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Thyroid Hormone Receptors and Cancer

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Abstract

Background—Thyroid hormone receptors (TRs) are ligand-dependent transcription factors that mediate the actions of the thyroid hormone (T3) in development, growth, and differentiation. The *THRA* and *THRB* genes encode several TR isoforms that express in a tissue- and development-dependent manner. In the past decades, a significant advance has been made in the understanding of TR actions in maintaining normal cellular functions. However, the roles of TRs in human cancer are less well understood. The reduced expression of TRs because of hypermethylation, or deletion of TR genes found in human cancers suggests that TRs could function as tumor suppressors. A close association of somatic mutations of TRs with human cancers further supports the notion that the loss of normal functions of TR could lead to uncontrolled growth and loss of cell differentiation.

Scope of Review—In line with the findings from association studies in human cancers, mice deficient in total functional TRs ($Thra1^{-/-}Thrb^{-/-}$ mice) or with a targeted homozygous mutation of the *Thrb* gene (denoted PV; $Thrb^{PV/PV}$ mice) spontaneous develop metastatic thyroid carcinoma. This review will examine the evidence learned from these genetically engineered mice that provided strong evidence to support the critical role of TRs in human cancer.

Major Conclusions—Loss of normal functions of TR by deletion or by mutations could contribute to cancer development, progression and metastasis.

General Significance—Novel mechanistic insights are revealed in how aberrant TR activities lead to carcinogenesis. Mouse models of thyroid cancer provide opportunities to identify molecular targets as potential treatment modalities.

Keywords

Thyroid hormone receptor mutant; Thyroid cancer; Phosphatidylinositol 3 kinase; Src kinase; β-catenin; Mouse models

1. Introduction

Molecular cloning of thyroid hormone receptors (TRs) cDNA in 1986 ushered in an exciting era in the understanding of the structure, expression, functions, and transcription regulation of TRs [1, 2]. Two human TR genes, *THRA* and *THRB,* located on different chromosomes, encode thyroid hormone (T3) binding TR isoforms (TRα1, β β2, and β3). Similar to other members of the nuclear receptor superfamily, these TR isoforms have an amino-terminal A/

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B domain, a central DNA-binding domain, and a carboxyl-terminal ligand-binding domain. These TR isoforms share extensive sequence homology in the DNA and ligand-binding domains, but differ in the length and amino acid sequence at the amino terminal A/B domain. Comparison of X-ray crystallographic structures of rat TRα and human TRβ ligand binding domains shows a close structural similarity [3, 4]. However, there are subtype-dependent differences in the ligand-binding pocket that allow selective recognition of certain ligands [4].

The transcriptional activity of TRs is regulated by multiple mechanisms including the type of thyroid hormone response elements (TREs) located on the promoters of T3 target genes, the tissue- and development-dependent expression of TR isoforms, and a host of nuclear co-repressors and co-activators [5]. In the absence of T3, TRs recruit corepressor proteins, such as nuclear receptor co-repressor 1 (NCOR1) and silencing mediator of retinoid and thyroid hormone receptors (SMRT), and repress the transcription of target genes. In the presence of T3, TRs undergo a conformational change and that results in the exchange of co-repressors for co-activators, such as p160/ steroid receptor co-activator-1 (SRC-1) family, to activate transcription of target genes. In addition to transcriptional stimulation, TRs also repress gene expression in a T3-dependent manner by binding to negative TREs [5]. However, recent advances have expanded this T3-dependent corepressor-coactivator exchange model and shown that NCOR1 and SMRT play a role in determining T3-sensitivity *in vivo*, suggesting that corepressors can be recruited to TR in the presence of T3 [6–8].

In spite of significant progress in understanding the molecular mechanisms by which TR functions in maintaining normal physiological T3-mediated homeostasis, the roles of TR in human cancers are less well understood. Early evidence to suggest that mutated TR could be involved in carcinogenesis came from the discovery that TRα1 is the cellular counterpart of the retroviral v-ERBA involved in the neoplastic transformation leading to acute erythroleukemia and sarcomas [2, 9]. It is a highly mutated chicken TRα1 that does not bind T3 and loses the ability to activate gene transcription. V-ERBA competes with TR for binding to TREs and interferes with the normal transcriptional activity of liganded-TR on several promoters [10, 11]. Early direct evidence that the v-ERBA oncoprotein can promote neoplasia in mammals through its dominant negative activity was provided by the finding that male transgenic mice overexpressing v-ERBA develop hepatocellular carcinomas [12].

The notion that the loss of TR functions could be involved in the development of human cancers gained further support by association studies. Loss in the expression of the *THRB* gene because of the truncation/deletion of chromosome 3p where the *THRB* gene is located was reported in many malignancies including lung, melanoma, breast, head and neck, renal cell, uterine cervical, ovarian, and testicular tumors [13–18]. The *THRA* locus undergoes frequent loss of heterozygosity (LOH) in sporadic breast cancer, and rearrangement of the *THRA* gene has also been reported in leukemia, breast, and stomach cancer [19–21]. Somatic mutations of TRs have been found in human hepatocellular carcinoma [22], renal clear cell carcinoma [23, 24], breast cancer [25], pituitary tumor [26, 27], and thyroid cancer [28] (Table 1). Many of these TR mutants have lost T3 binding activity and transcription capacity, and some exhibit dominant negative activity [23, 28] (Table 1).

Decreased expression due to silencing of the *THRB* gene by promoter hyermethylation has been found in human cancer including breast, lung, and thyroid carcinoma [29–32]. A recent study has provided evidence that the expression of the *THRB* gene could also be repressed via micro RNAs regulatory mechanisms in papillary thyroid cancer [33]. The findings from these association studies raised the possibility that TRs could function as tumor suppressors in human cancers. However, this possibility could not be directly tested until genetically engineered mouse models became available. This article will briefly review the current

mouse models of thyroid cancer caused by the loss of total functional TRs (*Thra1*$^{-/-}$*Thrb*$^{-/-}$ mice) or a targeted homozygous mutation of the *Thrb* gene (denoted PV; *Thrb*$^{PV/PV}$ mice) and its derived strains. The altered molecular pathways leading to carcinogenesis due to the PV mutation or loss of TRs will be highlighted and the underlying mechanisms discussed.

2. Mouse models of thyroid cancer

To test the hypothesis that the loss of normal functions of TRβ contributes to cancer development and progression, several mutant mice have been developed, each of which was designed to explore a specific aspect of the roles TRs play in cancer.

2.1. The Thrb$^{PV/PV}$ mouse

The *Thrb*$^{PV/PV}$ knockin mouse, created by targeting the PV mutation to the *Thrb* gene locus, was initially intended to study an inheritable disease with reduced tissue sensitivity to thyroid hormone known as resistance to thyroid hormone (RTH) [34]. The PV mutation was identified in an RTH patient with a frameshift mutation in the C-terminal 14 amino acids of TRß (Figure 1), resulting in a complete loss of T3 binding and transcriptional capacity [35]. This *Thrb*$^{PV/PV}$ mouse faithfully recapitulates human RTH with the dysregulation of the hypothalamus-pituitarythyroid axis, leading to elevated serum thyroid hormone accompanied by nonsuppressible high serum thyroid-stimulating hormone (TSH) [34]. As *Thrb*$^{PV/PV}$ mice age, they spontaneously develop follicular thyroid carcinoma, resembling the pathological progression of human thyroid cancer [36, 37]. Pathological changes progress from hyperplasia, capsular invasion, vascular invasion, and anaplasia to eventually distant metastasis (Figure 2). Metastasis occurs mainly in the lung and occasionally in the endocardium, but not in the local lymph nodes [36, 37]. The findings that *Thrb*$^{PV/PV}$ mice spontaneously develop follicular thyroid carcinoma similar to human cancer indicate that these mice could be used as a model to understand how the loss of normal functions of TRß could lead to cancer phenotypes and elucidate the molecular genetic changes underlying follicular thyroid carcinoma.

2.2. The Thrb$^{PV/-}$ mouse

The critical role of TRß in cancer is further illustrated by the generation of the *Thrb*$^{PV/-}$ mouse. This double mutant mouse was derived from the cross-breeding of heterozygous *Thrb*$^{PV/+}$ mice with *Thrb* knockout mice [38]. Remarkably, in contrast to the heterozygous *Thrb*$^{PV/+}$ mice, *Thrb*$^{PV/-}$ mice spontaneously develop follicular thyroid carcinoma with a pathological progression similar to that of *Thrb*$^{PV/PV}$ mice [38]. These findings indicate that one mutated *Thrb* allele in the absence of the other wild-type allele is sufficient to induce spontaneous thyroid carcinoma. Thyroid carcinoma occurs either when both *Thrb* alleles are mutated or when one allele is mutated and there is ablation of the other wild-type allele. Thus, this *Thrb*$^{PV/-}$ mouse model has provided direct *in vivo* evidence to indicate that the *Thrb* gene can function as a tumor suppressor and raises the possibility that the *THRB* gene could serve as a novel therapeutic target in thyroid cancer.

2.3. The TR-double knockout (Thra1$^{-/-}$Thrb$^{-/-}$) mouse

Association studies show that the expression of TRs is frequently silenced in human cancers [23, 28, 30, 32, 33]. To further test the hypothesis that a lack of TRs is deleterious, Zhu et al. used *Thra1*$^{-/-}$*Thrb*$^{-/-}$ mice [39–41] to delineate whether a total loss of all functional TRs could lead to cancer development. Remarkably, these mice spontaneously develop follicular thyroid cancer with pathological progression from hyperplasia to capsular invasion, vascular invasion, anaplasia, and metastasis, similar to human thyroid cancer [42]. These findings provided direct *in vivo* evidence to show that functional loss of both *Thra1* and *Thrb* genes promotes thyroid tumor development and metastasis. Importantly, this mouse model

provides an opportunity to identify the common pathways shared by the loss of TR functions due to mutations (*Thrb*$^{PV/PV}$ mice) or due to deletion of both TR genes (*Thra1*$^{-/-}$ *Thrb*$^{-/-}$ mice) in thyroid carcinogenesis. Moreover, by comparison of the gene expression profiles and the altered signaling pathways between these two mouse models of thyroid cancer, it would be possible to discern the different molecular actions of thyroid carcinogenesis resulting from TRβ mutation and deletion of total functional TRs.

3. Lessons learned from mouse models of thyroid cancer

The mouse models of thyroid cancer described above allowed elucidation of molecular actions of a mutated TRβ, as well as new insight into how deficiency of TR leads to thyroid carcinogenesis. cDNA microarray analyses indicate complex alterations of multiple pathways in thyroid carcinogenesis of *Thrb*$^{PV/PV}$ mice [43] suggesting PV could act as an oncogene via multiple mechanisms to promote cancer progression. The following sections will highlight what has been learned about the roles of TR in thyroid cancer via the loss-of-function approach by TRβ mutations and deletion of TRs.

3.1. Oncogenic actions of PV via transcription regulation

3.1.1. Down-regulation of the peroxisome proliferator-activated receptor γ (PPARγ)—PV was identified in an RTH patient and has been shown to mediate RTH phenotypes via its dominant negative activity by interfering with transcription activity of wild-type TRs (WT-TRs) [44, 45]. Thus, it was hypothesized that TR-positively regulated tumor suppressor genes in the thyroids would be repressed by PV. The repressed expression of the tumor suppressors in the thyroids would facilitate cancer progression. This PV-mediated action is exemplified by the regulation of PPARγ [46, 47]. The importance of PPARγ signaling in thyroid carcinogenesis was made clear by the identification of the PPARγ-paired box gene 8 (PAX8) fusion gene in approximately 35% of human follicular thyroid carcinomas [46]. This fusion gene loses its ability to stimulate PPARγ-ligand dependent transcription and inhibits PPARγ transcriptional activity [46]. These observations raised the possibility of PPARγ as a tumor suppressor in thyroid carcinogenesis.

Consistent with these findings, *Pparγ* mRNA levels are markedly decreased in thyroid carcinogenesis of *Thrb*$^{PV/PV}$ mice [37]. PPARγ protein levels are also reduced in thyroid tumors and are totally lost in lung metastases. In addition to its inhibition of expression, PV also acts to repress the ligand-dependent transcription activity of PPARγ [47]. Biochemical and cell-based studies indicate that similar to WT-TRβ, PV competes with PPARγ for binding to the peroxisome proliferator responsive element (PPRE) as homodimers or as heterodimers with PPARγ, thereby repressing the transcription activity of PPARγ (See “i” of Figure 3). PPRE–bound PV recruits the nuclear co-repressor NCOR1 to the promoter of PPARγ target genes, independent of T3. Such constitutive association of PV with co-repressors prevents the recruitment of the SRC-1 to the PPRE-bound PV/PPARγ complexes, in spite of the presence of PPARγ ligands (e.g., troglitazone). Thus, in the thyroids of *Thrb*$^{PV/PV}$ mice, decreased expression and repression of transcriptional activity of PPARγ lead to reduced expression of PPARγ downstream tumor suppressor target genes, thereby promoting the progression and development of thyroid cancer. A recent study further confirmed the tumor suppressor role of PPARγ in another mouse model in which the *Pax8-Pparγ* (*PPFP*) gene was targeted to the thyroid deficiency in PTEN (phosphatase and tensin homologue deleted from chromosome 10). Treatment of this mouse with another PPARγ ligand, pioglitazone, decreased thyroid growth and prevented metastatic disease [48].

3.2. Oncogenic actions of PV via novel extra-nuclear actions

Recent studies have indicated that TRs could also mediate T3 biological activities beyond TRE-mediated gene transcription. These extra-nuclear actions were reported to regulate ion channels, glucose transporters, protein kinases, and phospholipid metabolism that affect cellular functions [5]. The extra-nuclear TR actions could initiate signal transduction via direct protein-protein interaction. One prominent example is TR-mediated activation of phosphatidylinositol 3-kinase (PI3K) activity in human endothelial cells reported by Simoncini et al. [49]. This activation is through direct physical interaction of TR with the p85α subunit of PI3K, leading to the phosphorylation and activation of Akt and endothelial nitric oxidase synthase [50]. This TR-mediated PI3K activation has also been demonstrated in other cell types such as human fibroblasts, neonatal rat cardiomyocytes, and human and rat insulinoma cell lines [51–55]. These studies suggest that non-TRE-dependent effects of TR via protein-protein interactions could be an important mode of TR actions. Likewise, mutations of TR that perturb the normal protein-protein interactions *in vivo* could lead to deleterious consequences as exemplified by the aberrant interaction of PV with several key regulators critical for cell proliferation, apoptosis, motility, and invasion in the thyroids of $Thrb^{PV/PV}$ mice. The key regulators that aberrantly interact with PV and their impact on cellular signaling are highlighted below.

3.2.1. Sustained activation of signaling pathways via direct interaction of PV with key cellular regulators

3.2.1.1. Phosphatidylinositol 3-kinase signaling: The PI3K signaling pathway controls a wide variety of cellular processes including cell death and survival, cell migration, protein synthesis, and metabolism. Aberrant PI3K-dependent signaling, mediated by Akt kinase, is well-known to contribute to abnormal cell growth and cellular transformation in a variety of neoplasms, including thyroid cancer [56, 57]. PI3Ks consist of a catalytic subunit with a molecular weight of about 110 kD (p110) and a tightly associated regulatory subunit (p85α, p85β, or p55γ). The regulatory subunits do not possess an intrinsic enzymatic activity, but are responsible for the activation and subcellular localization of the catalytic subunits. These molecules contain rho-GTPase-activating protein homology domains, Src homology (SH) 2 domains, SH3 domains, and proline-rich motifs [58]. Upon activation by membrane receptors, PI3K phosphorylates phosphatidylinositol-4,5 biphosphate (PIP2) to form phosphatidylinositol-3,4,5-triphosphate (PIP3). Through phosphatidylinositol-dependent kinases, the immediate downstream effectors of PI3K, Akt is phosphorylated and activated to further phosphorylate downstream protein substrates. The activity of PI3K is negatively regulated by PTEN, a protein phosphatase that dephosphorylates PIP3 to form PIP2 [58].

Prompted by reports that the Akt activity is over-activated in thyroid cancer in humans and in $Thrb^{PV/PV}$ mice [56, 57, 59], Furuya et al. examined whether the increased Akt activity was mediated by the increased kinase activity of PI3K. Indeed, the activity of PI3K in the thyroids of $Thrb^{PV/PV}$ mice is increased up to 10-fold that in normal thyroids [60]. This increase is due to the direct physical interaction of the hormone binding domain of PV with the C-terminal SH2 domain of p85α subunit [60, 61] (see “ii” of Figure 3). The signal of PV-mediated PI3K activation is relayed via phosphorylation cascades of the downstream effectors, Akt, integrin-linked kinase, mammalian target of rapamycin (mTOR), and $p70^{S6K}$ to increase cell proliferation, migration, and invasion to promote thyroid carcinogenesis [60] (“ii” of Figure 3). That this novel oncogenic action of PV via direct protein-protein interaction with the p85α subunit is critical in thyroid carcinogenesis was further illustrated by the delay of cancer progression and the blockage of distant metastasis when PI3K activity was inhibited by treatment of $Thrb^{PV/PV}$ mice with a specific inhibitor, LY294002 [62]. Moreover, when the PI3K negative regulator, PTEN, is haplo-deficient in $Thrb^{PV/PV}Pten^{+/-}$

mice, thyroid tumor progression is greatly accelerated by further activation of PI3K-Akt signaling leading to shortening of survival and increased tumor size [63].

3.2.1.2. Integrin-Src-FAK signaling pathway: Invasion of the basement membrane (BM) and migration through the extracellular membrane (ECM) are critical processes in cancer progression and metastasis. These processes are mediated mainly by interactions of integrin receptors and BM/ECM components with subsequent degradation of ECM by metalloproteases (MMPs) to help the cancer cell invasion through the membrane barriers. After binding to their ligands, integrin receptors undergo conformational changes and activate intracellular signaling molecules such as Src kinase and focal adhesion kinase (FAK) [64, 65]. Aberrant expression of integrins has been reported in many human cancers including thyroid cancer [66, 67]. Overactivation of Src and its downstream effector, FAK, are frequent in human cancer and associated with invasive potential in thyroid cancer [68–70]. Recent studies showed increased protein abundance of integrins α5, αV, β1, and β3 and their ligand fibronectin in the thyroid of in $Thrb^{PV/PV}$ mice [71] (see "iii" of Figure 3). Importantly, PV was found to physically complex with integrins α5 and β1. PV also activated Src kinase pathway by increasing its phosphorylation, which activates FAK by increasing phosphorylation on several tyrosine sites [71]. Interestingly, PV also physically interacts with FAK, thus forming a large complex of PV-integrins-FAK-PI3K, leading to activation of downstream signaling of p38 mitogen-activated protein kinase (MAPK) via increased phosphorylation cascades to stimulate the expression of MMP-9 at the mRNA and protein levels ("iii" of Figure 3). The activation of cSrc-FAK is known to remodel the actin cytoskeleton in cancer cells that could underlie aberrant cell migration and invasion [72–74]. Lu et al. also found an increased protein abundance of β-actin and erzin, which links the cytoskeletal structure and the plasma membrane. The findings that PV also complexes with β-actin, as well as with erzin, uncover a novel mechanism by which PV could change cytoskeletal organization to promote cell migration and invasion ("iii" of Figure 3).

That PV-induced activation of Src-FAK-MAPK signaling via complex formation is critical in thyroid carcinogenesis was shown in a recent study in which Src-FAK signaling was also found to be highly activated in a more aggressive mouse model of $Thrb^{PV/PV}Pten^{+/-}$ mice [75]. Treatment of $Thrb^{PV/PV}Pten^{+/-}$ mice with a Src-specific inhibitor, SKI-606 (bosutinib), prevents tumor growth, blocks distant metastasis, and regains the differentiation state of thyroid follicular cells. These responses were accompanied by down-regulation of MAPK pathways and inhibition of epithelial-mesenchymal transition (EMT; "iii" of Figure 3). These findings suggest that one of the mechanisms by which PV act as an oncogene is via complexing with key cellular regulators in multiple signaling pathways to affect expression and activity of ECM, adhesion, migration, EMT, and differentiation of cancer cells in thyroid carcinogenesis [75].

3.2.2. Aberrant activation of signaling pathways via PV-mediated stabilization of key cellular regulators

3.2.2.1. Pituitary tumor-transforming gene (PTTG): PTTG, also known as securin, is a critical mitotic checkpoint protein that helps hold sister chromatids together before entering anaphase [76]. It was originally isolated from GH4 pituitary cells and shown to cause *in vitro* cell transformation and to induce tumor formation *in vivo* [77] and genetic instability in a variety of cells including thyroid cells [78–80]. Although overexpression of PTTG is evident in thyroid carcinomas [81, 82], how the over-expressed PTTG acts in thyroid carcinogenesis was not clear. The finding that the PTTG is over-expressed in the thyroid tumors of $Thrb^{PV/PV}$ mice provided a tool to probe its role in thyroid carcinogenesis [43]. The link of the over-expressed PTTG to chromosomal aberrations was directly demonstrated in cell lines derived from thyroid tumors of $Thrb^{PV/PV}$ mice [83]. Spectral karyotyping

analysis (SKY) of seven tumor cell lines showed abnormal karyotypes, and also exhibited a variety of structural chromosomal aberrations, including common recurrent translocations and deletions [83]. This SKY analysis suggested that the development and progression of follicular thyroid carcinoma in *Thrb*$^{PV/PV}$ mice comprise recurrent structural and numerical genomic changes, some of which mimic those described in human thyroid cancer. Ying et al. elucidated the molecular basis of PTTG-induced chromosomal instability during thyroid carcinogenesis of *Thrb*$^{PV/PV}$ mice [84]. PTTG protein was found to physically associate with TRβ as well as PV. Concomitant with T3-induced degradation of TRβ [85], PTTG proteins are degraded by the proteasomal machinery, but no such degradation occurs when PTTG is associated with PV (see "iv" of Figure 3). The degradation of the PTTG-TRβ complex is activated by the direct interaction of the liganded TRβ with the steroid receptor coactivator-3 (SRC-3) that recruits a proteasome activator (PA28γ) [84]. PV that does not bind T3, thus cannot recruit SRC-3-PA28γ complex to activate proteasome-mediated degradation, and as a result PTTG protein level is increased ("iv" of Figure 3). The accumulated aberrantly elevated PTTG impedes mitotic progression in cells expressing PV [84]. The PV-induced stabilization of PTTG protein, a critical regulator of chromosomal integrity, serves as a novel extra-nuclear action to contribute to thyroid carcinogenesis.

3.2.2.2. β-catenin: β-catenin is key regulator of the Wnt signaling pathway, which plays important roles not only in normal physiological processes in adults, but also in embryogenesis and carcinogenesis [86]. In the canonical pathway, stabilized β-catenin translocates from cytoplasm into the nucleus and interacts with T cell factor/lymphoid enhancer factor (TCF/LEF) family transcription factors to stimulate the expression of genes critical for normal cellular functions. The cellular level of β-catenin is regulated by two distinct proteolytic pathways involving axin/glycogensynthase kinase 3β (GSK-3β)/ adenomotous polyposis coli (APC) complex and p53 inducible pathway [87, 88]. Abnormally accumulated β-catenin has been reported in many human cancers, including thyroid cancer [89].

The aberrantly elevated β-catenin in thyroid tumors of *Thrb*$^{PV/PV}$ mice led to the discovery that the cellular stability of β-catenin is down-regulated by the liganded TRβ, but elevated by PV [90] (see "iv" of Figure 3). Cell-based studies showed that T3 induces the degradation of β-catenin in cells expressing TRβ via proteasomal pathways. In contrast, since PV does not bind T3, this T3-mediated regulatory mechanism is lost in PV, leading to accumulated β-catenin in PV-expressing cells ("iv" of Figure 3). Further analyses showed that β-catenin physically associates with the unliganded TRβ or PV. However, in the presence of T3, β-catenin is dissociated from TRβ/β-catenin complexes, but not from PV/β-catenin complexes. Thus, β-catenin signaling is repressed by T3 in TRβ-expressing cells through decreasing β-catenin-mediated transcription activity and target gene expression, whereas sustained β-catenin signaling is observed in PV-expressing cells [90] ("iv" of Figure 3). Importantly, an increased β-catenin level in thyroid tumors of *Thrb*$^{PV/PV}$ mice is associated with an increase in phosphorylated β-catenin (serine 552), accompanied by increased expression of β-catenin downstream target genes, cyclin D1, c-myc, and matrix metalloprotease (MMP)-1. Altogether, these studies show that β-catenin signaling is constitutively activated in the thyroids of *Thrb*$^{PV/PV}$ mice ("iv" of Figure 3). The stabilization of β-catenin, via association with a mutated TRβ, represents a novel activating mechanism of the oncogenic β-catenin that could contribute to thyroid carcinogenesis in *Thrb*$^{PV/PV}$ mice.

3.3. Oncogenic actions of PV via "Gain-of-function"

A critical issue in understanding the oncogenic actions of a mutated TRβ (i.e., PV) is whether PV acts simply via loss of the WT-TR tumor suppressor function or also results

from gain-of-function activities. As indicated in Section 2.3., *Thra1*$^{-/-}$*Thrb*$^{-/-}$ mice spontaneously develop follicular thyroid carcinoma [42]. The *Thrb*$^{PV/PV}$ and *Thra1*$^{-/-}$*Thrb*$^{-/-}$ mice exhibit similarly elevated serum levels of TSH and thyroid hormones [91], but intriguingly the *Thra1*$^{-/-}$*Thrb*$^{-/-}$ mice develop follicular thyroid carcinoma with a slower progression and a less aggressive malignant phenotype [36, 42]. These observations raised the possibility that in addition to the loss of normal tumor suppressor functions of WT-TRβ, PV could acquire additional oncogenic activity via gain-of-function through mutation. This possibility was tested by using cDNA microarrays to compare the gene expression profiles of laser-captured microdissected thyroid tumor cells of *Thrb*$^{PV/PV}$ mice and *Thra1*$^{-/-}$*Thrb*$^{-/-}$ mice [92]. Analysis of the cDNA microarray data between tumor cells of these two mutant mice showed contrasting global gene expression profiles. With stringent selection using 2.5-fold change in cDNA microarray analysis, 241 genes with altered gene expression were identified. Nearly half of the genes (n=113: 49% of total) with altered gene expression in thyroid tumor cells of *Thrb*$^{PV/PV}$ mice were associated with tumorigenesis and metastasis; some of these genes function as oncogenes in human thyroid cancers. The remaining genes were found to function in transcriptional regulation, RNA processing, cell proliferation, apoptosis, angiogenesis, and cytoskeleton modification. These results indicate that the more aggressive thyroid tumor progression in *Thrb*$^{PV/PV}$ mice was not due simply to the loss of tumor suppressor functions of TR via mutation but also, importantly, was a result of gain-of-function in the oncogenic activities of PV to drive thyroid carcinogenesis. Thus, the mechanisms by which PV acts as an oncogene are more diverse than previously envisioned in that as a mutated TRβ can evolve with an oncogenic advantage to promote thyroid carcinogenesis.

However, it also important to point out that there are common altered pathways shared in *Thra1*$^{-/-}$*Thrb*$^{-/-}$ and *Thrb*$^{PV/PV}$ mice during thyroid carcinognesis [42, 93–95]. Aberrant activation of Akt-mTOR-p70^{S6K} pathway, vascular growth factor and its receptors were observed in the thyroid tumors of both *Thra1*$^{-/-}$*Thrb*$^{-/-}$ and *Thrb*$^{PV/PV}$ mice [42, 59]. The over-expression of known tumor promoters such as the *Pttg1* gene and the suppression of tumor suppressors such as the PPARγ also were similarly detected in thyroid carcinogenesis in both mutant mice [42, 96]. These findings clearly show that functional loss of both *Thra* and *Thrb* genes by deletion or through mutation in PV promote thyroid tumor development and metastasis via some common pathways.

4. Summary and future directions

Studies of the oncogenic actions of mutant PV in thyroid carcinogenesis of *Thrb*$^{PV/PV}$ mice have provided direct *in vivo* evidence to show that TRβ mutations can lead to cancer development. Importantly, novel molecular mechanisms of TRβ mutations in carcinogenesis have been uncovered. PV acts as an oncogene via multiple molecular mechanisms. It can function by interfering with the transcription activity of WT-TR by abnormal repression in the expression of tumor promoters (e.g., PPARγ). PV can also act at the transcription level independent of TR, via “gain-of-function.” Importantly, PV can also function via extra-nuclear sites, for example by initiating the actions via direct protein-protein interaction with key cellular regulators such as PI3K, integrins, FAK, β-actin, and erzin. Via phosphorylation cascades, the signals initiated by the interaction of PV with these regulators are transduced to activate downstream pathways, such as PI3KAKT-mTOR signaling and MAPK signaling, to promote cancer cell proliferation, apoptosis, migration, and invasion. Moreover, new regulatory mechanisms of protein stability of key cellular regulators by PV have also been elucidated, such as the PV induced stabilization of PTTG, leading to genomic abnormalities. The discovery of these complex actions of PV revealed that thyroid carcinogenesis resulted from alterations of multiple signaling pathways. The identification of these altered cellular

pathways provides new opportunities for potential molecular targets for diagnosis and treatments.

Although the *Thrb*$^{PV/PV}$ mouse has provided a valuable tool to advance our understanding of how a mutated TRβ can lead to thyroid carcinogenesis, many challenging issues are yet to be elucidated. 1). At present, no thyroid cancer has been detected in heterozygous *Thrb*$^{PV/+}$ mice. Studies show that PV is oncogenic via collaboration with other key regulators in homozygous *Thrb*$^{PV/PV}$ mice to bring about thyroid cancer. It is important to identify the factors with which PV collaborates to invoke its oncogenic activity in heterozygous *Thrb*$^{PV/+}$ mice. Addressing this issue will shed new light on why RTH patients who are mostly heterozygous in the mutation of one single allele of the *THRB* gene have no increased propensity to develop thyroid cancer. 2). Another issue is whether the oncogenic action of a mutated TRβ is limited to a PV-specific mutated sequence or also extends to other mutations in the helix 11 and 12 of TRβ that can invoke oncogenic activity. Clarification of this central question would further advance our understanding of the role of TRβ mutations in thyroid cancer. 3). In addition to thyroid cancer, PV has been shown to play key roles in the development of pituitary tumors [91] and breast cancer [97], suggesting that the oncogenic actions of PV are not restricted to just the thyroid. Further identification of other target organs that are affected by the expression of PV could shed new light on the understanding of the somatic mutations in human cancers such as hepatocellular carcinomas [22] and renal carcinomas reported previously by others [24]. Addressing these challenges and others that may emerge subsequently will certainly lead to recognition and appreciation of the important roles of TR in cancer biology.

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Abbreviations

BM	basement membrane
ECM	extracellular membrane
EMT	epitherlial mesenchymal transition
FAK	focal adhesion kinase
MAPK	mitogenactivated protein kinase
LOH	loss of heterozygosity
NCOR1	nuclear receptor corepressor 1
MMP	metalloprotease
mTOR	mammalian target of rapamycin
PAX8	paired box gene 8
PIP2	phosphatidylinositol-4,5-biphosphate
PIP3	phosphatidylinositol-3,4,5-triphosphate
PI3K	phosphatidylinositol 3-kinase
PPARγ	peroxisome proliferator-activated receptor γ

PPRE	peroxisome proliferator responsive element
PPTG	pituitary tumor-transforming gene
PTEN	phosphatase and tensin homologue delated from chromosone 10
RTH	resistance to thyroid hormone
RXRα	retinoid X receptor α
SH	Src homology
SKY	spectral karyotyping analysis
SMRT	silencing mediator of retinoid and thyroid hormone receptors
SRC	steroid receptor coactivator
TREs	thyroid hormone response elements
TRs	thyroid hormone nuclear receptors
T3	triiodothyronine
TSH	thyroid stimulating hormone

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Highlights

- Mutations of thyroid hormone receptors (TR) are associated with human cancers
- Loss of TR normal functions by deletion or mutations contribute to cancer development
- Mice harboring a homozygous mutation of TRβ spontaneously develop thyroid cancer
- Nuclear and extra-nuclear actions of a TRβ mutant mediate thyroid carcinogenesis
- Mouse models of thyroid cancer allow uncovering novel molecular targets for treatment

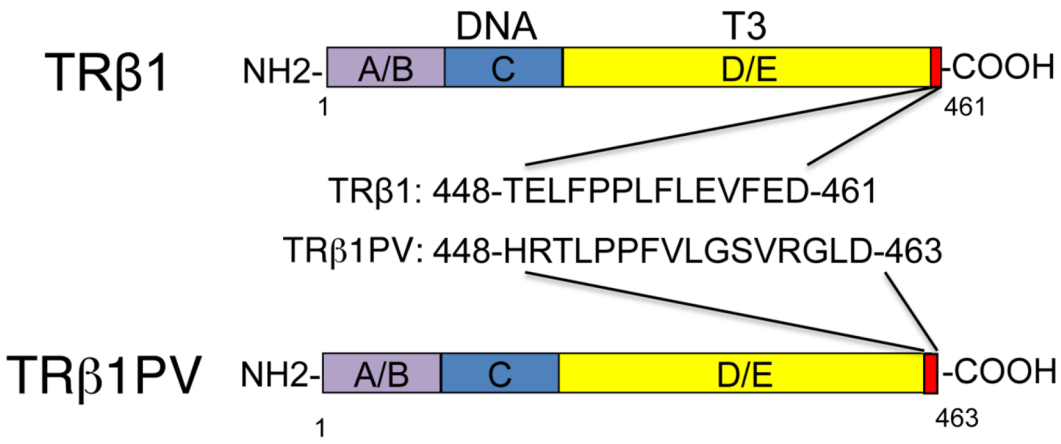

Figure 1. Schematic comparison of TRβ1 and TRβ1PV mutant

The TRβ1PV mutation has a frame-shift mutation in the C-terminal 14 amino acids of TRβ1 (461 amino acids), ending with an addition of two amino acids (463 amino acids). TRβ1PV has completely lost of T3 binding activity and transcription capacity. The carboxyl-terminal sequences of the TRβ1 and TRβ1PV mutant are shown. The domains are also indicated.

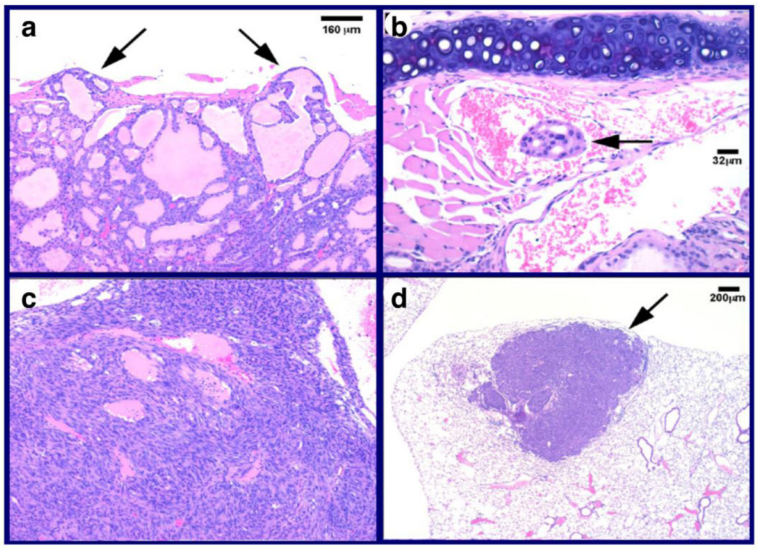

Figure 2. *Thrb*$^{PV/PV}$ mice spontaneously develop follicular thyroid carcinoma with pathological progression similar to human cancer

Representative examples of pathological features in thyroid carcinogenesis of *Thrb*$^{PV/PV}$ mice: capsular invasion in the thyroid (a) (arrows), vascular invasion in the thyroid (b) (arrow), anaplasia in the thyroid (c, same magnification as panel a) and metastatic thyroid carcinoma lesions in the lung, often in a follicular pattern (d) (arrow) [36]. Magnifications are as indicated.

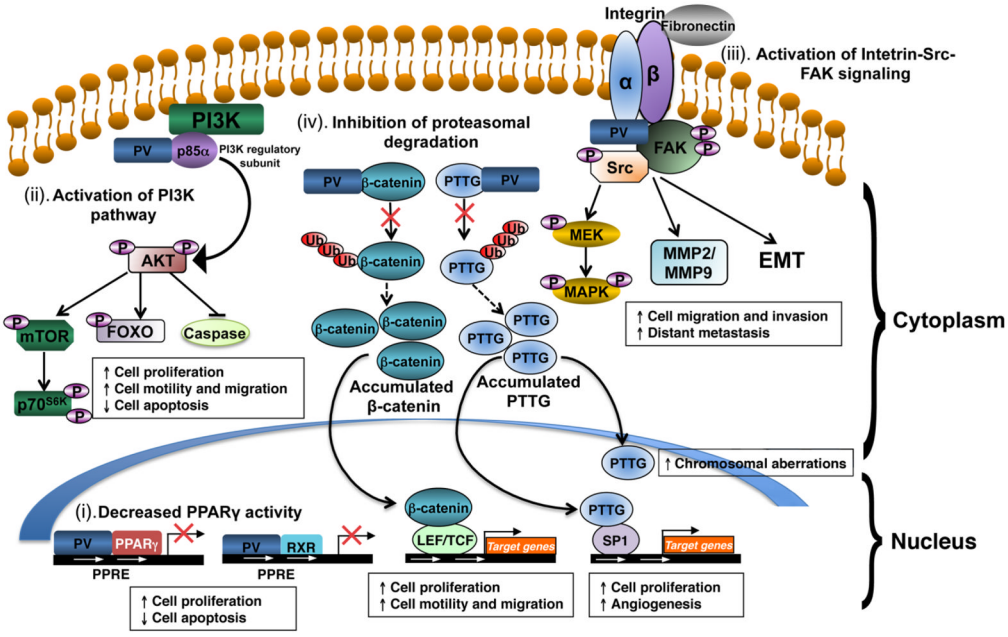

Figure 3. PV mediates oncogenic actions in thyroid carcinogenesis of *Thrb*$^{PV/PV}$ mice via multiple mechanisms

(i). PV decreases peroxisome proliferator–activated receptor γ (PPARγ) activity by competition with PPARγ for binding to PPARγ response elements (PPRE) as heterodimers with PPARγ or the retinoid X receptor (RXR). Repressed transcriptional activity of PPARγ leads to increase cancer cell proliferation and decrease apoptosis in thyroids of *Thrb*$^{PV/PV}$ mice. (ii) PV activates phosphatidylinositol 3-kinase (PI3K) signaling pathway by physical interaction with the regulatory subunit of PI3K (p85α). Over-activation of PI3K-Akt signaling increases proliferation and migration of cancer cells and decreases apoptosis via the downstream pathways as shown. (iii) PV activates integrin-Src-focal adhesion kinase (FAK) signaling cascade. PV interacts with integrin and activates phosphorylation of Src and FAK. Activated Src-FAK signaling pathway induces epithelial-mesenchymal transition (EMT) and activation of matrix metalloproteases (MMPs) to increase cell migration, invasion and distant metastasis. Activated Src also potentiates mitogen-activated protein kinase (MAPK) signaling to increase cell proliferation and EMT. (iv) PV inhibits proteasomal degradation of the pituitary tumor transforming gene (PTTG) and β-catenin. PV binds to PTTG and prevents its degradation by proteasome, resulting in accumulation of PTTG. Elevated PTTG impedes mitotic progression, leading to chromosomal aberrations. Elevated PTTG, via transcription activation of its target genes, increases cell proliferation and augments angiogenesis. PV also increases the stability of β-catenin via direct protein-protein interaction, thereby inhibiting protesomal degradation of β-catenin. Elevated β-catenin protein levels increase transcription activation of its downstream target genes that affect cell proliferation and migration.

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Table 1

Somatic mutations of thyroid hormone receptor genes in human cancers

Type of cancer	Gene	Mutation Sites	Impaired activity		Dominant negative activity	Reference
			T3 binding	DNA binding		
Hepatocellular carcinoma	*THRB*	234G insertion	ND	ND	ND	22
		D211N	No	No		
		R153L	No	No		
		R194G	ND	ND		
		M27I, C102R, T363N	Yes	Yes		
		K258E	No	No		
		S38L, C441R	ND	ND		
		M308I	Yes	No		
		H400Y, F434N	Yes	No		
		K108N, T324P	Yes	No		
		S38P, I54T, P273S, P273L, E306G	Yes	No		
		K23E, I187V	Yes	Yes		
	THRA	A225G, T227N	ND	ND		
		A225G, D246N, G350K	Yes	No		
		S40T, K136R, L251P, V390A	Yes	No		
		K74E, A264V	Yes	Yes		
		K74R, M150T, E159K	Yes	No		
		N179I	ND	ND		
		S38Q, Q108K, F112L, I299V	Yes	Yes		
		C110Y, C254A	Yes	Yes		
		G24E, M256V, E343A, P269L	Yes	No		
Renal cell carcinoma	*THRB*	S99R, W129L, F451I	Yes	No	Yes	23, 24
		Y321H	Yes	No	No	
		F451S	Yes	No	No	
		Q252R, A387P, F417L	Yes	No	No	
		K155E, K411E	No	Yes	No	
		Δ1–26, S380F	Yes	No	No	

Type of cancer	Gene	Mutation Sites	Impaired activity		Dominant negative activity	Reference
			T3 binding	DNA binding		
		E299K, H412R, L456S	Yes	No	No	
	THRA	S183N, H184Q, R228H, K288E	Yes	Yes	No	
		I116N, M388I	Yes	Yes	No	
		I116N, A225T, M388I	Yes	Yes	No	
Breast cancer	*THRB*	deletion (123–242) deletion (319–366) truncated at 167 deletion (166–402) deletion (181–382)	ND	ND	ND	25
Pituitary tumor	*THRB*	R438H	Yes	No	ND	26, 27
		H450Y	Yes	No	Yes	
Thyroid cancer	*THRB*	V109A, I431T	Yes		Yes	28
		R185K, T273A, L456S	Yes		Yes	
		M32V	No		No	
		E34G, P141L	Yes		Yes	
		A318D, F451I	Yes		Yes	
		N76D, S81L, I135V, Q136H, R201X	Yes		Yes	
		F403L. C446R	Yes		Yes	
		K91R, K289M	Yes		Yes	
		Q235X, M379T, D427G	Yes		Yes	
		K411E	Yes		Yes	
		Q205L	Yes		Yes	
		K103R	Yes		Yes	
		M32T, L373P	Yes		Yes	
		K411E, H435R	Yes		No	
		S99R	Yes		Yes	
	THRA	T80I, L109P	Yes		Yes	
		E213D	Yes		Yes	

Type of cancer	Gene	Mutation Sites	Impaired activity		Dominant negative activity	Reference
			T3 binding	DNA binding		
		S305P, K337R	Yes		Yes	
		G57E	Yes		Yes	
		K29T, C97X	Yes		No	
		Y352C	Yes		Yes	
		S183N, H184Q, Q187X, R228H, E245V, K288E	Yes		Yes	
		S183N, H184Q, R228H, M369V	Yes		Yes	
		S183N, H184Q, R228H	Yes		Yes	
		S271I	Yes		Yes	

ND. Not determined.