Lack of mutation in exon 10 of *p53* gene in thyroid tumors

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Ausencia de mutaciones del exón 10 del gen *p53* en tumores tiroideos

Antecedentes: p53 es una proteína nuclear que tiene un rol importante en la regulación de la proliferación celular y comanda cascadas de señalización para la reparación de ADN y apoptosis. En muchos tipos de cáncer, hay una alta frecuencia de mutaciones de p53. Estas mutaciones también son muy prevalentes en el cáncer indiferenciado de tiroides, pero no se encuentran en tumores benignos y son infrecuentes en el cáncer bien diferenciado. La mayor parte de las mutaciones se localizan en los exones 5 a 8 del gen. Recientemente se ha descrito una mutación de la línea germinal del exón 10 en el codón 337 del p53, en niños brasileños con tumores suprarrenales. Objetivo: Buscar mutaciones del codón 337, del exón 10 de p53 en tumores tiroideos. Material y métodos: Se estudiaron 74 tumores tiroideos (5 carcinomas foliculares incluyendo 3 altamente invasivos, 22 carcinomas papilares incluyendo 6 variantes con células altas, 11 adenomas foliculares, 1 carcinoma medular y 35 bocios benignos). El ADN se extrajo de una sección central de los tumores y desde tejidos tiroideo normal contralateral o sangre en 38 pacientes. Los productos de PCR para el exón 10 de p53 fueron examinados por análisis de conformación de polimorfismos de hebra simple. Se secuenciaron 2 muestras en que se sospechó la presencia de bandas con migración aberrante y 3 productos de PCR adicionales provenientes de muestras de tumor con patrones normales de polimorfismo, pero no se detectaron mutaciones. Resultados: En todas las muestras estudiadas, no se detectaron mutaciones. Conclusiones: El exón de p53 no presenta mutaciones en los tumores tiroideos. Esto sugiere que esta mutación es específica para tumores suprarrenales. (Rev Méd Chile 2004; 132: 1513-6)

The tumor suppressor gene *p53* is a transcription factor that acts in cell cycle regulation, inducing cell cycle arrest or cell death in response to DNA-damaging agents, such as viral infection, radiation and chemotherapeutics[1]. The *p53* protein resides primarily in the nucleus, binds to specific DNA sequences, and functions at least in part as a transcriptional regulator[2]. Inactivated *p53* mutations have been described in some 50% of human cancers and are believed to be a major determinant of the phenotype of many forms of cancer[1-3].

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Several studies, both with immunocytochemical and genetic analyses, have shown that *p53* mutations are highly prevalent in poorly differentiated and undifferentiated thyroid carcinomas, as well as thyroid cancer cell lines[4-6]. However, they are not found in benign tumors and are infrequent in well-differentiated cancers, suggesting that mutational inactivation of *p53* occurs at a late stage of thyroid tumor progression[7]. These data suggest that mutational inactivation of the *p53* gene may be a key event in the progression from differentiated to anaplastic carcinoma[4,7]. There is also evidence that *p53* may interfere with thyroid cell differentiation. Introduction of a mutated *p53* markedly impairs the differen-

tiated gene expression of PCC13 thyroid cells[8]. By contrast, wild-type *p53* reintroduction into an undifferentiated thyroid carcinoma cell line leads to reexpression of thyroid peroxidase, a characteristic differentiated marker of the thyroid cell[9].

Typically, mutations in *p53* gene are located in exons 5-8, a highly conserved DNA binding domain of *p53*. Recently, a distinct nucleotide substitution in the exon 10 of *p53* was identified at a high frequency, 77 to 97% of children with benign and malignant adrenocortical sporadic tumors investigated by 2 distinct groups[10,11]. This germline mutation leading to an Arg337His mutation of exon 10 was also identified in asymptomatic relatives of the patients but in none of the unrelated controls, suggesting that the mutation is a risk factor associated with adrenocortical tumors rather than a benign polymorphism commonly found in southern Brazil[10,11].

Sporadic tumors often appear to have the same gene mutations as their familial counterparts. Many germline mutations have been demonstrated to be associated with sporadic tumors, including thyroid cancer[12-16]. We recently showed that a polymorphism at codon 72 of exon 4 of *p53* was associated with sporadic thyroid carcinomas[17].

Because of the high prevalence of the codon 337 of exon 10 of *p53* mutation in southern Brazilian population and the possibility that this polymorphism could be also associated to other cancers, we designed this study to screen a large amount of samples for this *p53* mutation in thyroid tumors.

MATERIAL AND METHODS

Subjects. The Ethics Committee of the University Hospital - School of Medicine of the State University of Campinas (HC-FCM/UNICAMP) approved the study and informed written consent was obtained from a total of 74 subjects (55 females, 19 males, 16 to 81 years old, 49±21 years old) that were consecutively referred to thyroid surgery because of thyroid nodules that presented clinical or epidemiological suspicion of cancer. The diagnosis of thyroid carcinoma was established by fine-needle aspiration cytological study and confirmed by the histological analysis of thyroid tissues. There were 28 thyroid malignant tumors: 5 follicular carcinomas (3 widely invasive and 2 minimally invasive); 22 papillary carcinomas (14 of the classic variant, 2 follicular variants, 6 tall cell variants) and 1 medu-

llary carcinoma. Other 46 cases (35 females, 11 males, 21 to 75 years old, 47±19 years old) of benign goitres included 19 follicular adenomas, 22 multinodular goitres and 5 Basedow-Graves disease. Thyroid tissue samples were obtained at the time of surgery at the University Hospital and immediately frozen in liquid N2. Besides collecting a central portion of all tumors, we obtained samples from the contra lateral normal thyroid lobe of 26 patients with thyroid cancer. In addition, peripheral blood samples were collected from 18 different patients with benign goitres. Tumor stage and degree of differentiation were obtained from surgical and pathological records. Experienced pathologists of the University Hospital of the Faculty of Medical Sciences of the State University of Campinas (UNICAMP) confirmed all diagnoses.

Methods. Genomic DNA was extracted from frozen tumors using a standard phenol-chloroform method. We used the same primers described by Latronico et al[10]. PCR was performed in 25 μl volumes of a mixture containing 100 ng DNA, 50 nM of each primer (5'-CTGAGGCACAAGAATCAC-3' and 5'-TCC-TATGGCTTTCCAACC-3'), 10 mM Tris- HCl (pH 8.0), 1.5 mM $MgCl_2$, 100 uM of each dinucleotide triphosphate and 0.5 U Taq DNA polymerase. Amplifications were carried out for 35 cycles of 94°C for 45 seconds, 62°C for 45 seconds and 72°C for 1 min, with an initial denaturation step of 94°C for 2 min and a final extension step of 72°C for 7 min using a Perkin-Elmer 9600 GeneAMp PCR system. The amplified 447 bp DNA fragments were examined on a 2% agarose gel, containing ethidium bromide. After confirming amplification, the samples were mixed with 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol and 50 mM NaOH, denatured at 94°C for 10 min, and loaded on to 6% polyacrylamide gels. The electrophoresis was conducted at 2-5 W at room temperature overnight. The gel was then stained with silver nitrate. DNA samples homo and heterozygous for the Arg337His mutation, obtained from adrenocortical tumors, were used as positive controls of the gels.

RESULTS

Figure 1 depicts an example of our results. All samples showed the same pattern of running, with no significant differences. Two samples suspected of presenting aberrant migrating bands were exci-

sed from the gel and purified using a commercial kit according to the manufacturer's instructions (Life Technologies, Paisley, UK). PCR products were sequenced with the ABI prism big dye sequencing kit (Perkin Elmer, Warrington, Cheshire, UK) using an ABI 377 Prism DNA Sequencer (Perkin Elmer). In all cases a wild-type sequence was found. In addition, we directly sequenced 3 additional PCR products from tumor samples with normal SSCP patterns, and all were wild type.

DISCUSSION

The *p53* gene is one of the best studied tumor suppressor genes, located on chromosome 17p13.1. Its mutation has been reported mainly in aggressive forms of tumors, especially anaplastic carcinomas[18]. It has been found in up to 40% of dedifferentiated and undifferentiated thyroid carcinomas and in less than 10% of the differentiated thyroid tumors[19]. However, mutant *p53* protein has also been detected in follicular and papillary carcinomas[20]. More recently, *p53* mutant protein was also demonstrated in 11 out of 66 nodular hyperplasia cases (16.7%) and in 7 out of 50 (14%) cases of follicular adenomas[21].

Although somatic mutations of *p53* are the most common genetic changes observed to date, the frequency of germline *p53* mutations is found to be very low in sporadic malignant tumors[4]. It has been postulated that *de novo* germline *p53* mutations may occur in a substantial population of patients in the pediatric age group, who die of their disease and do not propagate the mutation[22,23]. On the other hand,

recent reports suggest that germline *p53* splicing mutations have been described infrequently in the literature because the method of mutation detection, in many studies, does not include all splice junctions24. The low figures reported in the literature might also reflect the use of less-sensitive mutation detection methods and, certainly, the fact that most researches focused on exons 5-8, within the DNA-binding domain of *p53*, instead of screening all 11 exons of T*P53*[24]. Indeed, because 85% of *p53* mutations are expected to occur in exons 5 through 8, thyroid tumor screening efforts, in almost all reports, were restricted to these regions of the gene (http://www.iarc.fr/*p53*/; http://cancergenetics.org/*p53*.htm).

The spectrum and frequency of cancers associated with germline *p53* mutations are uncertain. Some cancers like breast carcinoma, soft tissue sarcomas, osteosarcoma, brain tumors, adrenocortical carcinoma, Wilms' tumor and phyllodes tumor are strongly associated with germline *p53* mutations while carcinoma of pancreas is moderately associated and leukaemia and neuroblastoma are weakly associated[25].

Screening exon 10 by PCR-SSCP and by direct sequencing, we did not find mutations in a large number of thyroid samples. These results support the concept that germline T*P53* mutations do not simply increase general cancer risk. Instead, they promote tissue-specific effects. Although our results are constrained by the fact that we did not screen poorly differentiated or undifferentiated tumors, they suggest that the Arg337His germline mutation described in Brazilian children is restricted to adrenocortical tumors.
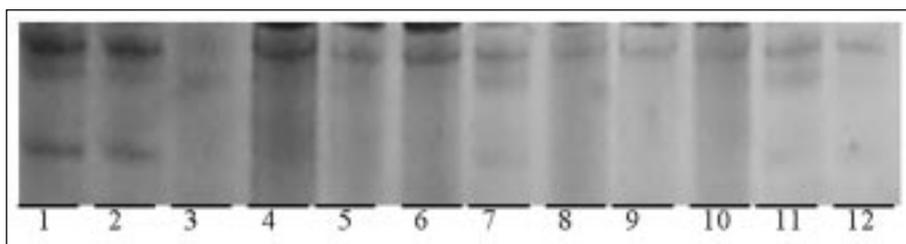

FIGURE 1. Gel of single-stranded conformation polymorphism analysis of PCR products (PCR-SSCP) representative of our results for exon 10 of p53 gene screening for mutations. Lanes 1 and 2 were loaded with the positive controls for the homo- and the heterozygous Arg337His mutation of exon 10 of the p53 gene, respectively. Lanes 3-7 and 8-12 were loaded with PCR products from follicular and papillary carcinomas, respectively.

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