Advances in Brief

Mutations in the Human Homologue of Drosophila patched (PTCH) in Basal Cell Carcinomas and the Gorlin Syndrome: Different in Vivo Mechanisms of PTCH Inactivation

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Abstract

The nevoid basal cell carcinoma (Gorlin) syndrome (NBCCS) is an autosomal dominant disorder characterized by multiple developmental defects and cancer susceptibility, in particular to basal cell carcinoma. The human homologue of Drosophila patched (PTCH) was recently identified, mapped to the NBCCS locus on chromosome 9q22.3, and found mutated in patients with NBCCS and also in sporadic basal cell carcinomas. Here we show germ-line PTCH mutations in three families with NBCCS. We demonstrate that a germ-line PTCH frameshift deletion in one patient with NBCCS was accompanied by loss of the normal copy of PTCH in a tumor developed in the same patient. Another basal cell carcinoma from this patient did not show the loss of the normal copy of PTCH, instead a missense mutation in a highly conserved residue was identified in the nondeleted allele, illustrating two different mechanisms of PTCH inactivation in different tumors derived from the same NBCCS patient. We also show somatic PTCH mutations in 4 basal cell carcinomas identified by analyzing 18 non-NBCCS patients with sporadic tumors. These data provide further support for PTCH as an important tumor suppressor gene in the development of the most common human cancer.

Introduction

BCCs of the skin is the most common cancer in humans (1). The estimated annual incidence in defined populations more than doubled over the last 15 years (2, 3), and the treatment of BCCs consumes considerable resources. Screening of BCC samples for LOH 22 autosomes (4) revealed LOH at the chromosomal region 9q as the most common event. This suggested the presence of an important tumor suppressor gene in this region, and furthermore, that this putative gene may also be related to the developmental defects seen in the BCC-predisposing Gorlin syndrome, a genetic disease mapping to the same chromosome.

The Gorlin syndrome (basal cell nevus syndrome or NBCCS, MIM 109400; reviewed in Ref. 5) is an autosomal dominant disorder characterized by multiple developmental abnormalities and susceptibility to a variety of tumors, in particular to BCCs. BCCs seen in NBCCS tend to develop earlier in life and are often seen in large numbers at multiple sites. The NBCCS locus was mapped to the long arm of chromosome 9 by genetic linkage analysis (4, 6, 7). Fine linkage mapping placed the NBCCS locus in an about 2.5-cM interval (8–10) at 9q22.3, facilitating positional cloning approaches of candidate disease genes.

One candidate gene for NBCCS was recently identified (11–13) as a human homologue (PTCH, formerly PTC) of Drosophila patched, an important developmental regulator, initially found as a segment polarity gene (14). By genetic analysis, patched was implicated in the transcriptional repression of genes encoding members of the transforming growth factor β and Wnt protein families and in the signal transduction pathway of Drosophila hedgehog. The human PTCH gene, which is expressed in target tissues of sonic hedgehog (11), encodes a putative transmembrane protein exhibiting 40% identity to Drosophila patched (12, 13). The genomic sequence of human PTCH is about 34 kb and encompasses at least 23 exons (12, 13). Germ-line mutations were recently identified in eight patients with NBCCS and also in three sporadic BCCs, suggesting that PTCH may indeed play an important role as a tumor suppressor (12, 13). Here we have analyzed Swedish NBCCS families and a series of sporadic BCCs for PTCH mutations using SSCP analysis of genomic DNA.

Materials and Methods

Tumor Samples. A total of 22 BCC samples from 19 patients (10 males and 9 females) were studied. The mean age of patients at diagnosis was 61 ± 20 (± SD), ranging between 30 and 80 years (median, 68). Eighteen patients had tumors located at sun-exposed areas. Sixteen patients had facial tumors, 2 patients had chest tumors, and 1 patient had a scrotal BCC. There were four solid BCCs and four nodular tumors; the remaining tumors were of mixed histopathology. Six tumors were classified as infiltrative. Seventeen patients had sporadic BCCs; 1 patient, not classified as NBCCS, showed multiple BCCs, and 1 patient had typical NBCCS (NBCCS1 in Table 1).

NBCCS Patients. Patients with NBCCS were ascertained through the Department of Dermatology, Karolinska Hospital, Stockholm. The diagnostic criteria for patients with NBCCS were as described previously (15).

DNA Extraction. DNA was extracted from blood samples using the Wizard genomic DNA purification kit (Promega Corp.). Extraction of DNA from paraffin-embedded tumor samples was carried out using established protocols (16). Paraffin-embedded tissue was examined microscopically to ensure the presence of at least 50% of tumor cells as a source material for DNA extraction, as described previously (17). The DNA samples BCC10 and BCC11 were isolated from the forehead BCCs developed in patient III/2 (pedigree 2 of Fig. 2 in Ref. 8).

PCR-SSCP Analysis and Sequencing. The oligonucleotide primers were used as described (12) except for: exon 4, forward 5'-GAG AAA TTT TGG TCT GTG CTT TTC C-3' and reverse 5'-CAT GTA CCA TGT ACG CAA CTT TGT C-3'; exon 9, forward 5'-GTC GCT CGG AGT CTT GCT TCT C-3' and reverse 5'-AGA AGA AGG AGG AGA CTT C-3'; exon 10, forward 5'-TTC GGC TTT TGT TCT GTG C-3' and reverse 5'-CCG GTG GCA TTT GTC AAC-3';
and exon 21, forward 5'-CAG GTA AAT GGA CAA GAA C-3' and reverse
DNA, 0.25 @LM of each primer, 100 @i of each deoxynucleotidetriphosphate,
described previously (18, 19). Briefly, the PCR reaction contained 100 ng of
5'-CTG TGT GTG GTG CTGCTC-3'. The PCR amplifications were as
95% formamide, 0.05% bromphenol blue, 0.05% xylene cyanol, and 50 mM
Elmerthermocycler (GeneAmp System 9600). The samples were mixed with
annealing at 52—58°C for 45 s, and extension at 72°C for 30 s in a Perkin-

sis was conducted at 2—5 W at room temperature overnight. The gels were
radic BCCs, one was found in a non-NBCCS patient with multiple
lyzed on SSCP gels, and those with altered mobility were sequenced. Altogether, eight different mutations were found: three were in spo-

Identification of PTCH Somatic Mutations in BCCs. The PTCH
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tide primer pairs flanking each exon. The PCR products were ana-
yzed on SSCP gels, and those with altered mobility were sequenced. Altogether, eight different mutations were found: three were in sporadic BCCs, one was found in a non-NBCCS patient with multiple BCCs, and three were germ-line mutations in families with NBCCS (Table 1; Figs. 1—3). In addition, one BCC from an NBCCS patient with a germ-line mutation was found to contain a mutation in the second PTCH allele.

Among sporadic BCCs, a 2-bp insertion was found in sample BCC25 (Fig. 1; Table 1). This frameshift mutation would be predicted to introduce a stop codon, resulting in truncation of the gene product. This mutation was absent in normal tissue from the same patient (Fig. 1B), and the PCR-SSCP analysis showed the absence of the wild-type PTCH allele in tumor DNA (data not shown). A nonsense mutation in another sporadic BCC, found at nucleotide position 1149 in sample BCC5 (Fig. 2; Table 1), creates a stop codon, leading to premature termination in the extracellular portion of the molecule. This GC to AT transition is characteristic for UV-induced mutations, found in the TP53 gene in BCCs (21). The normal copy of PTCH was also lost in this tumor sample. The third somatic nucleotide change was found at position —8 of the 5' splice junction of intron 10 in sample BCC8.

This T—C transition was not found by analyzing 50 chromosomes by SSCP analysis; however, we could not exclude a rare polymorphism in this consensus splice site. No mRNA sample was available from this tumor. The last mutation was a 1-bp insertion in BCC6.

Germ-line Mutations in NBCCS and Differential Inactivation of PTCH in BCCs Derived from a Single Patient with NBCCS. A 23-bp PTCH deletion, identified originally in two tumor DNAs (BCC10 and BCC11 in Table 1), which were extracted from two different forehead tumors of patient NBCCS1, was found also in the patient's normal tissue (Fig. 3; Table 1). This germ-line deletion would introduce a stop codon after a run of 40 amino acids downstream, resulting in premature termination of the translation product and removal of all putative transmembrane domains. The PCR-SSCP analysis showed that the normal copy of PTCH was lost in tumor DNA BCC10 (Fig. 3A). This result provides the direct evidence that the inactivated PTCH allele is retained, whereas the wild-type PTCH is absent in tumor DNA of an NBCCS patient. The second tumor from the same patient (BCC11 in Table 1) did not exhibit loss of the normal

Table 1 Summary of PTCH mutations in BCCs and NBCCs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Predicted protein change</th>
<th>Mutation type</th>
<th>LOH at 9q22.3 in tumor DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCC5 (sporadic)</td>
<td>8</td>
<td>1149G—A</td>
<td>W387X</td>
<td>Somatic</td>
<td>Yes</td>
</tr>
<tr>
<td>BCC6 (sporadic)</td>
<td>10</td>
<td>1382insC</td>
<td>Frameshift</td>
<td>Somatic</td>
<td>Yes</td>
</tr>
<tr>
<td>BCC8 (multiple)</td>
<td>11</td>
<td>1493-8T—C</td>
<td>Splice defect?</td>
<td>Somatic</td>
<td>Yes</td>
</tr>
<tr>
<td>BCC25 (sporadic)</td>
<td>7</td>
<td>1051ins2</td>
<td>Frameshift</td>
<td>Somatic</td>
<td>Yes</td>
</tr>
<tr>
<td>BCC11 from NBCCS1</td>
<td>8</td>
<td>1067T—G</td>
<td>L360R</td>
<td>Somatic</td>
<td>No</td>
</tr>
<tr>
<td>NBCCS1</td>
<td>2</td>
<td>253delG</td>
<td>Frameshift</td>
<td>Germ-line</td>
<td>NA</td>
</tr>
<tr>
<td>BCC10 from NBCCS1</td>
<td>2</td>
<td>loss of PTCH</td>
<td>Loss of Pch</td>
<td>Somatic</td>
<td>Yes</td>
</tr>
<tr>
<td>NBCCS2</td>
<td>18</td>
<td>3232delG</td>
<td>Frameshift</td>
<td>Germ-line</td>
<td>NA</td>
</tr>
<tr>
<td>NBCCS3</td>
<td>16</td>
<td>2788insG</td>
<td>Frameshift</td>
<td>Germ-line</td>
<td>NA</td>
</tr>
</tbody>
</table>

a The numbering of nucleotides as in Ref. 12.
b The numbering of amino acids as in Ref. 13.
c LOH was determined either directly at the site of mutation by PCR-SSCP (see "Materials and Methods") or by flanking microsatellite markers, as described previously (17).
d Deleted sequence (5'-AAACF000TAGAAACATFCAAAA-3') was flanked by a 5-bp direct repeat.
e Deleted sequence was 5'-CCCGTG-3'. NA, not applicable.
f Duplicated sequence was 5'-TCGGC-3'. The nomenclature of mutations is according to Beaudet and Tsui (22).

Results and Discussion

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coding region was amplified from DNA samples using oligonucleo-
tide primer pairs flanking each exon. The PCR products were ana-
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Fig. 1. Somatic PTCH mutation in tumor sample BCC25. A, a 2-bp insertion in tumor sample BCC25; B, normal tissue of the patient. Inserted nucleotides are boxed.
PTCH MUTATIONS IN BASAL CELL CARCINOMA AND GORLIN SYNDROME

Gene in different tumors derived from the same NBCCS patient carrying a germ-line PTCH mutation: a loss of normal copy of PTCH in BCC10 and a somatic missense mutation in BCC11. These findings strongly implicate PTCH as a tumor suppressor gene in BCCs.

Two more germ-line mutations were found by PCR-SSCP analysis of blood DNA in patients with NBCCS (NBCCS2 and NBCCS3) in whom tumor samples were not available for analysis (Table 1). One was a 5-bp duplication at nucleotide position 2788; the other was an in-frame deletion of six nucleotides at position 3232 (Table 1). The in-frame deletion involves highly conserved proline and valine residues, components of the putative transmembrane domain.

Phenotypic Diversity of NBCCS. The phenotype of the NBCCS index case with the 23-bp deletion was severe, with 100 BCCs and 10 keratocysts at the age of 33. The patient had palmar and plantar pits and calcification in falx cerebri, i.e., all four major features of NBCCS. In addition, hypertelorism, enlarged head circumference with prominent frontal bossing, scoliosis, pectus excavatus, and bifid ribs were present. His affected mother, however, has a mild phenotype with only three BCCs at the age of 58, with the first presentation of BCC at the age of 40. She has slightly enlarged head circumference and frontal bossing. Two affected daughters of the index case presented with frontal bossing and enlarged head circumference with strabismus early in infancy. The fact that the NBCCS segregates with the deletion in this three-generation family (data not shown) indicates that the intrafamilial phenotypic diversity of the disease must be explained by factors other than the truncating PTCH mutation, either environmental or genetic.

The remaining cases with NBCCS carrying PTCH mutations were also familial. Although the phenotype of the index case with the 5-bp insertion (NBCCS3) was a classical one with a number of BCCs and keratocysts in the affected father and her daughter, the disease was milder in the family with the in-frame 6-bp deletion (NBCCS2). In this family, the affected mother has been treated for 10 BCCs and 2

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Fig. 2. Somatic PTCH mutation in tumor sample BCC5. A, stop-codon containing allele in tumor sample BCC5; B, control genotype. Arrow, the nucleotide substitution.

Fig. 3. Germ-line 23-bp deletion in PTCH in patient NBCCS1. A, SSCP analysis of tumor BCC10 (Lane t) and normal tissue (Lane n) together with five controls on the left side; B, sequence analysis of the patient (upper panel) and a control (lower panel). Arrows, deletion breakpoints.
jaws, whereas her daughter, who is now 42, has been treated for 10 BCCs and 7 keratocysts. No affected family member in this family was found with such a severe phenotype similar to the index case in the family with 23-bp deletion. This finding suggests a less pronounced functional defect of Ptch caused by the small in-frame deletion and the emerging genotype-phenotype relations in NBCCS.

The observations of tumor-related phenotypic variability within and between families with NBCCS are consistent with a large number of susceptibility loci and environmental factors implicated in the development of common human cancers. It will be interesting to identify additional factors in this Mendelian phenotype that are responsible for these variations. Such families will provide a useful resource for future studies aiming at the genetic dissection of human complex traits such as susceptibility to BCC development.

Acknowledgments

We thank the patients with NBCCS and their family members for providing their blood samples for this study.

References

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