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Are *RET/PTC* Rearrangements in Benign Thyroid Nodules of Biological Significance?

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Dear Editor:

R*ET/PTC* are chimeric oncogenes generated by the fusion of the catalytic domain of the tyrosine kinase receptor *RET* to the 5′ terminal region of heterologous genes. While initially *RET/PTC* expression was considered restricted to papillary thyroid cancer (PTC), highly sensitive detection techniques disclosed the presence of this oncogene also in nonmalignant thyroidal diseases like Hashimoto's thyroiditis, adenomas, and benign colloid nodules (1). To date, the biological significance of *RET/PTC* in benign thyroid lesions controversial. While it could represent an event in the transition to malignancy, it might affect cell behavior in other ways. Here, we present a patient with a thyroid nodule that was benign on cytology and histopathology. Molecular analysis revealed that it harbored *RET/PTC*. Because of the juxtaposition of this with a distinctive clinical course, we speculated that *RET* rearrangements may be associated with more rapid nodular growth.

The patient was a 69-year-old woman who we followed at our University Hospital for 12 years. She presented to us in 1998 for a slight increase in volume in the anterior region of the neck. She had been subjected to subtotal thyroidectomy at the age of 40 years with a histological postoperative diagnosis of benign nodular goiter and since then had been on continuous replacement therapy with L-thyroxine. Physical examination of the neck revealed a mobile hard left thyroidal mass about 2 cm of diameter. There was no palpable cervical lymphadenopathy and she had no cervical compressive symptoms. The results of thyroid function tests were in the normal range and thyroid auto-antibodies were absent. Thyroid ultrasonography revealed recidivism of multinodular goiter. The dominant nodule was in the left lobe. It was solid and hypoechoic on ultrasonography with a mean volume of 2.34 mL. Fine-needle aspiration cytology of the nodule was benign and Thy 2 according to cytological classification of the British Thyroid Association. Taking into account of the benign cytology, the absence of compressive symptoms, and the age of the patient, we decided to follow her with annual ultrasonographic evaluation. The nodule size was stable until 2004, but between 2005 and 2009 the nodule rapidly increased in dimension, reaching the final volume of 7.48 mL (Supplementary Fig. S1; Supplementary Data are available online at www.liebertonline.com/thy).

A second fine-needle aspiration cytology was performed in late 2009, which confirmed the previous cytologic diagnosis of benign lesion. Nevertheless, because of the increasing size of the nodule and the onset of compressive symptoms, we advised surgery for the patient, which she consented to. The thyroidectomy specimen was independently examined by three pathologists who agreed with the diagnosis of benign hyperplastic nodule and excluded the presence of any foci of malignancy or features of lymphocytic thyroiditis (Supplementary Fig. S2). Studies for *RET* rearrangements by Southern blot on reverse transcriptase–polymerase chain reaction products were performed as previously described (2) on both cytological and histological specimens (Supplementary Table S1). Southern blot clearly showed the presence of *RET/PTC1* (Supplementary Fig. S3). The presence of the oncogene was confirmed by dideoxy sequencing of material from the nodule in the thyroidectomy specimen.

Although a direct correlation between the rapid size increase of the nodule and the appearance of *RET/PTC1* cannot be demonstrated, it is possible that the rapid nodule growth that occurred in this patient was empowered by the presence of *RET/PTC1*. Interphase fluorescence *in situ* hybridization analysis has demonstrated that subclonal *RET* rearrangements are very frequent in PTC (3). Even if *RET/PTC* is present only in a sub-set of cells, it might influence the entire nodular mass or a large part of it. Chemokines, small cytokines that bind to 7-transmembrane receptors present on the cell surface, activate a kinase cascade leading to activation of mitogen-activated protein kinases and ultimately in cell migration and proliferation. Expression of the chemokines CXCL1 and CXCL10 and their corresponding receptors is induced by *RET/PTC*, therefore modulating cell proliferation also by an autocrine/paracrine mechanism (4). Therefore, the oncogene *RET/PTC* might stimulate proliferation of the neighbor cells by a paracrine loop, extending his effect to the oncogene-negative cells. Although to date there is no evidence that the

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presence of *RET* rearrangements worsen the prognosis of nodules with benign cytology, we advance the hypothesis that it could promote more rapid nodule enlargement. This hypothesis may be difficult to test. It would require long-term follow-up of cohorts of *RET/PTC*-positive and -negative benign thyroid nodules. Reliable sampling to confirm the *RET/PTC*-positive and -negative status of thyroid nodules without interfering with their natural history, however, is problematic.

Acknowledgments

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References

1. Ishizaka Y, Kobayashi S, Ushijima T, Hirohashi S, Sugimura T, Nagao M 1991 Detection of retTPC/PTC transcripts in thyroid adenomas and adenomatous goiter by an RT-PCR method. Oncogene **6:**1667–1672.
2. Elisei R, Romei C, Vorontsova T, Cosci B, Veremeychik V, Kuchinskaya E, Basolo F, Demidchik EP, Miccoli P, Pinchera A, Pacini F 2001 RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated, malignant and benign thyroid lesions in children and adults. J Clin Endocrinol Metab **86:**3211–3216.
3. Unger K, Zitzelsberger H, Salvatore G, Santoro M, Bogdanova T, Braselmann H, Kastner P, Zurnadzhy L, Tronko N, Hutzler P, Thomas G 2004 Heterogeneity in the distribution of RET/PTC rearrangements within individual post-Chernobyl papillary thyroid carcinomas. J Clin Endocrinol Metab **89:** 4272–4279.
4. Melillo RM, Castellone MD, Guarino V, De Falco V, Cirafici AM, Salvatore G, Caiazzo F, Basolo F, Giannini R, Kruhoffer M, Orntoft T, Fusco A, Santoro M 2005 The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells. J Clin Invest **115:** 1068–1081.

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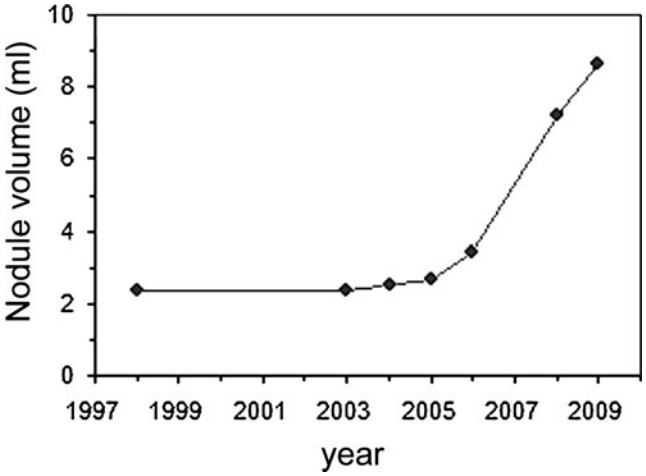
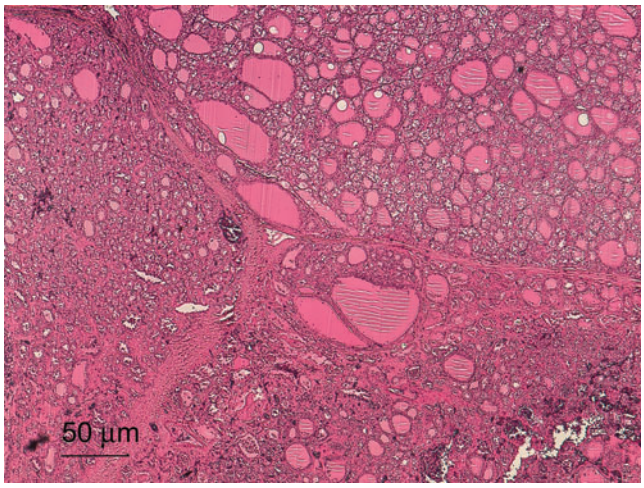
Supplementary Data



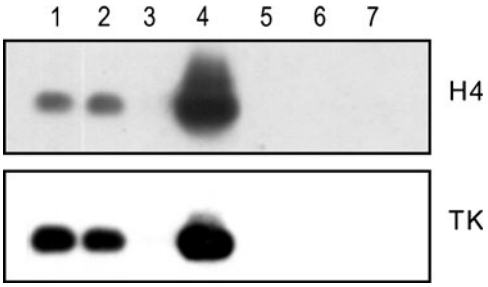
SUPPLEMENTARY FIG. S1. Progressive increase of nodule size determined by ultrasonography. Ultrasonography was performed using a 7.5–10 MHz linear transducer (Esaote, Genoa, Italy). The nodule volume was calculated according to the formula of the ellipsoid model: width× length×thickness×0.52.



SUPPLEMENTARY FIG. S2. Histological appearance of the thyroid nodule stained with hematoxylin and eosin.

Supplementary Table S1. Primers and Probes Used for RET/PTC Screening

Gene	*Primer sequences (5′–3′)*	*Gene*	*Oligoprobes (5′–3′)*
RET/PTC-1	For: GCTGGAGACCTACAAACTGA	H4	GGCACTGCAGGAGGAGAACCGCGA- B
	Rev: GTTGCCTTGACCACTTTTC	TK	GGAATTCCCTCGGAAGAACT-B
RET/PTC-3	For: AAGCAAACCTGCCAGTGG	Ele1	GGTCGGTGCTGGGTATGTAAGGA-B
	Rev: CTTTCAGCATCTTCACGG	TK	GGAATTCCCTCGGAAGAACT-B



SUPPLEMENTARY FIG. S3. RET/PTC1 rearrangement detected by Southern blot on RT-polymerase chain reaction products of cytology specimens. RNA was extracted from TPC-1 cells harboring RET/PTC1, WRO harboring wild-type RET, or cytology specimens. Lanes: 1, patient nodule; 2 and 3, PTC nodules; 4, TPC-1 cells; 5, WRO cells; 6 and 7, nodules with benign histology diagnosis. Fifteen follicular thyroid carcinomas were negative (not shown). The blots were hybridized with probes for the TK domain of RET or H4 exon 1.