American Journal of Pathology, Vol. 160, No. 6, June 2002

Commentary

Molecular Rearrangements and Morphology in Thyroid Cancer

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Tumor development within the human thyroid gland provides an attractive experimental model with which to consider the pathogenesis of carcinoma, the most common and clinically significant cancer. Thyroid tumors are readily accessible to morphological and molecular study because their primary treatment is surgical. Investigations of thyroid cancer have expanded our knowledge of carcinoma biology. Thyroid carcinoma is the only adult epithelial malignancy in which specific chromosomal rearrangements have been identified.[1–3] *RET* and *PPARγ* rearrangements in thyroid carcinoma create fusion proteins that are hypothesized to play fundamental roles in thyroid oncogenesis.[3,4] The fusion protein pathways are prime targets for new strategies directed at improving thyroid cancer diagnosis and treatment.[5–7]

The *RET* proto-oncogene is interesting because it is mutated by different mechanisms in different (endocrine and neuroendocrine) thyroid carcinomas. *RET* rearrangement was discovered in papillary thyroid carcinoma,[2] and it is an important pathogenic event in this cancer.[4,8] On the other hand, activating *RET* point mutations/insertions are pathogenic in medullary thyroid carcinoma,[9,10] which can manifest in either sporadic or familial form such as the multiple endocrine neoplasia type 2 syndrome.[11–13] The molecular mechanisms associated with *RET* mutations in cancer are incompletely understood.

Our current concepts of chromosomal rearrangements in cancer are based primarily on investigations of blood cell malignancies. Chromosomal rearrangements in leukemias are genetic hits with complex functional consequences. Fusion proteins encoded by both derivative chromosomes have activities that often contribute uniquely to the neoplastic process.[14,15] The fusion proteins can also inhibit their wild-type counterparts in a dominant-negative manner.[17–19] Chromosomal rearrangements are early events that seem to require additional collaborating mutations and cellular alterations for cancer induction.[16,20,21] Future studies will determine the extent to which rearrangement mechanisms are similar in carcinomas and noncarcinomas and the degree to which *RET* and other gene fusions may be useful in the management of thyroid cancer.

A study reported by Fusco and colleagues[22] in this issue of *The American Journal of Pathology* correlates *RET* rearrangements with specific morphological patterns in thyroid tumors. The motivations are laudable but the undertaking is difficult because thyroid and other epithelial tumors typically consist of neoplastic cells intermingled irregularly with normal (connective tissue and vascular) and reactive (stromal and immune) cells in a solid tumor mass. Robust methods to analyze specific cell subpopulations within fresh and fixed epithelial tumors are needed.[23,24] A main reason that blood cell malignancies have been so amenable to new molecular genetic approaches is that >90% pure populations of well-defined, viable tumor cells can often be obtained.[25–28]

Immunohistochemistry was performed by Fusco and colleagues[22] with an affinity-purified polyclonal antiserum to detect RET protein expression. Because RET is not synthesized in normal follicular epithelial cells, immunoreactivity is thought to correlate with overexpression of the rearranged *RET* gene. However, the possibilities of confounding wild-type RET protein within thyroid tumors[29–31] and/or cross-reactivity of the antiserum with receptors bearing homologous tyrosine kinase domains need to be kept in mind. The immunohistochemistry was coupled with reverse transcriptase-polymerase chain reaction and laser capture microdissection of paraffin-embedded tumor tissues to assess *RET* rearrangement status. Degraded mRNA in paraffin-embedded tissue reduces sensitivity and in this study control housekeeping transcripts were detected in only 7 of 14 cases, a highly select sample of the original 46 cases. Such high-cycle nested polymerase chain reaction and hybridization protocols are prone to contamination and low specificity, although appropriate negative and positive controls were reported. A common source of contamination/false-positives is purified plasmids and/or cell lines harboring rearrangements that are used to test assay sensitivity or to

Accepted for publication April 4, 2002.

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perform functional studies. Low specificity is often suggested by artifactual bands and smearing seen with ethidium bromide staining. The androgen receptor clonality assay was also conducted on DNA obtained from manually dissected paraffin-embedded tissue, and it is somewhat surprising that all four follicular adenomas were polyclonal. Nearly all follicular adenomas in the literature are monoclonal.[32–34] On the other hand, this may be a special mixed group of tumors. Two of the hyperplastic nodules were monoclonal and two were polyclonal, consistent with the clonality reported in the literature.[35–37] Based on these considerations, this study must be interpreted conservatively and additional experiments with fresh tissue (fragments or frozen sections) and complimentary methods such as fluorescence *in situ* hybridization will be needed to corroborate *RET* rearrangement at the DNA level.

Putting aside potential technical caveats, the data of Fusco and colleagues[22] show that *RET* rearrangement can occur within "morphologically benign" thyroid nodules. Two main tumor groups were seen. In the first group, three tumors exhibited *RET* rearrangement in focal areas of well-developed papillary carcinoma-like morphological change (type A), consistent with the idea that these are small papillary carcinomas arising within benign thyroid nodules. This contention would not be surprising to most pathologists who often observe small papillary carcinoma-like foci dispersed widely within thyroid tissue in both surgery and autopsy material. The distribution of these foci within the nodules seems inconsistent with the theoretical possibility that they preceded growth of the remaining tumor. In fact, all three tumors had foci with *RET/PTC1* rearrangement, which is the most prevalent rearranged *RET* form in the small classic and occult/microcarcinoma[38–40] papillary carcinoma subtypes. Two of these three nodules were polyclonal, as would also be expected if they contained co-existing tumors. Thus, tumor morphology and molecular genetics seem concordant in this group.

In the second group, two of six tumors with widespread but weak papillary carcinoma-like morphological change (type D) exhibited *RET/PTC3* rearrangement, consistent with the idea that these are carcinomas with variable papillary nuclear morphology. In support of this possibility, one tumor was monoclonal, as would be expected for a papillary carcinoma. The other tumor could not be tested. Two other tumors with type D morphology were negative for *RET* rearrangement and the remaining two tumors were not tested. Thus, tumor morphology and molecular genetics seem discordant in this group.

Interestingly, other recent studies affirm a discordance between *RET* rearrangement status and papillary thyroid carcinoma-like morphology in thyroid tumors. For example, up to 50% of Hurthle cell thyroid tumors harbor *RET* rearrangements,[41,42] despite the widely held view that they are more closely related to follicular tumors than to papillary carcinoma. In addition, papillary carcinomas containing *RET/PTC3* rearrangement exhibit a spectrum of histologies (classic, solid, and tall cell) that have different biological tendencies.[43–45] Thyroid tumors with mixed morphological and clinical features of papillary and follicular carcinoma have also been described.[46] These findings suggest that there is an imprecision in our morphological categorization of thyroid tumors and that unknown cellular factors cooperate with *RET* rearrangement to determine papillary carcinoma morphology and biology. The overall findings of Fusco and colleagues[22] suggest that we have much to learn regarding thyroid cancer pathogenesis and the degree to which thyroid tumor morphology correlates with underlying molecular genetic events.

The imprecise nature of papillary carcinoma morphology makes pathological diagnosis of thyroid tumors difficult. The issue is complicated by varied approaches of pathologists to follicular-patterned thyroid tumors.[47–49] Even so, it is likely such imprecision has little clinical impact because nearly all low-stage thyroid cancers have excellent prognosis. Whereas it might be informative from a biological perspective to better define the biology of the tumors in this study, from a clinical perspective they will likely behave at worst like well-differentiated thyroid cancer, a readily curable disease. In fact, 80 to 90% of thyroid nodules now removed by surgery are benign, and we therefore may actually be overtreating many patients with thyroid nodules to exclude the possibility of cancer. The most rational long-term clinical goal is to increase our ability to differentially diagnose benign from malignant/precursor thyroid nodules before surgery so that appropriate, more individualized treatments can be rendered.

In summary, the results of Fusco and colleagues[22] suggest that some thyroid nodules with a predominance of benign morphological features have *RET* rearrangement. Techniques such as fluorescence *in situ* hybridization will be needed to document the existence, frequency, and geographic distribution of *RET* rearrangements in these relatively rare tumors. The study highlights an imprecision in our morphological classification of papillary carcinoma-like tumors, making it more likely that molecular endocrine tumor markers will help us subdivide thyroid and other endocrine tumors into more distinct biological subgroups. Regardless of whether morphological or molecular markers are considered, their clinical utility is dependent strictly on tumor biology and clinical context. It is therefore critical that well-organized clinical databases containing comprehensive patient information and clinical follow-up data be constructed to rigorously define clinicopathological correlates of putative biomarkers identified with the new molecular genetic techniques.[50] Thyroid cancer is no exception in this respect.

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