Thyroid Hormone Nuclear Receptor TRα1 and Canonical WNT Pathway Cross-Regulation in Normal Intestine and Cancer

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A pivotal role of thyroid hormones and their nuclear receptors in intestinal development and homeostasis have been described, whereas their involvement in intestinal carcinogenesis is still controversial. In this perspective article we briefly summarize the recent advances in this field and present new data regarding their functional interaction with one of the most important signaling pathway, such as WNT, regulating intestinal development and carcinogenesis. These complex interactions unveil new concepts and will surely be of importance for translational research.

Keywords: thyroid hormones, thyroid hormone receptors, intestinal epithelium, intestinal carcinogenesis, canonical WNT pathway

INTRODUCTION

The role of the Thyroid Hormones (THs) in intestinal development have been established since the beginning of the 20th century based on the observations on amphibian metamorphosis. Indeed, during this postnatal developmental process the gastrointestinal tract is dramatically remodeled, with a first phase of apoptosis followed by a strong increase of cell proliferation correlating with an increase of circulating THs (1).

THE INTESTINAL EPITHELIUM

The intestinal epithelium is a tissue which combines the absorptive properties of a single layered/high-surface epithelium with the protective advantages of a constantly renewing barrier. It comprises six different mature cell types, which are separated into absorptive (enterocytes and M cells) and secretory (goblet, enteroendocrine, tuft and paneth cells) lineages. The different cell types allow functions such as the nutrient uptake, metabolic control and immune regulation. Thus, the intestinal epithelium is a multifunctional dynamic tissue highly dependent on continuous supply of all cell types in appropriate ratios (2). The epithelial lineages are derived from Intestinal Stem Cells (ISCs) which are located at or near the base of the intestinal crypts (3). The crypts are invagination of the intestinal wall, where the ISCs are well protected from the hazards of the digestive process occurring in the lumen. Within the crypts, self-renewing ISCs give rise to progenitor cells that
rapidly proliferate and commit into epithelial lineages (2, 3). These cells are then pushed out of the crypts, they differentiate while migrating and die by apoptosis at the apex of the villi. This system efficiently secludes the ISCs and exposes to the hazards of the digestive tract only postmitotic cells that are programmed to die (2, 3). Paneth cells represent the exception to this rule, since they migrate downwards to the base of the crypts where they reside with a lifespan of approximately 30 days (2).

Homeodomain transcription factors (such as Cdx genes) and several pathways (including WNT, Hedgehog, Notch, BMP) play crucial roles in diverse developmental and homeostatic processes (2, 4). While the molecular basis of their action is well understood, the complex cross-regulation that occurs between them and/or with the environment (i.e. fibroblasts, myofibroblasts, immune cells, microbiota) still need to be better defined.

FROM HOMEOSTASIS TO CANCER: THE CANONICAL WNT PATHWAY IN THE INTESTINE

Among the signalling pathways mentioned in the previous paragraph, the WNT pathway is highly conserved and fundamental for intestinal development, cell proliferation and differentiation, ISCs maintenance. No other pathway plays such an important role on the self-renewal/proliferative capability and cell fate of ISCs (2, 5). Indeed, the Tcf7l2-knockout mice (the Tcf7l2 gene encodes for TCF4, the transcriptional activator of the canonical WNT signalling) show a strong impairment in epithelial renewal and the ISC compartment is entirely absent (6). Other two papers reported that blocking WNT pathway by the targeted overexpression of the WNT inhibitor Dickkopf-1 in the intestinal epithelium, induces in vivo the complete loss of the proliferative compartment (7, 8). On the other side, van de Wetering and colleagues concluded that the β-catenin/Tcf4 transcriptional complex constitutes the master switch that controls proliferation versus differentiation in healthy and malignant intestinal epithelial cells (9). In the context of cancer, the WNT signalling pathway is well known for its role as a key driver of intestinal tumorigenesis. In fact, among the most frequently mutated genes, the very early event in cell transformation depends on the uncontrolled activation of the WNT/β-catenin pathway. This is driven very frequently by loss-of-function (LOF) mutations of the APC/Apc gene, whose product in a wild type (WT) context, blocks the functionality of the β-catenin and thus the activity of the pathway (10, 11). Other genes participating in this pathway frequently mutated in cancer include AXIN2/Axin2 (LOF) as well as CTNNB1/Ctnnb1 gain-of-function (GOF), the last encoding for the β-catenin protein. Specifically, CTNNB1/Ctnnb1 mutations are responsible for a stabilized oncogenic form of the protein (10, 11). It is important to underline that several signalling pathways can synergize with and induce WNT pathway activation to accelerate the early steps of tumorigenesis. Indeed, the Notch (12), Shh (13), Yap/Taz (14) are examples of signalling pathways which trigger APC-dependent tumorigenesis. Finally, we have demonstrated that THs via their Nuclear Receptor α1 (namely Thyroid hormone Receptor α1, TRα1) can be included in this list (15, 16).

TRs IN THE INTESTINAL DEVELOPMENT AND HOMEOSTASIS

The action of THs in the nucleus depends on the ability of TRs to bind the hormone T3, which is then considered as the active hormone and the cellular effector of THs. TRs activate or repress the transcription of target genes by binding to specific DNA sequences called thyroid hormone response elements (TREs) (17). During the amphibian metamorphosis different TRs are distinctly involved. Indeed, the Thyroid hormone Receptor α (TRα) is expressed at low level in the tadpole pre-metamorphic intestine whereas the Thyroid hormone Receptor β (TRβ) is strongly increased after the surge in the TH level and they both play a fundamental role during the gut remodeling process (18). Moving to mammals, the generation of specific murine models helped to elucidate the function of TRs in multiple organs and tissues including the intestinal epithelium (19). In mammals, the postnatal maturation consists of an increase in mucosal growth coupled to a burst in cell proliferation (19). Interestingly, THs level increases significantly in rodents at the weaning period (20), when the intestine undergoes this structural and functional reshaping. This increase has been also correlated with the mucosal growth and the onset of adult-type digestive enzymes expression in the enterocytes (21). Detailed analyses in murine models helped to define the different specific actions of TRα1 in intestinal epithelium progenitor/stem cell physiology. First, their proliferation strongly correlates with T3 levels and TRα1 expression (21, 22). Second, the activity of the ISCs at homeostasis and the regenerative properties of the epithelium after γ-ray irradiation are strongly affected by the lack of TRα1 expression (23, 24). Third, the targeted overexpression of TRα1 in the intestinal epithelium (vil-TRα1 mice) results in crypt hyperplasia and enhanced proliferation up to adenoma development (15).

THs-TRs AND COLORECTAL CANCERS: STILL MORE WORK NEEDED

Epidemiologic studies attempted to define a correlation between altered THs status in patients and cancer development (25). Generally speaking, there is still a lack of consensus since both a stimulating/blocking action of THs or mutated TRs have been described in several tumor types (25–28). Accordingly, hypothyroidism is a beneficial factor for ocular melanoma and mammary cancer (29) whereas it represents an aggravating risk in the case of hepatocarcinomas (26). Nevertheless, an increased risk for colon, lung, prostate, and breast cancer with increased THs has been demonstrated, even suggesting a TH-dose effect on cancer occurrence (30). The ambiguity from epidemiological studies on
THs and colorectal cancer (CRC) may arise from important missing information on: (i) mutational status of oncogenes (APC, KRAS, TP53) when hypo- or hyper-thyroidism appears, (ii) combination of mutations and/or expression of key genes and/or activation or repression of signalling pathways in different cell types influencing the biology of the cancer cells, (iii) local availability of the hormone (31) which is not directly correlated with the circulating levels of the THs but it is dependent on the levels of the TH-activating/inactivating enzymes and transporters (32). Indeed, the deiodinases selenoenzymes such as Dio2 (which converts T4 into T3), Dio3 (which converts T4 and T3 into revT3 and T2) appear important actors of THs activity (32). A complex interplay between Dio2 (i.e., high T3), Dio3 (i.e. low T3) and Shh (33) or WNT (34) has been described in skin tumors or in colon cancer, respectively. In this last case, Dio3 is upregulated by WNT signal resulting in a positive effect on cell proliferation (34) and, contrary to our observations in animals (15, 16, 24), a report also indicates a negative effect of T3 on cancer stem cells maintenance in vitro (35). Concerning the TRs, it has been shown that their mutation or aberrant expression is associated with gastrointestinal tumors (25). In particular, TRβ gene is frequently methylated and its expression strongly decreased in colon cancer (36) whereas it is still unclear whether in this same context TRα gene expression is altered.

More work is clearly needed. In particular, the studies reported above do not take into account the complexity and the heterogeneity of the tumors. Hypo- or hyper-thyroidism per se are probably not directly involved in inducing mutations, but stimulate or inhibit cellular processes that can facilitate tumor development in the presence of a favorable genetic background.

TRα1/WNT CROSSTALK IN THE PHYSIO-PATHOLOGY OF THE INTESTINE

TRα1 controls the proliferation of the mouse intestinal epithelium precursor cells by modulating, directly or indirectly, the expression of genes involved in cell cycle control, some of them being related to the WNT pathway (20, 37). For instance, TRα1 is a direct transcriptional regulator of the Ctnnb1 gene. The increased expression of β-catenin, in turn, activates its targets such as cyclins D1 and D2 as well as c-Myc which can be considered TRα1 indirect targets (20). Another TRα1 direct target in the intestinal epithelium is the secreted frizzled-related protein (Sfrp2), which is another component of the WNT pathway (38). Interestingly, through its regulation by TRα1, we showed that SFRP2 stabilizes β-catenin in intestinal progenitors in vivo and in primary cells (39). These genes have been identified through a transcriptional analysis performed in cells isolated from the intestinal crypts of WT, TRα1/0 and TRβ1/− mice (37). Moreover, the constitutive TRα1 overexpression in the intestinal epithelium (vil-TRα1 mice) not only confirmed their regulation but showed an increased cell proliferation and adenoma development even though it was not able per se to induce tumorigenesis (15). Interestingly, the TRα1 overexpression enhanced the intestinal tumorigenic process in the ApcY1638N (40) animals (vil-TRα1/ApcY1638N mice, hereafter designated as vil-TRα1/Apc) (15) while TRβ-KO in the same Apc-mutated background retarded tumor development (16). One of the most interesting molecular features of the vil-TRα1/Apc mice is the increased activity of the WNT pathway compared with that of the Apc-simple mutants, which is likely responsible of the earlier onset of tumor development in the vil-TRα1/Apc mice (15). Importantly, looking for the mechanisms involved, we characterized the molecular signature of TRα1-expressing murine tumors that includes Wnt and Notch pathways as well as stem cell markers (16). We also demonstrated in cohorts of CRC patients a significant up-regulation of TRα1 and a positive highly significant correlation between TRα1 expression and WNT pathway activity, therefore validating the relevance of the fundamental observations in clinics (16). Finally, the modulation of TRα1 expression in human colon adenocarcinoma cell lines, directly correlated with proliferation, migration and WNT pathway activity (41).

In order to further investigate and shed more light in this cross-talk we performed cellular and molecular analyses in animals as well as in cells (methodological details in Supplementary Materials and Methods). As shown in Figure 1, the mRNA expression of WNT targets such as Ccnd1 and c-Myc (Figure 1A) is up-regulated in vil-TRα1 intestines compared to the WT and their level is similar to the normal part of the vil-TRα1/Apc double mutant mice. However, in the lesions of these animals there is a further up-regulation, probably due to the WNT pathway hyperactivation in the context of the synergistic TRα1 overexpression and Apc mutation (15, 16). The mRNAs encoding for the WNT transcriptional effectors Tcf7l2 and Lef1 present an expression pattern similar to those of their molecular targets (Figure 1A). The expression of TRα1 direct targets Ctnmb1 and Sfrp2 behave in different manner and, surprisingly, their expression is diminished in the double mutant intestines (normal or lesions) compared to the simple vil-TRα1 mice (Figure 1B). TRα1 and β-catenin/Tcf4 bind to specific genomic sequences called TRE and WNT Responsive Elements (WRE), respectively. We decided to analyze the reciprocal influence of TRα1, β-catenin and Tcf4 in a cellular test in vitro using the respective luciferase-driven reporters DR4/TRE (20) and TopFlash/WRE (42). For this aim, we transfected Caco2 cells with the specific reporters and observed that the DR4-luciferase activity dependent on TRα1 can be impaired by the co-transfection of β-catenin and Tcf4 (Figure 1C). On the contrary, TRα1 transfection had a small but significant positive effect on the TopFlash-driven luciferase response (Figure 1C). All these experiments were conducted in cells in the presence of serum, thus containing physiological concentrations of THs (43). FopFlash and mut-DR4 have been used as negative control and, as expected, we could not detect any modulation of the luciferase activity (data not shown). Intriguingly, the luciferase response was also present in the absence of T3 (our unpublished observation), indicating that TRα1 functionally interacts with the β-catenin/Tcf4 pathway.
FIGURE 1 | Continued
complex independently from the hormone. These results in cells pushed us to investigate the eventual presence of the three proteins on the chromatin, by using an in vivo chromatin immunoprecipitation (ChIP) approach to analyze the TREs and WREs of the specific target genes of TRα1 and β-catenin/Tcf4, respectively. As shown in Figure 1D, in the WT intestine, TRα1 binds to the promoter region of the Sfrp2 and Ctnnb1 genes containing TRE elements (37). As the Sfrp2 and Ctnnb1 expression profiles suggested, the TRα1 chromatin occupancy changed between the WT and mutant intestine. In fact, in vil-TRα1/Apc mice, the TRα1-specific DNA binding on the regulatory regions is lost, when compared with the WT animals. To analyze the specific DNA binding of β-catenin/Tcf4 on their target genes and the eventual presence of TRα1 in the same regions, we decided to look at WREs described within the Axin2 (44) and c-Myc (45), two classical direct WNT targets. In both cases (Figure 1D) TRα1 was not present on the WRE regions in the WT intestine but was clearly enriched on them, both in normal mucosae and the tumors of vil-TRα1/Apc mice (Figure 1D). As we could not find any TREs within the Axin2 or c-Myc genomic regions in proximity of the WREs analyzed, we speculate that TRα1 might be present as a protein partner to
amplify the transcriptional response dependent upon the WNT effectors β-catenin/Tcf4.

**DISCUSSION AND CONCLUSIONS**

Complex cross-talks between signaling pathways and hormones, such as T3, have been described in several physiological models and in cancers (41, 46, 47). We reported here recent literature and also included new data on the intriguing relations between THs/TRα1 and WNT/β-catenin pathway in normal intestine and in tumors. From these different findings we propose the model summarized in Figure 2. Indeed, in a normal intestine TRα1 and the WNT effectors control their own target genes (Wild Type panel). When TRα1 is up-regulated in a normal context (vil-TRα1 mice) it stimulates its target genes including β-catenin, resulting in the activation of the WNT pathway and, by consequence, increased crypt proliferation, through the mechanisms we have already described (41, 48). In the case of the tumors (panel vil-TRα1/Apc), the stronger increase of the β-catenin/Tcf4 complex not only induces the WNT pathway activity but may also act to “displace” TRα1 from its own targets to the WNT targets. We may speculate that the “displacement” of TRα1 can be considered the molecular counterpart of the WNT hyperactivation resulting from the interaction of this two signalling pathways. Indeed, in the intestinal tumors of vil-TRα1/Apc mice the transcriptional activity of TRα1 on its own target is similar to that observed in WT condition, despite the nuclear receptor is expressed at augmented levels comparable with those of vil-TRα1/Apc mice. It is worth noting that TRβ or other nuclear receptors and β-catenin/Tcf4 (alone or in combination) have been shown to form protein complexes (49–52). We can speculate that TRα1 interacts with β-catenin/Tcf4 complex on WNT targets and that this process might be independent from T3. In addition, this interaction could contribute to the WNT pathway hyperactivation. In the case of the normal mucosae of vil-TRα1/Apc mice, the molecular phenotype is even more intriguing, given that these portions of the intestines have histological appearance similar to that of the vil-TRα1, but show molecular features close to the tumors.

According to the literature and also considering our work, THs-TRs definitively play a pivotal role in intestinal development and homeostasis in both mouse and amphibians by regulating the expression of a large panel of genes, including those belonging to the WNT pathway and cell cycle regulators (37, 53, 54). It is important to underline that all these studies provide data that can be now used in bioinformatics and statistics analyses for a direct comparison of TH-TR dependent mechanisms in the intestine of different species. Such a study will aim to identify common/uncommon mechanisms, genes directly or indirectly regulated in developmental, homeostatic and pathological conditions. This comparative analysis will surely be of importance for translational research. Indeed, when considering the tumor heterogeneity and cell plasticity within the CRCs (55–57), one of the future challenges will be to fully define the genes and signalling networks influencing the activity of TRα1 and, vice-versa, the cascade of regulations depending on THs-TRα1.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

**ETHICS STATEMENT**

The animal study was reviewed and approved by Ministère de l’Enseignement Supérieur et de la Recherche, Direction Générale pour la Recherche et l’Innovation, Sécrétariat “Autorisation de projet” (agreement #02847.01).

**AUTHOR CONTRIBUTIONS**

MS participated in the design of the study, performed all the experiment, carried out the data analysis and drafted the manuscript. LC helped in design the manuscript, figure preparation and revised the manuscript. MP conceived and supervised the study, carried out the data analysis drafted and revised the manuscript. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.725708/full#supplementary-material
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