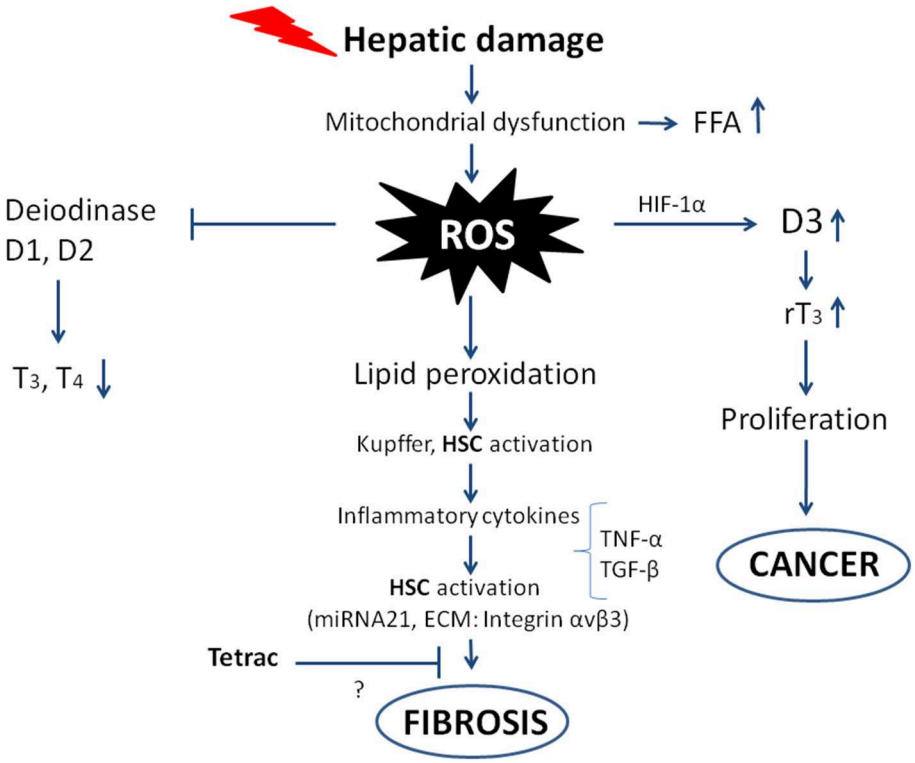

FIGURE 2 | Scheme showing the possible pathways from hepatic damage to fibrosis leading to cancer. The oxidative stress and ROS production follow mitochondrial dysfunction and hepatic damage. The direct consequences are impairment of deiodinase activity leading to decreased T3 production, on the other hand deiodinase 3 is imbalanced with increased activity and increased rT3, which stimulates the proliferation of tumor cells. The ROS produced give rise to inflammatory cytokines that increase the ROS and activate Hepatic Stellate Cells (HSC), leading to fibrosis and eventually cancer. The inhibition by tetrac of the fibrogenic process is only suggested as shown by the question mark, with integrin αvβ3 being among the ECM components involved in the "activation" of the Hepatic Stellate Cells.

(43, 57–59). Hypothyroidism is also associated with human cancer, although contradictory results have been reported relating cancer progression and thyroid hormones (60, 61). For example, primary hypothyroidism has been associated with a decreased risk of breast cancer (61). This effect may depend on non-genomic actions of thyroid hormones because mutant TRs inhibit transactivation activity in glioma and breast cancer (62). In fact, hypothyroidism is involved in different metabolic pathologies, such as obesity, type 2 diabetes, insulin resistance and cancer (60). The impairment of thyroid hormone homeostasis is not considered sufficient for HCC development, but other liver pathologies must be present in order to impair this equilibrium and eventually start a pro-carcinogenic process, such as inflammation, fibrosis or cirrhosis (3, 63, 64).

The downregulation of nuclear thyroid hormone receptors may act as a signal for tumorigenesis, supporting the concept that thyroid hormone receptors inhibit tumorigenesis (3). In fact, a switch from hypo- to hyper-thyroid conditions can be antitumorigenic (60). This clearly indicates that thyroid hormone signaling and thyroid hormone receptors are important for HCC progression (40). In particular T3 seems to have oncosuppressor properties, although it stimulates proliferation in hepatocytes and other cell types, but at the same time it inhibits the growth of hepatoma cells by increasing the time of the G1 phase of the cell cycle. This is related to a decreased expression of the cell cycle mediator cyclin-dependent kinase 2 and cyclin E, and increased gene expression of transforming growth factor TGF-β (65). Other studies confirm these effects of TH receptor β, although contradictory results have also been reported for a human hepatoma cell line (66).

The liver is a major target for thyroid hormones, and in fact, a higher number of mutations of the thyroid hormone receptors α and β have been found in the liver, also in association with the development of liver cancer. However, so far no clear indication has been found, as the situation of the signaling of the thyroid hormone receptor appears to be quite complicated, not only because of the two different typologies of signaling, non-genomic and genomic, but also because non-genomic and genomic effects of thyroid hormones can cross-talk. In addition, TR-α knockout mice are protected from diet-induced hepatic steatosis and hepatic insulin resistance (67). The TRα mutants in HCC act as dominant negative inhibitors in spite of the concentration of T3, impairing gene transcription (3, 68, 69). At variance with these results, the TRβ mutants play a dominant negative effect only at low-intermediate concentrations. In conclusion, TR mutants may have different effects and roles in the development of cancer (60).

TRβ1 can inhibit the nuclear signaling pathways in HCC and breast cancer cells (70). In agreement with these data, a

new role for TRβ1 as an anti-metastatic factor has been shown because it inhibits activation of both ERK and PI3K pathways (3, 69, 71). Mutants of TRα1 and TRβ1 from HCC show many alterations from the WT receptors, which indicate that these mutants may act as repressors or activators of specific genes. A similar situation has also recently been shown in the development of renal clear cell carcinoma, and this causes resistance to thyroid hormones (72). In any case, hypothyroidism is associated with the development of cancer in human beings, probably by decreasing apoptosis, while v-ErbA transgenic mice develop liver cancer because v-ErbA may be a dominant-negative receptor (73). As mentioned above, hypothyroidism is also a risk factor for other pathologies, such as NASH (74), and also for viral hepatitis and alcoholic liver disease (52).

The demonstration that v-ErbA can give rise to tumor formation first came from Barlow et al. (73) who created a transgenic mouse with an ectopic expression of v-ErbA. These animals are affected by hypothyroidism, reduced fertility, decreased body weight and abnormal behavior. The male mice also developed hepatocellular carcinoma. v-ErbA has oncogenic potential through its ability to increase the transformation capability of other oncogenes. Parallel studies have also shown that v-ErbA promotes tumorigenesis by interfering with the AP-1 pathway because v-ErbA prevents the inhibition of the AP-1 pathway through thyroid hormone receptors. Estrogens may block the v-ErbA effect and this could explain the protective effects of estrogens toward neoplastic transformation in females. At variance with this, androgens would be permissive toward oncogenic transformation due to v-ErbA (73). Typically, glucocorticoids inhibit the activation of AP-1 and in this way they become potent anti-inflammatory agents (75).

As to the link between viral hepatitis and hypothyroidism, it was found that in patients with chronic hepatitis there was an emergency response shown by the increased levels of thyroperoxidase antibodies (AbTPO), and the subjects positive for AbTPO had a higher risk of hypothyroidism. In cells in culture, HCV infection had a role in thyroid autoimmunity, suggesting an interaction between HCV and the thyroid. At variance with this, patients with chronic hepatitis B virus infection were less prone to autoimmune thyroid disease (76, 77).

Several studies have suggested that thyroid hormones stimulate tumor growth because they stimulate cell proliferation in several types of cancer cells. Thyroid status also affects tumor progression and metastasis both in animals and human beings (60).

Hercbergs et al. showed that hypothyroxinemia can be a compassionate strategy to prolong the life expectancy of terminal tumor patients (78). This is based on a methimazole therapy, to keep low Thyroxine, free T4 and TSH levels, and at the same time to have a normal euthyroid condition through the administration of T3. T3 inhibits tumor cell growth, but an impairment of TH homeostasis alone is not enough to decrease HCC development and invasion. In the liver in particular, HCC develops after a slow progression from liver fibrosis, chronic liver injury and cirrhosis, up to the pre-cancerous alterations with the pre-symptomatic feature being the downregulation of nuclear receptors, TRα and TRβ (3). Therefore, there is no contradiction

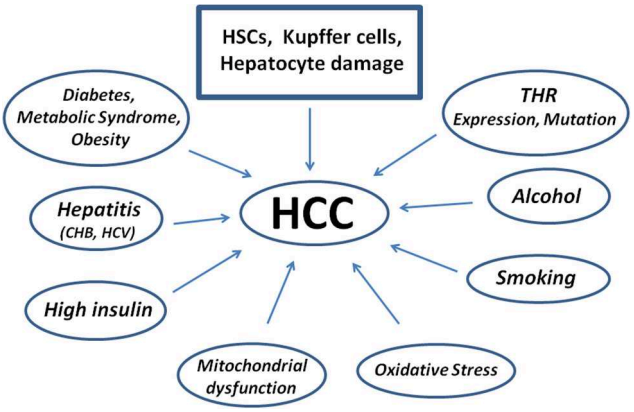

FIGURE 3 | Scheme showing diseases and factors leading to the pathogenesis of Human Hepatocellular Carcinoma starting from Hepatic Stellate Cells damage. Kupffer cells contribute to repair, but may also impair the damage as well as hepatocytes. CHB, Chronic Hepatitis B; HCV, Hepatitis C Virus.

between the data reported by Hercbergs et al. and the finding of an association between hypothyroidism and liver pathologies such as cancer (3, 78). Actually, among the patients participating in the study of Hercbergs et al. only one was affected by a liver tumor, and not all tissues behave in a similar way. The compassionate therapy reported by Hercbergs et al. that can be induced by either thyroidectomy or pharmacologically (by PTU or methimazole, perhaps also TR-KO) is in agreement with the association between thyroid hormone levels and liver cancer. In fact, hypothyroidism leads to a delay in hepatic regeneration as reported before (79, 80). It is difficult to summarize all the contributions dealing with thyroid hormones and liver cancer development. We refer the reader to an excellent review on the epidemiology of liver cancer (81), and another very good recent review where both *in vitro* studies as well as preclinical and clinical studies report on thyroid hormones and cancer (82).

The factors possibly leading to HCC are reported in **Figure 3**. As to possible genetic markers, microRNA and their dysregulation are genetic factors strongly associated to the pathogenesis of tumor growth and HCC. Exosomes with high levels of miRNA exit the cell and contribute to the spread and invasion of the tumor through activation of the Phosphatase and tensin homolog/3-Phosphoinositide-dependent protein kinase 1/Akt signaling pathway, better known as PTEN/PDK1/Akt (83).

METABOLISM OF CANCER CELLS AND THYROID HORMONES: PKM2

The progression of cancer also depends on metabolism. In particular, tumor cells have an increased aerobic glycolysis and lactic acid production; for cancer cells this process is called the Warburg effect (84). It was later found that there is a connection between mitochondria and thyroid hormones in the modulation of this process (85, 86). Suhane and Ramanujan evaluated different metabolic parameters and activities in breast cancer cells, such as lactate generation, oxygen consumption,

mitochondrial viability by the MTT assay, and hexokinase activity as the first step of glycolysis. They found that T3 directly increases the metabolism of mitochondria in breast cancer cells and also the expression of one of the isoforms of pyruvate kinase that is responsible for the Warburg effect (85).

Pyruvate kinase catalyzes the last step of glycolysis that converts the phosphoenolpyruvate to pyruvate through the transfer of one phosphate group to ADP (86, 87). Mammals have four isoforms encoded by two genes: PKL is found in liver and other tissues; PKM is present in two isoforms, PKM1 and PKM2, that show the same catalytic activity. PKM2 is more active in regenerating tissue, embryogenesis and cancer, but is also present in non-proliferating and differentiated tissues (88, 89). Therefore, active PKM2 is important for cancer cell metabolism and survival. Cells transformed to express PKM1 instead of PKM2 switched from aerobic glycolysis to mitochondrial respiration and were unable to give rise to tumor formation (90).

Recently Zhao et al. used a xenograft in a murine model to show that PKM2 is able to activate the nuclear transcription factor SREBP-1a, leading to cell proliferation and increased tumor progression. The interaction appears to be highly specific for this type of SREBP-1a (91, 92). This is an important result as it confirms the role of lipid accumulation in cancer progression. Gnoni et al. showed that in HepG2 cells, T3 activates SREBP and the effect is inhibited by tetrac (93).

Hedgehogs (Hh) are a morphogen family that represents an evolutionary highly conserved pathway, from *Drosophila* to human beings. These proteins are able to move from the cell membrane to the nucleus; they have an essential role in embryonic development, and dysregulation of Hh may lead to tumor development (94). The cAMP/PKA pathway is an important negative modulator of the Hh pathway. PKA is important for Sonic Hedgehog (Shh), the main Hh paralog, in fact it phosphorylates Gli (glioma-associated oncogenes) transcription factors repressing gene transcription (95).

Thyroid hormones are tumor suppressors and inhibitors of Shh signaling in Basal Cell Carcinoma. This inhibition may be mediated by deiodinase cross-talk, in particular an increase in deiodinase 3 via Shh/Gli2 leading to a decrease in T3 and increase of rT3, as reported above. Hedgehog-depleted mice show elevated thyroid hormone levels because thyroid hormones are tumor suppressors and inhibitors of Shh signaling in Basal Cell Carcinoma (96). The cAMP/PKA pathway has the opposite effects on Shh signaling. The same Hedgehog pathway is activated in many other pathologies affecting the human liver, including NAFLD and liver fibrosis (51, 97). The increase in deiodinase 3 leads to decreased T3 levels available to modulate gene expression, including the conversion of PKM2 from tetramer conformation to the dimer/monomer conformation that slows cancer progression (88). cAMP (or forskolin) has the opposite effects, activating D2 and therefore the production of T3 from T4 (49, 88). In conclusion, hypothyroidism is a condition that may lead to cancer progression due to rT3 stimulation of cell proliferation, but also because the decrease in T3 leads to an increased activity of the glycolytic pathway typical for cancer cells (88).

MOLECULAR MECHANISMS MODULATED BY THYROID HORMONES, THROUGH INTEGRIN αVβ3 INVOLVED IN LIVER CANCER

The family of metalloproteinases consists of more than 20 structurally related, zinc-dependent endopeptidases, that are able to degrade (but also activate) different components of the ECM, such as growth factors, cytokines, and chemokines that reside in the ECM, nowadays considered an important player in cancer progression (98). Through their proteolytic activity they play a role in cancer metastasis and invasion by regulating the signaling pathways involved in cell growth, survival, metastasis and invasion, but also angiogenesis and inflammation (99). Therefore, Matrix Metalloproteinases (MMPs), in particular MMP-2 and MMP-9, are involved in cancer metastasis, and inhibitors of MMP are studied as possible antitumor tools. One of the possible effects is the lysis of the ECM components. Thyroid hormones increase the expression of these MMPs, and a nano-formulation of tetraiodothyroacetic acid, Nano-diamino-tetrac, is able to downregulate the expression of MMP-2 and MMP-9 (100, 101).

The growth of normal tissues, as well as the growth of tumors, depend on the local formation of vasculature, and research on cancer treatment has focused on vascular targets and related growth factors, such as VEGF and basic fibroblasts growth factors (bFGF). Both T3 and T4 are pro-angiogenic as shown when using the chick-egg chorioallantoic membrane model (102). MMP, VEGF, and other angiogenic growth factors acting via miR-126 may be important therapeutic targets in liver cancer (103). In the context of growth factors and cross-talk with thyroid hormones, EGF should also be mentioned because liver cells express high levels of the receptor for this growth factor, which is associated with drug resistance and the angiogenic processes.

Many papers have shown the role of microRNA in cancer progression. MiR-21 and miR-15A play a role in metastasis, but their expression is also modulated by thyroid hormones (101, 104–107) and these effects on cancer progression start at the integrin αvβ3. Tetrac also seems to act effectively on the miRNAs (101); more information on this topic can be found in a very recent review on miRNA in HCC (108).

Thyroid hormones can interact with and modulate the action of growth factors and this may also be related to cancer metastasis. Among growth factors, TGF-β seems to be involved in liver fibrosis and cancer development. TGF-β is a pro-fibrogenic cytokine upregulated in liver disease (109) and apparently there is a direct relationship between thyroid hormones and TGF-β in fibrosis (110). The role of TGF-β in oncogenic transformation has been widely revised and appears to be mediated by the activation of MAPKs and interaction with several types of integrins such as integrin αvβ3 (111). The possible therapeutic approach has also recently been evaluated (112).

Experiments carried out on a pituitary cell line, GH4C1, showed the opposite effect of T3 on the SMAD binding element (SBE) with respect to TGF-β in promoting transcriptional

activation of SBE. A more recent paper shows that HEP-G2 treatment with Hexachlorobenzene (HCB), a hormone interferent that gives rise to hypothyroidism, may be reverted by statins through TGF-β, and it is also able to inhibit deiodinase 1, which is highly expressed in the liver, thus decreasing the production of T3 from T4. This could be responsible for the inhibitory effect on tumor promotion caused by statins (and T3 also) (113).

T4 promotes Epithelial Mesenchymal Transition (EMT) through integrin αvβ3, and induction of β-catenin and nanotetrac inhibits this pathway (114). Wnt/β-catenin is a pathway involved in fibrosis and hepatic tumor, as reported above. Wnt signaling inhibits glycogen synthase kinase (GSK-3β), which prevents β-catenin phosphorylation, leading to cytoplasmic accumulation of non-phosphorylated β-catenin that can enter the nucleus to regulate gene expression (114, 115). We have recently shown that tetrac and Nanotetrac downregulate β-catenin and High Mobility Group A2 in colon cancer and the immune checkpoint PD/PD-L1 (106, 116–119). It has been reported by Alvarado et al. (120) that T3, and the agonist GC-1, stimulate cell proliferation in normal hepatocytes, and the effect is dependent on β-catenin activation, Wnt signaling and PKA activation. At the same time, pre-treatment with either T3 or GC-1 after partial hepatectomy leads to a higher increase in cell proliferation with respect to non-treated cells (120). Wnt signaling is also involved in liver fibrogenesis, a recognized risk factor for liver cancer (3, 121). In this case stroma (Hepatic Stellate Cells, macrophages, endothelial cells) activation arising from inflammation due to liver damage in turn leads to increased proliferation and contractility, altered secretion and activity of ECM, leading to a microenvironment that may favor the development of cancer cells (19, 35).

Chemosensitization of cancer cells by tetrac, particularly those resistant to other cancer therapeutic treatments, has been reported. P-glycoprotein (P-gp, MDR1, ABCB1) is a plasma membrane pump that gives rise to the efflux of cancer therapeutic agents. This pump is mainly responsible for the cell chemoresistance in HCC (122, 123). Thyroid hormones are important modulators of this pump by increasing the transcription of *MDR1*, thus increasing the activity of the pump. The mechanism of this stimulation is not yet known in detail, but it is known that thyroid hormones support chemoresistance. Tetrac, instead, increases the retention time of doxorubicin. Thyroid hormones stimulate the Na/H exchanger, the integral plasma membrane protein that exchanges sodium and protons according to the concentration gradient, thus increasing intracellular pH, and tetrac inhibits it, giving rise to cell acidification and inhibition of MDR function and expression (124). Several other factors inhibit the activity of the P-glycoprotein, increasing the retention time of chemotherapeutic agents (i.e., doxorubicin) besides tetrac, osteopontin, VEGF, and calcium channels blockers (124). Most of these effects of thyroid hormones in cancer are blocked by integrin αvβ3 inhibitors (**Table 1**).

DO EXOSOMES HAVE SOMETHING TO DO WITH THYROID HORMONE'S ACTIONS IN LIVER CANCER?

Exosomes are vesicles, structures derived from cells that are able to modulate intercellular communication. They may contain a wide variety of molecules: cytokines, growth factors and nucleic acids. Exosomes are pivotal elements that make communication between cells easier through "cargos" whose content may change during diseases, particularly cancer, and this can be important to understand the response to disease. The exosomes impact the recipient cell by either epigenetic or translational and transcriptional changes (130). They modulate tumor cell function through apoptosis, differentiation, angiogenesis, or metastasis. The exchange of small molecules such as miRNA is a main object of study, in fact these miRNAs can be biomarkers with a wide range of applications in the management of pathologies such as cancer (131, 132). Modulation of exosomal miRNA represents a target of the personalized medicine intensely pursued nowadays. In Hepatic Stellate Cells treated with exosomes derived from HCC, it was found that the exosomes were able to convert Hepatic Stellate Cells to Cancer Associated Fibroblasts (CAF). Exosomes from HCC, through miRNA-21, were able to activate Hepatic Stellate Cells through the PTEN/PDK1/Akt pathway, thus promoting cancer progression through the secretion of cytokines that stimulated angiogenesis, such as VEGF, MMP-2, and MMP-9 (83).

To our knowledge, modulation of thyroid hormones' effect through exosomes has not been reported on to date. However, elements of the signaling of thyroid hormones, such as the integrin αvβ3 and the already mentioned PKM2 are known. PKM2, as reported above, is important for tumor cell metabolism, helping the switch from oxidative phosphorylation to the glycolytic pathway, typical of tumor cells. The thyroid hormone is an inhibitor of PKM2 that catalyzes the last step of glycolysis producing pyruvate and ATP (88). PKM2 is a tetramer able to activate STAT3 by phosphorylation, and also SNAP-23, important for the secretion of exosomes (133).

Other pathways involving integrin αvβ3 can be modulated by the exosomes, in connection with the delivery of a cargo of Oviductosome (OVS) to modulate sperm capacitation and fertility (134, 135). Integrin αvβ3 and heparan sulfate–proteoglycan in Hepatic Stellate Cells represent new receptors for the exosomes of these cells (134). In prostate cancer the integrin αvβ3 has been proposed as an easy marker of this type of tumor (136).

Embryonic endothelial progenitor cells are able to produce exosomes through stimulation of folliculogenesis in thyroid cells due to the expression of laminin −1α (137). It has been proposed that extracellular vesicles combined with iPSCs (EV-iPSCs) may represent an easy method to slow down or inhibit liver fibrosis. As reported before, there are many mechanisms involved in Hepatic Stellate Cells activation such as cell injury, altered ECM components, immune defense, metabolic dysregulation, infection, and membrane signaling pathways such as kinases and integrin αvβ3. Therefore, possible inhibitors of integrin

TABLE 1 | Mechanisms of reported and possible chemotherapeutic actions of tetrac/Nanotetrac/Nano-diamino-tetrac.

Action	Example	Effects	References
Chemosensitization	Efflux of doxorubicin, P-gp effect;	↓	(81, 99, 117)
	Efficiency of chemotherapeutic agents	↑	(124)
Radiosensitization	Repair of radiation-induced DSB. Radiation-induced activation of integrin αvβ3	↓	(101, 125)
Cell survival gene expression	Antiapoptotic genes (*XIAP, MCL-1*)	↓	(101, 102)
	Proapoptotic genes (e.g., *CASP2, BC2L14*)	↑	(106)
	Stress-defense genes (e.g., *HIF-1α*)	↓	(5, 106, 122)
	Oncogene K-ras WT and mutated	↑	(126, 127)
Cell cycle	Cyclins and cyclin-dependent protein kinase genes	↓	(106)
Growth factors pathways	*EGFR* gene expression and function	↓	(102, 122)
	Vascular calcification, ectopic mineralization	↓	(102, 106, 107)
	Wnt/β-catenin	↓	(106)
Cytokines	IL-1α, IL-1β, IL-6	↓	(101, 106)
	IL-11	↑	
Chemokines	CXCL2, CXCL3, CX3CL1, CCL20, CCL26, CXCL12	↓	(128, 129)
	CXCL10	↑	
miRNA	miRNA15A	↑	(101, 104, 106)
	miRNA21	↓	(105)
Immunotherapy	Immune checkpoint PD-1/PDL-1, HMGA2	↓	(116–119)

Modified from Davis et al. (101). ↑ increase, ↓ decrease.

αvβ3 such as tetrac or Nanotetrac could prevent or inhibit the fibrogenic process (138, 139).

THE FUTURE OF PHYSIOLOGY OR THE PHYSIOLOGY OF THE FUTURE?

Monolayer cell cultures have long been used to study the physiological and pathological mechanisms of cells and tissues or organs and have also shown their limitations in not being fully comparable to whole tissues, making them a limited model. That is why, over the years, many different strategies have been developed to cultivate cells that more closely resemble the tissue or organ. The technology of 3D cultures has therefore improved, with the idea of being more similar to the structure and physiology of a tissue that is either healthy or cancerous. These models are supposed to overcome the limitation of monolayer cell cultures. Under defined culture conditions, cells may self-assemble into 3D structures called spheroids. But they may also reproduce the embryonic development and give rise to 3D cultures called organoids. Both spheroids and organoids reproduce the morphology and physio-pathological properties of normal and tumor cells, providing new tools for research, drug discovery, and precision medicine. Among the different strategies we mention the one developed and reported by Takebe et al. (140) starting from human induced pluripotent stem cells (iPSCs). For an extensive review on models for development and diseases with organoids see (141). The liver is an extensively studied tissue in the research of biomaterials (141), in particular for the cytotoxicity of drugs and possible drugs (142). The organoids first became popular in the 70s up to the 90s and were first used to study Developmental Biology. Recently, there has been a revival of organoids as 3D structures derived from stem cells made from various organs—a specific cell type and self-organizing in a specific structure (141).

At present the reports showing effects of thyroid hormones on organoids or spheroids are quite limited. An earlier paper on Endocrinology shows the modulation by T3 of the mRNA of 5′-deiodinase reported either in primary hepatocytes or on positively charged dishes for spheroid cultures. The activation by T3 of mRNA D1 was higher in the spheroid structures than in primary hepatocytes and was unaffected by the inhibitor of protein synthesis cycloheximide (143). A few years later the same group showed an increase of responsiveness to T3 in a spheroid culture with respect to isolated hepatocytes, while the different response may involve the Thyroid hormone response element (TRE) complex (144). Another paper in the same years shows that in a 3D collagen gel-culture, thyroid cells easily give rise to folliculogenesis with the proper orientation and polarization, whereas in the usual monolayer that is not possible (145). A paper on recent advances in 3D cell cultures assessing liver physiology and pathology was reported by Calitz et al. (146). For very recent reviews on 2D, 3D, organoids and spheroids see (147, 148) as well as an extensive Chapter on liver culture models by LeCluyse et al. (149). A very interesting work on the construct of hepatocyte aggregation on chitin-based substrates made of butterfly wings opens a new area of study with biomaterials (150).

The effects of thyroid hormones and tetrac mediated by integrin αvβ3 on spheroids made of HuH7 cells integrin αvβ3-negative HCC, compared to mesenchymal stem cells (MSCs) invasion, migration and differentiation has been reported by Schmohl et al. (151) also suggesting a possible therapeutic approach based on tetrac.

CONCLUSIONS

Let us summarize the main information available on the role of thyroid hormones in hepatocyte growth and liver cancer, one of the world's more common fatal diseases, and the research that focuses on therapeutic tools in order to minimize this burden.

A role of thyroid hormones in the pathogenesis of liver cancer has been studied for many years and can be due to thyroid hormones' status, dysregulation of deiodinases, THR mutations or integrin αvβ3 dysregulation.

Tetrac and its derivatives, counteracting many actions of integrin αvβ3, inhibit and prevent many of the weak points of cell metabolism and functions typical of tumor cells, leading to the inhibition of tumor cell growth. This could represent a feasible therapeutic approach for liver cancer as well.

The new strategies of biotechnological research represented by 2D and 3D culture systems, organoids, spheroids and biomaterials, studying the mechanisms relating to thyroid hormones and liver cancer, may represent new frontiers of models in Physiology and Physio-pathology research.

AUTHOR CONTRIBUTIONS

FG, PDV, SI, and JZP wrote the manuscript. VP, H-YL, and PJD revised the draft. All the authors approved the manuscript.

REFERENCES

1. Yen PM. Physiological and molecular basis of thyroid hormone action. *Physiol Rev*. (2001) 81:1097–142. doi: 10.1152/physrev.2001.81.3.1097
2. Vasudevan N, Ogawa S, Pfaff D. Estrogen and thyroid hormone receptor interactions: physiological flexibility by molecular specificity. *Physiol Rev*. (2002) 82:923–44. doi: 10.1152/physrev.00014.2002
3. Perra A, Plateroti M, Columbano A. T3/TRs axis in hepatocellular carcinoma: new concepts for an old pair. *Endocr Relat Cancer*. (2016) 23:R353–69. doi: 10.1530/ERC-16-0152
4. Bergh JJ, Lin HY, Lansing L, Mohamed SN, Davis FB, Mousa S, et al. Integrin alphaVbeta3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology*. (2005) 146:2864–71. doi: 10.1210/en.2005-0102
5. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol*. (2016) 12:111–21. doi: 10.1038/nrendo.2015.205
6. Incerpi S, Davis PJ, Pedersen JZ, Lanni A. Nongenomic actions of thyroid hormones. In: *Principles of Endocrinology and Hormone Actions'. Endocrinology*. Belfiore A, Le Roith D, editors. Cham: Springer International Publishing (2017), p. 259–84. doi: 10.1007/978-3-319-44675-2_32
7. Plow EF, Haas TA, Zhang L, Loftus J, Smith JW. Ligand binding to integrins. *J Biol Chem*. (2000) 275:21785–8. doi: 10.1074/jbc.R000003200
8. Lin HY, Sun M, Tang HY, Lin C, Luidens MK, Mousa SA, et al. L-Thyroxine vs. 3,5,3'-triiodo-L-thyronine and cell proliferation: activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. *Am J Physiol Cell Physiol*. (2009) 296: C980–91. doi: 10.1152/ajpcell.00305.2008
9. Hammes SR, Davis PJ. Overlapping nongenomic and genomic actions of thyroid hormone and steroids. *Best Pract Res Clin Endocrinol Metab*. (2015) 29:581–93. doi: 10.1016/j.beem.2015.04.001
10. Casella C, Taglietti V. *Le funzioni del fegato in 'Principi di Fisiologia' Vol I*. Pavia: La Goliardica Pavese (1993).
11. Gilgenkrantz H, Collin de l'Hortet A. Understanding liver regeneration: from mechanisms to regenerative medicine. *Am J Pathol*. (2018) 188:1316–27. doi: 10.1016/j.ajpath.2018.03.008
12. Candelotti E, De Vito P, Ahmed RG, Luly P, Davis PJ, Pedersen JZ, et al. Thyroid hormones crosstalk with growth factors: Old facts and new hypotheses. *Immun Endoc Metab Agents Med Chem*. (2015) 15:71–85. doi: 10.2174/1871522215011507101315 34
13. Sinha RA, Singh BK, Yen PM. Direct effects of thyroid hormones on hepatic lipid metabolism. *Nat Rev Endocrinol*. (2018) 14:259–69. doi: 10.1038/nrendo.2018.10
14. Araki O, Ying H, Zhu XG, Willingham MC, Cheng SY. Distinct dysregulation of lipid metabolism by unliganded thyroid hormone receptor isoforms. *Mol Endocrinol*. (2009) 23:308–15. doi: 10.1210/me.2008-0311
15. Radenne A, Akpa M, Martel C, Sawadogo S, Mauvoisin D, Mounier C. Hepatic regulation of fatty acid synthase by insulin and T3: evidence for T3 genomic and nongenomic actions. *Am J Physiol Endocrinol Metab*. (2008) 295: E884–94. doi: 10.1152/ajpendo.90438.2008
16. Davidson NO, Powell LM, Wallis SC, Scott J. Thyroid hormone modulates the introduction of a stop codon in rat liver apolipoprotein B messenger RNA. *J Biol Chem*. (1988) 263:13482–5.
17. Ness GC. Thyroid hormone. Basis for its hypocholesterolemic effect. *J Fla Med Assoc*. (1991) 78:383–5.
18. Patsenker E, Stickel F. Role of integrins in fibrosing liver diseases. *Am J Physiol Gastrointest Liver Physiol*. (2011) 301: G425–34. doi: 10.1152/ajpgi.00050.2011
19. Leonardi GC, Candido S, Cervello M, Nicolosi D, Raiti F, Travali S, et al. The tumor microenvironment in hepatocellular carcinoma (review). *Int J Oncol*. (2012) 40:1733–47. doi: 10.3892/ijo.2012.1408
20. Eshraghian A, Hamidian Jahromi A. Non-alcoholic fatty liver disease and thyroid dysfunction: a systematic review. *World J Gastroenterol*. (2014) 20:8102–9. doi: 10.3748/wjg.v20.i25.8102
21. Chi HC, Chen CY, Tsai MM, Tsai CY, Lin KH. Molecular functions of thyroid hormones and their clinical significance in liver-related diseases. *Biomed Res Int*. (2013) 2013:601361. doi: 10.1155/2013/601361
22. Ramaiah SK, Rittling S. Pathophysiological role of osteopontin in hepatic inflammation, toxicity, and cancer. *Toxicol Sci*. (2008)103:4–13. doi: 10.1093/toxsci/kfm246
23. Sahai A, Malladi P, Melin-Aldana H, Green RM, Whitington PF. Upregulation of osteopontin expression is involved in the development of nonalcoholic steatohepatitis in a dietary murine model. *Am J Physiol Gastrointest Liver Physiol*. (2004) 287:G264–73. doi: 10.1152/ajpgi.00002.2004
24. Pearce EN. Thyroid hormone and obesity. *Curr Opin Endocrinol Diabetes Obes*. (2012) 19:408–13. doi: 10.1097/MED.0b013e328355cd6c
25. Pacifico L, Anania C, Ferraro F, Andreoli GM, Chiesa C. Thyroid function in childhood obesity and metabolic comorbidity. *Clin Chim Acta*. (2012) 413:396–405. doi: 10.1016/j.cca.2011.11.013
26. Altinova AE, Törüner FB, Aktürk M, Bukan N, Cakir N, Ayvaz G, et al. Adiponectin levels and cardiovascular risk factors in hypothyroidism and hyperthyroidism. *Clin Endocrinol*. (2006) 65:530–5. doi: 10.1111/j.1365-2265.2006.02628.x
27. Benomar Y, Wetzler S, Larue-Achagiotis C, Djiane J, Tomé D, Taouis M. *In vivo* leptin infusion impairs insulin and leptin signalling in liver and hypothalamus. *Mol Cell Endocrinol*. (2005) 242:59–66. doi: 10.1016/j.mce.2005.07.003
28. Erion MD, Cable EE, Ito BR, Jiang H, Fujitaki JM, Finn PD, et al. Targeting thyroid hormone receptor-beta agonists to the liver reduces cholesterol and triglycerides and improves the therapeutic index. *Proc Natl Acad Sci USA*. (2007) 104:15490–5. doi: 10.1073/pnas.0702759104
29. Singh BK, Sinha RA, Yen PM. Novel Transcriptional Mechanisms for Regulating Metabolism by Thyroid Hormone. *Int J Mol Sci*. (2018) 19:E3284. doi: 10.3390/ijms19103284

30. Wajner SM, Goemann IM, Bueno AL, Larsen PR, Maia AL. IL-6 promotes nonthyroidal illness syndrome by blocking thyroxine activation while promoting thyroid hormone inactivation in human cells. *J Clin Invest.* (2011) 121:1834–45. doi: 10.1172/JCI44678
31. Mancini A, Di Segni C, Raimondo S, Olivieri G, Silvestrini A, Meucci E, et al. Thyroid Hormones, Oxidative Stress, and Inflammation. *Mediators Inflamm.* (2016) 2016:6757154. doi: 10.1155/2016/6757154
32. De Vito P, Incerpi S, Pedersen JZ, Luly P, Davis FB, Davis PJ. Thyroid hormones as modulators of immune activities at the cellular level. *Thyroid.* (2011) 21:879–90. doi: 10.1089/thy.2010.0429
33. De Vito P, Balducci V, Leone S, Percario Z, Mangino G, Davis PJ, et al. Nongenomic effects of thyroid hormones on the immune system cells: New targets, old players. *Steroids.* (2012) 77:988–95. doi: 10.1016/j.steroids.2012.02.018
34. Manka P, Coombes J, Bechmann L, Dollé L, Swiderska-Syn M, Briones-Orta MA, et al. Thyroid hormone receptor regulates hepatic stellate cell activation. *J Hepatol.* (2017) 66:s582. doi: 10.1016/S0168-8278(17)31587-8
35. Higashi T, Friedman SL, Hoshida Y. Hepatic Stellate Cells as key target in liver fibrosis. *Adv Drug Deliv Rev.* (2017) 121:27–42. doi: 10.1016/j.addr.2017.05.007
36. Vázquez CMP, Costa JO, Bomfim LGS, Pires LV, da Silva DG, Fukutani KF, et al. Oxidized Low-Density Lipoprotein (Ox-LDL) and Triggering Receptor-Expressed Myeloid Cell (TREM-1) levels are associated with cardiometabolic risk in nonobese, clinically healthy, and young adults. *Oxid Med Cell Longev*. (2019) 2019:7306867. doi: 10.1155/2019/7306867
37. Anania C, De Luca E, De Castro G, Chiesa C, Pacifico L. Liver involvement in pediatric celiac disease. *World J Gastroenterol.* (2015) 21:5813–22. doi: 10.3748/wjg.v21.i19.5813
38. Collins D, Wilcox R, Nathan M, Zubarik R. Celiac disease and hypothyroidism. *Am J Med*. (2012) 125:278–82. doi: 10.1016/j.amjmed.2011.09.003
39. Hrncir T, Hrncirova L, Kverka M, Tlaskalova-Hogenova H. The role of gut microbiota in intestinal and liver diseases. *Lab Anim.* (2019) 53:271–280. doi: 10.1177/0023677218818605
40. Manka P, Coombes JD, Boosman R, Gauthier K, Papa S, Syn WK. Thyroid hormone in the regulation of hepatocellular carcinoma and its microenvironment. *Cancer Lett*. (2018) 419:175–86. doi: 10.1016/j.canlet.2018.01.055
41. Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, et al. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr Rev*. (2008) 29:898–38. doi: 10.1210/er.2008-0019
42. Luongo C, Dentice M, Salvatore D. Deiodinases and their intricate role in thyroid hormone homeostasis. *Nat Rev Endocrinol.* (2019) 15:479–88. doi: 10.1038/s41574-019-0218-2
43. Goemann IM, Romitti M, Meyer ELS, Wajner SM, Maia AL. Role of thyroid hormones in the neoplastic process: an overview. *Endocr Relat Cancer*. (2017) 24:R367–85. doi: 10.1530/ERC-17-0192
44. Goemann IM, Marczyk VR, Romitti M, Wajner SM, Maia AL. Current concepts and challenges to unravel the role of iodothyronine deiodinases in human neoplasias. *Endocr Relat Cancer*. (2018) 25:R625–45. doi: 10.1530/ERC-18-0097
45. Kester MH, Toussaint MJ, Punt CA, Matondo R, Aarnio AM, Darras VM, et al. Large induction of type III deiodinase expression after partial hepatectomy in the regenerating mouse and rat liver. *Endocrinology.* (2009) 150:540–5. doi: 10.1210/en.2008-0344
46. Ciavardelli D, Bellomo M, Crescimanno C, Vella V. Type 3 deiodinase: role in cancer growth, stemness, and metabolism. *Front Endocrinol.* (2014) 5:215. doi: 10.3389/fendo.2014.00215
47. Lin HY, Tang HY, Leinung M, Mousa SA, Hercbergs A, Davis PJ. Action of reverse T3 on cancer cells. *Endocr Res.* (2019) 3:1–5. doi: 10.1080/07435800.2019.1600536
48. Huang SA, Dorfman DM, Genest DR, Salvatore D, Larsen PR. Type 3 iodothyronine deiodinase is highly expressed in the human uteroplacental unit and in fetal epithelium. *J Clin Endocrinol Metab*. (2003) 88:1384–8. doi: 10.1210/jc.2002-021291
49. Dentice M, Luongo C, Huang S, Ambrosio R, Elefante A, Mirebeau-Prunier D, et al. Sonic hedgehog-induced type 3 deiodinase blocks thyroid hormone action enhancing proliferation of normal and malignant keratinocytes. *Proc Natl Acad Sci USA.* (2007) 104:14466–71. doi: 10.1073/pnas.0706754104
50. Dentice M, Luongo C, Ambrosio R, Sibilio A, Casillo A, Iaccarino A, et al. β-Catenin regulates deiodinase levels and thyroid hormone signaling in colon cancer cells. *Gastroenterology.* (2012) 143:1037–47. doi: 10.1053/j.gastro.2012.06.042
51. Bohinc BN, Michelotti G, Xie G, Pang H, Suzuki A, Guy CD, et al. Repair-related activation of hedgehog signaling in stromal cells promotes intrahepatic hypothyroidism. *Endocrinology.* (2014) 155:4591–601. doi: 10.1210/en.2014-1302
52. Reddy A, Dash C, Leerapun A, Mettler TA, Stadheim LM, Lazaridis KN, et al. Hypothyroidism: a possible risk factor for liver cancer in patients with no known underlying cause of liver disease. *Clin Gastroenterol Hepatol.* (2007) 5:118–23. doi: 10.1016/j.cgh.2006.07.011
53. Bano A, Chaker L, Plompen EP, Hofman A, Dehghan A, Franco OH, et al. Thyroid function and the risk of nonalcoholic fatty liver disease: The Rotterdam study. *J Clin Endocrinol Metab*. (2016) 101:3204–11. doi: 10.1210/jc.2016-1300
54. Sorvillo F, Mazziotti G, Carbone A, Morisco F, Cioffi M, Rotondi M, et al. Increased serum reverse triiodothyronine levels at diagnosis of hepatocellular carcinoma in patients with compensated HCV-related liver cirrhosis. *Clin Endocrinol* (Oxf). (2003) 58:207–12. doi: 10.1046/j.1365-2265.2003.01697.x
55. Fonseca TL, Fernandes GW, McAninch EA, Bocco BM, Abdalla SM, Ribeiro MO, et al. Perinatal deiodinase 2 expression in hepatocytes defines epigenetic susceptibility to liver steatosis and obesity. *Proc Natl Acad Sci USA.* (2015) 112:14018–23. doi: 10.1073/pnas.1508943112
56. Fernandes GW, Bocco BMLC, Fonseca TL, McAninch EA, Jo S, Lartey LJ, et al. The Foxo1-Inducible Transcriptional Repressor Zfp125 Causes Hepatic Steatosis and Hypercholesterolemia. *Cell Rep*. (2018) 22:523–34. doi: 10.1016/j.celrep.2017.12.053
57. Liao CH, Yeh CT, Huang YH, Wu SM, Chi HC, Tsai MM, et al. Dickkopf 4 positively regulated by the thyroid hormone receptor suppresses cell invasion in human hepatoma cells. *Hepatology*. (2012) 55:910–20. doi: 10.1002/hep.24740
58. Chen J, Rajasekaran M, Hui KM. Atypical regulators of Wnt/β-catenin signaling as potential therapeutic targets in hepatocellular carcinoma. *Exp Biol Med*. (2017) 242:1142–49. doi: 10.1177/1535370217705865
59. Chi HC, Liao CH, Huang YH, Wu SM, Tsai CY, Liao CJ, et al. Thyroid hormone receptor inhibits hepatoma cell migration through transcriptional activation of Dickkopf 4. *Biochem Biophys Res Commun*. (2013) 439:60–5. doi: 10.1016/j.bbrc.2013.08.028
60. Wu SM, Cheng WL, Lin CD, Lin KH. Thyroid hormone actions in liver cancer. *Cell Mol Life Sci.* (2013) 70:1915–36. doi: 10.1007/s00018-012-1146-7
61. Cristofanilli M, Yamamura Y, Kau SW, Bevers T, Strom S, Patangan M, et al. Thyroid hormone and breast carcinoma. Primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma. *Cancer*. (2005) 103:1122–8. doi: 10.1002/cncr.20881
62. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev.* (2010) 31:139–70. doi: 10.1210/er.2009-0007
63. Ledda-Columbano GM, Perra A, Loi R, Shinozuka H, Columbano A. Cell proliferation induced by triiodothyronine in rat liver is associated with nodule regression and reduction of hepatocellular carcinomas. *Cancer Res.* (2000) 60:603–9.
64. Perra A, Kowalik MA, Pibiri M, Ledda-Columbano GM, Columbano A. Thyroid hormone receptor ligands induce regression of rat preneoplastic liver lesions causing their reversion to a differentiated phenotype. *Hepatology.* (2009) 49:1287–96. doi: 10.1002/hep.22750
65. Yen CC, Huang YH, Liao CY, Liao CJ, Cheng WL, Chen WJ, et al. Mediation of the inhibitory effect of thyroid hormone on proliferation of hepatoma cells by transforming growth factor-beta. *J Mol Endocrinol.* (2006) 36:9–21. doi: 10.1677/jme.1.01911
66. Chen RN, Huang YH, Lin YC, Yeh CT, Liang Y, Chen SL, et al. Thyroid hormone promotes cell invasion through activation of furin expression in human hepatoma cell lines. *Endocrinology.* (2008) 14:3817–31. doi: 10.1210/en.2007-0989

67. Jornayvaz FR, Lee HY, Jurczak MJ, Alves TC, Guebre-Egziabher F, Guigni BA, et al. Thyroid hormone receptor-α gene knockout mice are protected from diet-induced hepatic insulin resistance. *Endocrinology*. (2012) 153:583–91. doi: 10.1210/en.2011-1793
68. Chan IH, Privalsky ML. Thyroid hormone receptors mutated in liver cancer function as distorted antimorphs. *Oncogene*. (2006) 25:3576–88. doi: 10.1038/sj.onc.1209389
69. Chan IH, Privalsky ML. Thyroid hormone receptor mutants implicated in human hepatocellular carcinoma display an altered target gene repertoire. *Oncogene*. (2009) 28:4162–74. doi: 10.1038/onc.2009.265
70. García-Silva S, Martínez-Iglesias O, Ruiz-Llorente L, Aranda A. Thyroid hormone receptor β1 domains responsible for the antagonism with the ras oncogene: role of corepressors. *Oncogene*. (2011) 30:854–64. doi: 10.1038/onc.2010.464
71. Martínez-Iglesias O, Garcia-Silva S, Tenbaum SP, Regadera J, Larcher F, Paramio JM, et al. Thyroid hormone receptor β1 acts as a potent suppressor of tumor invasiveness and metastasis. *Cancer Res.* (2009) 69:501–9. doi: 10.1158/0008-5472.CAN-08-2198
72. Harrus D, Déméné H, Vasquez E, Boulahtouf A, Germain P, Figueira AC, et al. Pathological interactions between mutant thyroid hormone receptors and corepressors and their modulation by a thyroid hormone analogue with therapeutic potential. *Thyroid.* (2018) 28:1708–22. doi: 10.1089/thy.2017.0551
73. Barlow C, Meister B, Lardelli M, Lendahl U, Vennström B. Thyroid abnormalities and hepatocellular carcinoma in mice transgenic for v-erbA. *EMBO J.* (1994) 13:4241–50. doi: 10.1002/j.1460-2075.1994.tb06744.x
74. Liangpunsakul S, Chalasani N. Is hypothyroidism a risk factor for non-alcoholic steatohepatitis? *J Clin Gastroenterol.* (2003) 37:340–3. doi: 10.1097/00004836-200310000-00014
75. Jonat C, Rahmsdorf HJ, Park KK, Cato AC, Gebel S, Ponta H, et al. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell*. (1990) 62:1189–204. doi: 10.1016/0092-8674(90)90395-U
76. Ding WJ, Wang MM, Wang GS, Shen F, Qin JJ, Fan JG. Thyroid function is associated with non-alcoholic fatty liver disease in chronic hepatitis B-infected subjects. *J Gastroenterol Hepatol*. (2015) 30:1753–8. doi: 10.1111/jgh.12998
77. Kabir A. Decreased serum total T3 level in hepatitis B and C related cirrhosis by severity of liver damage. *Ann Hepatol*. (2013) 12:506–7. doi: 10.1016/S1665-2681(19)31017-8
78. Hercbergs A, Johnson RE, Ashur-Fabian O, Garfield DH, Davis PJ. Medically induced euthyroid hypothyroxinemia may extend survival in compassionate need cancer patients: an observational study. *Oncologist*. (2015) 20:72–6. doi: 10.1634/theoncologist.2014-0308
79. Alisi A, Demori I, Spagnuolo S, Pierantozzi E, Fugassa E, Leoni S. Thyroid status affects rat liver regeneration after partial hepatectomy by regulating cell cycle and apoptosis. *Cell Physiol Biochem*. (2005) 15:69–76. doi: 10.1159/000083639
80. López-Fontal R, Zeini M, Través PG, Gómez-Ferrería M, Aranda A, Sáez GT, et al. Mice lacking thyroid hormone receptor β show enhanced apoptosis and delayed liver commitment for proliferation after partial hepatectomy. *PLoS ONE*. (2010) 5:e8710. doi: 10.1371/journal.pone.0008710
81. Kim DY, Han KH. Epidemiology and surveillance of hepatocellular carcinoma. *Liver Cancer*. (2012) 1:2–14. doi: 10.1159/000339016
82. Krashin E, Piekiełko-Witkowska A, Ellis M, Ashur-Fabian O. Thyroid hormones and cancer: a comprehensive review of preclinical and clinical studies. *Front Endocrinol.* (2019) 10:59. doi: 10.3389/fendo.2019.00059
83. Zhou Y, Ren H, Dai B, Li J, Shang L, Huang J, et al. Hepatocellular carcinoma-derived exosomal miRNA-21 contributes to tumor progression by converting hepatocyte stellate cells to cancer-associated fibroblasts. *J Exp Clin Cancer Res*. (2018) 37:324. doi: 10.1186/s13046-018-0965-2
84. Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat Rev Cancer*. (2011) 11:325–37. doi: 10.1038/nrc3038
85. Suhane S, Ramanujan VK. Thyroid hormone differentially modulates Warburg phenotype in breast cancer cells. *Biochem Biophys Res Commun*. (2011) 414:73–8. doi: 10.1016/j.bbrc.2011.09.024
86. Silvestri E, Coppola M, Cioffi F, Goglia F. Proteomic approaches for the study of tissue specific effects of 3,5,3'-triiodo-L-thyronine and 3,5-diiodo-L-thyronine in conditions of altered energy metabolism. *Front Physiol.* (2014) 5:491. doi: 10.3389/fphys.2014.00491
87. Jurica MS, Mesecar A, Heath PJ, Shi W, Nowak T, Stoddard BL. The allosteric regulation of pyruvate kinase by fructose-1,6-bisphosphate. *Structure*. (1998) 6:195–210. doi: 10.1016/S0969-2126(98)00021-5
88. Dayton TL, Jacks T, Vander Heiden MG. PKM2, cancer metabolism, and the road ahead. *EMBO Rep*. (2016) 17:1721–30. doi: 10.15252/embr.201643300
89. Dayton TL, Gocheva V, Miller KM, Israelsen WJ, Bhutkar A, Clish CB, et al. Germline loss of PKM2 promotes metabolic distress and hepatocellular carcinoma. *Genes Dev*. (2016) 30:1020–33. doi: 10.1101/gad.2785 49.116
90. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature*. (2008) 452:230–3. doi: 10.1038/nature06734
91. Zhao X, Zhao L, Yang H, Li J, Min X, Yang F. Pyruvate kinase M2 interacts with nuclear sterol regulatory element-binding protein 1a and thereby activates lipogenesis and cell proliferation in hepatocellular carcinoma. *J Biol Chem.* (2018) 29:6623–34. doi: 10.1074/jbc.RA117.000100
92. Nickels JT Jr. New links between lipid accumulation and cancer progression. *J Biol Chem.* (2018) 293:6635–6. doi: 10.1074/jbc.H118.002654
93. Gnoni GV, Rochira A, Leone A, Damiano F, Marsigliante S, Siculella L. 3,5,3'triiodo-L-thyronine induces SREBP-1 expression by non-genomic actions in human HEP G2 cells. *J Cell Physiol*. (2012) 227:2388–97. doi: 10.1002/jcp.22974
94. Varjosalo M, Taipale J. Hedgehog: functions and mechanisms. *Genes Dev*. (2008) 22:2454–72. doi: 10.1101/gad.1693608
95. Skoda AM, Simovic D, Karin V, Kardum V, Vranic S, Serman L. The role of the Hedgehog signaling pathway in cancer: a comprehensive review. *Bosn J Basic Med Sci.* (2018) 18:8–20. doi: 10.17305/bjbms.2018.2756
96. Luongo C, Ambrosio R, Salzano S, Dlugosz AA, Missero C, Dentice M. The sonic hedgehog-induced type 3 deiodinase facilitates tumorigenesis of basal cell carcinoma by reducing Gli2 inactivation. *Endocrinology*. (2014) 155:2077–88. doi: 10.1210/en.2013-2108
97. Swiderska-Syn M, Suzuki A, Guy CD, Schwimmer JB, Abdelmalek MF, Lavine JE, et al. Hedgehog pathway and pediatric nonalcoholic fatty liver disease. *Hepatology.* (2013) 57:1814–25. doi: 10.1002/hep.26230
98. Cathcart J, Pulkoski-Gross A, Cao J. Targeting Matrix metalloproteinases in cancer: bringing new life to old ideas. *Genes Dis.* (2015) 2:26–34. doi: 10.1016/j.gendis.2014.12.002
99. Bauvois B. New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: outside-in signaling and relationship to tumor progression. *Biochim Biophys Acta.* (2012) 1825:29–36. doi: 10.1016/j.bbcan.2011.10.001
100. Cohen K, Flint N, Shalev S, Erez D, Baharal T, Davis PJ, et al. Thyroid hormone regulates adhesion, migration and matrix metalloproteinase 9 activity via αvβ3 integrin in myeloma cells. *Oncotarget.* (2014) 5:6312–22. doi: 10.18632/oncotarget.2205
101. Davis PJ, Glinsky GV, Lin HY, Leith JT, Hercbergs A, Tang HY, et al. Cancer cell gene expression modulated from plasma membrane integrin αvβ3 by thyroid hormone and nanoparticulate tetrac. *Front Endocrinol* (Lausanne). (2015) 5:240. doi: 10.3389/fendo.2014.00240
102. Davis FB, Mousa SA, O'Connor L, Mohamed S, Lin HY, Cao HJ, et al. Proangiogenic action of thyroid hormone is fibroblast growth factor-dependent and is initiated at the cell surface. *Circ Res*. (2004) 94:1500–6. doi: 10.1161/01.RES.0000130784.90237.4a
103. Jing BQ, Ou Y, Zhao L, Xie Q, Zhang YX. Experimental study on the prevention of liver cancer angiogenesis via miR-126. *Eur Rev Med Pharmacol Sci.* (2017) 21:5096–100. doi: 10.26355/eurrev_201711_13825
104. Leng J, Song Q, Zhao Y, Wang Z. miR-15a represses cancer cell migration and invasion under conditions of hypoxia by targeting and downregulating Bcl-2 expression in human osteosarcoma cells. *Int J Oncol.* (2018) 52:1095–104. doi: 10.3892/ijo.2018.4285
105. Pfeffer SR, Yang CH, Pfeffer LM. The Role of miR-21 in Cancer. *Drug Dev Res*. (2015) 76:270–7. doi: 10.1002/ddr.21257

106. Mousa SA, Glinsky GV, Lin HY, Ashur-Fabian O, Hercbergs A, Keating KA, et al. Contributions of thyroid hormone to cancer metastasis. *Biomedicines*. (2018) 6:E89. doi: 10.3390/biomedicines6030089
107. Stryker ZI, Rajabi M, Davis PJ, Mousa SA. Evaluation of angiogenesis assays. *Biomedicines*. (2019) 7:E37. doi: 10.3390/biomedicines7020037
108. Li N, Zhu Y. Targeting liver cancer stem cells for the treatment of hepatocellular carcinoma. *Therap Adv Gastroenterol*. (2019) 12:1–21. doi: 10.1177/1756284818821560
109. Lee UE, Friedman SL. Mechanisms of hepatic fibrogenesis. *Best Pract Res Clin Gastroenterol*. (2011) 25:195–206. doi: 10.1016/j.bpg.2011.02.005
110. Alonso-Merino E, Martín Orozco R, Ruíz-Llorente L, Martínez-Iglesias OA, Velasco-Martín JP, Montero-Pedrazuela A, et al. Thyroid hormones inhibit TGF-β signaling and attenuate fibrotic responses. *Proc Natl Acad Sci USA*. (2016) 113:E3451–60. doi: 10.1073/pnas.1506113113
111. Xiong S, Klausen C, Cheng JC, Leung PCK. TGFβ1 induces endometrial cancer cell adhesion and migration by up-regulating integrin αvβ3 via SMAD-independent MEK-ERK1/2 signaling. *Cell Signal*. (2017) 34:92–101. doi: 10.1016/j.cellsig.2017.03.010
112. Schuppan D, Ashfaq-Khan M, Yang AT, Kim YO. Liver fibrosis: Direct antifibrotic agents and targeted therapies. *Matrix Biol*. (2018) 68–69:435–51. doi: 10.1016/j.matbio.2018.04.006
113. Ridruejo E, Romero-Caími G, Obregón MJ, Kleiman de Pisarev D, Alvarez L. Potential molecular targets of statins in the prevention of hepatocarcinogenesis. *Ann Hepatol*. (2018) 17:490–500. doi: 10.5604/01.3001.0011.7394
114. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. *Nat Rev Genet*. (2004) 5:691–701. doi: 10.1038/nrg1427
115. Waisberg J, Saba GT. Wnt-/-β-catenin pathway signaling in human hepatocellular carcinoma. *World J Hepatol*. (2015) 7:2631–5. doi: 10.4254/wjh.v7.i26.2631
116. Nana AW, Chin YT, Lin CY, Ho Y, Bennett JA, Shih YJ, et al. Tetrac downregulates β-catenin and HMGA2 to promote the effect of resveratrol in colon cancer. *Endocr Relat Cancer*. (2018) 25:279–93. doi: 10.1530/ERC-17-0450
117. Lin HY, Chin YT, Shih YJ, Chen YR, Leinung M, Keating KA, et al. In tumor cells, thyroid hormone analogues non-immunologically regulate PD-L1 and PD-1 accumulation that is anti-apoptotic. *Oncotarget*. (2018) 9:34033–37. doi: 10.18632/oncotarget.26143
118. Lin SJ, Chin YT, Ho Y, Chou SY, Sh Yang YC, Nana AW, et al. Nano-diamino-tetrac (NDAT) inhibits PD-L1 expression which is essential for proliferation in oral cancer cells. *Food Chem Toxicol*. (2018) 120:1–11. doi: 10.1016/j.fct.2018.06.058
119. Davis PJ, Mousa SA, Lin HY. Tetraiodothyroacetic acid (tetrac), integrin αvβ3 and disabling of immune checkpoint defense. *Future Med Chem*. (2018) 10:1637–39. doi: 10.4155/fmc-2018-0123
120. Alvarado TF, Puliga E, Preziosi M, Poddar M, Singh S, Columbano A, et al. Thyroid hormone receptor β agonist induces β-catenin-dependent hepatocyte proliferation in mice: implications in hepatic regeneration. *Gene Expr*. (2016) 17:19–34. doi: 10.3727/105221616X691631
121. Heindryckx F, Gerwins P. Targeting the tumor stroma in hepatocellular carcinoma. *World J Hepatol*. (2015) 7:165–76. doi: 10.4254/wjh.v7.i2.165
122. Davis PJ, Lin HY, Sudha T, Yalcin M, Tang HY, Hercbergs A, et al. Nanotetrac targets integrin αvβ3 on tumor cells to disorder cell defense pathways and block angiogenesis. *Onco Targets Ther*. (2014) 7:1619–24. doi: 10.2147/OTT.S67393
123. Chan KT, Lung ML. Mutant p53 expression enhances drug resistance in a hepatocellular carcinoma cell line. *Cancer Chemother Pharmacol*. (2004) 53:519–26. doi: 10.1007/s00280-004-0767-4
124. Davis PJ, Incerpi S, Lin HY, Tang HY, Sudha T, Mousa SA. Thyroid hormone and P-glycoprotein in tumor cells. *Biomed Res Int*. (2015) 2015:168427. doi: 10.1155/2015/168427
125. Hercbergs AH, Lin HY, Davis FB, Davis PJ, Leith JT. Radiosensitization and production of DNA double-strand breaks in U87MG brain tumor cells induced by tetraiodothyroacetic acid (tetrac). *Cell Cycle*. (2011) 10:352–7. doi: 10.4161/cc.10.2.14641
126. Nana AW, Wu SY, Yang YS, Chin YT, Cheng TM, Ho Y, et al. Nano-diamino-tetrac (NDAT) enhances resveratrol-induced antiproliferation by action on the RRM2 pathway in colorectal cancers. *Horm Cancer*. (2018) 9:349–60. doi: 10.1007/s12672-018-0334-9
127. Lee YS, Chin YT, Yang YSH, Wei PL, Wu HC, Shih A, et al. The combination of tetraiodothyroacetic acid and cetuximab inhibits cell proliferation in colorectal cancers with different K-ras status. *Steroids*. (2016) 111:63–70. doi: 10.1016/j.steroids.2016.03.006
128. Davis PJ, Glinsky GV, Lin HY, Incerpi S, Davis FB, Mousa SA, et al. Molecular mechanisms of actions of formulations of the thyroid hormone analogue, tetrac, on the inflammatory response. *Endocr Res*. (2013) 38:112–8. doi: 10.3109/07435800.2013.778865
129. Davis PJ, Glinsky GV, Lin HY, Mousa SA. Actions of thyroid hormone analogues on chemokines. *J Immunol Res*. (2016) 2016:3147671. doi: 10.1155/2016/3147671
130. Ung TH, Madsen HJ, Hellwinkel JE, Lencioni AM, Graner MW. Exosome proteomics reveals transcriptional regulator proteins with potential to mediate downstream pathways. *Cancer Sci*. (2014) 105:1384–92. doi: 10.1111/cas.12534
131. Braicu C, Tomuleasa C, Monroig P, Cucuianu A, Berindan-Neagoe I, Calin GA. Exosomes as divine messengers: are they the Hermes of modern molecular oncology? *Cell Death Differ*. (2015) 22:34–45. doi: 10.1038/cdd.2014.130
132. Abudoureyimu M, Zhou H, Zhi Y, Wang T, Feng B, Wang R, et al. Recent progress in the emerging role of exosome in hepatocellular carcinoma. *Cell Prolif*. (2019) 52:e12541. doi: 10.1111/cpr.12541
133. Wei Y, Wang D, Jin F, Bian Z, Li L, Liang H, et al. Pyruvate kinase type M2 promotes tumour cell exosome release via phosphorylating synaptosome-associated protein 23. *Nat Commun*. (2017) 8:14041. doi: 10.1038/ncomms14041
134. Al-Dossary AA, Bathala P, Caplan JL, Martin-DeLeon PA. Oviductosome-sperm membrane interaction in cargo delivery: detection of fusion and underlying molecular players using three-dimensional super-resolution structured illumination microscopy (SR-SIM). *J Biol Chem*. (2015) 290:17710–23. doi: 10.1074/jbc.M114.633156
135. Bathala P, Fereshteh Z, Li K, Al-Dossary AA, Galileo DS, Martin-DeLeon PA. Oviductal extracellular vesicles (oviductosomes, OVS) are conserved in humans: murine OVS play a pivotal role in sperm capacitation and fertility. *Mol Hum Reprod*. (2018) 24:143–57. doi: 10.1093/molehr/gay003
136. Krishn SR, Singh A, Bowler N, Duffy AN, Friedman A, Fedele C, et al. Prostate cancer sheds the αvβ3 integrin *in vivo* through exosomes. *Matrix Biol*. (2019) 77:41–57. doi: 10.1016/j.matbio.2018.08.004
137. Degosserie J, Heymans C, Spourquet C, Halbout M, D'Auria L, Van Der Smissen P, et al. Extracellular vesicles from endothelial progenitor cells promote thyroid follicle formation. *J Extracell Vesicles*. (2018) 7:1487250. doi: 10.1080/20013078.2018.1487250
138. Chen L, Brigstock DR. Integrins and heparan sulfate proteoglycans on Hepatic Stellate Cells (HSC) are novel receptors for HSC-derived exosomes. *FEBS Lett*. (2016) 590:4263–74. doi: 10.1002/1873-3468.12448
139. Povero D, Pinatel EM, Leszczynska A, Goyal NP, Nishio T, Kim J, et al. Human induced pluripotent stem cell-derived extracellular vesicles reduce hepatic stellate cell activation and liver fibrosis. *JCI Insight*. (2019) 5:125652. doi: 10.1172/jci.insight.125652
140. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*. (2013) 499:481–4. doi: 10.1038/nature12271
141. Clevers H. Modeling development and disease with organoids. *Cell*. (2016) 165:1586–97. doi: 10.1016/j.cell.2016.05.082
142. Andersson TB. Evolution of novel 3D culture systems for studies of human liver function and assessments of the hepatotoxicity of drugs and drug candidates. *Basic Clin Pharmacol Toxicol*. (2017) 121:234–8. doi: 10.1111/bcpt.12804
143. Menjo M, Murata Y, Fujii T, Nimura Y, Seo H. Effects of thyroid and glucocorticoid hormones on the level of messenger ribonucleic acid for iodothyronine type I 5'-deiodinase in rat primary hepatocyte cultures grown as spheroids. *Endocrinology*. (1993) 133:2984–90. doi: 10.1210/endo.133.6.8243326
144. Menjo M, Yamaguchi S, Murata Y, Hayashi Y, Nagaya T, Ohmori S, et al. Responsiveness to thyroid hormone is enhanced in rat hepatocytes cultured

as spheroids compared with that in monolayers: altered responsiveness to thyroid hormone possibly involves complex formed on thyroid hormone response elements. *Thyroid.* (1999) 9:959–67. doi: 10.1089/thy.1999.9.959

145. Toda S, Koike N, Sugihara H. Cellular integration of thyrocytes and thyroid folliculogenesis: a perspective for thyroid tissue regeneration and engineering. *Endocr J.* (2001) 48:407–25. doi: 10.1507/endocrj.48.407
146. Calitz C, Hamman JH, Fey SJ, Wrzesinski K, Gouws C. Recent advances in three-dimensional cell culturing to assess liver function and dysfunction: from a drug biotransformation and toxicity perspective. *Toxicol Mech Methods*. (2018) 28:369–85. doi: 10.1080/15376516.2017.1422580
147. Muller FA, Sturla SJ. Human *In vitro* models of non alcoholic fatty liver disease. *Curr Opin Toxicol.* (2019) 16:9–16. doi: 10.1016/j.cotox.2019.03.001
148. Brazovskaja A, Treutlein B, Camp JG. High-throughput single-cell transcriptomics on organoids. *Curr Opin Biotechnol.* (2019) 55:167–71. doi: 10.1016/j.copbio.2018.11.002
149. LeCluyse EL, Witek RP, Andersen ME, Powers MJ. Organotypic liver culture models: meeting current challenges in toxicity testing. *Crit Rev Toxicol.* (2012) 42:501–48. doi: 10.3109/10408444.2012.682115
150. Elbaz A, Gao B, He Z, Gu Z. Hepatocyte aggregate formation on chitin-based anisotropic microstructures of butterfly wings. *Biomimetics*. (2018) 3:E2. doi: 10.3390/biomimetics3010002
151. Schmohl KA, Müller AM, Wechselberger A, Rühland S, Salb N, Schwenk N, et al. Thyroid hormones and tetrac: new regulators of tumour stroma formation via integrin αvβ3. *Endocr Relat Cancer*. (2015) 22:941–52. doi: 10.1530/ERC-15-0245

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

Advantages of publishing in Frontiers

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

FAST PUBLICATION
Around 90 days from submission to decision

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

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

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

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

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

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership