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PAX8-PPARγ fusion protein in thyroid carcinoma

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Abstract

Thyroid carcinoma is the most common endocrine malignancy, and the incidence of thyroid carcinoma has been progressively increasing. Most thyroid carcinomas contain one of a small number of mutually exclusive driver mutations, such as BRAFV^{600E}, RAS mutations, *RET* gene fusions, or *PAX8/PPARG* gene fusions. The PAX8/PPARG gene fusion results in production of a PAX8-PPARγ fusion protein, denoted PPFP, and is found in ~30 – 35% of follicular thyroid carcinomas as well as a subset of follicular variant of papillary thyroid carcinomas. *In vitro* and *in vivo* evidence indicate that PPFP can act as an oncoprotein. Although the specific mechanism of PPFP action is yet to be defined, PPFP is considered to act as a dominant negative inhibitor of wild type PPARγ and/or as a unique transcriptional activator of subsets of PPARγ and PAX8 responsive genes. Detection of the fusion transcript in thyroid nodule biopsy specimens can aid clinical decision-making when cytological analyses are indeterminate. The PPARγ agonist pioglitazone is highly therapeutic in a transgenic mouse model of PPFP thyroid carcinoma, suggesting that PPARγ agonists may be therapeutic in patients with PPFP thyroid carcinomas.

Introduction

Thyroid carcinoma is the most common endocrine malignancy, and the incidence of thyroid carcinoma has more than doubled since 1990. This increased diagnosis may be a result of the increased use of imaging techniques (computed tomography, ultrasound, etc.) that enable the incidental detection of small non-palpable thyroid nodules, as well as the increased use of ultrasound-guided fine needle aspiration (FNA) biopsy of these nodules. Still, at least part of the increase is from finding more large tumors.[1] The American Cancer Society estimates that in the United States in 2014 there will be about 62,980 new cases of thyroid carcinoma (47,790 in women and 15,190 in men) and about 1,890 deaths from thyroid carcinoma (1,060 women and 830 men).[2]

Although thyroid carcinomas rarely produce thyroid hormone, at least 95% of these tumors arise from thyroid hormone-producing follicular epithelial cells. These carcinomas are broadly categorized as papillary thyroid carcinomas (PTC, ~85% prevalence), follicular thyroid carcinomas (FTC, ~10% prevalence) and anaplastic (undifferentiated) thyroid

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carcinomas (ATC, 1 – 2% prevalence) based upon histological characteristics. PTCs and FTCs usually are well differentiated, although some are more poorly differentiated. Histological subtypes of PTC include classical, follicular variant, tall cell and others. ATC is the rarest of all the thyroid carcinomas but the most aggressive type, with a median survival of only ~6 months. In the future, the classification of follicular cell-derived thyroid carcinomas may be based upon underlying genetic changes as well as histological features. In addition to carcinomas, thyroid nodules may be due to benign hyperplasia or benign follicular adenomas. It is unclear whether adenomas have malignant potential. In addition to the above tumors, ~ 3–5% of thyroid carcinomas originate from the calcitonin-producing parafollicular C cells (medullary thyroid carcinomas).

Population studies suggest that 3 – 8% of asymptomatic adults have thyroid nodules.[3-10] These thyroid nodules can be detected by palpation or more commonly imaging, especially in adults of increased age.[3, 4, 7-9, 11, 12] Approximately 95% of thyroid nodules are benign, and an important clinical challenge is to accurately identify the ~5% that are malignant. Presently, FNA biopsy of thyroid nodules followed by cytological examination provides an accurate diagnosis of malignant or benign in most cases, but about 25% of all nodules cannot be accurately diagnosed by FNA cytology.[3-10] As our understanding of the molecular pathology of thyroid cancer improves, this is translating into new molecular diagnostic tests that have the potential to improve the cytological interpretation of FNA biopsies. In addition, advances in our understanding of the molecular pathology of thyroid cancer are leading to the development of better prognostic markers and novel targeted therapies.

Gene mutations in thyroid carcinoma

The vast majority of thyroid cancers contain one of a small number of driver mutations, such as $BRAF^{V600E}$, RAS mutations, *RET* gene fusions, or *PAX8/PPARG* gene fusions. $BRAF^{V600E}$ is the most common driver mutation in thyroid cancer,[13-15] and approximately 40 – 45% of PTCs contain this mutation. It is especially common in tall cell PTC and also is found in classic PTC, but it is uncommon in follicular variant PTC.[16,17] *RET* gene fusions also are found in PTC and are particularly common in radiation-induced cancers.[18-21] RAS mutations are found primarily in follicular variant PTCs, FTCs and benign follicular adenomas.[22-24]

The *PAX8/PPARG* gene fusion is found in 30 – 35% of FTCs[25-27] as well as a substantially smaller fraction of follicular variant PTCs.[25, 28, 29] This rearrangement also is occasionally found in follicular adenomas.[26, 27, 30] Thus, RAS mutations and *PAX8/PPARG* gene fusions are found in the same histological types of thyroid tumors, although RAS mutations are substantially more common in follicular adenomas.

Another chromosomal rearrangement involving *PPARG*, in this case resulting in a gene fusion with *CREB3L2*, has been found in a very small number of FTCs.[31] The existence of two different fusion partners with *PPARG* in FTCs suggests that the PPARγ portion of the resulting fusion proteins is important to the mechanism of carcinogenesis.

Additional mutations accumulate as thyroid carcinomas become less differentiated. Anaplastic carcinomas probably arise from preexisting well differentiated carcinomas

through the acquisition of abnormalities such as p53 mutations[32-35] or abnormalities in β-catenin signaling.[36-38] Activation of the phosphatidylinositide 3-kinase (PI3K)/AKT pathway also is common, and can occur through a number of mechanisms such as activating mutations in PIK3CA or AKT1, or loss of PTEN expression through genetic or epigenetic mechanisms.[39-43] PI3K/AKT pathway activation also is frequent in FTCs.[42, 44, 45]

This review will focus on the *PAX8/PPARG* rearrangement. The mechanism of oncogenesis, the role of fusion transcript detection in the diagnostic evaluation of thyroid nodules, and the potential of the resulting PAX8/PPARγ fusion protein (PPFP) as a therapeutic target will be discussed.

Function and structure of PAX8

PAX8 belongs to the paired box family of transcription factors. It is necessary for normal thyroid development, and in the mature thyrocyte PAX8 drives the expression of many thyroid-specific genes such as those encoding thyroglobulin, thyroid peroxidase and the sodium iodide symporter.[46] Although PAX8 also is expressed in the developing brain and kidney, the only abnormality in *Pax8*$^{-/-}$ mice is the absence of a thyroid gland. *PAX8* mutations also are a known cause of congenital hypothyroidism in humans.[47]

The structure of the *PAX8* gene and the resulting mRNA and proteins are shown schematically in Figure 1. *PAX8* has 12 exons, with the translational start codon being derived from exon 2. The DNA binding domain (paired domain) of PAX8 is found at the amino terminus of the protein, encoded by exons 3, 4 and the beginning of exon 5. Alternative splicing of exons 8-10 results in the production of 4 or 5 RNA transcript and protein isoforms, denoted PAX8A, B, C, D and E. PAX8A is the longest isoform and includes all codons from exons 2-12. PAX8B is deleted in exon 9, but its sequence (NM_013951) has been suppressed from GenBank due to insufficient evidence of its existence. However, as will be noted below, one of the known splice variants of PPFP is of the PAX8B type (i.e., deleted in exon 9). Deletion of exon 9 does not alter the reading frame of the downstream exons. PAX8C utilizes an internal exon 9 5' splice site, resulting in a smaller exon 9 and a reading frame shift that alters and shortens the carboxyl terminal end of the protein, with the stop codon now being in exon 11. PAX8D is deleted in exons 8 and 9, and PAX8E is deleted in exons 8, 9 and 10. Both of these result in truncated proteins shorter than PAX8C but with a reading frame shift identical to that of PAX8C. PAX8A and PAX8B contain a serine, threonine and tyrosine-rich transcriptional activation domain encoded by exons 10-11 that is not present in the other isoforms.[48] Accordingly, transfection data indicate that PAX8A and PAX8B have greater transcriptional activity than PAX8C.[49]

Function and structure of PPARγ

PPARγ belongs to the nuclear receptor family of transcription factors. It is the master regulator of adipogenesis as well as a potent modulator of whole-body lipid metabolism and insulin sensitivity.[50, 51] Fatty acids and eicosanoids such as 15 deoxy $\Delta^{12,14}$ prostaglandin J2 can act as endogenous ligands for PPARγ.[52-56] Synthetic ligands such as thiazolidinediones are potent activators of PPARγ with robust insulin sensitizing activity and are used in the treatment of type 2 diabetes mellitus.[57] PPARγ has additional functions, such as anti-

inflammatory activity.[58] There is evidence that PPARγ may be a tumor suppressor and this will be discussed subsequently.

The structure of the relevant portion of the *PPARG* gene and the resulting protein are shown schematically in Figure 2. PPARγ has the classic structure of a nuclear hormone receptor with an amino terminal regulatory "AB" domain, a centrally located zinc finger DNA binding domain, and a ligand binding/transcriptional regulatory domain that occupies the carboxyl terminal half of the protein. The *PPARG* gene has two promoters that drive the transcription of two different first exons, resulting in the production of the protein isoforms PPARγ1 and PPARγ2. PPARγ2 is identical to PPARγ1 except that it has a unique 30 amino acid addition at its amino terminus. The proteins appear to be functionally similar in transfection experiments,[59] but their expression patterns differ. PPARγ1 is broadly expressed, whereas PPARγ2 is expressed specifically in adipocytes.

Structure of the PAX8/PPARG fusion gene, transcript and protein

The *PAX8/PPARG* rearrangement is created by a translocation between chromosomal regions 2q13 and 3p25.[60] This translocation results in a fusion transcript wherein most of the coding sequence of PAX8 (2q13) is fused in frame with the entire coding exons of PPARγ1 (3p25). The *PAX8* promoter, which is highly active in thyroid follicular cells, drives the expression of the fusion transcript, resulting in high level expression of the fusion transcript and protein (PPFP).[61]

The fusion gene, transcript and protein are represented schematically in Figure 3. Typically, the translocation fuses *PAX8* intron 10 with the intron immediately preceding the first coding exon of PPARγ1. The resulting PPFP consists of an N-terminal PAX8 fragment encoded by exons 2-10 fused in frame to full length PPARγ1. The PAX8 portion is essentially a truncated version of PAX8A, missing much of the C-terminal activation domain. However, alternative splicing has resulted in the detection of multiple RNA isoforms within the same tumor.[60] Specifically, transcripts with PAX8 exons 1-8, 1-9, and 1-10 with exon 9 deleted have been detected fused to the first coding exon of PPARγ1, and all of these maintain the PPARγ1 reading frame. Some studies have reported expression of one of these shorter isoforms without expression of isoforms containing exon 10. In these cases it is not known if the actual chromosomal translocation is still in *PAX8* intron 10, or in intron 8 or 9.

Detection of PPFP in thyroid tumors

Since PPARγ is expressed at very low levels in the normal thyroid, high-level expression of PPARγ by immunohistochemistry provides evidence for the presence of PPFP. However, this is not entirely specific, and definitive testing for PPFP requires either fluorescent in situ hybridization (FISH) or RT-PCR. Based upon these techniques, PAX8/PPARγ rearrangements have been found in about 1/3 of FTCs and substantially less frequently in other thyroid tumors.[26-30, 60, 62-75] Although the sample size is small, a comparison of FTCs with and without PPFP showed no significant differences in the extent of capsular or vascular invasion, disease-free survival or overall survival.[75]

PPFP as an oncoprotein

Several *in vitro* studies have provided evidence that PPFP can act as an oncoprotein. Both transient and stable transfection of PPFP in Nthy-ori 3-1 cells (human SV40 large T antigen-immortalized thyrocytes) have shown accelerated growth rates and lower numbers of cells in the G_0/G_1 resting state compared to empty vector-transfected cells.[76] PPFP also decreased the rate of apoptosis, suggesting that the reduced apoptosis may be partially responsible for the accelerated growth.[76] In addition, PPFP expression in FRTL-5 rat thyroid cells resulted in increased DNA synthesis as evidenced by ^{3}H-thymidine incorporation.[77] Nthy-ori 3-1 cells[76] and rat thyroid PCCL3 cells[78] that stably express PPFP exhibit increased anchorage-independent growth (colony formation in soft agar), a hallmark of cellular transformation. Expression of either PPFP or CREB3L2/PPARγ in primary human thyroid cells resulted in an increased number of cells and increased DNA synthesis,[31] suggesting that the common PPARγ moiety is at least partially responsible for the oncogenic actions of these fusion proteins. Although little else is known regarding the function of CREB3L2/PPARγ, further comparison of this fusion protein with PPFP has the potential to reveal important insights into the mechanisms of oncogenesis. In the osteosarcoma U2OS cell line, transfected PPARγ transactivated PPARγ response elements whereas PPFP was ineffective.[60] Furthermore, coexpression of PPFP with PPARγ abrogated PPARγ-mediated reporter gene expression in a dominant negative fashion. This is important because there is evidence that PPARγ may have anti-tumor or tumor suppressor properties, which would imply that PPFP may be oncogenic by inhibiting the activity of endogenous PPARγ. For example, addition of a PPARγ agonist to several PPARγ-positive ATC cell lines led to an increased portion of cells in G_0/G_1 with a reduced number of cells in G_2/M and S phase, suggesting decreased cell proliferation.[79] In addition, DNA synthesis was slowed down, evident from decreased ^{3}H-thymidine incorporation in these cells while expression of cell-cycle progression inhibitors $p21^{cip1}$ and $p27^{kip1}$ was increased.[79] Overexpression of PPARγ by transfection into PPARγ-positive or –negative cell lines similarly decreased colony formation in soft agar and triggered nuclear condensation, fragmentation of chromatin and apoptosis, with G_0/G_1 cell cycle arrest in several human thyroid cancer cell lines.[62, 79-81] These data provide evidence that PPARγ has a tumor suppressive effect in some thyroid cell lines, and similar data have been obtained in several nonthyroidal cell lines.[82-87]

There also is *in vivo* evidence that PPARγ may have anti-tumor properties. For example, mice with a homozygous knock in mutation in thyroid hormone receptor beta were unexpectedly found to develop thyroid carcinoma, and the disease became more aggressive in the presence of a single allele deletion of *Pparg*.[88]

Although the above data suggest a simple story that endogenous PPARγ is a thyroid cancer tumor suppressor and that PPFP is oncogenic by virtue of dominant negative activity against PPARγ, the actual situation is likely more complex. Firstly, PPARγ is expressed at extremely low levels in the normal thyroid[89] and it is not known if it has any function in that organ. Second, microarray data from human thyroid carcinomas strongly suggest that PPFP has PPARγ-like activity in these tumors.[72, 90] For example, compared to non-PPFP thyroid carcinomas, two of the most highly induced genes in PPFP carcinomas are *AQP7* and *ANGPTL4*, both of which are well known to be induced by PPARγ in adipocytes.

Furthermore, PPFP activates the *AQP7* promoter in a PPARγ-like manner in thyroid and non-thyroid cell types based on transfection data. In addition, high level expression of endogenous PPARγ appears to contribute to the aggressive behavior of human ATCs, at least when assessed in cell culture and orthotopic injections in mice.[91] Finally, PPARγ antagonists have antiproliferative effects on a wide variety of cancer cell lines.[92]

The expression of PPFP in transgenic mice is apparently not sufficient to cause thyroid cancer, which is consistent with the observation that benign thyroid adenomas occasionally express PPFP. However, transgenic mice with thyroid-specific expression of PPFP and thyroid-specific homozygous deletion of Pten develop metastatic thyroid carcinoma.[93] Deletion of *Pten* was added to the PPFP mouse model because it results in increased phosphorylated (activated) AKT (pAKT), as observed in human PPFP carcinomas.[94] Increased pAKT is common in FTCs in general and is not specific for PPFP carcinomas,[42] and can result from a variety of genetic or epigenetic events.

Importantly, the PPARγ agonist pioglitazone dramatically decreased the thyroid carcinoma size and completely prevented metastatic disease in this mouse model of PPFP thyroid carcinoma.[93] Most remarkably, pioglitazone caused an adipogenic response in the remaining thyroid cancer cells as manifested by lipid accumulation and induction of a broad array of adipocyte PPARγ target genes (PPARγ is the master regulator of adipogenesis). This indicates that, in the presence of pioglitazone, PPFP is very strongly PPARγ-like. It should be noted that mice with *Pten* deletion alone (without PPFP expression) develop benign thyroid hyperplasia that is unaffected by pioglitazone.[93]

Interestingly, there also is evidence that PPFP may inhibit tumorigenesis *in vivo* by inhibiting angiogenesis.[95] Although ectopic PPFP expression caused immortalized thyrocytes to exhibit increased growth and decreased apoptosis *in vitro*, these cells showed decreased mouse xenograft tumor growth *in vivo*. Further investigation of the xenograft tumors identified reduced CD31 staining and VEGF expression, suggesting that PPFP inhibits neovascularization. Expression of tissue inhibitor of metalloproteinase 3 (TIMP3), an inhibitor of angiogenesis, was increased, and this was considered a potential explanation for the findings. However, TIMP3 is repressed, not induced, in PPFP FTCs obtained from patients.[72, 90]

Mechanisms of cellular transformation and oncogenesis by PPFP

Although the specific mechanism of PPFP action is yet to be defined, PPFP can act as a dominant negative inhibitor of wild type PPARγ and/or as a unique transcriptional activator of a subset of PPARγ responsive genes. It is expressed in thyroid carcinomas 10-50 fold above endogenous PPARγ.[72, 90] PPFP also has mixed actions on PAX8 target genes in transfection experiments. It is important to note that PPFP has the DNA binding domains of both PAX8 and PPARγ. Since it contains transcriptional regulatory domains of both PAX8 and PPARγ, it has the potential to bring inappropriate transcriptional coregulatory proteins to PAX8 and PPARγ target genes. Therefore, a plausible mechanism of oncogenesis is the modulation of the downstream pathways of PAX8 or PPARγ.

PAX8 is required for normal thyroid development and is important in the maintenance of differentiated follicular cell function. When PPFP was expressed in human thyroid cancer cell lines, PAX8 responsive genes (sodium iodide symporter (*SLC5A5*), thyroid peroxidase (*TPO*), thyroid stimulating hormone receptor (*TSHR*), and thyroglobulin (*TG*)) were variably stimulated or inhibited. *SLC5A5* gene expression was stimulated in response to PPFP alone in one study[77] whereas this stimulatory effect required cotransfection of PPFP with wild type PAX8 in another study.[96] TSHR expression was inhibited[96] while *TPO* transcription was increased by PPFP.[77] Repression of the *TG* promoter was observed in response to PPFP[77] but cotransfection with PPFP and PAX8 was required for this inhibitory effect in another study.[96] In both these studies, PPFP inhibited PAX8 mediated transcription of *TG* in a dominant negative fashion, while the addition of a PPARγ agonist did not reverse this dominant negative effect.[77, 96] The molecular basis for the variable effects of PPFP on PAX8-responsive genes is not known. Other PAX transcription factors are associated with cancers (reviewed in 97). For example, gene fusions of *PAX3* or *PAX7* with FKHR underlie alveolar rhabdomyosarcomas, gene fusions of *PAX5* with *IGH* are found in lymphomas, and over expression of PAX2 is found in a variety of cancers. Therefore it is possible that the structurally-related PAX8 portion of PPFP is actively involved in its oncogenic action.

PPARγ is a nuclear hormone receptor that is expressed at very low levels in the normal thyroid and has no as-yet-identified function in that organ. *In vitro* studies indicate that PPFP can inhibit wild type PPARγ function, and the concept that PPFP may be oncogenic by inhibition of postulated tumor suppressor activities of endogenous PPARγ remains attractive, as discussed above. However, it also is clear that PPFP can act in a PPARγ-like manner.[72, 90] *In vitro*, PPFP stimulates the promoters of some PPARγ target genes and represses others.[72, 76, 77]

Analysis of gene expression profiling data of FTCs that express PPFP versus those that do not demonstrated that PPFP cancers have a distinct transcriptional signature.[72, 90, 98] The studies by Giordano et al[72] and Lacroix et al[90] yielded highly concordant data. These studies identified numerous adipocyte PPARγ target genes such as *AQP7* and *ANGPTL4* as being upregulated in PPFP carcinomas, and gene ontology pathways such as fatty acid metabolism and beta oxidation were highly enriched. In addition, a number of genes known to be associated with cancer were identified in these data sets. For example, both studies found the cancer-associated genes *MCYL1* and *NRG1* to be induced in PPFP tumors, and found the angiogenic factors *FGFBP1*, *PGF* and *ANGPTL4* to be induced and the anti-angiogenic factor *TIMP3* to be repressed. However, for unclear reasons, the PPFP thyroid carcinoma gene expression profile identified by Lui et al[98] has virtually no overlap with the above two studies. Lui et al[98] found that PPFP tumors are associated with upregulation of genes associated with signal transduction, cell growth and translational control, and repression of ribosomal protein and translational associated genes. A limitation in the interpretation of gene expression profiling data from PPFP thyroid carcinomas is that the specific genes that are essential for oncogenesis are not known.

Stable expression of PPFP in the rat thyroid cell line PCCL3 was found to activate the Wnt/TCF pathway in a subset of cells.[78] This TCF-active fraction was enriched in the ability to grow under anchorage independent conditions and to invade through Matrigel, a synthetic

basement membrane. In addition, the thyroids from the previously described mouse model of PPFP carcinoma were found to have induction of a number of Wnt/TCF target genes. These are potentially important observations because the Wnt/TCF pathway plays a central role in the cell biology of normal stem cells as well as in cancer stem cells from a variety of organs such as the colon. In fact, the PCCL3/PPFP cells were hierarchically organized such that TCF-active cells gave rise to both TCF-active and –inactive cells, whereas TCF-inactive cells only gave rise to TCF–inactive cells. This hierarchical organization is typical of stem cells. Thus, it is possible that PPFP activates thyroid cancer stem cells via the induction of TCF-responsive genes.

PPFP as a diagnostic tool and a therapeutic target

PPFP is primarily found in FTCs and follicular variant PTCs. It is not possible to diagnose FTCs by cytological analysis of FNA biopsies because the diagnosis of FTC requires evidence of invasion. This is a major reason why ~20 to 25% of thyroid FNA biopsies are considered cytologically indeterminate. Evidence is accumulating that the use of molecular analyses in this situation results in better discrimination of benign versus malignant thyroid nodules. For example, in one study, 5 nodules with indeterminate cytology were found to contain PPFP RNA in the biopsy specimens, and subsequent surgical pathology indicated all 5 were malignant.[99] The implication is that the expression of PPFP in a thyroid nodule biopsy with indeterminate cytology indicates the need for surgery. Thus, analysis of PPFP along with other driver mutations is a promising approach to enhance clinical decision making in patients with indeterminate thyroid nodule biopsies.

As described above, the PPARγ agonist pioglitazone is highly therapeutic in a mouse model of PPFP thyroid carcinoma, greatly reducing the size of the primary tumor and eliminating metastatic disease.[93] These data suggest that pioglitazone may be therapeutic in patients with PPFP thyroid carcinoma, a hypothesis that is being tested in a phase II clinical trial in patients with advanced disease not amenable to cure by surgery or radioiodine (clinicaltrials.gov identifier NCT01655719). If successful this would be especially notable because pioglitazone is FDA-approved for the long-term therapy of type 2 diabetes and has very little toxicity compared with other targeted chemotherapies such as tyrosine kinase inhibitors. As a ligand for PPFP, pioglitazone turns this fusion protein into a strongly PPARγ-like transcription factor, and this results in trans-differentiation of the mouse PPFP thyroid cancer cells into adipocyte-like cells. It is presumed that the cancer cells lose malignant character as they gain differentiated character. This is supported by limited observations that a non-adipogenic PPARγ agonist with full insulin sensitizing properties has no anti-tumor effect in the mouse model of PPFP carcinoma.[78] Thus, although the proadipogenic actions of pioglitazone are considered an unwanted side effect in the therapy of diabetes, this action probably is integral to the therapeutic action of pioglitazone in PPFP thyroid carcinoma.

Conclusions

The t(2;3)(q13;p25) chromosomal translocation that produces PPFP is a driver mutation found in ~30-35% of FTCs as well as a subset of follicular variant PTCs. PPFP can act as a

dominant negative inhibitor of PPARγ or as a PPARγ-like transcription factor depending on the target gene and cellular context, and it also has variable effects on PAX8 target genes. It is presumed that modulation of subsets of these pathways underlies the oncogenic actions of PPFP. The identification of PPFP as an oncoprotein has important clinical implications. Cytological criteria are insufficient to differentiate FTCs from benign lesions in thyroid biopsy specimens. The molecular detection of PPFP in thyroid biopsies with indeterminate cytology provides evidence that the nodule is highly likely to be malignant and that surgery is indicated. Although the majority of thyroid cancer patients are cured by surgery with or without radioiodine therapy, patients with locally recurrent or distant metastatic disease can be difficult or impossible to cure. Therefore, novel therapeutic approaches are necessary for the management of these patients. Since pioglitazone results in a highly therapeutic effect in a mouse model of PPFP thyroid carcinoma, this drug may be therapeutic in patients with PPFP thyroid carcinoma. This currently is being tested in clinical trial NCT01655719. It is likely that the pro-adipogenic actions of pioglitazone as a PPFP ligand underlie its therapeutic efficacy, a point which needs to be kept in mind if additional PPARγ agonists are tested in this disease.

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Key points

- PPFP is a consequence of a chromosomal translocation found in ~1/3 of follicular carcinomas and substantially smaller subsets of follicular variant papillary carcinomas and benign follicular adenomas.
- *In vitro* and *in vivo* evidence indicate that PPFP acts as an oncoprotein.
- PPFP can act as a dominant negative inhibitor of wild type PPARγ and/or as a PPARγ-like transcription factor, and similarly can activate or repress PAX8-responsive genes.
- The PPARγ agonist pioglitazone has a powerful therapeutic effect in a mouse model of PPFP thyroid carcinoma, and is currently being tested in a phase II clinical trial.

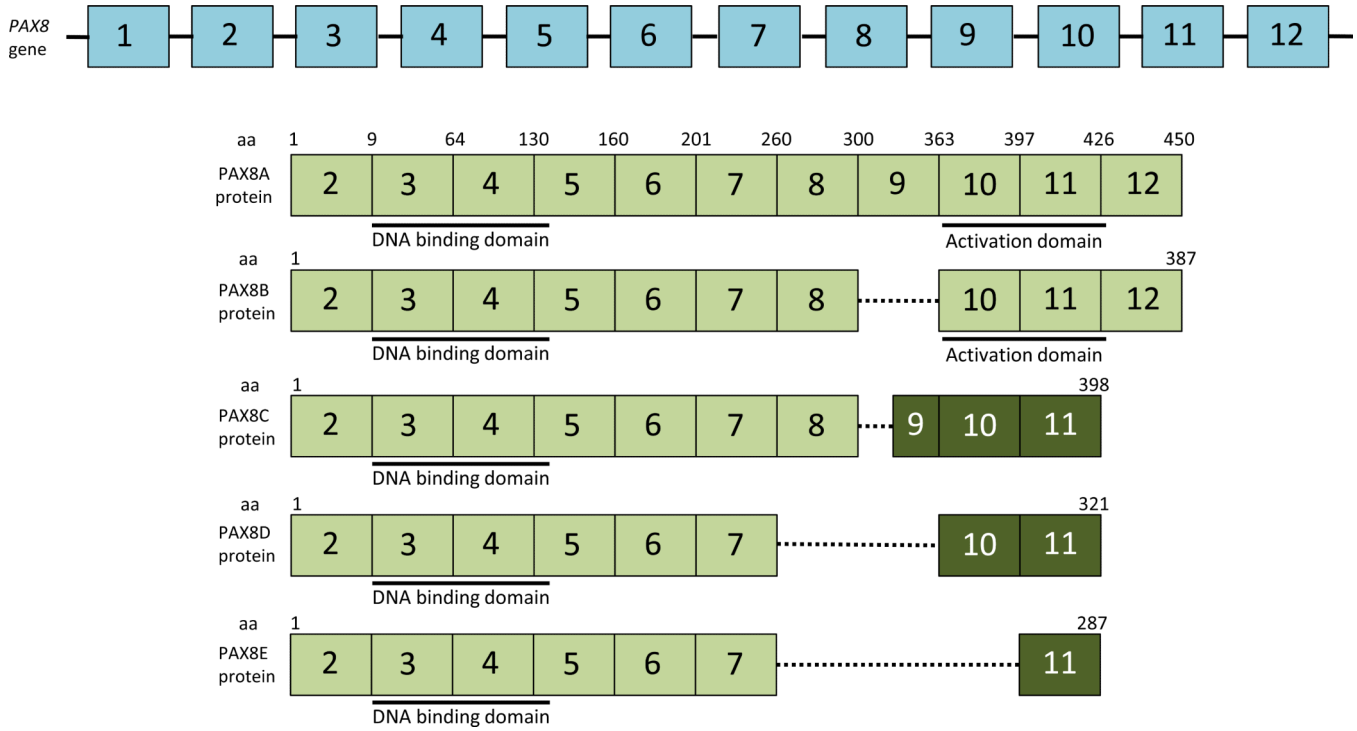

Figure 1.

Schematic representation of the PAX8 gene, mRNA and protein isoforms. The exons are numbered 1 -12 (not drawn to scale). PAX8A mRNA is depicted below the gene and contains all 12 exons. Coding exons in the mRNA are shown in green, noncoding in white. The PAX8A protein is depicted below its mRNA. The DNA binding domain (paired domain) is found at the amino terminus of the protein, encoded by exons 3, 4 and the 5' end of exon 5. A carboxyl terminal transcriptional activation domain is encoded by exons 10 and 11 and is rich in serine, threonine and tyrosine. Alternative splicing results in the isoforms PAX 8B, 8C, 8D and 8E. In PAX8B, exon 9 is deleted but this deletion does not alter the reading frame downstream. PAX8C utilizes an internal exon 9 5' splice site, resulting in a smaller exon 9 and a reading frame shift that alters and shortens the carboxyl terminal end of the protein, resulting in a stop codon in exon 11. PAX8D is deleted in exons 8 and 9, and PAX8E is deleted in exons 8, 9 and 10. Both of these result in truncated proteins shorter than PAX8C but with a reading frame shift identical to that of PAX8C. The reading frame shift is depicted by a darker shade of green within the exons. aa, amino acid.

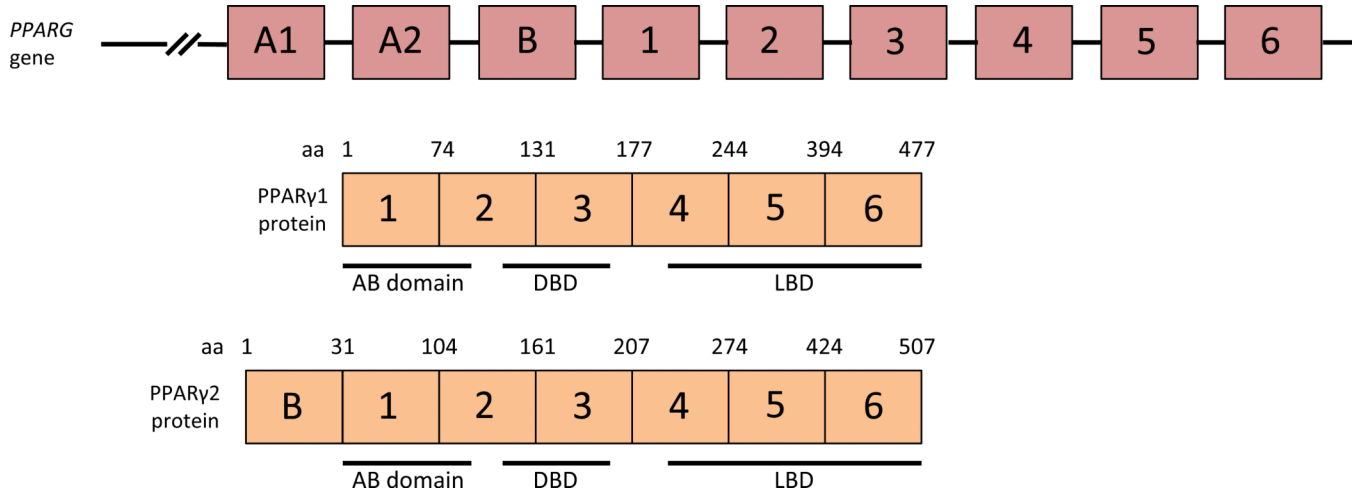

Figure 2.

Schematic representation of the *PPARG* gene and PPARγ1 protein. Only the *PPARG* exons that contribute to the PPARγ1 protein are shown, and for convenience they are numbered starting at 1 (the gene has several additional upstream exons). The exons are not drawn to scale. The PPARγ1 protein has an N-terminal regulatory "AB" domain, a zinc finger DNA binding domain (DBD) and C-terminal ligand binding domain/transcriptional regulatory domain (LBD).

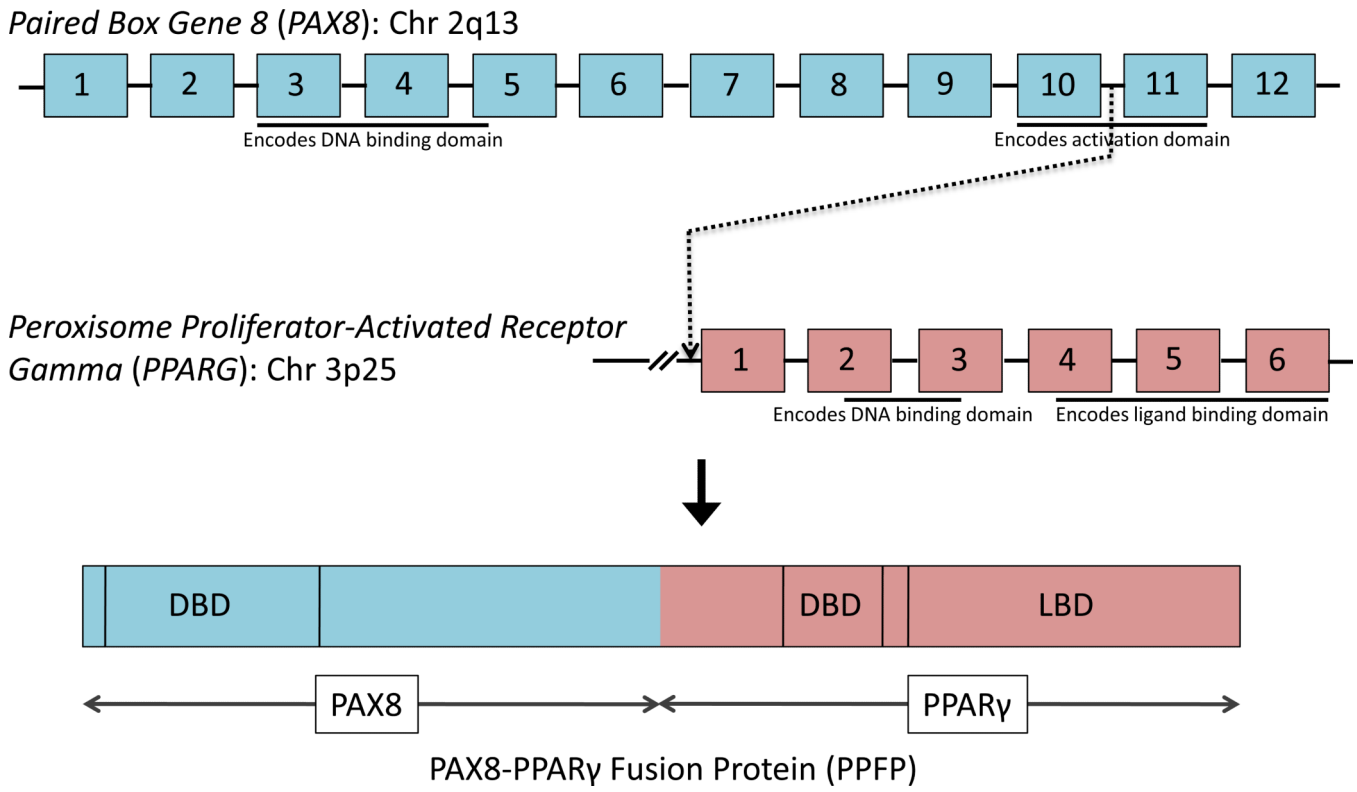

Figure 3.

The *PAX8/PPARG* rearrangement is created by a translocation between chromosomal regions 2q13 and 3p25. This translocation results in a fusion transcript wherein most of the coding sequence of PAX8 (2q13) is fused in frame with the entire coding exons of PPARγ1 (3p25). The resulting fusion protein, denoted as PPFP, contains the DNA binding domain and part of the C-terminal activation domain of PAX8, as well as the DNA and ligand binding domains (DBD, LBD) of PPARγ. Not shown, PAX8 variants result in alternative isoforms of PPFP that only include PAX8 exons 1-8, or 1-9, or 1-10 without exon 9.