INTRODUCTION

Nuclear receptors comprise a large family of ligand-inducible transcription factors that are critically important for growth, differentiation, development, and maintenance of metabolic homeostasis. They regulate the expression of target genes by binding to the specific DNA sequences at the promoters to mediate the biological effects. Many nuclear receptors have multiple isoforms with over-lapping functions or isoform-specific functions (1, 2). The expression of these receptor isoforms is regulated in a tissue- and development-dependent manner. A host of coregulatory proteins that influence the ligand selectivity and DNA binding capacity further modulates the transcriptional activities (3, 4).

Abnormal expression and/or aberrant functions of sex steroid nuclear receptors are known to be involved in the development and progression of such endocrine cancers as breast, ovarian, endometrium, and prostate, but less is known about the role of nuclear receptors in the carcinogenesis of the thyroid. Progress in this area has recently been made as a result of the discoveries of the fusion gene of PAX8 with the peroxisome proliferator-activated receptor γ (PPARγ; PAX8-PPARγ) in follicular thyroid carcinoma and of the spontaneous development of follicular thyroid carcinoma in the homozygous knock-in mutant mice harboring a mutated thyroid hormone β receptor (TRβ). This review will first examine the latest findings on the possible roles of several sex steroid nuclear receptors in thyroid carcinogenesis. It will then discuss the molecular actions of the mutant TRβ in carcinogenesis, particularly in relation to a unique knock-in mouse model of thyroid cancer.
Abnormal expression of estrogen and progesterone receptors in thyroid cancer

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Thyroid carcinoma is more common in women than in men (5). For 2003, the estimate of new cases of thyroid cancer has a female predominance with a 2.9:1 ratio (6). This predominance suggests that estrogens may play a critical role in the development of thyroid carcinoma. In the past two decades, efforts have been made to demonstrate the presence of estrogen receptors (ERs) in thyroid tumors and to correlate tumor malignancy with ER expression. Using a dextran-coated charcoal method and analysis by the method of Scatchard, Miki et al. did not detect ER in the cytosol of normal thyroid, but they found a significantly higher ER in the neoplastic and hyperplastic thyroid tissues (7). Using different biochemical methods, Mizukami et al. (8) and Yane et al. (9) also showed a higher expression of ER in neoplastic thyroid lesions than in normal thyroids or in adjacent normal tissues. Lewy-Trenda examined 72 thyroid glands for the expression of ER by using immunochemical assays with anti-ER antibodies. Positive staining occurred in the nuclei of differentiated thyroid cancer cells (24%), but not in non-neoplastic cells. A small number of oxyphillic (4%) and follicular adenomas (6%) also stained positive for ERs (10).

Consistent with these findings, several studies showed that estrogens stimulate the proliferation of thyroid carcinoma cells (11–13), whereas the antiestrogen, tamoxifen, inhibits the proliferation of a tumor cell line derived from medullary thyroid carcinoma (11). These studies clearly showed that cell proliferation induced by estrogens is mediated by ERs, but little is known about the specific molecular pathways. One study suggested that activation of the mitogen-activated protein kinase by phosphorylation might be one of the key steps in the estrogen-mediated cell proliferation of thyroid cancer cells (13).

The relevance of the increased expression of ERs in thyroid tumorigenesis is not obvious, particularly given that there is no clear correlation in the extent of expression of ER to age, sex, presenting clinical or pathological features, or, in cases of carcinoma, to subsequent metastatic potential (10, 14, 15). Furthermore, the failure of several studies to detect a greater expression of ERs in thyroid tumors than in normal tissues casts further doubt on the significance of expression of ERs in thyroid tumor development and progression (16–19). It is unclear whether the discrepancy among studies is due to the sensitivity of the detection or the intrinsic variability in the expression of ERs in tumor samples. Plainly, more studies are needed to understand whether estrogens and ERs are the major factors that contribute to thyroid cancers predominance in females.

Fewer studies have investigated the roles of progesterone receptors (PRs) in thyroid carcinogenesis. Because of the interest in understanding thyroid cancer’s predominance in females, the expression of PRs in thyroid tumors has been evaluated by means of ligand binding assays, enzyme immunoassays, and/or immunohistochemistry. In a few limited studies, the presence of PR and ER was assessed concurrently in the same samples. In 135 thyroid lesions that included papillary, follicular, medullary, and Hurthle cell carcinomas, van Hoeven detected the presence of PR in 51% of the cases, with the highest abundance in papillary carcinomas, particularly in male patients and women older than 50 years (15). In that same study, ER was found in 46% of the
samples. In other studies, however, a higher frequency of ER than PR was found in papillary carcinomas (7, 10, 14). Similar to the findings for ER, no correlations were observed between the expression of PR and age, sex, tumor size, presence of capsular or vascular invasion, or lymph node status (10, 14). Still, how the expression of PR is involved in the development of thyroid cancer has not been assessed.

ALTERED EXPRESSION OF THE RETINOIC ACID RECEPTORS IN THYROID CANCER

Retinoic acids (RAs) are essential for many biological processes including proliferation, development, differentiation, carcinogenesis, and apoptosis. These biological effects are mediated through their receptors (RARs). The retinoids, both the natural and synthetic analogs, have been shown to be effective in preventing several cancers in experimental animals and in reversing pre-neoplastic lesions in humans (20, 21). Whether the retinoids could be effective in re-differentiating thyroid cancer cells to be amenable to radioiodide or TSH-suppressive T4 therapy has prompted several investigators to study the expression of RAR in cancer cell lines and tissues. Using Northern blot analysis, del Senno found that the expression of RARα mRNA was lower in thyroid carcinoma cells than in normal thyroid follicular cells. Moreover, del Senno demonstrated that RA reduces the proliferation and function of thyroid follicular cells (22, 23). These findings were confirmed in a larger study. Using immunohistochemistry and Western blotting, Rochaix et al. compared the expression of RARβ in 40 normal/benign tissues, 16 papillary carcinomas, and two follicular carcinomas, immunostaining was detected in the nuclei, but was limited to the normal epithelial thyroid tissue. A dramatic decrease in RARβ immunostaining was observed in all 16 papillary carcinomas, but in only one follicular carcinoma (24).

Because the feasibility of retinoid-induced differentiation therapy in thyroid cancer hinges on functional RARs, Schmutzler et al. not only examined the expression of mRNA in several human thyroid carcinoma cell lines and tissues, but also assessed the ligand and DNA binding activities (25). Functional RARs were clearly detectable in the two human thyroid carcinoma cell lines (FTC-133 and FTC-238) and two anaplastic thyroid carcinoma cell lines (HTH74 and C643). Intriguingly, variable levels of mRNA were observed in these cell lines, an observation probably indicative of dysregulation of receptor expression in thyroid cancer (25). These results suggest the heterogeneity in the expression of RARs and the association of the dysregulation of the expression of RAR with thyroid carcinogenesis. However, the available expressed functional RAR seems to be able to respond to RA treatment. In a pilot study, patients with advanced thyroid cancer and without the therapeutic options of operation or radioiodide therapy were treated with 13-cis-retinoic acid (1.5 mg/kg body weight daily for 5 weeks). Overall, tumor regression was observed in 19 patients (38%). However, response to retinoid therapy did not always correlate with increased radioiodine uptake (a re-differentiation marker), and so other direct antiproliferative effects could also be involved (26). These encouraging clinical findings warrant additional studies on the RA-based treatment of thyroid cancer. At present, however, little is known about either the molecular mechanisms by which the expression of RAR
is dysregulated during thyroid carcinogenesis or the RA-induced-redifferentiation of follicular cells. Elucidation of these mechanisms should help in the design of an effective treatment of thyroid carcinomas that uses the retinoids.

**ABNORMALITIES OF THYROID HORMONE RECEPTORS IN THYROID CANCER**

The thyroid hormone receptors (TRs) mediate the pleiotropic activities of the thyroid hormone (T3) in growth, development, and differentiation and in maintaining metabolic homeostasis. The two TR genes, α and β, are located on human chromosomes 17 and 3, respectively. Alternative splicing of the primary transcripts gives rise to five major TR isoforms (α1, α2, β1, β2, and β3). TRα1, TRβ1, TRβ2, and TRβ3 differ in their lengths and amino acid sequences at the amino terminal A/B domain, but they bind T3 with high affinity to mediate gene regulatory activity. By contrast, TRα2, which differs from the other TR isoforms in the C-terminus, does not bind T3, and its precise functions have yet to be elucidated. The expression of TR isoforms is tissue-dependent and developmentally regulated (1, 2).

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 that is involved in the neoplastic transformation leading to acute erythroleukemia and sarcomas (27, 28). The oncogenic role of v-erbA was subsequently demonstrated in mammals in that male transgenic mice overexpressing v-erbA developed hepatocellular carcinoma (29).

In recent years, increasing evidence suggests that aberrant expression and mutation of the TR genes could be associated with human neoplasias. Somatic point mutations of TRα1 and TRβ1 were found in 65% (11/17 tumors) and 76% (13/17 tumors), respectively, of human hepatocellular carcinomas. Many of these mutated TRs have lost T3-binding activity and exhibit aberrant DNA-binding activity (30). Aberrant expression and mutations of TR genes were also found in renal clear cell carcinomas (31). Cloning of TRs from 22 renal clear cell carcinomas and 20 surrounding normal tissues identified somatic mutations in 32% and 14% of cloned TRβ1 and TRα1 cDNAs, respectively (32). Most of the mutations were localized in the hormone-binding domain that leads to loss of T3-binding activity and/or impairment in binding to TREs. Similar to the mutated TRs detected in hepatocellular carcinoma (30, 33), the mutated TRs identified in renal clear cell carcinomas exhibit dominant negative activity (32). These studies suggest that mutated TR plays an important role in the development of these human cancers.

**Abnormal expression and somatic mutations of TRs in thyroid cancer**

Similar to the expression levels reported for ER, PR, and RAR, an altered expression of TRs was detected in thyroid carcinomas. Comparison of the mRNA expression levels of TR isoforms in normal, hyperplastic, and neoplastic human thyroid tissues indicated that TRβ1 mRNA is significantly lower in papillary and follicular carcinomas than in normal thyroid. No differences, however, were found in the expression levels of TRα1 and TRα2 mRNA (34, 35). These findings suggest an association of the reduced expression of TRβ1 mRNA with the development of thyroid carcinomas.
These studies, however, did not determine whether TRβ1, TRα1, and TRα2 were altered at the protein level.

In addition to the reduced expression of TRβ1 mRNA, a lower expression of TRα1 mRNA was found in 16 papillary thyroid carcinomas from Polish patients. The TRβ1 and TRα1 protein levels, however, were higher in cancerous tissues than in nearby healthy tissues, an indication of the complexity in the regulation of TR expression in these tumors (36). To understand the nature of TRs in these papillary thyroid carcinomas, cDNAs were cloned concurrently from both the tumor lesions and the healthy thyroids as controls. Sequence analyses indicated that 93.8% and 62.5% of papillary thyroid carcinomas had mutations in TRβ1 and TRα1, respectively. In contrast, no mutations were found in healthy thyroid controls, and only 11.1% and 22.2% of thyroid adenomas had mutations in TRβ1 and TRα1, respectively. Functional analysis indicated that these mutated TRs lose their transactivation function and exhibit dominant negative activity (36).

The reduced expression of TRβ1 mRNA in papillary thyroid carcinomas was further confirmed in a more recent study of 16 Japanese patients (37). In contrast to the Polish patients, no amino acid-substitution-mutations were detected in the TRβ1 cDNAs cloned from these papillary thyroid carcinomas. The reasons for the different propensity in the mutations of the TRβ gene in these two groups of patients are not entirely clear. One possibility is that the Polish patients were from the post-Chernobyl population and that radiation exposure is a contributing factor to the high frequency of TR mutations. Indeed, five of the 16 Polish patients with mutated TRs were in their teens when the Chernobyl accident occurred. One of 16 patients received radiation treatment during her childhood because of another disease. The age of other patients ranged from 32–58 years old at the time of the Chernobyl accident (Monika Puzianowska-Kuznicka; personal communication). The validation of this hypothesis would require a cohort study with a larger number of patients and a detailed knowledge of irradiation dose received by the patients.

Another possibility is that the propensity of mutations of TRβ1 in papillary thyroid carcinomas could be affected by the patient’s ethnic origin. Genetic variation between different populations occurs frequently. For example, a wide variation in the frequency of RET/PTC rearrangements, a hallmark of papillary thyroid carcinoma, has been reported, ranging from a few percent in Japanese (38) and Saudi Arabian patients (39), to 18.8% in Italian patients (40), to 70% in New Caledonian and 85% in Australian patients (41). The frequency of polymorphisms associated with thyroid diseases also differs in Japanese and Caucasian populations (42). Clarification of the issue of whether genetic background affects the frequency of TRβ1 mutations in papillary thyroid carcinoma awaits additional analyses in patients with different ethnic origins.

**Germline mutations of the TRβ gene in thyroid cancer: lessons learned from a unique mouse model of thyroid carcinogenesis**

So far, the TR mutants identified in human cancers including thyroid carcinoma are somatic mutations. A knock-in mouse that harbors a germline mutation of the TRβ gene has been created (43). The mutation was targeted to the TRβ gene locus
via homologous recombination and the Cre/loxP system. The mutation is called PV (TRβPV mouse) after a patient with the mutation who suffers from the disease known as resistance to thyroid hormone (RTH) (44, 45). RTH is a syndrome characterized by the elevated levels of circulating thyroid hormone that are associated with non-suppressible TSH. Some of the clinical features include attention-deficit hyperactivity disorder, mental retardation, short stature, decreased weight, tachycardia, and hearing abnormalities (44, 45). PV has a unique mutation in exon 10, a C-insertion at codon 448, which produces a frame shift of the carboxyl-terminal 14 amino acids of TRβ1. In vitro studies revealed that PV has completely lost T3-binding activity, lacks transcriptional capacity, and exhibits potent dominant negative activity (46). Extensive characterization of the phenotype indicates that the TRβPV mouse faithfully reproduces the human RTH (43). This TRβPV mouse provides a valuable model for clarifying the role of germline mutations of the TRβ gene in carcinogenesis.

In addition to the phenotypes of RTH, homozygous TRβPV (TRβPV/PV) mice exhibited the phenotype of age-dependent increased mortality. By the age of about 10 months, 50% had died, and by the age of 14–15 months, all mice were dead. In contrast, the heterozygous (TRβPV/+ ) mice did not exhibit such abnormalities. Morphological examinations of the moribund TRβPV/PV mice indicate that as these mice aged, they spontaneously developed thyroid carcinoma (47). Histological evaluation of thyroids of 27 moribund TRβPV/PV mice showed capsular invasion (85%), vascular invasion (74%), anaplasia (37%), and metastasis to the lung and heart (26%) but not to lymph nodes (Table 1).

Representative examples of the pathological features of capsular invasion (Panel A), vascular invasion (Panel B), anaplasia (Panel C), and metastasis to the lung (Panel D) are shown in Figure 1. The histological features and the metastatic patterns indicate that the thyroid carcinoma developed in TRβPV/PV mice is follicular. Thus TRβPV/PV mice provide the first animal model for studying the molecular genetics underlying follicular thyroid carcinogenesis.

Using microarrays consisting of 20,000 mouse cDNAs, Ying et al. recently profiled the global alterations in gene expression in the thyroids of TRβPV/PV mice at 6 months of age, at which time metastasis had begun (48). They found that 185 genes were up-regulated (2- to 17-fold) and 92 were down-regulated (2- to 20-fold). The majority (~60%) of these altered genes are unnamed. Functional clustering of named genes with reported functions (100 genes) indicated that ~39% were tumor-, metastasis/invasion-, and cell cycle-related. Importantly, several tumor-related genes, such as cyclin D1, pituitary tumor transforming gene-1, cathespin D, and transforming growth factor α, that have been reported to be over-expressed in human thyroid

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Table 1. Histologic progression of thyroid neoplasia in 5-14 month-old TRβPV/PV mice
cancers were found to be activated in the arrays (49–53). Analyses of the gene profiles suggested that the signaling pathways mediated by TSH, peptide growth factors, transforming growth factor-β, tumor necrosis factor-α, and nuclear factor κB were activated, whereas pathways mediated by peroxisome proliferator-activated receptor γ (PPARγ) were repressed (48). These findings suggest that the expression of the TRβ mutant directly and indirectly alters multiple signaling pathways that could contribute to the development of thyroid cancer and that thyroid carcinogenesis is mediated by multiple genetic events.

The frequent occurrence of the somatic mutations in several human cancers (30, 32, 36, 54, 55) and the development of follicular thyroid carcinoma in TRβ<sup>PV/PV</sup> mice (47) raise the question of whether PV could function to initiate carcinogenesis. On the basis of observations that TRβ<sup>PV/PV</sup> but not TRβ<sup>PV/+</sup> mice develop follicular thyroid carcinoma, it is unlikely that PV could act alone to initiate thyroid carcinogenesis. One of the significant differences in phenotypes between TRβ<sup>PV/PV</sup> and TRβ<sup>PV/+</sup> mice is that the circulating serum TSH concentration in TRβ<sup>PV/PV</sup> mice is ~275-fold higher than that in TRβ<sup>PV/+</sup> mice (43). TSH is the main regulator of thyrocyte differentiation and proliferation, and the possibility that it is an initiator of thyroid carcinogenesis has been intensively studied (56, 57). Recent clinical and biochemical studies, however, do not support the role of TSH as an initiator of follicular carcinoma (58, 57). Additional genetic changes need to occur for the transformation of the hyperproliferative thyroid cells to cancer cells. On the basis of these considerations, it is reasonable to propose

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Figure 1. Pathological features in thyroid glands and metastasis in the lung of TRβ<sup>PV/PV</sup> mice. Histologic sections from tissues of TRβ<sup>PV/PV</sup> mice showed evidence of capsular invasion in thyroid (A) (arrows), vascular invasion in thyroid (B)(arrows), anaplasia in thyroid (C) and metastatic thyroid carcinoma lesions in lung (arrow).
that mutation of the two alleles of the TRβ gene could be one of the genetic changes leading to the transformation of the hyperproliferative thyroid cells to cancer cells. This hypothesis needs to be tested in future studies.

ABNORMALITIES OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ IN THYROID CANCER

PPARγ is a nuclear receptor that is involved in a wide range of cellular processes including adipogenesis, inflammation, atherosclerosis, cell cycle control, apoptosis, and carcinogenesis (59, 60). PPARγ mRNA is abundantly expressed in adipose tissue, large intestine, and hematopoietic cells, and it is moderately expressed in kidney, liver, and small intestine (61). It was recently found also to express in the thyroid (48). PPARγ inhibits cell growth, and one of the mechanisms in inhibition of cell proliferation is by reducing E2F/DP DNA-binding and transcriptional activity (62). Consistently, activation of PPARγ signaling by its ligands has been shown to block cell proliferation of various malignant cells and, in some cases, to induce differentiation and apoptosis (63–68). Ohta et al. reported that PPARγ mRNA is expressed in human papillary thyroid carcinoma cell lines (69). Significant, but variable expression of PPARγ mRNA was detected in four of the six cell lines studied. Consistent with findings in other cancer cell lines (63–68), cell proliferation was inhibited and apoptosis was induced by treatment with troglitazone. Ohta et al. also found that troglitazone significantly reduced tumor growth and prevented distant metastasis of BHP18–21 tumors in nude mice in vivo (69). In a more recent study, Martelli et al. also evaluated whether PPARγ is involved in the growth regulation of normal and tumor thyroid cells (70). No mutations were detected in PPARγ exons 3 and 5 in human thyroid carcinoma cell lines and tissues. The growth of thyroid carcinoma cells was inhibited by treatment with PPARγ agonists, but no growth inhibitory effect was observed in NPA cells by PPARγ agonists that did not express PPARγ. Growth inhibition induced by PPARγ agonists or by overexpression of the PPARγ gene in thyroid carcinoma cells was associated with increased p27 protein levels and apoptotic cell death (70).

TRβPV/PV mice provide an unprecedented opportunity to study the role of PPARγ in thyroid carcinogenesis in vivo. Using quantitative real-time PCR and Northern blotting, Yin et al. found that the expression of PPARγ mRNA was repressed 50%–60% in the thyroids of TRβPV/PV mice at the ages of 4, 6, and 12 months (71). Immunohistologic analysis demonstrated that the expression of PPARγ protein in the primary lesions of TRβPV/PV mice was less than that in the thyroids of wild-type mice and was not detectable in the metastasis in the lung (unpublished results), an indication that the expression of PPARγ protein remained low during thyroid carcinogenesis. Moreover, PV was found to abolish ligand (troglitazone)-dependent transcriptional activity of PPARγ in primary cultured thyroid cells from wild-type mice (71). The PV-induced transcriptional repression could be due to PV’s competition with PPARγ for binding to the peroxisome proliferator-activated receptor response element (PPRE) present in the PPARγ downstream target genes. Indeed, gel shift assay showed that the in vitro translated PV protein could bind to PPRE. This notion is supported by the
finding that the lipoprotein lipase (LpL) gene, a known PPARγ downstream target gene (72), was repressed ~5-fold, as shown by cDNA microarrays (48). Subsequent analyses by quantitative real-time PCR further demonstrated that the expression of the LpL gene was down-regulated (Panel B; Figure 2) concurrently with PPARγ mRNA (Panel A; Figure 2) in the thyroid glands of TRβ<sup>PV/PV</sup> mice at the ages of 6 and 12 months, thus confirming the repression of PPARγ signal pathways during thyroid carcinogenesis (71). These results indicate that reduced expression of PPARγ mRNA and repression of its transcriptional activity are associated with thyroid carcinogenesis.
and raise the possibility that PPARγ can be tested as a potential molecular target for prevention and treatment of follicular thyroid carcinoma.

That the attenuation of the PPARγ signaling pathways is associated with the development and progression of follicular thyroid carcinoma is also supported by the findings that the PAX8-PPARγ rearrangement occurs frequently in human follicular thyroid carcinomas, less frequently in adenomas, but not at all in papillary thyroid carcinomas (73–76). Even though the molecular actions of the PAX8-PPARγ rearrangement, particularly in its relation to the thyroid follicular carcinoma, has yet to be clarified, it is known that the fusion of PAX8, a thyroid transcription factor, to the amino terminus of PPARγ results in the loss of the transcriptional activity of PPARγ (73). Moreover, PAX8-PPARγ protein acts to inhibit thiazolidinedione-induced transactivation by PPARγ in a dominant negative manner (73). Taken together, these studies suggest that suppression of PPARγ signaling is closely linked to the development and progression of follicular thyroid carcinoma.

CONCLUDING REMARKS

Studies in the past few decades have clearly established that nuclear receptors play significant roles in the development and progression of several endocrine tumors, such as breast and prostate cancers. Progress in understanding the role of nuclear receptors in thyroid carcinoma lags behind that in breast and prostate cancers. Studies so far indicate that altered expression of ER, PR, RAR, TR, or PPARγ is associated with thyroid carcinomas. More studies are warranted to clarify the functional consequences of altered expressed receptors and to elucidate their signaling pathways in relation to carcinogenesis of the thyroid. These efforts will not only advance our understanding of the molecular genetics of thyroid cancer, but also provide opportunities to develop novel strategies for prevention and treatment.

The discovery that TRβ1PV/PV mice spontaneously develop follicular thyroid carcinoma indicates that, in addition to altered expression of nuclear receptors, mutation of nuclear receptors is another abnormality that could contribute to thyroid carcinogenesis. It is currently unknown whether, in addition to the TRβ gene, mutations of other nuclear receptors could also contribute to the development and progression of thyroid cancer. The finding that the TRβ1PV/PV mouse can be used as an animal model of follicular thyroid carcinoma opens the door to further study of the molecular genetic events underlying carcinogenesis, to identifying signature genes during different stages of tumor progression for clinical diagnosis, and to the testing of drugs and other treatment modalities.

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REFERENCES

9. Abnormalities of nuclear receptors in thyroid cancer


