Increased expression of endothelin-1 converting enzyme in human thyroid carcinoma

Endothelin-1 (ET-1), a 21-amino-acid peptide possessing vasoconstrictive and mitogenic properties, is principally released by the vascular endothelium (Yanagisawa et al., 1988; Donckier et al., 1991) but can also be produced by thyroid follicular cells (Lenziardi et al., 1995). In the September 2003 issue of Clinical Endocrinology, we demonstrated that both ET-1 mRNA and ET-1 peptide as well as the effector receptor ETAR mRNA levels were increased in thyroid papillary carcinoma and Hashimoto's thyroiditis (Donckier et al., 2003). ET-1 peptide is synthesized through successive proteolytic cleavage of a 203-amino-acid precursor to obtain Big-endothelin-1 (1-38Big-ET-1). During secretion, 1-38Big-ET-1 is cleaved by a specific membrane-bound metalloproteinase, the ET-1 converting enzyme (ECE-1) that leads to the equimolar secretion of the ET-1 active form (1-21ET-1) and the C-terminal fragment (22-38CTF or 22-38Big-ET-1) (Kido et al., 1997). The physiological importance of this cleavage is indicated by the reported 140-fold increase in vasoconstrictor potency upon cleavage to 1-21ET-1 (Kimura et al., 1989). Because the amounts of cross-reactivity from our anti-ET-1 antibody were 100%, 87% and 0%, respectively, for 1-21ET-1, 1-38Big-ET-1 and 22-38CTF, our study suggested that the increased ET-1 immunostaining, as evaluated by counting the number of positive cells among 1000 follicular cells, may result from mixed labelling of 1-21ET-1 and 1-38Big-ET-1. In this respect, we considered it of importance to demonstrate that increased expression of the ET-1 peptide could be related to an increased expression of the ECE-1.

The study was performed in human thyroid samples:
- normal thyroid (n = 7)
- papillary thyroid carcinoma (n = 12)
- Hashimoto's thyroiditis (n = 9)
- benign nontoxic nodular goitre (n = 11)

A newly developed assay using the real-time quantitative polymerase chain reaction (RTQ-PCR) technique was used, as described previously (Donckier et al., 2003), with:
- forward primer ATCATCAAGCACCTCCTCGAA
- reverse primer TCGATCCTGGTCTCGTTCATG
- probe F-AGCGTGAGCGAGGCAGAGAGAAAGG-T
- slope -3.16
- correlation -0.99
- NM_001397 474-575

The results are expressed using the ΔΔCt method.

As shown in Fig. 1, ECE-1 mRNA expression levels were found to be increased (P < 0.001) in thyroid papillary carcinoma and in Hashimoto's thyroiditis compared with normal thyroid. No change was evident in nodular goitre.

Thus, in addition to the increased expression of the ET-1 and its mitogenic receptor ETAR, our current study also demonstrates overexpression of ECE-1. Consequently, we can assume that the increased peptide detected by immunochemistry (and quantified by morphometry) in human thyroid carcinoma tissue most probably encompasses the ET-1 active form (1-21ET-1). This reinforces the concept of a possible outburst of the endothelin system in the pathogenesis of thyroid carcinoma.

Fig. 1 mRNA relative expression of ECE-1 determined by RTQ-PCR in normal thyroid, thyroid papillary carcinoma, Hashimoto's thyroiditis and nodular goitre. Note the overexpression of ECE-1 in the thyroid papillary carcinoma and Hashimoto's thyroiditis. The results are expressed as mean ± SD. ***p < 0.001 vs. normal (Kruskal-Wallis and Mann-Whitney tests).

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References


