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NOVEL ONCOGENIC ACTIONS OF TRβ MUTANTS IN TUMORIGENESIS

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Abstract

The thyroid hormone, T3, plays important roles in metabolism, growth, and differentiation. Germline mutations in thyroid hormone receptor beta (TRβ) have been identified in many individuals with resistance to thyroid hormone, a syndrome of reduced sensitivity to T3. A close association of somatic mutations of TRβ with several human cancers has become increasingly apparent, but how TRβ mutants could be involved in the carcinogenesis *in vivo* has not been addressed. The creation of a mouse model ($TR\beta^{PV/PV}$ mouse) that harbors a knockin mutation of TRβ (denoted TRβPV) has facilitated the study of the molecular actions of TRβ mutants *in vivo*. The striking phenotype of thyroid cancer and the development of pituitary tumors exhibited by $TR\beta^{PV/PV}$ mice have uncovered novel functions of a TRβ mutant in tumorigenesis. It led to the important findings that the oncogenic action of TRβPV is mediated by both genomic and non-genomic actions to alter gene expression and signaling pathways activity.

Keywords

thyroid hormone receptor mutants; thyroid cancer; pituitary tumor; non-genomic action; TRβPV; phosphatidylinositol 3-kinase; pituitary tumor transforming gene; β-catenin

INTRODUCTION

Thyroid hormone receptors (TRs) belong to the superfamily of ligand-dependent transcription factors. Two TR genes, *THRA* and *THRB*, located on two different chromosomes encode four T3-binding receptors: TRα1, TRβ1, TRβ2, and TRβ3. They bind the thyroid hormone (T3) that plays critical roles in differentiation, growth, and metabolism (1). Although abnormal expression and aberrant activity of sex steroid nuclear receptors are well known to be involved in the development and progression of cancers, much less is known about the possible involvement of TRs in tumorigenesis. However, increasing evidence suggests that TRs could also play a role in tumor progression. Indeed, studies using cancer cell lines show that wild-type TRs can regulate cell proliferation, cell differentiation, and cell migration (2-9). Besides, abnormal expression and somatic mutations of TRs have been described in human cancers, such as those of the liver (10), kidney (11,12), pituitary (13-15), breast (16,17), colon (18), and thyroid (19-21).

The precise contribution of TR mutants to tumorigenesis is not fully understood, but their high frequency in human cancers suggests that they have a contributory role. The first evidence to support this hypothesis was the observation that a TRα1 mutant, initially identified as the *v-erbA* oncogene in an avian retrovirus, could cause hepatocellular

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carcinomas when expressed in transgenic mice (22). Altogether, these observations suggest that partial loss of normal TR function due to reduced expression, or complete loss or alteration of TR activity, provides an opportunity for cells to proliferate, invade, and metastasize. In this context, TR could act as a tumor suppressor.

Although a correlation between TR abnormalities and the development of cancers has been established, the target genes and signaling pathways affected by TR mutants are not well known. In addition, there is still limited knowledge about the molecular mechanisms by which the TR mutants alter the activity of the affected genes and signaling pathways to mediate carcinogenesis.

The creation of a knockin mouse harboring a C-terminal 14 amino acid frameshift mutation in TRβ (*TR*$\beta^{PV/PV}$ mice) provides a valuable tool to explore *in vivo* the molecular mechanisms that are altered by a TR mutant to drive tumorigenesis (23). The TRβPV mutation was initially identified in a patient (called PV) who has the syndrome of resistance to thyroid hormone (RTH). RTH is characterized by a reduced sensitivity of tissues to the action of thyroid hormones, and it is frequently associated with the mutation of one copy of *THRB*. Studies using reporters in cultured cells have shown that the TRβPV mutation has completely lost T3 binding capacity and displays dominant negative activity (24,25). Consistent with the phenotype of RTH patients, *TR*$\beta^{PV/+}$ and *TR*$\beta^{PV/PV}$ mice faithfully reproduce human RTH. Strikingly, as *TR*$\beta^{PV/PV}$ mice but not *TR*$\beta^{PV/+}$ age, they spontaneously develop follicular thyroid carcinoma with tumor progression similar to human cancer (26). In addition, *TR*$\beta^{PV/PV}$ mice spontaneously develop thyroid-stimulating hormone (TSH)-secreting tumors (TSHomas) (27). Thus, the phenotype of the *TR*$\beta^{PV/PV}$ mice indicates that mutations of the TRβ gene extend beyond RTH and that TRβ mutants could act as oncogenes.

Extensive molecular studies were performed to understand the mechanisms behind the development and progression of tumors mediated by TRβPV. Comprehensive cDNA micro-array analysis of gene expression in the thyroids of *TR*$\beta^{PV/PV}$ mice show a dramatic alteration in the expression of genes involved in different signaling pathways, including TSH, Wnt-β-catenin, transforming growth factor β, tumor necrosis factor α, and nuclear factor κB peroxisome-proliferator-activated receptor γ (PPARγ) pathways (28). These results suggest that complex alterations of multiple signaling pathways induced by TRβPV contribute to thyroid carcinogenesis. However, the mechanisms by which TRβPV alters gene profiles to mediate thyroid carcinogenesis in *TR*$\beta^{PV/PV}$ mice were unknown. Our initial studies showed that TRβPV exerts dominant-negative actions and thereby alters gene transcription to mediate thyroid cancer and pituitary tumor in *TR*$\beta^{PV/PV}$ mice (27,29-31). Most recent studies further indicate that TRβPV mediates its oncogenic actions in the thyroid *via* non-genomic mechanisms (32-34). This review will highlight the currently known mechanisms mediating the oncogenic actions of TRβ mutants in *TR*$\beta^{PV/PV}$ mice.

GENOMIC ACTIONS OF MUTATED TRs IN CARCINOGENESIS

Although significant progress has been made in understanding the mechanisms by which wild-type TRs act in regulating gene transcription, how TR mutants affect transcription activity to drive tumorigenesis is far less understood. Typically, wild-type TRs bind to specific DNA sequences on specific DNA recognition motifs usually located in the promoters of T3-target genes (denoted thyroid hormone response elements, or TREs) as monomers, homodimers, or, more frequently, heterodimers with RXR (1). Binding of TRs to their DNA recognition motif is ligand-independent. The regulation of TR transcriptional activity is complex, and it depends not only on the presence of T3 but also on the type of TREs on the promoter of T3-target genes. Classically, unliganded TRs recruit co-repressors

such as NCoR and mediate basal transcriptional repression of target genes. Conversely, the binding of T3 induces conformational changes of TRs and results in the release of co-repressors, the recruitment of co-activators such as those from the p160/SRC1 family, and transcriptional activation. TRs can also regulate target genes indirectly through protein-protein interaction with other transcription factors.

Several studies showed that TR mutations identified in cancer cell lines frequently lead to the loss of T3 binding, confer TR dominant negative activity, and impair the binding to DNA recognition motifs, thereby leading to abnormal transcriptional activity (12,14,15,21,35). In addition, some TR mutations lead to alterations in TR affinity for co-repressors, even in the absence of T3 (35). Although these studies provided further insights into the mode of actions of TR mutants in cancer cells, the *TRβ*$^{PV/PV}$ mouse offers the unique opportunity to study *in vivo* the mechanisms underlying the molecular action of a TRβ mutant that spontaneously leads to thyroid and pituitary tumors.

1. Decreased activity of PPARγ signaling pathway mediated by TRβPV in thyroid cancer

We made the interesting finding that the thyroid tumors of *TRβ*$^{PV/PV}$ mice display a significant decrease in *PPARγ* mRNA levels (29). Our *in vivo* studies using *TRβ*$^{PV/PV}$*PPARγ*$^{+/-}$ mice further show that thyroid carcinogenesis progresses significantly faster from increased cell proliferation and reduced apoptosis (30). In addition, biochemical and cell-based studies show that TRβPV acts to abolish the ligand (troglitazone)-mediated transcriptional activity of PPARγ (29). Identification of the repressed PPARγ signaling pathway during thyroid carcinogenesis in *TRβ*$^{PV/PV}$ mice is particularly relevant in view of the work of Kroll et al. (36) that reported the identification of a chromosomal rearrangement yielding a *PAX8-PPARγ1* fusion gene in human follicular carcinomas. When fused to *PAX8*, *PPARγ1* not only loses its capability to stimulate *PPARγ*-ligand (thiazolidinedione)-induced transcription but also acts to inhibit *PPARγ1* transcriptional activity (36), raising the possibility that PPARγ could act as a tumor suppressor in thyroid carcinoma.

Our studies aimed at deciphering the molecular mechanisms underlying the inhibition of PPARγ ligand-mediated activity transcriptional activity by TRβPV provided further insights into how the TRβPV mutation could interfere with the PPARγ signaling pathway (31). Similar to TRβ1, TRβPV competes with PPARγ for binding to the peroxisome proliferator responsive element (PPRE) as homodimers or heterodimers with PPARγ or RXR, thereby competing with PPARγ for PPRE binding and for sequestering RXR (29,31) (Figure 1). Unliganded TRβ1 and TRβPV recruit the nuclear co-repressor NCoR to the promoter of PPARγ-target genes *in vivo*. However, although T3 can relieve the repression effect of unliganded TRβ1/PPARγ on troglitazone-dependent transcriptional activity of PPARγ by releasing NCoR, it cannot relieve the repression effect of TRβPV/PPARγ because TRβPV cannot bind T3. Further analyses indicate that the constitutive association of TRβPV with co-repressors prevents the recruitment of the steroid hormone coactivator -1 (SRC-1) to the PPARγ/TRβPV complexes in the presence of troglitazone. In the *TRβ*$^{PV/PV}$ thyroid, reduced *PPARγ* expression and decreased PPARγ transcriptional activity could lead to a reduction in the expression of PPARγ-downstream tumor suppressor genes and/or an increase in the expression of tumor promoter genes, thereby promoting the progression and development of thyroid cancer. Importantly, these findings highlight a dominant negative action of TRβPV that can mediate thyroid carcinogenesis by altering PPARγ signaling.

2. Up-regulation of cyclin D1 mRNA levels by TRβPV in pituitary tumors

Somatic mutations of *THRB* were identified in several patients with TSHomas (13,15). This disease represents about 2% of all pituitary adenomas in humans. Patients with TSHomas have high serum TSH despite elevated thyroid hormone levels, indicating that TSHomas

exhibit a defect in the negative regulation of TSH by thyroid hormone. The TRβ mutants identified in TSHomas show impaired T3 binding and exhibit dominant negative activity (13,15,37). Some TRβ mutants were found to interfere with the normal regulation of the glycoprotein hormone α-subunit and TSHβ genes, which encode the subunits of TSH (14,15).

The *TR*β$^{PV/PV}$ mice spontaneously develop TSHomas, indicating that the mutation of TRβ is one of the genetic events that can mediate the development of this tumor. We therefore explored the mechanisms underlying the development of TSHomas using the *TR*β$^{PV/PV}$ mouse as a model.

Similar to the patients with TSHomas, *TR*β$^{PV/PV}$ mice exhibit severe dysregulation of the pituitary-thyroid axis with highly elevated TSH associated with increased T3 (23,27). Mice deficient in both TRα and TRβ (*TR*α$^{-/-}$*TR*β$^{-/-}$ mice) have similarly elevated serum TSH and thyroid hormone levels as those of *TR*β$^{PV/PV}$ mice. However, *TR*α$^{-/-}$*TR*β$^{-/-}$ mice do not develop TSHomas. These findings indicate that the dysregulation of the pituitary-thyroid axis alone is not sufficient to mediate the pathogenesis of TSHomas (27). That these two mutant mice have a similar degree of resistance to thyroid hormone in the pituitary, but have contrasting phenotypes in displaying TSHomas has facilitated the comparison of gene expression profiles. cDNA microarray studies show the up-regulation of growth and proliferation-related genes in the pituitary of *TR*β$^{PV/PV}$ mice but not in that of *TR*α$^{-/-}$*TR*β$^{-/-}$ mice (27). Among the proliferation-related genes, *Ccdn1* encoding cyclin D1 is up-regulated in the pituitary of *TR*β$^{PV/PV}$ mice (27). Additional studies confirmed the up-regulation of *Ccdn1* at the mRNA levels and further showed that cyclin D1 protein is overexpressed. The over-expression is accompanied by concurrent activation of the cyclin-dependent kinase (cdk)/retinoblastoma (Rb) protein/E2F pathway and increased cellular proliferation in the pituitary (27,38).

The molecular mechanism by which TRβPV activates the expression of *Ccdn1* was studied. Multiple factors are known to regulate the activity of the cyclin D1 promoter, including cAMP-response element-binding protein (CREB) (38,39). We found that liganded TRβ represses *Ccdn1* expression via tethering to the *Ccdn1* promoter through binding to CREB. This repression effect is lost in TRβPV, thereby resulting in constitutive activation of *Ccdn1* in *TR*β$^{PV/PV}$ mice (27) (Figure 1). Thus the TRβPV mutation, by altering *Ccdn1* expression, induces aberrant cellular proliferation in the pituitary that contributes to TSHomas.

NON-GENOMIC ACTIONS OF TRs IN CARCINOGENESIS

More recently, several reports showed that thyroid hormones exert rapid actions on cell functions through non-genomic mechanisms. The non-genomic effects may occur through signal transduction mechanisms initiated by binding of hormones to TRs located in the plasma membrane, in the cytoplasm, or in the mitochondria (for review, ref. 40). These non-genomic actions were reported to regulate ion channels, glucose transporters, protein kinase (PI3K, PKC, PKA, ERK/MAPK), and phospholipid metabolism by activation of phospholipase C and D (41). Whether TR mutations could impair cell signaling *via* non-genomic mechanisms was not known. We therefore took advantage of the *TR*β$^{PV/PV}$ mouse model to study the possibility that a TRβ mutation could mediate thyroid carcinogenesis via non-genomic mechanisms. We made the remarkable discovery that the phosphatidylinositol 3-kinase (PI3K)-AKT pathway, the β-catenin signaling pathway, and PTTG activity are altered by TRβPV in thyroid cancer through novel mechanisms involving protein-protein interaction (32-34,42).

1. Overactivation of phosphatidylinositol 3-kinase (PI3K)-AKT signaling by TRβPV in thyroid cancer

PI3Ks consist of a catalytic subunit of about 110 kD (p110) and a regulatory subunit (p85α, p85β or p55γ) that is encoded by at least three mammalian genes. PI3K phosphorylates phosphatidylinositol-4,5 biphosphate [PIP2] to produce phosphatidylinositol-3,4,5-triphosphate [PIP3]. The major effector of PI3K is the AKT kinase, which is activated upon PIP3-mediated membrane recruitment and in turn phosphorylates target proteins regulating cell proliferation, cell survival, cell size, and mRNA translation.

The abnormal activation of PI3K-AKT signaling contributes to abnormal cell growth and cellular transformation in a variety of neoplasms, including thyroid cancer (43,44). As in human thyroid cancer, the PI3K-AKT signaling pathway is overactivated in the thyroid tumors of $TR\beta^{PV/PV}$ mice (32). Consistent with a major role of PI3K signaling in thyroid cancer, the treatment of $TR\beta^{PV/PV}$ mice with the potent PI3K inhibitorLY294002 significantly delays thyroid tumor progression and metastatic spread (42,45).

We investigated the mechanisms by which TRβPV alters the PI3K signaling pathway to mediate thyroid carcinogenesis in $TR\beta^{PV/PV}$ mice. Previous reports showed the interaction of wild-type TRs with p85α to activate PI3K signaling pathway in human fibroblasts and vascular endothelial cells (46,47). We found that TRβ1 and the TRβPV mutant can physically interact with the C-terminal SH2 (Src homology 2) domain of p85α *in vitro* and in thyroid extracts (32) (Figure 1). Importantly, the binding of p85α with TRβPV is two to three times stronger than that with TRβ1, resulting in a greater increase of PI3K activity. Consistent with a higher activity of AKT, the downstream phosphorylation cascade of effectors, mTOR and $p70^{S6k}$, is also concurrently increased. Interestingly, the interaction of TRβPV with p85α occurs in both the nuclear and the cytoplasmic compartments of thyroid extracts to activate AKT and downstream signaling pathways in both compartments (32). That the regulation of PI3K signaling by TRβPV also occurs in the nuclear compartment is consistent with previous studies showing the presence of components of the PI3K signaling pathway in the nucleus (48,49).

Further insights into the mechanism underlying the activation of PI3K in the thyroid of $TR\beta^{PV/PV}$ mice led to the remarkable finding that the nuclear co-repressor NCoR is involved in the modulation of TRβPV-induced PI3K activation (42). In addition to its action in regulating the genomic actions of unliganded TRs (50), NCoR has been reported to be involved in transcription-independent mechanisms; NCoR is found not only in the nucleus, but also in the cytoplasm (51,52). Notably, we found that NCoR physically interacts with p85α and that NCoR and TRβ or TRβPV interact with the same region in the C-terminal SH2 domain of p85α, thereby competing with each other for binding to p85α. Additional *in vitro* studies showed that TRβPV interacts with p85α with a relatively higher affinity than does TRβ or NCoR (42). That led us to test the possibility that alterations in NCoR protein abundance could modulate the activation of PI3K by TRβPV. Indeed, overexpression of cellular NCoR protein levels leads to a consistent reduction in PI3K signaling. Conversely, knocking down cellular NCoR with small interfering RNA (siRNA) increases PI3K activity. In thyroid tumors of $TR\beta^{PV/PV}$ mice, NCoR protein abundance is markedly decreased as compared with wild-type thyroids. Altogether, our results indicate that the reduction in NCoR protein abundance in the thyroids of $TR\beta^{PV/PV}$ mice favors the interaction between p85α and TRβPV to activate PI3K signaling (42). Therefore, NCoR, via protein-protein interaction, is a novel regulator of PI3K signaling that could modulate thyroid tumor progression.

2. Increased activity of pituitary tumor-transforming gene (PTTG) by TRβPV in thyroid cancer

The search for genes underlying the chromosomal aberrations in *TR*β$^{PV/PV}$ mice using cDNA microarray led to the finding that *Pttg* mRNA levels are significantly increased in thyroid cancer of *TR*β$^{PV/PV}$ mice (28). In addition, cellular PTTG protein levels are markedly increased in the primary lesions of thyroid as well as lung metastases of *TR*β$^{PV/PV}$ mice (33). PTTG functions as a securin during cell cycle progression and inhibits premature sister chromatid separation. PTTG is involved in multiple cellular pathways, including cell proliferation, DNA repair, cell transformation, angiogenesis induction, invasion, and the induction of genetic instability. Consistent with its functions, aberrant PTTG overexpression is found in a wide variety of endocrine and non-endocrine tumors [for review, (53)].

The finding that PTTG abundance is increased in the thyroids of *TR*β$^{PV/PV}$ mice prompted us to test whether it could play a role in the thyroid carcinogenesis mediated by TRβPV. Indeed, our cell-based studies showed that aberrant accumulation of PTTG induced by TRβPV inhibits mitotic progression (33). Besides, although PTTG loss does not prevent the initiation of thyroid cancer in *TR*β$^{PV/PV}$*Pttg*$^{-/-}$ mice, their thyroid glands are smaller with decreased thyrocyte proliferation and reduced thyroid cancer aggressiveness as compared with *TR*β$^{PV/PV}$*Pttg*$^{+/+}$ mice (54).

The mechanism involved in the increased abundance of PTTG in the thyroids of *TR*β$^{PV/PV}$ mice was not known. It might reflect, at least partially, the increased *Pttg* mRNA levels. However, TRs and PTTG are known to be involved in proteasome-mediated degradation pathways, and therefore we tested the possibility that TRβ and TRβPV could regulate PTTG protein levels through such mechanisms (33).

A series of cell-based and molecular studies showed that the DNA binding domain of TRβ1 or TRβPV interacts with the amino-terminal region (amino acid 1-119) of PTTG (33). Moreover, T3 induces the degradation of TRβ1 concomitantly with that of PTTG *via* a mechanism involving the proteasomal machinery (33). T3 does not, however, induce TRβPV degradation, a finding consistent with the fact that this TRβ mutant has lost T3 binding capacity. In TRβPV-expressing cells, PTTG protein levels are not altered by T3 treatment and remain high (33). Our results thus support the idea that TRβ1 regulates PTTG degradation through T3 binding. This regulatory function is completely lost by TRβPV that fails to bind T3.

We next sought to understand how TRβPV fails to regulate the stability of PTTG as the liganded TRβ1 does. We considered the possibility that the protein complexes TRβ1/PTTG and TRβPV/PTTG recruit proteasome activators differently. Steroid receptor co-activator-3 (SRC-3) is degraded via 19S proteasome through its physical interaction with proteasome activator 28γ (PA28γ), an activator of the trypsin-like activity of the proteasome (55). Similar to other steroid receptors (56), the liganded TRβ1 recruits SRC-3, but the unliganded TRβ1 does not (33). In contrast, TRβPV does not bind SRC-3 whether T3 is present or not. We therefore tested the possibility of the existence of a differential recruitment of TRβ/PTTG and TRβPV/PTTG complexes by SRC-3/PA28γ. We found that the liganded TRβ1/PTTG complex recruits SRC-3/PA28γ through the direct interaction of TRβ1 with SRC-3, whereas the unliganded TRβ1 and TRβPV fail to recruit SRC-3/PA28γ (33). This study indicates that the regulation of PTTG degradation by the proteasome pathway is impaired by TRβPV *via* protein-protein interaction and thereby results in mitotic abnormalities, contributing to thyroid carcinogenesis (Figure 1).

3. Stabilization of β-catenin by TRβPV in thyroid cancer

The aberrant cellular abundance of β-catenin in thyroid tumors of *TR*$\beta^{PV/PV}$ mice provided us with the opportunity to understand how TRβ and the TRβPV mutant regulate the cellular levels of β-catena *in vivo* (34). β-catenin is the central mediator of the Wnt signaling pathway, which is critical for various cellular processes, including oncogenesis (57). Stabilized β-catenin protein accumulates in the nucleus and complexes with the T cell factor/lymphoid enhancer factor (TCF/LEF) family of DNA-binding transcription factors to enhance the expression of a variety of genes, including critical regulators of cell cycle progression (cyclin D1, c-myc) and invasion (matrix metalloprotease-1, MT1-MMP). Aberrant accumulation of β-catenin has been reported in a number of human cancers, including thyroid (58).

We sought to determine the molecular mechanisms involved in the increased stability of β-catenin as well as the consequences of such an accumulation on thyroid cancer development and progression in *TR*$\beta^{PV/PV}$ mice. The cellular levels of β-catenin are controlled by two distinct adenomatous polyposis coli (APC)-dependent proteasomal pathways. One includes the glycogen synthase kinase 3β (GSK3β)-regulated pathway involving the APC-axin complex (59) and the other is a p53-inducible pathway involving APC-Siah-1 (60). An additional mode of β-catenin cellular level regulation is mediated by nuclear receptors, namely the retinoid X receptor α (RXRα) and peroxisome proliferators-activated receptor γ (PPARγ) that belong to the same nuclear receptor superfamily as TRs. RXR and PPARγ regulate β-catenin protein abundance via APC/GSK3β/p53-independent mechanisms (61,62).

We found that, similar to RXRα and PPARγ2, TRβ and TRβPV physically interact with β-catenin *in vitro* and in cells. The regulation of the β-catenin protein level by TRβ and TRβPV also occurs via APC/GSK3β/p53-independent mechanisms. The complexing of TRβ with β-catenin is weakened, however, by the binding of T3 to TRβ, thereby allowing more uncomplexed β-catenin to be degraded by the proteasomal pathway. In contrast, since TRβPV does not bind T3, the association of TRβPV with β-catenin is independent of T3. Our results indicate that the constitutive association of TRβPV with β-catenin prevents the degradation of β-catenin, and hence leads to its accumulation in the thyroids of *TR*$\beta^{PV/PV}$ mice (Figure 1).

The consequence of the aberrant stabilization of β-catenin in the development and progression of thyroid cancer was investigated in *TR*$\beta^{PV/PV}$ mice. We first studied the cellular abundance of β-catenin phosphorylated on serine 552 (P552-β-catenin), as it reflects β-catenin nuclear translocation and transcriptional activity (63). We found that the increased β-catenin cellular levels in the thyroids of *TR*$\beta^{PV/PV}$ mice are associated with an increased cellular abundance of P552-β-catenin. Consistently, the β-catenin transcriptional downstream targets (c-myc, cyclin D1, and MT1-MMP) display elevated mRNA and/or protein levels. Our data indicate that TRβPV prevents β-catenin degradation by physical interaction with β-catenin, thereby leading to constitutive β-catenin signaling in the thyroids of *TR*$\beta^{PV/PV}$ mice.

SUMMARY AND FUTURE DIRECTIONS

The creation of a knockin mutant mouse harboring a mutated TRβ has revealed new insights into the molecular mechanisms by which TR mutants contribute to tumorigenesis. We found that the deleterious effects of TRβ mutants in causing thyroid cancer and pituitary tumor are mediated, at least in part, by interfering with the transcriptional activity of wild-type TRs (Figure 2) (27,29,31,63). TRβPV interferes with the normal regulation of transcription activity, thereby leading to abnormal repression of tumor suppressors (PPARγ) in thyroid

cancers and to the constitutive activation of tumor promoters (cyclin D1) in pituitary tumors (Figure 2).

The striking phenotype of thyroid cancer manifested by *TR*β$^{PV/PV}$ mice led to the recent identification of new modes of action of TRβ mutants that are beyond nucleus-initiated transcription (Figure 1B and Figure 2). TRβPV interacts with the PI3K regulatory subunit p85α, leading to the overactivation of PI3K-AKT signaling and increased PI3K-AKT downstream signaling to affect cell proliferation, apoptosis, migration, and metastasis (32). The direct protein-protein interaction of TRβPV with PTTG or β-catenin affects their degradation by the proteasomal pathways, thus leading to PTTG and β-catenin aberrant accumulation (33,34). Increased PTTG abundance contributes to chromosomal aberration and genomic instability. Constitutively active β-catenin signaling, by altering downstream gene expression, affects cell proliferation and migration. Therefore, the development and progression of thyroid tumors in *TR*β$^{PV/PV}$ mice not only involve aberrant transcriptional activity but also derailed post-transcriptional mechanisms. As suggested by our earlier studies (28), these findings support the hypothesis that tumorigenesis mediated by TRβPV in *TR*β$^{PV/PV}$ mice involves the complex alterations of multiple signaling pathways.

While much has been learned about the oncogenic actions of a TRβ mutant by using *TR*β$^{PV/PV}$ mice, the question remains as to whether the oncogenic actions of TRβ mutants are limited only to TRβPV or could be extended to other TRβ mutants with different mutation sites. Studies in several human cancers have identified somatic mutations at various sites in the *TR*β gene (10-12), suggesting that the oncogenic actions of TRβ mutants most likely are not limited to the C-terminal frameshifted mutation as in TRβPV. At present, there is another reported TRβ knockin mouse that harbors a Δ337T dominantly negative mutation (64). However, whether the TRβΔ337T homozygous knockin mouse develops cancer is currently unknown. To address the question of whether other TRβ mutations are also oncogenic, it would be necessary to develop other knockin mutant mice harboring different mutation sites. Developing these knockin mutant mice would certainly advance our understanding of the role of TRβ mutations in human cancers.

It has long been established that nuclear receptors play a significant role in the development and progression of endocrine tumors. For instance, aberrant activation of estrogen receptor (ER) and androgen receptor (AR) signaling has been reported to favor the development and progression of breast and prostate tumors, respectively [for review, (65,66)]. The finding that both nuclear receptors act as tumor promoters in their target tissues led to the development and use of anti-estrogen and anti-androgen strategies for breast and prostate cancer prevention and treatment.

Although our studies as well as several others provide lines of evidence to indicate that wild-type TRs act as tumor suppressors in the thyroid and pituitary, several studies reported that thyroid hormone stimulates breast and prostate cancer cell proliferation, suggesting that TRs could act as tumor promoters (2-6). These contrasting findings suggest that TRs may have opposite effects on cell functions depending on the target tissues. On the other hand, it is also possible that exposure of cells to supraphysiological doses of thyroid hormones favors induction of proliferation, as is the case with androgens and estrogens. In this regard, a future challenge would be to clarify the role of TRs on tumor development and progression in target tissues by development of pertinent *in vivo* models. These efforts will provide opportunities to develop better strategies for prevention and treatment of endocrine cancers.

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Abbreviations

NCoR	nuclear receptor co-repressor
PI3K	phosphatidylinositol 3-kinase
PPARγ	peroxisome proliferator-activated γ
RTH	resistance to thyroid hormone
RXRα	retinoid X receptor α
SRC-1	steroid receptor co-activator-1
SRC-3	steroid receptor co-activator-3
TRs	thyroid hormone nuclear receptors
T3	thyroid hormoney
TSH	thyroid-stimulating hormone

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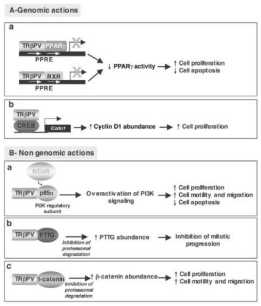

Figure 1. Proposed molecular mechanisms by which TRβPV mediates its oncogenic actions

A- Genomic actions of TRβPV contributing to thyroid (a) and pituitary tumors (b) in *TR*β$^{PV/PV}$ mice. (a) TRβPV competes with PPARγ for the binding to PPARγ responsive elements (PPRE) as homodimers or heterodimers with PPARγ or RXR, thereby decreasing PPARγ transcriptional activity to increase cell proliferation and to decrease cell apoptosis in the thyroid of *TR*β$^{PV/PV}$ mice. (b) TRβPV interacts with CREB in the promoter of the Cyclin D1 gene (*Ccdn1*), leading to the constitutive activation of Cyclin D1 and increased cell proliferation in the pituitary of *TR*β$^{PV/PV}$ mice. B- Non genomic actions of TRβPV contributing to thyroid tumor in *TR*β$^{PV/PV}$ mice. TRβPV physically interacts with other cellular proteins , thereby altering their activity or abundance. (a) TRβPV physically interacts with the regulatory subunit of PI3K, p85α, to activate PI3K signaling, thereby affecting cell proliferation, motility, migration, and apoptosis. TRβPV competes with NCoR to interact with p85α, but NCoR protein abundance is lower in thyroid cancer of *TR*β$^{PV/PV}$ mice than in normal thyroid, thus favoring the physical interaction of TRβPV with p85α, resulting in overactivation of PI3K signaling. (b) TRβPV physically interacts with PTTG and inhibits PTTG degradation by protesome pathways, leading to increased PTTG abundance and inhibition of mitotic progression. (c) TRβPV strongly interacts with β-catenin, and thereby prevents its degradation by protesome pathways. That leads to β-catenin accumulation and to increased cell proliferation, cell motility and migration (see text for detailed explanations).

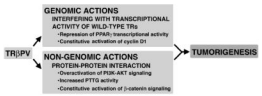

Figure 2. Scheme summarizing the genomic and non-genomic actions of a TRβ mutant in tumorigenesis

The TRβPV mutant interferes with the transcriptional activity of wild-type TRs, thereby altering the transcriptional activity of the tumor suppressor PPARγ in the thyroid (29,31) and activating cyclin D1 expression in the pituitary (27). TRβPV also exerts its oncogenic actions in the thyroid by acting through non-genomic mechanisms involving protein-protein interaction, thereby leading to PI3K-AKT overactivation (32) and accumulation of PTTG (33) and β-catenin proteins (34) (see text for detailed explanations).