Editorial: RET/PTC Rearrangement—A Link between Hashimoto’s Thyroiditis and Thyroid Cancer...or Not

RET/PTC rearrangement has been known for almost two decades as one of the most common molecular alterations in thyroid papillary carcinoma. It was discovered by Fusco et al. (1) in 1987 using a transfection assay on NIH3T3 cells, which revealed the transforming activity of DNA isolated from papillary carcinomas. The new oncogene, named RET/PTC, was subsequently found to be a fusion between the RET gene coding for transmembrane receptor tyrosine kinase and the H4 gene (2). As of today, 11 different fusion partners of RET have been identified, giving rise to specific types of RET/PTC fusions (reviewed in Refs. 3 and 4). The two most common rearrangement types are RET/PTC1 and RET/PTC3, both of which are intrachromosomal inversions involving the long arm of chromosome 10. The rearrangement is likely to be predisposed by close positioning of the RET and its fusion partners within the nuclei of normal thyroid cells (5, 6). The fusion results in constitutive activation of the truncated tyrosine kinase portion of RET and signaling along the MAPK pathway. RET/PTC activation is believed to be oncogenic for thyroid follicular cells, because it transforms cells in vitro and results in the formation of thyroid tumors in transgenic mice (7–9).

RET/PTC is found in 20–40% of adult sporadic papillary carcinomas and with higher prevalence in children and in patients exposed to radiation (3, 4). Originally, the rearrangement was considered to be specific for papillary thyroid carcinomas because it was not found in other thyroid tumors in a large series of cases studied using Southern blot analysis (10). Southern blot is a highly reliable method for the detection of chromosomal rearrangements, which, however, has limited sensitivity and requires at least approximately 10% of tumor cells within the sample for successful detection. More recently, the specificity of RET/PTC for papillary carcinomas has been challenged by several observations that reported its detection in benign thyroid follicular adenomas, benign and malignant oncocytic (Hurthle cell) tumors, and even in some nonneoplastic conditions such as Hashimoto’s thyroiditis (11–16). Most of those reports used for the analysis a highly sensitive RT-PCR technique, which is capable of the detection of a very small proportion of cells carrying a particular genetic event. Two of those studies have found both RET/PTC1 and RET/PTC3 rearrangements in virtually all thyroid glands affected by Hashimoto’s thyroiditis (12, 14). This led the authors to conclude that multiple occult papillary carcinomas exist in thyroid glands of most patients affected by this common disease, implying the necessity for their aggressive surgical treatment. However, both of these studies had substantial technical limitations because they used highly degraded RNA isolated from formalin-fixed and paraffin-embedded tissues. Furthermore, they used PCR with increased cycle numbers and more than one round of amplification, an approach that dramatically increases the sensitivity of detection but also carries a higher risk of false-positive amplification. The results of these studies have not been reproduced in other observations using standard-sensitivity PCR (17). Because of these limitations and the lack of reproducibility, the conclusions on the association between Hashimoto’s thyroiditis and the occurrence of multiple tumors in most thyroid glands were met with substantial skepticism. Nevertheless, these studies provided some evidence suggesting that RET/PTC may be present in a subpopulation of cells in the thyroid glands affected by Hashimoto’s thyroiditis.

These reports added a new dimension to the long-standing controversy regarding the association between Hashimoto’s thyroiditis and follicular cell-derived thyroid tumors. This association was first proposed more than 50 yr ago by Dailey et al. (18), who found a significantly higher frequency of thyroid carcinomas and adenomas in surgically removed glands with Hashimoto’s thyroiditis than in glands unaffected by this disease. Most of the malignancies found in their series were papillary carcinomas. The subsequent studies yielded conflicting results, reporting either very high (up to 23%) or low incidence of thyroid carcinoma in the removed thyroid glands with Hashimoto’s thyroiditis (reviewed in Ref. 19). The increase in risk of thyroid cancer in patients with Hashimoto’s thyroiditis was not found in the prospective case-control studies (20, 21). The high incidence of cancer reported in several studies could still be interpreted as evidence for a relationship between the two diseases, but it did not imply whether the putative relationship is causal or merely incidental. The findings of RET/PTC rearrangement in these glands could provide a strong pathogenetic link between the two conditions. However, this would require unequivocal and reproducible detection of RET/PTC, preferably using more than one method, as well as evidence that the occurrence of the rearrangement in few cells is carcinogenic and confers the cells with the phenotypical features of papillary carcinoma.

In this issue of JCEM, the paper by Rhoden et al. (22) reports a study of RET/PTC rearrangements in Hashimoto’s thyroiditis and papillary carcinomas using two detection techniques, interphase fluorescence in situ (FISH) and RT-PCR. The experiments used high-quality RNA extracted from snap-frozen thyroid tissue; microscopic examination by an experienced thyroid pathologist was undertaken to identify the papillary carcinoma foci. The authors report that in the majority of thyroid glands with Hashimoto’s thyroiditis, they were able to detect RET/PTC by FISH...
in rare follicular cells. Notably, by careful microscopic examination of the areas containing positive cells, they excluded the possibility that these cells were collected from the microscopic foci of papillary carcinoma. In many of those cases, the RT-PCR analysis confirmed a low-level of RET/PTC1 rearrangement. However, in some cases of Hashimoto’s thyroiditis positive for RET/PTC by FISH, RT-PCR did not reveal RET/PTC1 or RET/PTC3. This is despite the fact that RT-PCR is typically much more sensitive than FISH.

Indeed, in most studies using FISH for the detection of chromosomal rearrangements, a cut-off level of 5–10% positive cells has been used to separate cases from false-positives (23, 24). In the study by Rhoden et al. (22), the authors set up a quite low cut-off level of 3.5%, which is still significantly above the detection limit of standard RT-PCR. Incidentally, if a more widely accepted cut-off level of 10% was used, virtually all cases of Hashimoto’s thyroiditis would be considered negative for RET/PTC. This demonstrates the complexity of the detection of very low-level events using current molecular techniques. Indeed, although most of the commonly used detection methods are highly reliable when the target event is present in abundance, their results are frequently irreproducible or difficult to interpret when the detection methods are pushed to the limits of their sensitivity.

Despite these technical limitations, the importance of the paper by Rhoden et al. (22) is in providing additional evidence for the possibility of RET/PTC presence in a subpopulation of cells within thyroid glands affected by Hashimoto’s thyroiditis. However, the significance of this low-level event with respect to neoplastic transformation remains unclear. Although it is legitimate to speculate that the expression of an oncogene in a target cell could predispose it to carcinogenesis, many additional and still unknown steps are likely to be required for the full transformation. Therefore, it would be at least premature to equate the presence of the RET/PTC rearrangement in a single cell or in a small group of cells with the presence of a papillary carcinoma. In fact, this study provides evidence against such a conclusion by showing that cells expressing RET/PTC did not demonstrate the diagnostic microscopic features of papillary carcinoma. Moreover, among clinically significant papillary carcinomas developed in thyroid glands affected by Hashimoto’s thyroiditis, only a minority of cases were found to harbor a high-level RET/PTC rearrangement in this study and in other reports (17). This would be unexpected if we assumed that RET/PTC, which is commonly present in thyroid cells within these glands, was sufficient for oncogenic transformation.

The study by Rhoden et al. (22) provides another important conclusion. It shows the importance of quantitative detection of the RET/PTC rearrangement and cautions against using low-level RET/PTC detection as a diagnostic tool for papillary carcinoma, not only because of technical unreliability, but also because the biological significance of this finding remains unknown. The availability of novel research tools, such as expression arrays and proteomics, and more sophisticated in vitro and in vivo models of RET/PTC expression provides hope that in the near future we will learn much more about the effects of RET/PTC activation in thyroid cells and its relationship to carcinogenesis. Until then, we should carefully separate the proven findings from speculations and exercise caution, particularly when the well-being of patients is at stake.

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