The Role of the PAX8/PPARγ Fusion Oncogene in the Pathogenesis of Follicular Thyroid Cancer

Norman L. Eberhardt[1,2], **Stefan K.G. Grebe**[3], **Bryan McIver**[1], and **Honey V. Reddi**[1]

[1] Department of Medicine, Division of Endocrinology, Mayo Clinic & Foundation, Rochester, MN 55905

[2] Department of Biochemistry and Molecular Biology, Mayo Clinic & Foundation, Rochester, MN 55905

[3] Department of Laboratory Medicine and Pathology, Mayo Clinic & Foundation, Rochester, MN 55905

Abstract

When identified at early stages, most well-differentiated thyroid cancers are readily treated and yield excellent outcomes. Follicular thyroid cancer (FTC) however, when diagnosed at a late stage, may be very resistant to treatment, and exhibits 10-yr survival rates less than 40%. Despite substantial progress in recent years, we still have limited understanding of the molecular and biological interrelationships between the various subtypes of benign and malignant follicular thyroid neoplasms. In contrast to the wealth of information available regarding papillary thyroid carcinoma (PTC), the triggering mechanisms of FTC development and the major underlying genetic alterations leading to follicular thyroid carcinogenesis remain obscure. Recent studies have focused on a chromosomal translocation, t(2;3)(q13;p25), fusing PAX8, a transcription factor that is essential for normal thyroid gland development, with the peroxisome proliferator-activated receptor gamma (PPARγ), a member of the steroid/thyroid nuclear receptor family. This chromatin rearrangement results in the expression of a PAX8/PPARγ fusion protein, designated PPFP, whose incidence is relatively common in FTC and may represent an initiating event in the genesis of FTC. Here we review progress on the studies of PPFP that assess its involvement in FTC tumorigenesis.

INTRODUCTION

Thyroid cancer comprises 94.5% of all endocrine malignancies, and ranks 13th in frequency of occurrence among all cancers, accounting for 2.6% of all cases (Jemal et al., 2008). Thyroid cancer is more than three times more common in women than in men. The American Cancer Society predicted 37,340 new thyroid cancer cases for 2008 in the United States with an expected 1,590 deaths due to cause-specific mortality (Jemal et al., 2008). In recent years the incidence of thyroid cancer appears to be increasing, having nearly doubled since 2000 (Davies and Welch, 2006, Greenlee et al., 2000, Jemal et al., 2008, Ward et al., 2006, Wu et al., 2005), though the increased incidence in the United States is in large part due to increased

surveillance and detection, particularly of smaller papillary tumors (Davies and Welch, 2006).

Thyroid cancers are derived from thyroid follicular cells and most of the tumors are well-differentiated malignancies. Papillary carcinoma (PTC) is the most frequent morphotype, representing 80% of all thyroid tumors. The majority of PTC and well-differentiated, minimally invasive follicular thyroid carcinomas (FTC) are relatively easy to treat with excellent prognoses. In contrast, a smaller proportion of FTC and Hürthle cell carcinomas (HCC), which altogether represent about 20% of thyroid cancer, are often more aggressive, more advanced at the time of diagnosis, less responsive to traditional therapy, and more likely to cause both morbidity and mortality. FTC share similar cytologic features with benign follicular adenomas (FA) and are distinguished chiefly by the presence of invasion beyond the tumor capsule or into blood vessels upon postoperative histo-pathologic examination. This similar histological appearance of FA and FTC has been interpreted as evidence for an adenoma to carcinoma progression, with FA representing a pre-malignant lesion (Fig. 1).

Significant progress has been made in unraveling the oncogenic pathways involved in PTC pathogenesis. *RET and TRK* rearrangements have been studied extensively, along with their downstream signaling pathways (Jhiang et al., 1998, Jhiang, 2000, Le and Norton, 2000, Powell et al., 1998, Tallini and Asa, 2001). *RET/PTC* and the related genetic rearrangements result in constitutive tyrosine kinase activity, leading to constant and unregulated signaling along the RET-RAS-BRAF-MAPK pathway (Kimura et al., 2003, Knauf et al., 2003, Knauf et al., 2005, Leboeuf et al., 2008, Melillo et al., 2005, Motti et al., 2007, Nikiforova et al., 2003a, Ouyang et al., 2006) (Fig. 1). Support for the importance of this signaling pathway as a major PTC oncogenic trigger comes from the observations that activating mutations in BRAF (BRAF^{V600E}), and to a lesser degree, RAS are observed frequently in PTC (Kimura et al., 2003, Nikiforova et al., 2003a). RET-rearrangements, RAS and BRAF mutations collectively account for 60–70% of PTC and appear to be mutually exclusive, further emphasizing the pivotal role of this kinase-cascade in PTC development (Kimura et al., 2003). Compelling evidence that this pathway is involved in the pathogenesis of PTC include the findings that expression of RET/PTC1 (Jhiang et al., 1996) or BRAF^{V600E} (Knauf et al., 2005), a constitutively active BRAF mutant, in transgenic mice induces papillary thyroid carcinomas.

A substantial body of evidence suggests that the PTEN/PI3K/AKT pathway is frequently involved in the pathogenesis of FTC and ATC (Hou et al., 2007, Kim et al., 2005, Ringel et al., 2001, Saito et al., 2001, Santarpia et al., 2008) (Fig. 1). In addition, recent studies focusing on chromosomal rearrangements in FTC have implicated PPARγ-mediated pathways and are the focus of much research. However, the pathogenesis of FTC remains much less well defined than that of PTC. Since FTC are among the more aggressive tumors and account for a larger proportion of the mortality associated with the disease, continued effort to understand the molecular pathogenesis of FTC is required with the hope of establishing targets for development of alternative therapeutic strategies.

Chromosomal Rearrangements in Follicular Thyroid Cancer

An important, relatively recent development in the study of FTC tumorigenesis has been the identification of the *PAX8/PPARγ* fusion oncogene. This fusion oncogene is created by a balanced translocation between chromosomes 2 and 3 (Kroll et al., 2000). During the chromosomal exchange, the 2q13-qter region is translocated to 3p25, resulting in an in-frame fusion between most of the coding sequence of the thyroid-specific paired-box transcription factor *PAX8* (2q13) and the entire translated reading-frame of the gene of the liganded nuclear receptor-family member peroxisome proliferator activated receptor gamma (*PPARγ)* (3p25). Several studies have implicated the expressed novel PAX8/PPARγ fusion protein (PPFP) in the pathogenesis of FTC. Interestingly, PPFP is not the only PPARγ fusion mutation that occurs

in FTC. Recently the CREB3L2-PPARγ fusion, resulting from a t(3;7)(p25;q34) chromosomal rearrangement, was identified (Lui et al., 2008). The CREB3L2-PPARγ fusion encodes a protein containing the transactivation domain of CREB3L2 and, like PPFP, a full-length PPARγ-1 moiety. The PPFP and CREB3L2-PPARγ fusion proteins and their functional domain structures are depicted in Fig. 2. The PPFP fusions exhibit several different PAX8 breakpoints, although the PPARγ breakpoint appears to be constant (Kroll et al., 2000, Marques et al., 2002). A similar t(2;20;3)(p21;q11.2;p25) translocation has also been observed in one FA, in which a sequence of PPARγ is fused to exon 28 of the thyroid adenoma associated gene (THADA), whose function is unknown (Drieschner et al., 2006). However, in this case a fusion transcript cannot result as the two genes are transcribed in opposite directions. The finding of multiple fusions to PPARγ suggests that chromosome 3p25 contains a breakpoint hot spot region (Drieschner et al., 2006), a finding that correlates with our earlier finding that 3p25 is the site of frequent loss of heterozygosity in FTC (Grebe et al., 1997).

The finding of two fusion genes in thyroid cancer, both of which involve the fusion of two transcription factors containing complete PPARγ-1 moieties is intriguing and raises several questions. For example, since both fusion proteins contain a complete PPARγ-1 moiety, does this strongly implicate the PPARγ-1 moiety in tumorigenesis? Do transcription factor genes contain structural elements that promote chromosomal recombination? Since RET/PTC rearrangements in PTC have been shown to indicate a close structural proximity of the individual RET and PTC genes within the super-structural arrangement of chromosomes (Nikiforova et al., 2000), one would predict that a similar proximity of the PPARγ, PAX8 and/or CREB3L2 loci might exist in thyroid cells, raising an intriguing question of whether this spatial proximity is associated with an ongoing functional interrelationship between these genes? In addition, all of the studies to date have focused on the fusion gene products associated with these chromosomal rearrangements, but are there additional consequences of these genetic rearrangements? For example, what are the consequences of juxtaposition of regulatory elements, including miRNAs and locus control regions from distinct, normally separated chromosomal regions? Are there regions of duplication and/or deletion that amplify or remove other important regulatory factors? Also, what are the consequences of the resulting haploinsufficiency of each of the involved genes? Clearly, the existence of a fusion mutation family in thyroid cancer provides extensive opportunities for future research.

PAX8 plays an essential role in the terminal differentiation steps of thyrocyte development (Macchia et al., 1998) and, unlike other members of the PAX gene family, is a key regulator of terminally differentiated gene expression, including the sodium iodide symporter, thyroglobulin and the TSH receptor (Pasca di Magliano et al., 2000). Thus interference with PAX8 function by PPFP could represent a conceivable mechanism by which PPFP influences tumorigenesis. The *PAX8* gene promoter drives expression of the *PPFP* gene (Kroll et al., 2000). Accordingly, PPFP expression would be expected to correlate with the differentiation status of thyroid tumors, being high in well-differentiated tumors and low or absent in poorly differentiated tumors. *PPFP* has been identified in about 36% of FTC, 11% of follicular adenoma (FA), 13% of the follicular variant of PTC (FVPTC), and 2% of Hürthle cell carcinoma and is absent from PTC, anaplastic thyroid cancer (ATC), and benign nodular hyperplasia (reviewed in (Placzkowski et al., 2008)). The occurrence of PPFP in FA, a benign condition, raises questions whether PPFP causes cancer; however, if there is an FA to FTC progression, PPFP may play a role in the progression pathway (Fig. 1). On the basis of its occurrence, its expected PAX8-dependent expression pattern, and its oncogenic functions, it has been proposed that *PPFP* may represent an early FTC-specific oncogene (French et al., 2003, Gregory Powell et al., 2004, Kroll et al., 2000, Marques et al., 2002, Nikiforova et al., 2002, Nikiforova et al., 2003a).

PPFP-mediated interference with PPARγ function is another potential target for PPFP's oncogenic actions. PPARs are comprised of four principle subtypes, α, β, δ and γ, and form a sub-family within the steroid/thyroid hormone nuclear receptor superfamily. While PPARγ is widely recognized to regulate adipogenesis and plays a major role in insulin sensitization, in recent years it has also been studied for its potential role in tumorigenesis (reviewed in (Krishnan et al., 2007, Wang et al., 2006)). In addition, PPARγ ligands have increasingly started to be evaluated as therapeutic agents in a variety of cancers (reviewed in (Matsuyama and Yoshimura, 2009, Rumi et al., 2004)), including thyroid cancer (reviewed in (Shen and Chung, 2005)). The influence of PPARγ in the pathogenesis of cancer may be mediated in part by its roles in regulating cell cycle control and apoptosis through its influence on gene expression involving multiple cell signaling pathways (reviewed in (Fajas et al., 2001a, Fajas et al., 2001b, Michalik et al., 2006, Rieusset et al., 1999, Schoonjans et al., 1997)).

Evidence that PAX8/PPARγ is an Oncogene

As summarized in Table 1, several *in vitro* studies from a number of laboratories have provided evidence that PPFP can act as an oncogene. Powel et al. have utilized both transient and stable transfection of PPFP in Nthy-ori 3-1 cells and have shown accelerated growth rates and lower numbers of cells in the G_0/G_1 resting state in PPFP-transfected cells compared to expression vector (Gregory Powell et al., 2004). Similar results on growth and apoptosis rates have been observed in cells that have been stably transfected with PPFP (Gregory Powell et al., 2004). In these studies transfection of wild-type PPARγ had no effect on Nthy-ori 3-1 cell growth, indicating that PPFP is not acting as wild-type PPARγ surrogate. The over-expression of both PPFP and CREB3L2/PPARγ stimulated the proliferation of primary human thyroid cells (Lui et al., 2008), suggesting that the common PPARγ moiety is an essential component of these fusion genes' dominant oncogenic mechanism of action.

PPFP transfection also reduced dramatically the rates of apoptosis in the PPFP-positive cells, suggesting reduced apoptosis may explain much of the enhanced growth (Gregory Powell et al., 2004). Confirming these results, Espidinal et al. also observed a significant increase in Nthy-ori 3-1 cell viability (ratio live/dead cells) after PPFP transfection (Espadinha et al., 2007). Also, PPFP expression in FRTL-5 cells leads to enhanced proliferation as assessed by ^{3}H-thymidine incorporation and soft agar assays (Au et al., 2006). Finally, Nthy-ori 3-1 cells that stably express PPFP exhibited increased colony-formation on soft agar (Gregory Powell et al., 2004), indicating that in addition to enhanced growth, PPFP-transformed cells have an attachment-independent growth phenotype, a hallmark of malignant transformation. Taken together these studies suggest that PPFP expression may provide a significant growth advantage as well as promoting attachment-independent growth, both factors that contribute to tumorigenesis.

At present the only *in vivo* evidence that PPFP can influence tumorigenesis comes from the work of Yin et al., who have shown that PPFP-mediated inhibition of PPARγ increases estrogen receptor-dependent tumor specification in breast cancer (Yin et al., 2009). While PPFP did not directly affect tumorigenesis in this model, the studies provide strong evidence that PPFP acts as a dominant negative inhibitor of wild-type PPARγ and effects breast epithelial cell behavior and carcinogen-induced tumorigenesis, as discussed in more detail below.

While the above studies demonstrate that PPFP exerts oncogenic functions *in vitro*, there is as yet no demonstration of PPFP's ability to affect tumorigenesis *in vivo*. Thus the impact of PPFP on the behavior and biology of human FTC remains to be established. Also, it remains to be seen, whether PPFP acting alone is sufficient to promote tumorigenesis, or whether additional oncogenic events are required for its action.

Several studies have attempted to assess the interaction of PPFP with other genetic factors that occur frequently in FTC, including activating point mutations in the Ras gene (Banito et al., 2007, Castro et al., 2006, Di Cristofaro et al., 2006, Foukakis et al., 2006, Nikiforova et al., 2003b, Zhu et al., 2003), microchromosomal abnormalities, particularly loss of heterozygosity (Grebe et al., 1997, Ward et al., 1998), and aneuploidy (reviewed in (Grebe and Hay, 1995)). Table 2 summarizes studies showing that co-expression of RAS mutations and PPFP is rare in FA, FTC, and FVPTC. On the basis of differential expression of HBME-1 and galectin-3, in FTC expressing mutant RAS or PPFP, it was concluded that RAS and PPFP operate via distinct pathways (Nikiforova et al., 2003b). A novel and rare widely infiltrating, angioinvasive glomeruloid variant of FTC was shown to contain both RAS mutation and PPFP rearrangement (Cameselle-Teijeiro et al., 2008), but the contributions of both factors to this tumor's growth phenotype are unknown. Finally, one study has shown that the co-existence of PPFP and aneuploidy was relatively more frequent among well differentiated tumors with 60% of FAs and 14% of FTCs harboring PPFP also exhibiting aneuploidy (Banito et al., 2007), suggesting that co-existence of PPFP and aneuploidy is not associated with a particularly aggressive phenotype (Banito et al., 2007). Clearly, additional studies will be required to understand the importance of these other factors and their interrelationships, if any, to PPFP-mediated tumorigenesis.

Mechanism of PPFP Action

While the precise mechanism of PPFP action remains to be elucidated, Table 3 lists the different studies that demonstrate that PPFP can act as a dominant negative inhibitor of wild-type PPARγ activity and/or as a unique transcriptional activator of PPARγ– and PAX8-responsive genes. In the original description of PPFP Kroll used transient transfection assays in PPARγ-null osteosarcoma U2OS cells to assess the ability of PPFP to stimulate transcription from a variety of PPARγ response elements (PPREs) linked to a luciferase reporter, including a multimerized perfect DR1 PPRE, a multimerized PPRE from the acyl CoA oxidase gene, and a native PPRE from the aP2 enhancer (Kroll et al., 2000). PPFP was unable to stimulate transcription on its own from any of these PPREs and was unresponsive to PPARγ agonists. However, when transfected with a PPARγ expression vector all of these PPREs exhibited strong troglitazone-induced transcriptional activity that was abolished when PPFP was co-transfected at a ratio of 1:1, indicating that PPFP can act by dominant negative inhibition of wild-type PPARγ (Kroll et al., 2000). Subsequently we demonstrated that identical results were obtained when PPFP and wild-type PPARγ were co-transfected into an immortalized human thyroid cell line, Nthy-ori 3-1 (Gregory Powell et al., 2004). Thus PPFP on its own lacked transcriptional activity with the aP2 PPRE in the presence or absence of troglitazone, but it was able to completely abolish the troglitazone-induced PPARγ activity (Gregory Powell et al., 2004), indicating that similar activity is observed in thyroid cell lines.

Further evidence that PPFP acts as a dominant negative inhibitor of wild-type PPARγ comes from the finding that the PPARγ antagonist GW9662 demonstrated a dose-dependent increase in cell growth of Nthy-ori 3-1 cells (Gregory Powell et al., 2004). Treatment of PPFP-transfected cells with GW9662 did not result in further increase in growth compared to GW9662, suggesting that both agents act upon the same pathway. Similar effects were observed upon expression of the 12 amino acid C-terminal deletion mutant of PPARγ (Kishida et al., 2001, Masugi et al., 1999), a potent dominant negative inhibitor of wild-type PPARγ, in Nthy-ori 3-1 cells (SKG Grebe et al., unpublished) Loss of contact inhibition and anchorage dependence, which also correlate with malignant transformation, were also observed upon over-expression of PPFP.

Recently PPFP has been shown to function as a dominant negative inhibitor of PPARγ function *in vivo* in a model of progestin/7,12-dimethylbenz(a)anthracene–induced mammary

carcinogenesis. PPFP transgenic mice were generated with mammary gland–directed expression of PPFP using the MMTV promoter (Yin et al., 2009). MMTV-PPFP transgenic mice lacked spontaneous tumorigenesis, suggesting that PPFP may not be a primary oncogene capable of promoting tumorigenesis on its own at least in breast epithelial cells. Nevertheless, mammary epithelial cells from MMTV-PPFP mice expressed a greater percentage of $CD29^{hi}/CD24^{neg}$, CK5+, and double-positive CK14/CK18 cells along with reduced PTEN and increased Ras, ERK and AKT activation. PPFP transgenic animals were highly susceptible to progestin/7,12-dimethylbenz(a)anthracene–induced mammary carcinogenesis and the resulting tumors exhibited high tumor multiplicity and the appearance of predominantly estrogen receptor A–positive (ER^+) ductal adenocarcinomas in striking contrast to the control mice. Tumors had similar phenotypes as mammary cells from PPFP transgenic mice with respect to PTEN, pERK, and pAKT expression. Importantly, tumorigenesis in MMTV-PPFP mice was insensitive to the chemopreventive effect of PPARγ agonists. While the relationship of these studies to thyroid cancer is currently unknown, these studies provide the only known *in vivo* experiment that demonstrates the ability of PPFP to act as a dominant negative inhibitor of wild-type PPARγ. The studies also further support the involvement of PPARγ in tumorigenesis, especially the previously unrecognized role of PPARγ in the specification of mammary lineage and the development of ER^+ tumors (Yin et al., 2009). Also, it is interesting to note the effects of PPFP on PTEN, pERK and pAKT expression in the MMTV-PPFP mammary epithelial cells, as each of these genes have been implicated in thyroid cancer, particularly FTC (reviewed in (Paes and Ringel, 2008)). It remains to be determined whether there is parallel expression of these genes in thyroid tumors expressing PPFP.

While all of these studies implicate PPARγ-antagonism as a major oncogenic principle for PPFP, there is also some evidence that this effect might be dependent on cellular context and that in certain scenarios transcriptional activation via both PPREs and PAX8 response elements might be observed. For example, Au et al. have shown *in vitro* that PPFP does not solely act via dominant negative inhibition of PPARγ, but that it can also exert independent transcriptional functions, depending on the cellular context, including the ability to stimulate transcription from PAX8- and PPARγ-responsive promoters (Au et al., 2006). In HeLa cells PPFP stimulated transcription from sodium-iodide symporter (NIS) and thyroperoxidase (TPO) promoters similar to that of wild-type PAX8, but inhibited transcription from the thyroglobulin (Tg) promoter and partially inhibited the synergistic stimulation of the Tg promoter by wild-type PAX8 and thyroid transcription factor-1. Similar results were also obtained in the thyroid cell lines FRTL-5 and Nthy-ori 3-1 (Au et al., 2006). Au et al. found a strong cell-line dependence on the influence of PPFP on PPRE-dependent transcription (Au et al., 2006). PPFP exerted a dominant negative effect on PPRE expression in HeLa cells, but stimulated the expression of the PPRE-dependent promoter in a PPARγ ligand-dependent manner in FRTL-5 and Nthy-ori cells. These latter effects may be due to direct interaction of PPFP with the various promoters, as PPFP was shown to bind to a PPRE using gel mobility shift assays (Au et al., 2006).

The finding by Au et al. that PPFP stimulated transcription from a promoter containing a PPRE in a ligand-dependent manner is directly contrary to our own findings whereby PPFP lacked a transcriptional response in Nthy-ori 3-1 cells and was unresponsive to PPARγ agonists (Gregory Powell et al., 2004). While the reasons for this discrepancy are unknown, it is possible that PPFP may have different responses to different PPREs as Au et al. utilized the multimerized acyl coA oxidase PPRE (Au et al., 2006) and Powell et al. utilized the native aP2 PPRE (Gregory Powell et al., 2004). The cell line-dependence of these PPFP-mediated effects on transcription may be related to the different approaches toward cell immortalization. HeLa and Nthy-ori 3-1 cells have been immortalized with the HPV E6 and SV40 large T antigen, respectively. In contrast, FRTL5 cells are non-transformed rat thyroid cells that require thyroid-stimulating hormone for growth.

Espadinha et al. examined the influence of PPFP expression on endogenous NIS, Tg and thyroid-stimulating hormone receptor (TSHR, thyrotropin) mRNA in Nthy-ori 3-1 cells (Espadinha et al., 2007). Transfection of cells with a PAX8 expression vector increased Tg, NIS, and TSHR mRNA levels, while TTF-1 expression only stimulated Tg mRNA levels. PPFP-transfected cells exhibited a significant decrease in TSHR mRNA levels, but did not affect Tg or NIS mRNA expression. In contrast to the study of Au et al. (Au et al., 2006), PPFP alone did not stimulate endogenous NIS gene mRNA expression. However, co-expression of PPFP and PAX8 did produce a significant stimulation of NIS mRNA expression above the PAX8-stimulated level. As a possible mechanism for this stimulatory effect, the authors have suggested that PPFP may be acting as a PAX8 co-activator (Espadinha et al., 2007). However, co-expression of PPFP and PAX8 led to decreased Tg and TSHR mRNA levels, relative to cells transfected with PAX8 alone. Thus PPFP can stimulate NIS gene expression in the presence of excess PAX8 and can also inhibit PAX8 function on the Tg and TSHR promoters. The mechanism for this inhibition is not yet known, although it appears unlikely to be by competition with PAX8 protein for promoter binding, since mobility gel shift experiments were unable to detect PPFP binding to the Tg PAX8 response element (Espadinha et al., 2007). These studies elucidate further complexities in the mechanism of PPFP-mediated gene expression and reveal that the regulation of endogenous promoters may be different than with the exogenous PAX8- and PPARγ-responsive reporters that have been studied to date. Furthermore, the studies reveal that PPFP may have opposite effects on the expression of selected thyroid-specific genes, which could exert paradoxical pro- and anti-dedifferentiating effects on thyrocyte function.

It is of interest to note that another member of the PPAR family, PPARδ, may also play a role in regulating thyroid cell proliferation. Zeng et al. have reported that PPARδ is up-regulated in many human thyroid tumors and that it induces cell proliferation via a cyclin E1–dependent mechanism (Zeng et al., 2008). There is no known relationship between PPFP and PPARδ and PPARδ appears to be upregulated in FA, PTC and ATC, indicating that it is not restricted to a particular thyroid cancer morphotype. In light of the data suggesting a role for PPARγ ligands as potential therapeutic agents for the treatment of thyroid cancer (Copland et al., 2006, Matsuyama and Yoshimura, 2009, Rumi et al., 2004, Shen and Chung, 2005), further exploration of PPARδ antagonists appears to be warranted.

In summary, PPFP can act as a dominant negative inhibitor of wild-type PPARγ action both *in vitro* (Au et al., 2006, Espadinha et al., 2007, Gregory Powell et al., 2004, Kroll et al., 2000) and *in vivo* (Yin et al., 2009). PPFP is also capable of both stimulating and inhibiting selected PAX8-responsive genes (Espadinha et al., 2007). In addition, at least *in vitro* under some circumstances PPFP can exert independent, ligand-responsive transcriptional activation from both PAX8- and PPARγ-responsive promoters (Au et al., 2006). Further studies will be required to establish the mechanisms for each of these PPFP actions, especially under *in vivo* conditions, and which of these actions are most important for PPFP-mediated tumorigenesis.

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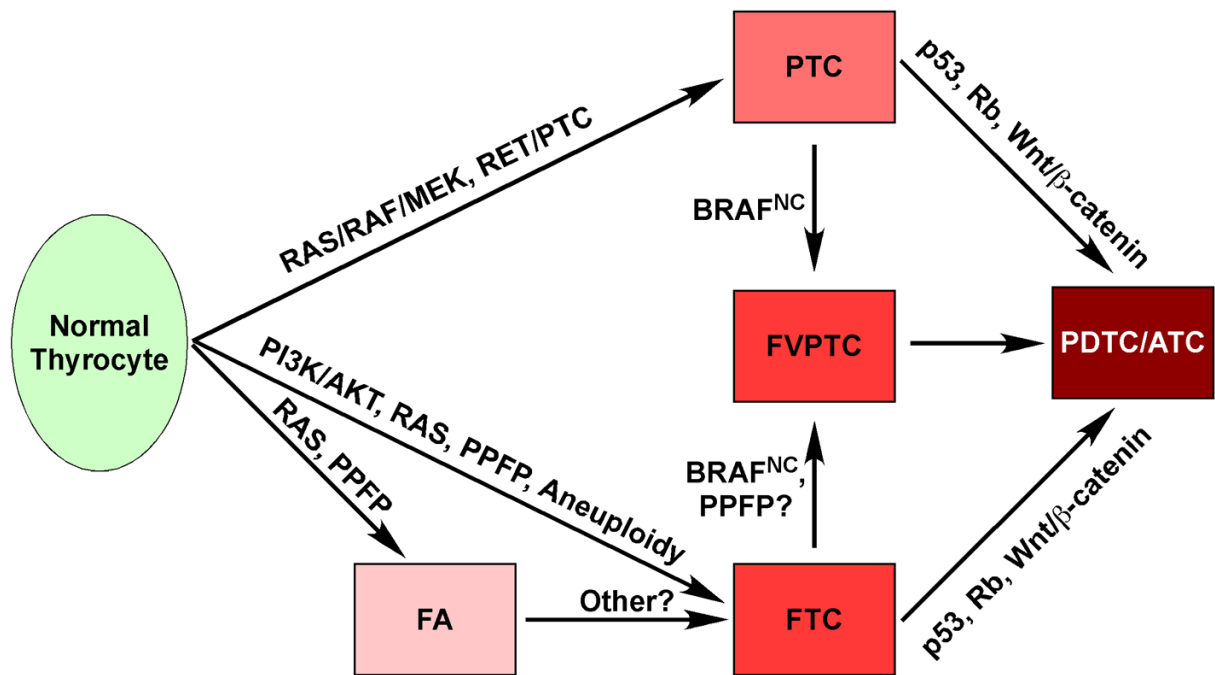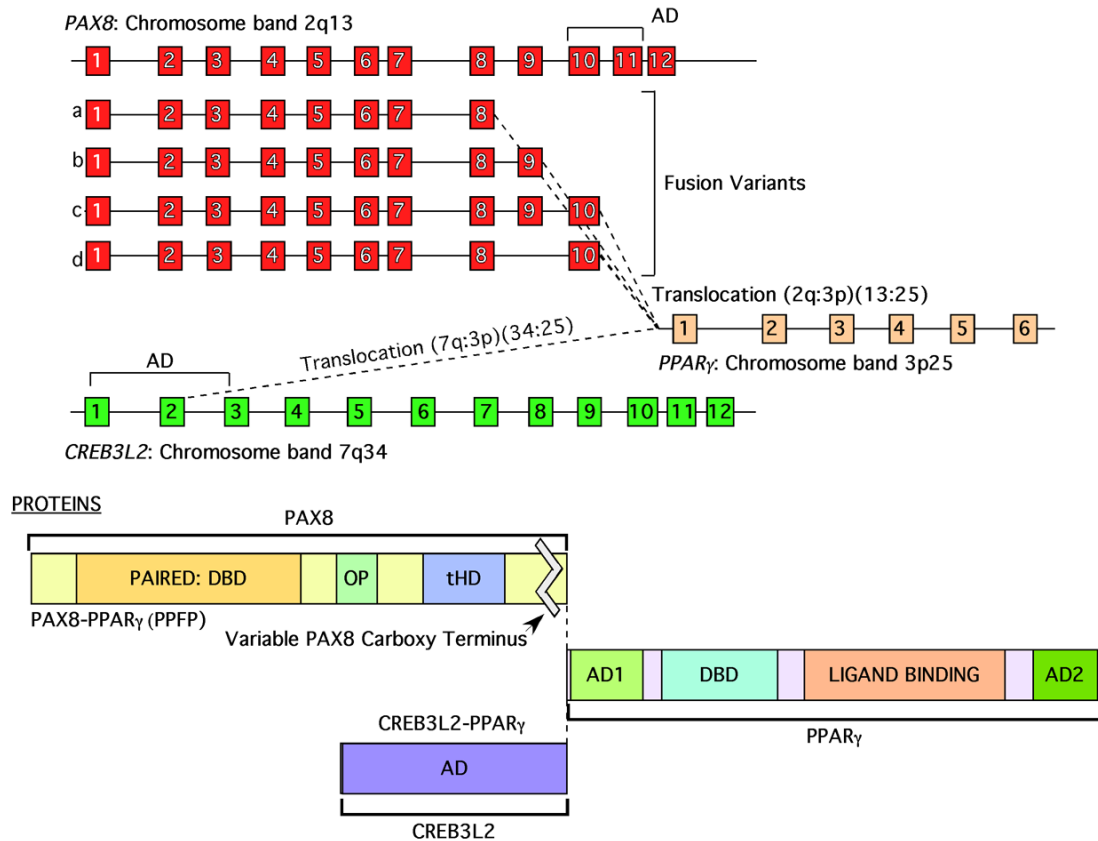
Figure 1.
Progression model of thyroid cancer indicating probable pathways that have been implicated in the pathogenesis of the different thyroid cancer morphotypes. FA, follicular adenoma; FTC, follicular thyroid carcinoma; PTC, papillary thyroid carcinoma; FVPTC, follicular variant of PTC; PDTC, poorly differentiated thyroid carcinoma; ATC, anaplastic thyroid cancer. BRAF[NC], non-conventional BRAF mutations (non-BRAF[V600E]), BRAF[A598V] (Santarpia et al., 2009), and BRAFK[601E] and BRAF[G474R] (Castro et al., 2006).

Figure 2.
The somatic PPARγ fusion mutation family in thyroid cancer. Schematic diagram of the *PAX8/PPARγ* (Kroll et al., 2000) and *CREB3L2/PPARγ* (Lui et al., 2008) rearrangements illustrating the genomic loci, exon arrangement, and sites of fusion of the two known fusion mutations. Exon numbering for PAX8 is in accord with NCBI Reference Sequence, NM_003466 with 12 exons. The PAX8 and CREB3L2 activation domains (AD) are indicated. In *PAX8/PPARγ*, the coding region for the PAX8 AD is eliminated in two of the known fusion variants (a and b) and the sequence for only a partial PAX8 AD is retained in the two other fusion isoforms (c and d). The CREB3L2 fusion sequence contains most, but not all, of the codons of the AD. The protein structures of the predicted fusion proteins are shown, including the PAX8 paired domain, containing the DNA binding domain (DBD), the octapeptide motif (OP), and the truncated homeodomain (tHD). All of the functional domains of PPARγ, including activation domains 1 and 2 (AD1 and AD2), DBD, and ligand binding domain are retained in both fusion proteins.

Table 1

Summary of evidence to delineate the potential oncogenic role of PPFP in FTC tumorigenesis.

Model Used	Conclusion	Study Reference
Nthy-ori 3-1 cells (human SV40 large T antigen- immortalized thyrocytes)	Increases cell growth, decreases apoptosis, induces anchorage independent growth	Powell et al., 2004
FRTL-5 (rat follicular thyrocytes)	Enhanced cell growth	Au et al., 2006
Nthy-ori 3-1 cells	Increased cell viability	Espadinha et al., 2007
Primary human thyrocytes	Increased cell proliferation	Lui et al., 2008

Table 2

Prevalence of RAS mutations and PAX8/PPAR rearrangements in follicular thyroid tumors.

	Presence of RAS mutations	Presence of PPFP	Presence of RAS and PPFP	Reference
FA	11/23	1/23	0/23	Nikiforova et al., 2003b
	1/7	1/7	0/7	Foukakis et al., 2006
	2/23	0/23	0/23	Di Cristofaro et al., 2006
	4/12	3/12	1/12	Castro et al., 2006
	4/34	5/40	0/32	Banito et al., 2007
	22/99 (22%)	**10/105 (9.5%)**	**1/97 (1%)**	
FTC	16/33	12/33	1/33	Nikiforova et al., 2003b
	4/25	5/25	0/25	Foukakis et al., 2006
	8/21	9/21	ND	Di Cristofaro et al., 2006
	6/27	10/22	3/22	Castro et al., 2006
	3/25	7/17	0/15	Banito et al., 2007
	37/131 (28.2%)	**43/118 (36.5%)**	**4/95 (4.2%)**	
FVPTC	13/30	0/30	0/30	Zhu et al., 2003
	3/12	1/12	ND	Di Cristofaro et al., 2006
	10/40	9/24	3/24	Castro et al., 2006
	5/17	1/17	0/17	Banito et al., 2007
	31/99 (31.3%)	**11/83 (13.2%)**	**3/71 (4.2%)**	

ND: Not determined

Table 3

Summary of evidence to delineate the mechanism of action of PPFP.

Function	Model Used	Conclusion	Study Reference
Dominant negative inhibition of PPARγ	U2OS cells (human osteosarcoma)	PPFP can inhibit PPARγ transcriptional activation	Kroll et al., 2000
Dominant negative inhibition of PPARγ and unique transcriptional activator (PAX8-responsive genes)	HeLa (human cervical cancer cells) Nthy-ori 3-1cells FRTL-5 (rat follicular thyrocytes)	PPFP function was cell line dependent	Au et al., 2006
Unique transcriptional activator (PAX8- and PPARγ-responsive genes)	JEG-3 cells (human choriocarcinoma), N2a cells (mouse preneuronal), FRTL-5 cells (rat thyrocytes), Primary dog thyrocytes	PPFP had unique transcriptional activity, though it could also function similar to either PAX8 or PPARγ	Giordano et al., 2006
Unique transcriptional activator (PAX8- responsive genes)	Nthy-ori 3-1 cells	Increased cell viability	Espadinha et al., 2007
Dominant negative inhibition of PPARγ	Transgenic mouse (mammary gland-specific expression of PPFP)	Inhibited PPARγ transcriptional activity, increasing susceptibility to carcinogen-induced tumorigenesis	Yin et al., 2009