

## Detection of the PAX8-PPAR $\gamma$ Fusion Protein in Thyroid Tumors

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Nobody wants to get cancer, but if you have to, thyroid cancer is a good one to get because of its excellent prognosis. Still, there is plenty of room for improvement in the current approaches to the diagnosis and therapy of this disease. Thyroid cancer is the most common endocrine malignancy. The American Cancer Society estimates 37 000 new cases of thyroid cancer were diagnosed in the US in 2008. The National Cancer Institute Surveillance Epidemiology and End Results database indicates that nearly 400 000 living Americans have a history of thyroid cancer and that 1 in every 127 Americans will be diagnosed with thyroid cancer during his or her lifetime. The vast majority of cases are so-called differentiated thyroid cancers, which are characterized histologically as papillary or follicular tumors. Although follicular cancers comprise only 10%–15% of thyroid cancers, they are of special interest for several reasons. First, it is not possible to distinguish follicular carcinomas from adenomas by biopsy. These tumors must be surgically removed to determine whether they are malignant, and approximately 80% of follicular neoplasms turn out to be benign. This situation contrasts with that for papillary thyroid cancers, which generally are straightforward to diagnose by fine-needle biopsy. Second, follicular thyroid cancers are more prone than papillary cancers to form distant (lung) metastases and are less prone to spread locally to neck lymph nodes. Thus, the detection of recurrences at an early, localized stage is more difficult. For both papillary and follicular thyroid cancers, there is no effective chemotherapy for patients not cured by the standard therapies of surgery and radioiodine.

Thyroid cancers usually are associated with specific genetic abnormalities. For example, close to half of papillary carcinomas have an activating mutation of the kinase BRAF (1). Activating RAS mutations, or gene fusions that cause inappropriate activation of the RET tyrosine kinase, also occur in papillary car-

cinomas. All of these mutations cause activation of mitogen-activated protein kinase signaling.

RAS mutations also are found in 30%–40% of follicular thyroid carcinomas and adenomas. A similar fraction of follicular carcinomas, but a much smaller fraction of adenomas, are found to harbor a t(2;3)(q13;p25) chromosomal translocation that fuses the PAX8<sup>2</sup> (paired box 8) gene with the PPARG (peroxisome proliferator-activated receptor gamma) gene (2). PAX8 is a transcription factor that is produced in high amounts in the thyroid. It is required for thyroid gland development, and it induces the expression of many thyroid-specific genes. PPAR $\gamma$  is a nuclear hormone receptor that plays important roles in carbohydrate and lipid metabolism, as well as in inflammation. It is produced at very low concentrations in the nonpathologic thyroid, however, and its function in this organ, if any, is unknown. The consequence of the t(2;3)(q13;p25) translocation is that the strong PAX8 promoter drives the production of a PAX8-PPAR $\gamma$  fusion protein (PPFP).<sup>3</sup> How PPFP contributes to thyroid neoplasia is not clear. Although PPFP can show dominant negative activity against PPAR $\gamma$  in transfection experiments (2), evidence also suggests it can function in a PPAR $\gamma$ -like manner, both in transfection experiments and in thyroid cancer (3, 4).

The ability to identify follicular thyroid tumors that produce PPFP is important for several reasons. Obviously, studying the mechanism of tumorigenesis and the biology of these cancers requires the ability to detect PPFP production and to compare PPFP-positive and -negative follicular cancers. The ability to detect PPFP also may have diagnostic importance, especially given that it is impossible to distinguish follicular carcinomas from adenomas by biopsy. In fact, this distinction can be difficult even in surgical samples. Although PPFP is occasionally found in follicular adenomas, it is many times more prevalent in follicular carcinomas. Furthermore, follicular adenomas with PPFP tend to have atypical features, such as increased proliferation

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<sup>2</sup> Human genes: PAX8, paired box 8; PPARG, peroxisome proliferator-activated receptor gamma; CREB3L2, cAMP responsive element binding protein 3-like 2.

<sup>3</sup> Nonstandard abbreviations: PPFP, PAX8-PPAR $\gamma$  fusion protein; RT-PCR, reverse-transcription PCR; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

markers and pleomorphism, that prompt a heightened concern for malignant potential (5, 6). Thus, detection of PFPF in a biopsy sample would provide a strong rationale for surgical excision of the nodule.

In this issue of *Clinical Chemistry*, Algeciras-Schimmich et al. describe and validate a clinically applicable reverse-transcription PCR (RT-PCR)-based assay for the detection of PFPF in formalin-fixed/paraffin-embedded tissues, fresh frozen tissues, and, potentially, fine-needle biopsy samples (7). The authors carefully compare their assay with detection by fluorescence in situ hybridization (FISH) with probes for *PPARG* and *PAX8* and with detection by immunohistochemistry (IHC) for PPAR $\gamma$  (because PFPF is produced at much higher concentrations than endogenous PPAR $\gamma$ , strongly positive IHC results for PPAR $\gamma$  suggest PFPF production). Although many publications have described the use of 1 or 2 of these techniques to detect PFPF (5, 6, 8–10), this report provides the most thorough validation and comparison of the 3 approaches. In addition, this RT-PCR assay allows the identification of different *PAX8* exon fusions to PPAR $\gamma$ . Although the importance of the different exon fusions is unknown, this area of research is an interesting one. The authors show that their RT-PCR assay results were in agreement with their FISH results for 53 of 56 tumors, with 1 case positive by FISH only and 2 positive by RT-PCR only. This degree of agreement is excellent, although the reasons for the occasional discrepancies were not investigated and thus we do not know how to interpret those cases. In contrast, a strong signal for PPAR $\gamma$  by IHC was sensitive but not as specific. All 9 follicular cancers that were positive for PFPF by RT-PCR also were positive by IHC, but 11 of 39 tumors that were negative by RT-PCR also were positive by IHC.

The concordance of the FISH and RT-PCR approaches is to be expected and essentially validates the RT-PCR method, which is technically much simpler and more amenable to use as a clinical diagnostic test. The fact that IHC detection of high PPAR $\gamma$  production was sensitive but not specific for PFPF detection replicates published data (5, 9). The low specificity of the PPAR $\gamma$  IHC results prompts Algeciras-Schimmich et al. to consider this approach inferior to RT-PCR. For the time being, this conclusion seems appropriate, but a final analysis would require knowing the biological importance of an increased PPAR $\gamma$  IHC signal in the absence of PFPF. The antibody used in these studies is not actually specific for PPAR $\gamma$ , because it also detects PPAR $\alpha$  and PPAR $\delta$ . In fact, PPAR $\delta$  is frequently increased in thyroid tumors (benign and malignant), where it increases proliferation by inducing cyclin E1 (11). The use of a truly PPAR $\gamma$ -specific antibody might decrease the false-positive rate for PFPF detection. Fur-

thermore, follicular cancers with gene fusions between *CREB3L2* (cAMP responsive element binding protein 3-like 2) and *PPARG* have been described (12), indicating that *PAX8* is not the only fusion partner for *PPARG*. The *CREB3L2*-PPAR $\gamma$  fusion transcript would, of course, not be detected with an RT-PCR assay for PFPF, but both fusion proteins are detected by IHC assays for PPAR $\gamma$ . Although the *CREB3L2*-*PPARG* fusion is rare, its existence points to a limitation of the PFPF RT-PCR assay if the goal is to detect PPAR $\gamma$  fusions as markers of potential thyroid malignancy.

It appears that the RT-PCR approach of Algeciras-Schimmich et al. will be appropriate for thyroid fine-needle biopsy samples, in that the authors were able to detect *PAX8* RNA in their biopsy samples. None of the tumors analyzed by biopsy turned out to contain the *PAX8*-PPAR $\gamma$  fusion, however, so we do not actually know the sensitivity and specificity of the assay in this setting. The analysis of fine-needle biopsy samples is a very important potential use of this assay. Only approximately 5% of thyroid nodules that get biopsied are malignant, however, and probably only approximately 5% of malignant nodules are PFPF-producing follicular carcinomas. Therefore, a very large number of samples will need to be studied to determine the utility of this (or any) assay for PFPF in a biopsy setting.

Algeciras-Schimmich et al. found PFPF to be present in 62% (13 of 21) of follicular carcinomas. This prevalence is higher than most investigators have observed. For example, the combined results of 4 studies (5, 6, 10, 13) show a prevalence of 34% (27 of 79 cases) for PFPF in follicular carcinomas. Most likely, the high PFPF detection rate by Algeciras-Schimmich et al. simply reflects variation due to the small numbers of cases, rather than methodologic differences.

The passage of time and additional studies will reveal the optimal way(s) to detect PFPF in thyroid tumors and will define when this assay should be performed. The work by Algeciras-Schimmich et al. is a welcome contribution along this road.

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