Brazilian Journal of Medical and Biological Research (2003) 36: 459-462
ISSN 0100-879X Short Communication

No evidence for mutations in exons 1, 8 and 18 of the patched gene in sporadic skin lesions of Brazilian patients

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Abstract

There is strong evidence that the patched (PTCH) gene is a gene for susceptibility to the nevoid basal cell carcinoma syndrome. PTCH has also been shown to mutate in both familial and sporadic basal cell carcinomas. However, mutations of the gene seem to be rare in squamous cell carcinomas. In order to characterize the role of the gene in the broader spectrum of sporadic skin malignant and pre-malignant lesions, we performed a polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis of genomic DNA extracted from 105 adult patients (46 females and 59 males). There were 66 patients with basal cell carcinomas, 30 with squamous cell carcinomas, 2 with malignant melanomas and 7 patients with precancerous lesions. Two tissue samples were collected from each patient, one from the central portion of the tumor and another from normal skin. Using primers that encompass the entire exon 1, exon 8 and exon 18, where most of the mutations have been detected, we were unable to demonstrate any band shift. Three samples suspected to present aberrant migrating bands were excised from the gel and sequenced directly. In addition, we sequenced 12 other cases, including tumors and corresponding normal samples. A wild-type sequence was found in all 15 cases. Although our results do not exclude the presence of clonal alterations of the PTCH gene in skin cancers or mutations in other exons that were not screened, the present data do not support the presence of frequent mutations reported for non-melanoma skin cancer of other populations.

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Presented at the XVII Annual Meeting of the Federação de Sociedades de Biologia Experimental, Salvador, BA, Brazil, August 28-31, 2002.

Publication supported by FAPESP.

Received April 12, 2002
Accepted November 26, 2002

Key words
- Skin
- Cancer
- PTCH
- Mutation

Non-melanoma skin cancer is the most common malignancy in human beings, and its incidence continues to increase. Basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) of the skin are both derived from the same cell type, the keratinocyte, and are most often related to exposure to ultraviolet radiation (1). Mutations of the human orthologue (PTCH) of the *Drosophila* patched gene, which maps to chromosome 9q22.3, have been implicated as the molecular defect in basal cell nevus syndrome (Gorlin syndrome), a cancer-prone dominant disorder characterized by multiple developmental defects and predisposition to BCC of the skin. PTCH is a tumor suppressor gene that encodes a transmembrane protein acting as a receptor for the sonic hedgehog signal transduction pathway (2). Mutations in the PTCH gene have been characterized in

a number of Gorlin syndrome families and have also been found in both familial and sporadic BCC (3,4). However, mutations in SCC seem to be restricted to individuals with a history of multiple BCC and to be related to ultraviolet light exposure (5). The patched gene is also involved in medulloblastomas and other tumors, suggesting that PTCH may be part of an important cancer pathway (6). The inactivation of the patched gene product has been proposed to be virtually necessary to step over the genetic threshold of the neoplastic process in the skin (7). In order to characterize the role of the gene in a broader spectrum of sporadic skin malignant and pre-malignant cancers we screened for PTCH mutations in the "hot spot" exons 1, 8 and 18, where most of the mutations have been detected (5,8,9).

The study was approved by the Ethics Committee of the University Hospital, School of Medicine, State University of Campinas, and informed written consent was obtained from a total of 105 patients (46 females and 59 males). DNA was extracted from a central homogeneous portion of tumor tissue that was carefully microdissected in order to minimize the possibility of contamination with normal tissue. In addition, a control autologous normal sample was obtained from each patient. There were 66 BCC, 30 SCC, 2 malignant melanomas and 7 precancerous lesions: 1 case of Bowen disease, 2 keratoacanthomas, 3 melanocytic nevi, and 1 neurofibroma. Stage and grade of differentiation of the tumors were obtained from surgical and pathological records. Diagnoses were confirmed by experienced pathologists of the University Hospital.

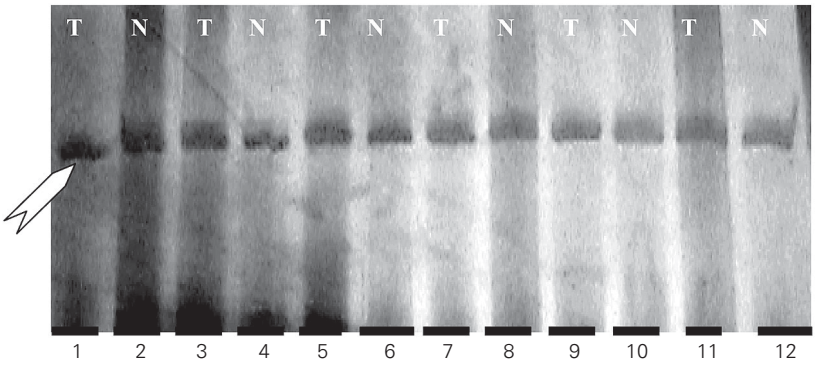

Figure 1. Polyacrylamide gel electrophoresis for single-strand conformation polymorphysm (SSCP) analysis representative of our results for exon 1 in the PTCH gene screening for mutations. The gel was loaded with 6 samples from tumors (T) and their corresponding normal (N) tissue. Lanes 1 to 6 display 3 paired samples of basal cell carcinoma and lanes 4 to 12 were loaded with 3 squamous cell carcinoma samples. The first lane (arrow), which was suspected to contain an aberrantly migrating band, was excised from the gel and sequenced directly. A wild-type sequence was obtained.

The sequence of oligonucleotide primers used for polymerase chain reaction-single-strand conformational polymorphism (PCR-SSCP) analysis were forward 5'-AAAGGCC TGGAGGCTATGAT-3' and reverse 5'-GG ACCTCACCACCTC GAGTA-3' for exon 1; for exon 8 and for exon 18, we used primers described in Refs. 4 and 7. PCR was performed in 25 µl containing 100 ng DNA, 50 nM of each primer, 10 mM Tris-HCl, pH 8.0, 100 µM of each dinucleotide triphosphate and 0.5 U Taq DNA polymerase. We used 1.5 mM $MgCl_2$ for exons 8 and 18 amplification while for exon 1, 1.0 mM $MgCl_2$ was employed. The annealing temperature was 61ºC for exon 1, 60ºC for exon 8, and 58ºC for exon 18. Amplifications were carried out for 35 cycles at 94ºC for 45 s, at the specific annealing temperatures for 50 s, and at 72ºC for 1 min, with an initial denaturation step at 94ºC for 2 min and a final extension step at 72ºC for 7 min using an MJ PTC-200 PCR system (MJ Research, Inc., Waltham, MA, USA). Negative control samples were included in all PCR and gel runs to detect possible contamination problems and gel loading inconsistencies. The PCR products were mixed with 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol and 50 mM NaOH, denatured at 94ºC for 10 min, and loaded onto 0.4 mm x 30 cm x 45 cm polyacrylamide gels. Electrophoresis was conducted at 2-5 W at room temperature overnight. The gels were then stained with silver nitrate. Screening of PTCH exons 1, 8 and 18 did not reveal any band shift, as depicted in Figure 1.

Three samples suspected of presenting

aberrant migrating bands were excised from the gel using the QIAquick gel extraction kit as described by the manufacturer (Qiagen, Hilden, Germany). PCR products were sequenced using the ABI prism big dye sequencing kit (Perkin Elmer, Warrington, Cheshire, UK) using the ABI 377 Prism DNA Sequencer (Perkin Elmer). In all 3 cases a wild-type sequence was found. In addition, we sequenced exons 1, 8 and 18 of PCR products from 12 other cases directly, including tumors (8 BCC and 4 SCC) and normal corresponding tissues, all of them presenting normal SSCP mobility patterns. A wild-type sequence was obtained in all cases.

Although the results of the present study do not exclude the possibility of mutations in a small proportion of skin carcinomas or mutations in other exons, these data do not support the view that deregulation of the patched-hedgehog signaling pathway plays a fundamental role in the pathogenesis of non-melanoma skin cancers. None of our 66 BCC samples presented mutations, in contrast to previous literature reports (2-4,7). The reason for this discrepancy is not known. The microdissected cell populations within tumors may be critical. SSCP is an established and cost-effective scanning method for detecting unknown mutations and is suitable for identifying small insertions/deletions and point mutations. This scanning method shows a high sensitivity for such a mutation pattern. However, PCR artifacts should also be considered, as the small amount of DNA template in the PCR amplification and the variability of Taq DNA polymerase terminal transferase activity can produce false-positive allelic imbalance (11). Indeed, most studies of PTCH gene were based on loss of heterozygosity analysis (10,12). Also, our negative results were strengthened by the use of normal control skin samples from all patients.

Our results provide no evidence for an important role of the PTCH-hedgehog signaling pathway in skin cancer development. It is unlikely that our result was due to a specific characteristic of the Brazilian population, which is genetically heterogeneous.

Acknowledgments

We thank Dr. Hamilton Ometto Stolf (Department of Medicine-Dermatology, UNESP) and Dr. Maria Aparecida Moraes (UNICAMP) for providing part of the samples used in this research. We thank Ms. Elaine C. Morari for continuous support during the study.

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