Is there a role for inherited TRβ mutation in human carcinogenesis?

Qual o papel da mutação do TRβ na carcinogênese da tireoide em humanos?

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SUMMARY

Resistance to thyroid hormone (RTH) is a rare autosomal dominant inherited disorder characterized by end-organ reduced sensitivity to thyroid hormone. This syndrome is caused by mutations of the thyroid hormone receptor (TR) β gene, and its clinical presentation is quite variable. Goiter is reported to be the most common finding. A close association of TRβ mutations with human cancers has become apparent, but the role of TRβ mutants in the carcinogenesis is still undefined. Moreover, higher TSH levels, described in RTH syndrome, are correlated with increased risk of thyroid malignancy, whereas TSH receptor stimulation is likely to be involved in tumor progression. We report here an illustrative case of a 29 year-old patient with RTH caused by a mutation in exon 9 (A317T) of TRβ gene, who presented multicentric papillary thyroid cancer. We review the literature on this uncommon feature, and discuss the potential role of this mutation on human tumorigenesis, as well as the challenges in patient follow-up. Arq Bras Endocrinol Metab. 2012;56(1):67-71

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SUMÁRIO

A síndrome da resistência aos hormônios tireoidianos (RHT) é caracterizada por redução da sensibilidade aos hormônios da tireoide. A apresentação clínica é variável, sendo a presença de bócio a manifestação mais frequentemente descrita. A associação de mutação no receptor β e neoplasias em humanos vem sendo demonstrada recentemente, porém o mecanismo pelo qual a mutação desse receptor está envolvida na carcinogênese não está completamente definido. Além disso, níveis elevados de TSH sérico, descritos na RHT, estão associados a aumento do risco de câncer de tireoide, e o estímulo do TSH está provavelmente envolvido na patogênese desses carcinomas. Este artigo relata o caso de um homem de 29 anos com RHT, com análise molecular demonstrando mutação no éxon 9, códon 317, e carcinoma papilar de tireoide. Revisamos a literatura dos casos relatados os quais descrevem associação entre RHT e câncer de tireoide e discutimos os desafios do tratamento e seguimento desses pacientes. Arq Bras Endocrinol Metab. 2012;56(1):67-71

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INTRODUCTION

Resistance to thyroid hormone (RTH) is a rare autosomal dominant inherited disorder characterized by end-organ reduced sensitivity to thyroid hormone. Although many steps are required for thyroid hormone to exert its action on target tissues, the first recognized and most common defect involves mutations in the beta-isoform of the thyroid hormone receptor (TRβ) gene. The exact incidence of RTH is unknown, because the condition may not be detected by routine screening for hypothyroidism. It is estimated to occur in approximately 1 per 40,000 newborns, and more than 1,000 affected subjects from 339 independent families have already been reported (1).

The clinical manifestations of RTH are extremely variable, the majority of patients being completely asymptomatic. The finding of elevated serum T4 levels, usually accompanied by high serum levels of T3, associated with non-suppressed serum TSH suggests the diagnosis. The majority of subjects are clinically euthyroid, but symptoms may vary not only among individuals, but also in different tissues of the same patient (1). As a consequence, clinical evidence of thyroid hormone (TH) deficiency and excess often coexist. Serum thyroglobulin concentration tends to be high, reflecting the level of TSH-induced thyroid gland hyperactivity (1). Mutational analysis of the thyroid hormone receptor beta gene (TRβ) allows the definitive diagnosis of RTH, enables family screening, and may help preventing potential misdiagnosis and inappropriate treatment.

Several experimental studies have demonstrated a role of TRβ mutation in carcinogenesis, and an association between TRβ mutations and several human cancers has become increasingly apparent (2-5). The mechanism by which TRβ mutants are involved in the carcinogenesis has not yet been defined, but it is likely to involve the TSH receptor. Indeed, a high frequency of TR mutations has been reported in human tumors (2). Higher serum TSH levels, typically described in RTH, are associated with increased risk of thyroid malignancy (6) whereas very low TSH levels or suppression are recommended as adjunctive treatment in differentiated thyroid cancer (7).

Here, we report a rare case of RTH and papillary thyroid carcinoma (PTC) in a 29 year-old man presenting a TRβ mutation. We review the literature on this uncommon feature, and discuss the potential role of this mutation in human tumorigenesis, as well as the challenges to patient follow-up, particularly the difficulty in achieving adequate serum TSH levels.

CASE REPORT

A 29 year-old man was referred to our Institution (Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil) for evaluation of a thyroid nodule and molecular diagnosis of RTH, diagnosed at 15 years of age, based on elevated serum thyroid hormone, normal TSH levels and positive family history. Laboratory evaluation at diagnosis was as follows: serum TSH 3.68 µUI/mL (reference range 0.27 – 4.2), and total T4 > 24 µg/dL (reference range 5.1 – 14.1 µg/dL). At our insti-

tution, thyroid function revealed TSH 7.45 µUI/mL, free T4 3.07 µg/dL (0.93 – 1.7 µg/dL), T3 226 ng/dL (80 – 200 ng/dL), thyroid peroxidase antibody 400 UI/mL. Past medical history included atrial fibrillation with a high ventricular frequency rate, optic nerve atrophy, lactose intolerance, gastroesophageal reflux, and neurosensory deafness. Psychomotor development was considered normal. The patient's father had a history of thyroid nodule, total thyroidectomy, and cardiac death around 45 years of age. His 32 year-old brother has also been diagnosed with RTH and underwent total thyroidectomy due to a thyroid nodule, later diagnosed as thyroid hyperplasia in the anatomopathological examination, was also obese, schizophrenic, and had dyslipidemia.

Physical examination revealed blood pressure of 120/78 mmHg and heart rate of 56 beats per minute. His height and weight were 1.69 m and 69 kg, respectively, and his body mass index was 24.15 kg/m². Cervical examination revealed a palpable and not enlarged thyroid, with a palpable hard nodule. A remarkable finding was gaze-evoked nystagmus with fixation. Cervical ultrasonography (US) showed two solid nodules (1.4 cm and 1.6 x 1.2 cm) in the right thyroid lobe, the larger one with microcalcifications and intense central blood flow. US also detected two additional smaller nodules in the right and left lobe, measuring 1.1 and 1.0 cm in diameter, respectively. Total thyroid volume was 21.5 cm³.

Total thyroidectomy was indicated after two unsatisfactory fine-needle aspiration biopsies. Intraoperative frozen-section analysis showed PTC and bilateral cervical lymph node dissection was performed. Histopathological examination revealed multicentric PTC without metastasis in 11 lymph nodes examined.

The molecular sequence analysis of the TRβ gene revealed the missense mutation GCT→ACT, in exon 9 (codon 317), causing the change of the amino acid alanine to threonine (A317T). Molecular analysis of the patient's brother revealed the same mutation.

Radioactive iodine (100 mci) was administered and ^{131}I whole-body scan uptake was detected only in the neck bed. After surgery, treatment with 200 µg/day of levothyroxine was started. Thirty days later, the patient became clinically hypothyroid, and laboratory analysis showed TSH 73.68 µUI/mL, total T4 19 µg/dL, free T4 3.5 ng/dL, and T3 133 ng/dL. Thyroid hormone replacement was increased to 400 mcg/day, and the patient was instructed to avoid lactose-rich food in

order to improve hormone absorption. Hypothyroid symptoms improved, but there was a new episode of atrial fibrillation. The patient's cardiologist decided to change the antiarrhythmic drug sotalol to propafenone. Five months after surgery and radioactive iodine treatment, thyroglobulin evaluation was negative, but anti-thyroglobulin was positive, 1:100. At this moment, TSH was 37.98 μUI/mL, T4 20.5 μg/dL, T4L 3.15 ng/dL, and T3 118.27 ng/dL. T3 replacement was initiated at 20 mcg every other day and three months later, TSH was 1.15 μUI/mL, T4 26.4 μg/dL, T4L 4.85 ng/dL, and T3 208.53 ng/dL. Reevaluation one year after surgery revealed similar laboratory findings, and cervical US did not show abnormalities.

METHODS

Laboratory investigation

Serum TSH, total T4, or free T4 and T3 were measured using ECLIA (Roche). Thyroid peroxidase antibody was determined by chemiluminescence (Siemens, Immulite 2000).

Molecular analysis

Genomic DNA was prepared from peripheral blood leukocytes by standard procedures. The $TR\beta$ gene was amplified using PCR (polymerase chain reaction) with specific primers for exon 9 (forward 5'-AGTGAATT-CACAGAAGGTTATTCCTATTGC-3' and reverse 5'-GATCTGCAGGCTCTTTGGATGCCCACTA-AC-3') and exon 10 (forward 5'-AGTCTGCAGAGG-CCTGGAATTGGACAAAGC-3' and reverse 5'-GGA-ATTATGAGAATGAATTCAGTCAGT-3'). Cycling conditions were as follows: initial denaturation (94°C, 5 min) and then 35 cycles (denaturation, 94°C for 30s; annealing, 54°C for 30s; synthesis, 72°C for 1 min), followed by a final extension of 5 min at 72°C.

Genotyping was performed using direct sequencing. For sequencing, PCR products were purified using the GFX PCR DNA purification kit (GE Healthcare, Buckinghamshire, UK), and submitted to direct sequencing using the Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA).

DISCUSSION

There is no specific clinical manifestation of the RTH syndrome, but goiter is the most common finding, re-

ported in 66%-95% of cases (1,8). Other characteristics are short stature, hyperactive behavior, attention disorder, anxiety, low intelligence quotient, and cardiac abnormalities (8-10). The reasons for the different phenotypes associated with the RTH syndrome are not entirely understood. Phenotypic expression may vary in patients with the same genotype (8,9), or even in members of the same family (8). Here, we present a case of a 29-year-old man harboring a mutation in exon 9, codon 317, presenting uncommon clinical features, such as optic nerve atrophy, neurosensory hearing loss, lactose intolerance gastroesophageal reflux, and atrial fibrillation. However, the most remarkable finding was the presence of multicentric PTC.

An association between mutations of TRβ and human cancers has become increasingly apparent since the identification of high frequencies of mutant TRs in several human cancers, including papillary thyroid carcinoma (3,4). However, the role of mutated TRβ in carcinogenesis is still unclear.

In an animal model of an inherited form of RTH in mice homozygousi for a negative mutant of TRβ (TRβ^PV, unique mutation at exon 10, codon 448), high levels of serum TSH (~400 fold) seemed to stimulate the proliferation of thyroid cells. These animals exhibited diffuse adenomatous hyperplasia as early as 3 weeks of age, and developed metastatic thyroid cancer, with both anaplastic and follicular patterns. On the other hand, heterozygous mice (TSH mildly increased, twofold) showed no detectable hyperplasia, suggesting that TSH-mediated hyperplasia could be an initial step in carcinogenesis (5). Gene profile analyses of TRβ^PV-mutated mice showed that the signaling pathways mediated by TSH, peptide growth factors (IGF-I, EGF, FGF), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α) and nuclear factor-kβ (NF-kβ) were activated, whereas pathways mediated by peroxisome proliferator-activated receptor γ (PPARγ) were repressed. This is particularly relevant in view of recent work suggesting that altered PPARγ expression and function could be an important risk factor in the development of human thyroid carcinomas. TRβ^PV deleterious effects causing thyroid cancer are, therefore, at least in part, mediated by interference with the transcriptional activity of wild-type TR, thereby leading to abnormal repression of tumor suppressors (PPARγ) (2).

Additional studies using mice with deletion of all functional TRs [TRα1 (-/-) and TRβ (-/-)] have demonstrated that these animals developed follicular

thyroid carcinoma with pathological progression from hyperplasia to capsular and vascular invasion, anaplasia and metastasis. Molecular analysis revealed that tumor promoters such as the pituitary tumor-transforming gene (PTTG) were activated, and tumor suppressors such as PPARγ and p53 were suppressed during the carcinogenesis process (11). Altogether, mouse models indicate that TR mutations could lead to other abnormalities beyond resistance to TH, including thyroid cancer, and may act as oncogenes. Tumorigenesis mediated by TRβ[PV] would involve complex alterations of multiple signaling pathways, aberrant transcriptional activity, and derailed post-transcriptional mechanisms (2). However, it is unlikely that mutated TRβ acts as an initiator of carcinogenesis. It probably cooperates with other pathways in the transformation of proliferative thyroid cells stimulated by TSH into cancer cells.

In addition to the data obtained from animal studies, clinical studies have demonstrated that the likelihood of thyroid cancer increases with higher serum TSH concentrations, even within normal ranges (6). Higher TSH levels have been associated with an advanced stage of thyroid cancer, whereas a relationship between decreased expression of TSH-R and poor prognostic scores has been demonstrated. Furthermore, overall survival is improved in thyroid cancer patients treated with suppressive doses of levothyroxine (12).

Based on the above considerations, high incidence of thyroid cancer could be expected in patients with RTH. Nevertheless, although goiter is a common finding in RTH, thyroid cancer is not, and few cases have been reported in the literature. In a large series of patients reported by Brucker-Davis and cols., we found the first report on the association between thyroid cancer and RTH syndrome (8). More recently, four other cases were described (13-16).

Table 1 summarizes the clinical and molecular characteristics of our case and previously reported ones. The series of cases reported by Brucker-Davis and cols. (8) did not describe individual phenotypes, and this limited our conclusions about their patient with RTH and thyroid cancer. All other cases reported the occurrence of differentiated thyroid cancer of the papillary type. A remarkable aspect is that both males and females were affected in a similar frequency. Except for our case, all patients tested for thyroid peroxidase antibody showed to be negative. All reports highlighted the difficulties of managing post-surgical hypothyroidism, and the need for higher than usual doses of levothyroxine (~3 μg/kg) to get near-normal TSH levels (13,14,16). A fascinating observation is that, despite the chronic TSH stimulation characteristic of RTH, disease presentation at diagnosis and follow-up of these patients suggests non-aggressive tumor behavior (13,14,16).

Another interesting aspect of the present case was the uncommon features of RTH syndrome presented by the patient, such as optic nerve atrophy, neurosensory hearing loss, and atrial fibrillation. The assumption that an aberrant thyroid hormone action is involved on the pathogenesis of these abnormalities is supported by interesting animal model studies. It has been shown that thyroid hormone action is required for normal cone opsin expression during mouse retinal development (17), whereas hearing impairment has been described in homozygous mutant mice (18). Moreover, although TRβ-mutant mice showed surprisingly little effect on cardiac function *in vivo* except for cardiac hypertrophy (19), decreased atrial depolarization velocity has been demonstrated in mutant mice (20).

Table 1. Clinical and molecular characteristics of patients with resistance to thyroid hormone and papillary thyroid carcinoma

Patient	Sex	Age (years)	TRβ mutation (exon/codon)	Tumor size (cm)*	Multicentric	LN Metastasis	Reference
1	NA	NA	NA	NA	NA	NA	Brucker-Davis and cols., 1995 (8)
2	F	46	10/R429Q	0.5	-	-	Taniyama and cols., 2001 (15)
3	F	26	NA	NA	NA	NA	Siristatidis and cols., 2004 (16)
4	F	38	9/M310T	0.4	+	-	Kim and cols., 2010 (13)
5	M	48	No mutation identified	NA[ε]	-	-	Paragliola and cols., 2011 (14); Patient 1
6	M	63	10/P453T	0.6	-	-	Paragliola and cols., 2011 (14); Patient 2
7	M	29	9/A317T	1.3	+	-	Weinert, 2011

NA: not available; F: female; M: male; LN: lymph nodes.
* If multicentric, the biggest tumor size is reported; [ε] TNM = pT2.

In conclusion, the reported case presents an interesting finding of simultaneous RTH and PTC, and highlights the possible common mechanism for RTH and carcinogenesis. Despite all accumulated data on mutated-TRβ or TSH mediated-effects on thyroid carcinogenesis, the reviewed cases illustrate the gaps in our understanding of the complex pathways of thyroid hormone action on human neoplasms.

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