Minireview: The Paired Box-8/Peroxisome Proliferator-Activated Receptor-γ Oncogene in Thyroid Tumorigenesis

Honey V. Reddi, Bryan McIver, Stefan K. G. Grebe, and Norman L. Eberhardt

Department of Medicine (H.V.R., B.M., N.L.E.), Division of Endocrinology, and Departments of Laboratory Medicine and Pathology (S.K.G.G.) and Biochemistry and Molecular Biology (N.L.E.), Mayo Clinic and Foundation, Rochester, Minnesota 55905

The American Cancer Society estimates 30,180 new cases of thyroid cancer in the United States in 2006. Of all thyroid cancers, 15–20% are follicular thyroid carcinoma (FTC), making this the second most common thyroid malignancy (after papillary carcinoma). A proportion of FTC has been found to be associated with a chromosomal translocation, t (2, 3)(q13; p25), which fuses the thyroid-specific transcription factor paired box-8 with the peroxisome proliferator-activated receptor-γ nuclear receptor, a ubiquitously expressed transcription factor. This fusion event causes expression of a paired box-8/peroxisome proliferator-activated receptor-γ fusion protein (PPFP). PPFP is detected in approximately 30% of FTC. In this report we review data on the role of PPFP in FTC, its mechanism of oncogenesis, and PPFP targeting as a strategy in thyroid cancer treatment. (Endocrinology 148: 932–935, 2007)

FOLLICULAR CELL-DERIVED thyroid carcinoma comprises several morphological subtypes: papillary (PTC), follicular (FTC), Hürthle-cell (HCC), and anaplastic (ATC) carcinomas. These subtypes are phenotypically distinct and exhibit extremes of malignant potential, from relatively indolent (PTC) to highly aggressive (ATC). FTC might arise in an adenoma-carcinoma sequence from follicular adenoma (FA), some of which are cytologically difficult to distinguish from FTC (1). The pathogenesis of HCC remains unclear, and there is controversy whether it represents a distinct morphotype or evokes on the background of PTC or FTC. FTC accounts for approximately 10–20% of all thyroid cancers and up to 40% of cause-specific deaths. ATC is the rarest but most aggressive thyroid cancer, with more than 90% 1 yr mortality, and probably arises from preexisting PTC, FTC, or HCC by acquisition of p53 mutation/inactivation, impaired Rb pathway function, or abnormalities in β-catenin signaling (1).

No signature oncogenic pathways have been identified in FTC until recently. RAS mutations are found in a subset of these tumors, but they have not been tied conclusively to a particular signaling pathway. However, the last 6 yr have seen the identification and partial molecular-mechanistic examination of a novel oncogenic fusion gene (2) in FTC. The resulting fusion protein, paired box-8 (PAX8)/peroxisome proliferator-activated receptor (PPAR)-γ fusion protein (PPFP) has been identified in almost 50% of FTC and a much smaller proportion of its putative precursor lesion, FA. It is absent in PTC and HCC, suggesting that PPFP may represent an early FTC-specific oncogene (2–7). A point to note is that in FTC, PAX8/PPARγ fusion and Ras gene activations rarely overlap in the same tumor, providing additional evidence that PPARγ-driven tumors represent a separate subset of tumors.

The absence of specific molecular markers available for the detection, diagnosis, or prognosis of FTC and the presence of PAX8/PPARγ rearrangements in a major proportion of FTC raises the possibility that study of this gene could provide insight into the main oncogenic pathways guiding FTC pathogenesis, ultimately improving the diagnosis, staging and treatment of FTC.

PPFP: A Putative Oncogene for FTC

PPFP is a somatic tumor genetic rearrangement, wherein most of the (long) q-arm of chromosome 2 is translocated to the (short) p-arm of chromosome 3, whereas in exchange the 3p25 terminal region is translocated to 2q13. This translocation creates a fusion transcript wherein the 5′-region of the thyroid-specific transcription factor pax8 gene (2q13) is fused in frame with exon1 of the PPARγ gene (3p25), a member of the thyroid hormone-steroid hormone nuclear receptor superfamily (2). The pax8 promoter, which is active in thyroid follicular cells, appears to drive expression of PPFP (8).

The predicted fusion protein and its putative functional domain structure are depicted in Fig. 1. PAX8 is a member of the paired-box family of transcription factors, which is necessary for normal thyroid development (9), whereas PPARγ is involved in adipocyte differentiation and lipid and carbohydrate metabolism as well as cell proliferation and differentiation (reviewed in Ref. 10).

PPFP encompasses an N-terminal PAX8 fragment, which
PPFP should clarify whether PPFP is sufficient on its own to precipitate follicular neoplasia or whether additional steps are required in that process.

Mechanism(s) of PPFP Action

Chromosomal translocations creating fusion genes could cause oncogenesis through a variety of mechanisms. The translocation could generate an abnormal pathogenic fusion protein, functioning in its own right as an entirely novel oncogene. Alternatively, deregulation of the genes involved in the translocation might abrogate their endogenous function, leading to oncogenesis, if one of these genes is a tumor suppressor. A third alternative, seen most prominently in PTC, is the overexpression of a protooncogene caused by alteration of a gene promoter system.

One possible mechanism of PPFP function could be modulation of the downstream pathways of one or other of its independent components, PAX8 and PPARγ. PAX8 is required for normal thyroid development and is involved in the maintenance of differentiated follicular cell function. However, as is typical for genes from the pax family, its expression is tightly regulated and controlled, so aberrant expression of PPFP might have oncogenic effects, simply by down-regulating endogenous PAX8 expression, akin to that observed for many other fusion genes from the pax family.

PPARγ, on the other hand, is a nuclear hormone receptor transcription factor that is expressed at very low levels in the thyroid and has no as yet identified function in that organ. In vitro studies indicate that PPFP function is mediated at least in part by inhibition of wild-type PPARγ function (2, 15, 16) or down-regulation of wild-type PPARγ expression (18). Data indicate that although ligand binding and activation of the ligand-gated activation function 2 domain increase the transcriptional activity of PPARγ, these same processes also induce ubiquitination and subsequent proteosomal degradation (19), providing a negative feedback system to balance PPARγ activity. It is possible that PPFP interferes in this delicate balance, pushing the cell toward proliferation (Fig. 2).

Gene transcription by PPARγ requires the formation of a heterodimer with retinoic X receptor (RXR), followed by binding to a PPARγ response element. Transactivation of the heterodimer is activated by ligands of PPARγ, RXR, or both. Because PPFP has the ability to independently bind RXR (16), it is possible that PPFP competes with wild-type PPARγ for
RXR, DNA binding sites, or both, thus preventing wild-type PPARγ-initiated transcriptional regulation (Fig. 2). In addition, the PPFP-RXR complex might still recruit cofactors, which are bound, but do not initiate transactivation, further diminishing the necessary resources for wild-type PPARγ action.

Analysis of gene expression array data of FTC that express PPFP demonstrated that these cancers have a distinct transcriptional signature (20–22). PPFP expression up-regulates genes associated with signal transduction, cell growth, and translational control, whereas a large number of ribosomal protein and translational associated genes are concurrently underexpressed. With respect to its ability to function as a novel protein, Giordano et al. (20) demonstrated that PPFP has unique transcriptional activities. They also demonstrated that PPFP has the potential to function in ways qualitatively similar to PAX8 or PPARγ, depending on the promoter and cellular environment (20). PPFP has been shown to disrupt normal transcriptional pathways of PAX8 in a cell type-specific manner (16), presumably through some form of negative feedback. Based on these observations, the hypothesis that PPFP functions primarily through the control of PPARγ requires reevaluation. Further analysis of the downstream regulatory pathways of PPFP will be essential as we begin to design strategies to intervene in the fundamental mechanisms of FTC.

PPFP as a Therapeutic Target

Currently therapeutic options for patients diagnosed with FTC include surgery followed in most cases by radioactive iodine treatment and thyroid hormone-suppressive therapy. These methods, however, are ineffective in the treatment of patients with metastatic cancer, in whom traditional chemotherapy is also usually ineffective (23). Consequently, novel therapeutic approaches are needed for these patients.

Molecular therapeutic approaches for FTC might be designed to target PPFP, keeping in mind the minimal knowledge available regarding PPFP-mediated oncogenesis (13). Because modulation of PAX8 and PPARγ transcriptional pathways appears to be PPFP’s primary mode of action, specific intervention might target one or more of those pathways, raising the possibility for highly selective, targeted therapy. Modulators of the PPARγ pathway (retinoic acid derivatives as well as thiazolidenediones) are already in clinical use for other diseases, generating hope that rapid progress can be made in the development of effective therapies that target the PPFP effect. PPARγ agonists have been shown to be modestly effective against ATC cell lines in vitro (24), raising the level of optimism for their function in FTC. The effect of PPFP on PAX8 might be altered by redifferentiation therapy with retinoic acid, an approach that has been tried in the past and found to be marginally useful (25). PPFP itself could be directly targeted using small-molecule compounds or monoclonal humanized antibodies against unique epitopes of the protein. The discovery of PPFP in FTC has not only generated hope for the early diagnosis of FTC but also gives us the opportunity to develop effective strategies based on an understanding of the disease process.

Acknowledgments

Received July 11, 2006. Accepted August 24, 2006.

Address all correspondence and requests for reprints to: Norman L. Eberhardt, Ph.D., Department of Medicine/Division of Endocrinology, 200 First Street SW, Mayo Clinic, Rochester, Minnesota 55905. E-mail: eberhardt mayo.edu.

This work was supported by National Institutes of Health Grant CA800117 and the Mayo Foundation.

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