RET/PTC1 Rearrangement in Hashimoto's Thyroiditis: Canonical WNT Expression Upregulation for Neoplastic Promotion

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ABSTRACT

Background: More than half a century has passed since the association between papillary thyroid carcinoma (PTC), and Hashimoto's thyroiditis (HT) has been posted. The discovery of the RET/PTC oncogene thirty years later has further strengthened this argument, followed by the associations made between hyalinizing trabecular adenoma (HTA) and both PTC and HT. Numerous researchers studied these relationships, with some debating a valid link.

Findings: This case of a 28-year-old woman with HT-associated PTC and a co-existing HTA, offers a unique opportunity to study these three diseases together in the same microenvironment. Fresh tissue analysis for gene expression showed that all three expresses RET/PTC1 transcripts, while this was not seen in the control normal thyroid tissue. There was also an increased expression of AKT, ERK1, nuclear β-catenin and TCF4 in both the HTA and PTC sampled tissue. AKT plays a central role in regulating cell proliferation and survival by inhibiting apoptotic processes, while ERK1 leads to cell proliferation. Both β-catenin and TCF4 are linked to the canonical WNT/β-catenin pathway.

Conclusion: The expression of RET/PTC1 highlights the link between inflammatory processes such as HT and neoplastic diseases such as HTA and PTC. The limited expression of TCF4, AKT, ERK1, and β-catenin supports the notion that the canonical WNT/β-catenin pathway plays a pivotal role in the development of this neoplasm subtype. To date, gene expression alone is not sufficient to make a distinction between benign and malignant thyroid tumors.

INTRODUCTION

The relationship between Hashimoto's thyroiditis (HT) and papillary thyroid carcinoma (PTC) was first reported by Dailey in 1955. This association was initially guided by the presence of thyroid antibodies and histopathological evidence of thyroiditis in cases of PTC, supporting that a sustained inflammatory response in HT may act as an initiating factor in carcinogenesis.

An association of both PTC and HT with hyalinizing trabecular adenoma (HTA) was also described. HTA is a rare diagnosis which can represent a diagnostic challenge on fine-needle aspiration cytology, as it shares nuclear features with PTC. But the two have very different prognoses. There are only a handful of case reports which document the presence of HTA in the background of HT.

RET/PTC is an oncogene activated in human papillary thyroid carcinoma, with up to 13 different rearrangements reported to date. Of these subtypes, only RET/PTC1 and RET/PTC3 variants have a significant role in PTCs; the others are only isolated in rare cases mostly related to radiation damage. However, RET/PTC activation, mainly RET/PTC1, was detected in other tumors of the thyroid gland such as HTA and Hurthle thyroid cell adenomas and carcinomas. RET/PTC activation has also been reported in non-neoplastic diseases such as HT.

This case shows the concurrence of both hyalinizing trabecular adenoma (HTA) and papillary thyroid carcinoma (PTC) in the background of Hashimoto's thyroiditis (HT). Representing an opportunity to review the traditional associations, and the biological basis of tumor progression, in this inflammatory context by gene expression analysis.

METHODS

Case Presentation

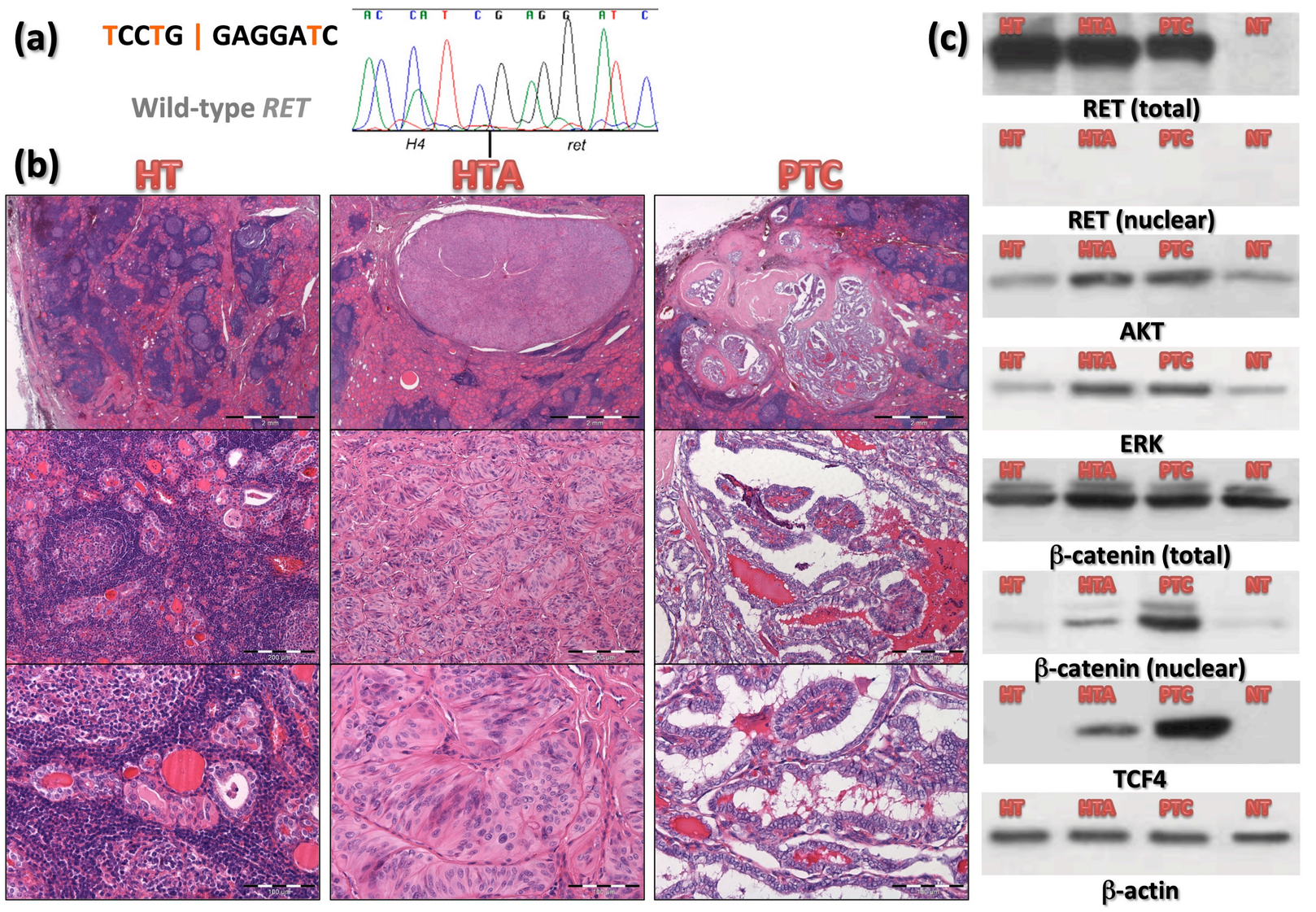
A 28-year old woman with an enlarged thyroid had a Fine Needle Aspiration Cytology (FNAC), the result was diagnostic of papillary thyroid carcinoma; RCPath classification: THY 5, Bethesda classification: VI.

The total thyroidectomy specimen was processed for histopathological evaluation. Excess fresh tissue was taken from the fresh specimen for molecular analysis.

Molecular Analysis

Before RNA extraction, representative sections of the frozen tissue were cut, manually microdissected, and examined histologically to confirm the adequacy of the sample and to estimate the proportion of follicular cells vs. stromal and inflammatory cells. The percentage of follicular cells in the perilesional samples of the standard set was always higher than 80%. The tumor samples contained at least 65% neoplastic follicular cells.

DNA was extracted from fresh frozen tissue, and following UV irradiation and nuclear protection of nuclei acid, RNA was extracted for reverse transcription. PCR products were then transformed by capillarity to a positively charged nylon membrane, immobilized by UV irradiation, and colorimetrically detected using NBT and BCIP.



(a) RET/PTC1 gene rearrangement observed in all thyroid samples. (b)The histopathological findings of Hashimoto's thyroiditis (HT) with dense multifocal lymphoplasmacytic infiltrate and scattered lymphoid aggregates. Hyalinizing trabecular adenoma (HTA) with a well-circumscribed tumor, composed of ribbons and nests of follicular cells with grooved palisading nuclei perpendicular to the membrane. Papillary thyroid carcinoma (PTC) with an infiltrative tumor showing papillary and follicular structures lined by neoplastic follicular cells. (c) Gene expression analysis of RET/PTC (total and nuclear), AKT, ERK, β-catenin (total and nuclear), TCF4 and β-Actin.

RESULTS

The microscopic assessment showed the presence of two distinct neoplasms in the background of HT. The bigger neoplasm showed features diagnostic of a classical papillary thyroid carcinoma. The second neoplasm had a thin fibrous connective tissue capsule, with the cells arranged in trabecular and insular patterns in a hyalinized stroma in-keeping with HTA. The background thyroid gland showed a dense multifocal chronic inflammation with mature plasma cells, lymphoid aggregates, and scattered germinal centers consistent with HT. DNA samples from the microdissected areas of inflammation (HT), HTA, and PTC revealed no somatic point mutations of *BRAF* (exons 11 and 15).

Differential gene expression analysis from the areas of HT, HTA, PTC, and normal thyroid tissue was performed, analyzing mRNA transcripts for both nuclear and total RET/PTC1, AKT, ERK, total and nuclear β-catenin, TCF4 and β-actin. Total RET/PTC1 transcripts were detected in tissues sampled from HT, HTA, and PTC only, with no signal for nuclear samples after UV irradiation (Figure 1c).

HTA and PTC revealed an unregulated expression of AKT, along with lower signals in samples from HT and the standard thyroid tissue. The ERK results were similar to those seen with AKT, as there were stronger bands in HTA and PTC in comparison with HT and the normal thyroid samples. Total β-catenin showed the same intensity in all sampled tissue, while the nuclear β-catenin was only seen in HTA and PTC, with stronger broader bands in the sampled PTC tissue. TCF4 transcripts were also limited to the HTA and PTC samples, with stronger signals in the PTC tissue. b-Actin transcripts were seen equally, with moderately intense bands, in all sampled tissue including the control normal thyroid tissue.

CONCLUSION

The gene expression analysis in our case revealed an upregulation of AKT and ERK, supporting an advantageous cell kinetic that result in cellular expansion by both blocking of apoptotic pathways and stimulation of proliferative activity. Unsurprisingly high levels of AKT, ERK, TCF4 and β-catenin transcription were detected in both HTA and PTC; while they were undetected in the HT and normal thyroid samples. These findings further support the association of the WNT/β-catenin pathway with the neoplastic process in thyroid tissue.

The RET/PTC rearrangement is not enough to explain the cellular and tumor phenotype, and additional genetic events are necessary for this context. Our results highlight that the canonical WNT pathway with nuclear b-catenin accumulation plays a central role in the development of autonomous clonal expansions of RET/PTC rearranged follicular cells, establishing a relevant difference in inflammatory and neoplastic conditions associated with RET/PTC rearrangements.

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ABSRACT TITLE:

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ABSTRACT TEXT

Objective: More than half a century has passed since the association between papillary thyroid carcinoma (PTC), and Hashimoto's thyroiditis (HT) has been posted. The discovery of the RET/PTC oncogene thirty years later has further strengthened this argument, followed by the associations made between hyalinizing trabecular adenoma (HTA) and both PTC and HT. Numerous researchers studied these relationships, with some debating a valid link.

Methods: This case of a 28-year-old woman with HT-associated PTC and a co-existing HTA, offers a unique opportunity to study these three diseases together in the same microenvironment. Total thyroidectomy specimen was routinely examined on H&E, and fresh tissue was analysed for gene expression.

Results: Fresh tissue analysis for gene expression showed that all three expresses RET/PTC1 transcripts, while this was not seen in the control normal thyroid tissue. There was also an increased expression of AKT, ERK1, nuclear β-catenin and TCF4 in both the HTA and PTC sampled tissue. AKT plays a central role in regulating cell proliferation and survival by inhibiting apoptotic processes, while ERK1 leads to cell proliferation. Both β-catenin and TCF4 are linked to the canonical WNT/β-catenin pathway.

Conclusion: This study is the first to conduct a biological analysis of these three diseases in a single specimen. The expression of RET/PTC1 highlights the link between inflammatory processes such as HT and neoplastic diseases such as HTA and PTC. The limited expression of TCF4, AKT, ERK1, and β-catenin supports the notion that the canonical WNT/β-catenin pathway plays a pivotal role in the development of this neoplasm subtype. To date, gene expression alone is not sufficient to make a distinction between benign and malignant thyroid tumors. Studying tumor morphology remains the gold-standard method for diagnosis and analyzing these processes is essential in developing drugs and predictive tests for treatments to inhibit this pathway in thyroid cancer.

Policy of full disclosure: /

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