HIGHLIGHT

A nuclear receptor in thyroid malignancy: is PAX8/PPARγ the Holy Grail of follicular thyroid cancer?

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Several types of thyroid cancer – classic papillary, anaplastic and medullary thyroid carcinoma – can be confidently diagnosed and distinguished through fine-needle aspiration cytology (FNAC) of a thyroid nodule, thanks to their characteristic morphology and additional immunocytochemistry. In contrast, the finding of follicular thyroid neoplasia, a cytological group diagnosis encompassing the three entities follicular adenoma, follicular carcinoma or follicular variant of papillary carcinoma, still represents a major diagnostic dilemma. Capsule or vascular invasion, the two decisive criteria of follicular thyroid malignancy, can be demonstrated or excluded only on histopathological investigation of the surgically removed specimen. As only 15–25% of all follicular thyroid neoplasia prove to be malignant, the majority of patients who undergo follicular FNAC diagnosis are unnecessarily subjected to cost-intensive surgery and its potential complications (1, 2).

For this reason, identification of appropriate markers that will allow highly specific and sensitive preoperative differential diagnosis of follicular neoplasia has a high priority. Encouraging results have been reported regarding altered thyroperoxidase immunoreactivity (TPOmAB47) or positive staining for glycoproteins CD44v6 and galectin-3 in the case of follicular thyroid cancer (3, 4).

Another contributor to the diagnostic dilemma presented by follicular neoplasia is undoubtedly the prevailing uncertainty about its aetiopathogenesis which, despite the impressive progress in identifying specific genetic alterations in other nodular thyroid diseases, remains unsolved.

In their recent study, Kroll et al. (5) suggested a novel pathomechanism in follicular thyroid malignancy, which follows up on previous reports of specific chromosomal translocations in these tumors (6). In a series of five follicular thyroid carcinomas with a karyotype showing a t(2;3)(q13;p25) chromosomal rearrangement, they were able to define specific translocation breakpoints on respective chromosomes using interphase fluorescence in situ hybridisation technology: the chromosome 2q13 breakpoint was located in the region coding for the thyroid transcription factor PAX-8 and the PAX-8 3q25 partner was identified as the peroxisome proliferator-activated receptor (PPAR) γ1. By means of rapid amplification of cDNA ends (RACE) PCR. Sequencing of the RACE products generated from cDNAs of the follicular thyroid carcinomas showed an in-frame fusion of four PAX-8 variants (exons 1–7, 1–8, 1–9 or 1–7 plus 9), presumably generated by alternate splicing, to PPARγ exons 1–6. This resulted in a PAX-8/PPARγ fusion protein comprised of the paired and partial homeobox DNA binding domains of PAX-8, but devoid of its transactivation domain, and the DNA binding, ligand binding and retinoid receptor X (RXR) dimerization and transactivation domains of PPARγ1 (Fig. 1). The expression of the fusion gene was demonstrated at the mRNA level by northern blot analysis with PPARγ (1.8 kb) and PAX-8 (3.1 kb) cDNA probes, both indicating a 3 kb transcript. Further investigation at the protein level, by immunoprecipitation with an antibody to wild-type PPARγ1, confirmed the presence of a 98 kDa protein, in agreement with the expected molecular mass of the fusion protein (87–98 kDa). Importantly, the PAX-8/PPARγ rearrangement was detected only in follicular carcinoma (5/8), but not in follicular adenoma (0/20), papillary carcinoma (0/10) or multinodular goitre (0/10) using nested-RT-PCR with primers located in PAX-8 exons 6 or 7 and PPARγ1 exon 1 respectively, for screening. In the remaining three follicular carcinomas, which were negative for the RT-PCR in this series, an alternate PAX-8/PPARγ1 fusion gene was suspected in one case with a proven t(2;3)(q13;p25) karyotype, and a fusion of PPARγ to a non-PAX-8 partner in another case with a 3p25 but absence of a corresponding 2q13 translocation. In the third follicular carcinoma, there was no indication of involvement of either PAX8 or PPARγ1.

Considering the established molecular components of thyroid disease, these novel data reported by Kroll et al. (5) are highly interesting for several reasons. Firstly, although limited by a small sample series and the presence of a particular karyotype, it is the first description of a specific molecular defect for follicular thyroid cancer. Secondly, it fits perfectly with an almost 10-year-old cytogenetics-based hypothesis by Hermann et al. (6) suggesting that disruption of a putative tumour-suppressor gene on chromosome 3p is peculiar to follicular thyroid malignancy (loss of heterozygosity for loci on 3p in six of six studied follicular cancers).
Three PPAR isotypes have been identified: PPAR dimerization with the RXR for target gene activation. Nuclear receptor superfamily, which requires heterodimeric receptors, they form a subgroup within the thyroid hormones, retinoid acid, vitamin D and the synthetic fibrates (7). Together with the receptors for some proliferation in rodents after stimulation with peroxisome proliferators, appear to mediate transcription resulting in peroxisome proliferations in rodents after stimulation with synthetic fibrates (7). Together with the receptors for thyroid hormones, retinoid acid, vitamin D and the orphan receptors, they form a subgroup within the nuclear receptor superfamily, which requires heterodimerization with the RXR for target gene activation. Three PPAR isoforms have been identified: PPARα (localized on chromosome 22), PPARβ (localized on chromosome 6) and PPARγ, which extends over 100 kb on chromosome 3 and exist in three transcripts, PPARγ1-3, generated by alternative promoter usage and splicing. These three PPARγ variants share six common exons. In addition, PPARγ1 (477 amino acids) contains two specific exons for the 5’ untranslated region. PPARγ2 comprises one further exon encoding 28 additional amino acids at the amino-terminus (505 amino acids), which increases susceptibility for ligand-independent receptor activation, and PPARγ3 encodes the same protein (477 amino acids) as PPARγ1 yet with a different promoter (7; Fig. 2). PPARγ1 and 2 are highly abundant in adipose tissue and mRNA for the former has also been demonstrated in various other tissues, including liver, heart, skeletal muscle, breast, prostate, colon and components of the immune system (monocytes, transformed B lymphocytes).

On the basis of the observation that PPARγ isoforms are the key regulators of adipocyte differentiation, a process paralleled by cell cycle arrest, and that treatment of cultured primary human liposarcoma cells with PPARγ agonists results in terminal cell differentiation (8), a more general role for PPARγ in regulation of differentiation and proliferation and, possibly, of altered PPARγ function in perturbances thereof, has been proposed. In this respect, thiazolidinediones, which are selective PPARγ agonists, may prevent formation of preneoplastic breast lesions in a mouse model (9), and appear to exert a growth inhibitory effect in addition to re-inducing differentiation in several human carcinoma tissues (breast, prostate, colon, lung and bladder) in vivo and partly also in vitro, concomitant with changes in the cell cycle machinery such as reduced cyclin D1 expression, downregulation of Bcl-2 and hypophosphorylation of the retinoblastomavirus in some tumors (10–16). However, ligand-dependent PPARγ activation can also be compromised by the phosphorylation of PPARγ that is observed in breast cancers with high mitogen-activated protein kinase activity (17, 18) or by mutations in the PPARγ gene itself, reported in some colon cancers (19).

What is the role of PAX-8/PPARγ1 in the thyroid?

To assess for the functional consequences of the...
PAX-8/PPARγ rearrangement in follicular thyroid cancer. Kroll et al. (5) expressed the fusion gene in U2OS cells and measured ligand-dependent activation of PPARγ response elements (PPRE) after stimulation with troglitazone. In this setting PAX-8/PPARγ was ineffective in inducing transactivation of several PPREs and completely abrogated wild-type PPARγ activity when co-expressed in the same cell, hence exerting a dominant negative effect. Immunohistochemistry of paraffin-embedded tissues with antibodies to wild-type PPARγ showed strong nuclear staining in the follicular thyroid cancers harbouring the rearrangement and only weak focal staining in all the other thyroid specimens investigated. Noteworthy was that, whereas wild-type PPARγ mRNA was demonstrated both in follicular adenoma and carcinoma on Northern blot analysis no wild-type PPARγ protein was detected upon immunoprecipitation of tissue extracts of follicular carcinoma or adenoma with a monoclonal PPARγ antibody. This suggests several points. Firstly, expression of PPARγ protein in the thyroid is normally very low. Secondly, there is no compensatory upregulation to counterbalance the overexpression of the PAX-8/PPARγ fusion gene and its putative pathophysiological effects in thyroid follicular cancer. Thirdly, the stark contrast between strong expression of PAX-8/PPARγ in follicular thyroid cancer and weak expression of wild-type PPARγ in the other thyroid tissues investigated does not easily suggest that abrogation of normal PPARγ function is the key component of follicular thyroid cancer. In this context, deregulation of PAX-8 function, which was not investigated in this particular study, could be another important consequence of the rearrangement.

Provided the rearrangement and its specificity for follicular cancer can be confirmed independently in larger series of thyroid samples and the relative frequency of the different PAX-8/PPARγ fusion genes in thyroid malignancy is known for iodine sufficient and deficient areas, in which the prevalence of follicular thyroid cancer is increased, the diagnostic implications of the described chromosomal translocations are obvious: demonstration of the most frequent rearrangement variants by molecular diagnostics at the DNA or protein level should be a feasible goal in routine preparation for FNAC and would allow confidence in the presurgical identification of a follicular thyroid cancer.

As for the pathogenetic role and potential therapeutic implications of the PAX-8/PPARγ rearrangement, these are less clear. Whether thiazolidinedione can attenuate the yet unknown PAX-8/PPARγ1 effects in the thyroid to achieve a beneficial course of a follicular thyroid cancer remains to be determined. Whatever the answers to these questions, the PAX-8/PPAR rearrangements reported by Kroll et al. (5) provide a long awaited first grasp of the molecular components of follicular thyroid cancer – and thus, with a little caution, enthusiasm for finally unravelling at least part of the Holy Grail of thyroid malignancy is possibly justified.

References


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