Thyroid hormone receptor β mutations in the ‘hot-spot region’ are rare events in thyroid carcinomas

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Abstract

Thyroid cancer constitutes the most frequent endocrine neoplasia. Targeted expression of rearranged during transfection (RET)/papillary thyroid carcinoma (PTC) and V600E V-raf murine sarcoma viral oncogene homolog B1 (BRAF) to the thyroid glands of transgenic mice results in tumours similar to those of human PTC, providing evidence for the involvement of these oncogenes in PTC. Kato et al. developed a mouse model that mimics the full spectrum of the human follicular form of thyroid cancer (FTC). FTC rapidly develops in these mice through introduction of the thyroid hormone receptor β (THRB)⁷PV mutant on the background of the inactivated THRβ wt locus. Our aim was to verify if, in the context of human follicular thyroid carcinogenesis, THRβ acted as a tumour suppressor gene. We screened for mutations of the THRβ gene in the hot-spot region, spanning exons 7–10, in 51 thyroid tumours and six thyroid cancer cell lines by PCR and direct sequencing. We did not find mutations in any of the tumours or cell lines analysed. Our findings suggest that, in contrast to the findings on the THRβ-mutant transgenic mice, THRβ gene mutations are not a relevant mechanism for human thyroid carcinogenesis.


Introduction

Well-differentiated thyroid carcinoma (WDTC) includes papillary and follicular types. Most WDTC behave in an indolent manner and carry an excellent prognosis (Kondo et al. 2006).

Several genes have been found to be involved in the development of papillary thyroid carcinoma (PTC). Rearrangements of the RET tyrosine kinase receptor gene (RET/PTC) are found in 13–43% and the V600E BRAF in 29–69% of sporadic PTC (Kondo et al. 2006). Targeted expression of RET/PTC and V600E BRAF to the thyroid glands of transgenic mice results in tumours, similar to human PTC, providing evidence for the involvement of these oncogenes in the initiation of PTC (Jhiang et al. 1996, Knauf et al. 2005). These two oncogenes are thought to exert their effect through activation of the mitogen activated protein kinase (MAPK) pathway (Kondo et al. 2006).

Accumulated evidence indicates that follicular thyroid carcinoma (FTC) arise through an oncogenic pathway distinct from that of PTC. At the molecular genetic level, both histological types share the presence of rat sarcoma viral oncogene (RAS) mutations, although at different frequencies: 0–21% for PTC and 40–53% for FTC (Kondo et al. 2006).

Paired box 8 (PAX-8)/peroxisome proliferators-activated receptor γ (PPAR-γ) rearrangement, present in 25–63% of FTC (Kondo et al. 2006) is only found at similar frequencies in the so-called follicular variant of PTC, being absent from the conventional PTC (Castro et al. 2006).

At variance with PTC, a mouse model for FTC was lacking until Kaneshige et al. (2000) described a mutant mouse with a mutation (PV) targeted to the thyroid hormone receptor β (THRβ) locus. In a heterozygous condition, these mice develop a phenotype similar to thyroid hormone resistance syndrome, whereas double-mutant mice spontaneously develop FTC which progress towards undifferentiated carcinoma. The authors proposed that the THRβ gene could function as a tumour suppressor gene in FTC (Kato et al. 2004).

In humans, germline inactivating mutations in THRβ have been associated with thyroid hormone resistance syndrome which is not associated with any type of thyroid carcinomas; however, in this condition, most of the patients are heterozygous for the mutated allele (Cheng 2005). Somatic THR mutations have been described in human malignancies, such as liver (Lin et al. 1997, 1999) and renal carcinomas (Puzianowska-Kuznicka et al. 2000). Low levels of expression have also been detected in a wide range of neoplasias.
(Gonzalez-Sancho et al. 2003). The absence of function of both TRHB alleles seems to play a role in carcinoma progression. This hypothesis is supported by the finding that the transgenic mice of TRHB-mutated gene only develop tumours when both alleles are inactivated (Kato et al. 2004).

Two reports describe a totally different situation regarding TRHB mutations in sporadic thyroid tumours: Puzianowska-Kuznicka et al. (2002) claimed a high frequency of mutations in PTC, and Takano et al. (2003) claimed the absence of mutations in the same group of tumours but, as far as we know, nobody has searched for TRHB mutations in FTC. In the present study, we attempted to clarify this controversy and to establish whether or not TRHB mutations contribute to thyroid carcinoma development/progression, with a particular interest on the FTC pathway. We studied by PCR and direct sequencing the TRHB exons 7–10, which are the hot-spot regions for the thyroid hormone resistance syndrome (Cheng 2005).

Materials and methods

Fifty-one paraffin-embedded thyroid tumours were retrieved from the files of Hospital São João/Medical Faculty of Porto and the University Hospital of the State University of São Paulo, Brazil. The analysed lesions were classified according to the criteria advanced by Rosai et al. (2003) into the following categories: FTC (n=28), classical PTC (n=16) and benign lesions (n=7), including three nodular goitres and four follicular adenomas. The histological evaluation was performed independently by two pathologists (I V C, J M).

Six thyroid cell lines (kindly provided by Professors Jacques Dumont and Marc Mareel) were also included in the present study. Two were PTC-derived cell lines (B-CPAP and TPC-1); four were derived from undifferentiated carcinomas (8505C, C643, NPA and Hth74).

Genomic DNA was obtained from microdissected areas of the tumour samples and from cell lines using the Puregene DNA isolation kit (GENTRA Systems, Minneapolis, MN, USA) following the manufacturer protocol.

One hundred samples of DNA from blood donors were used as control population for the variants/mutations.

Primers were designed to the intronic regions of exons 7–10, in order to analyse coding regions and exon/intron boundaries, the sequences are as follows: Exon 7F: gcactctgtgctcttgctc; Exon 7R: tgaggtagaaaacactggcata; Exon 8F: caaccttctattaatctttcttt; Exon 8R: acctctggaaactgatgaaactat; Exon 9F: ttgggttccctggtgcct; Exon 9R: agccgctagacaagcaaaagc; Exon 10F: taagctgctgaatggaca and Exon 10R: ggccatggcgaaatgaca.

PCR was performed for 35 cycles, with the appropriate annealing temperatures. The bands were excised from agarose gels and purified with GFX Amersham Kit following the manufacturer’s protocol.

The DNA obtained was subjected to automated sequencing using the ABI Prism Big Dye Terminator Ready Reaction Kit (Perkin-Elmer, Foster City, CA, USA) and an ABI Prism 3100 Genetic Analyser. Sequencing was performed on both strands using the primers described earlier. Whenever a sequence variant was found, both PCR and sequencing were repeated, in order to rule out possible polymerase introduced mistakes. Only reproducible results were considered valid.

Results

Mutation screening

Mutation screening of RAS, V600E BRAF, as well as PAX-8/PPAR-γ rearrangement detection was previously reported (Soares et al. 2004, Trovisco et al. 2005, Castro et al. 2006). Sequencing of TRHB exons 7–10 and adjacent intron boundaries did not disclose mutations in any of the samples studied, including the six thyroid cell lines.

DNA sequencing revealed the presence of three sequence variants, one of which had not been previously described in the literature.

The novel synonymous variant, an ACC→ACT transition in codon 232, was found in a nodular goitre. The two previously reported variants were: (a) a g/a transition within a non-coding region that was found in heterozygosity at position 367933 (single nucleotide polymorphism (SNP) ID: rs13063628) in four of the 28 FTC (14.3%) and in three of the 16 PTC (18.75%); two homozygotes for the A allele were found in two anaplastic cell lines (C643 and Hth74) (Fig. 1); (b) the transition at position 245 (SNP ID: rs3752874) was found in 43% of the tumour samples; in the latter, the frequency of the variant was found in a nodular goitre.

![Figure 1](https://example.com/figure1.png) A Electropherogram demonstrating the presence of 367933 g/a SNP in THRB gene in homozygosity and heterozygosity. The dotted line indicates the coding sequence of exon 9 and the arrow indicates the nucleotide that is variable.
rare T allele appeared to be overrepresented in the tumour samples when our data were compared with the data on record (NCBI: SNP database); however, analysis of the blood donor population revealed that the frequency was similar to that of the normal Portuguese population (39%).

The frequency of the variants did not differ according to the histology of the cases (Table 1).

Discussion

THRs belong to the superfamily of ligand-dependent transcription factors that regulate growth, differentiation and maintenance of metabolic homeostasis. THRs are encoded by β and α genes, located on chromosomes 3 and 17 respectively (Cheng 2005). Kaneshige et al. (2000) created a mutant mouse by targeting a mutation to the THR locus (THRBPV/+ mice). This mutation, found in heterozygosity in a patient with thyroid hormone resistance syndrome (PV), leads to a protein without triiodothyronine (T3)-binding activity that exhibits potent dominant negative activity (Kaneshige et al. 2000). Heterozygous PV mice develop thyroid hormone resistance syndrome while homozygous PV mice develop, as they age, hyperplasia, FTC with capsular and vascular invasion, anaplasia and, eventually, lung metastases (Kato et al. 2004).

In an attempt to verify whether THRβ is involved in human thyroid carcinoma we analysed, in a series of 44 PTC and FTC, exons 7–10 of THRβ gene. We did not detect any mutation in the regions analysed (only silent sequence variants); thus our results do not support a role for THRβ gene alterations in sporadic thyroid tumours in contrast with classical tumour suppressor gene in human FTC and show that THRβ is not a classical tumour suppressor gene in human FTC and show that THRβ gene mutations are not a relevant mechanism for human thyroid carcinogenesis.

<table>
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<tr>
<th>Table 1 Distribution of THRβ DNA variants in the different histotypes of thyroid lesions</th>
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<tr>
<td>Histotype</td>
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<td>Benign lesions (n = 7)</td>
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<tr>
<td>FTC (n = 28)</td>
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<td>PTC (n = 16)</td>
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Percentages refer to individuals homozygotes/heterozygotes to the less frequent allele. FTC, follicular thyroid carcinoma; PTC, papillary thyroid carcinoma.

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