"PTCH2, a Novel Human Patched Gene, Undergoing Alternative Splicing and Up-regulated in Basal Cell Carcinomas\(^1\)

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Abstract

By a combination of cDNA library screening, rapid amplification of cDNA ends analysis, and BAC sequencing, a novel human patched-like gene (PTCH2) has been cloned and sequenced. The genomic organization is similar to PTCH1 with 22 exons and, by radiation hybrid mapping, PTCH2 has been localized to chromosome 1p33-34, a region often lost in a variety of tumors. Several alternatively spliced mRNA forms of PTCH2 were identified, including transcripts lacking segments thought to be involved in sonic hedgehog binding and mRNAs with differentially defined 3' terminal exons. In situ hybridization revealed high expression of PTCH2 transcripts in both familial and sporadic basal cell carcinomas in similarity to what has been observed for PTCH1, suggesting a negative regulation of PTCH2 by PTCH1. This finding tightly links PTCH2 with the sonic hedgehog/PTCH signaling pathway, implying that PTCH2 has related, but yet distinct, functions than PTCH1.

Introduction

PTCH\(^4\) was recently identified as the gene responsible for the Gorlin’s syndrome, an autosomal dominant disorder characterized by multiple BCCs, medulloblastomas, and ovarian fibromas, as well as numerous developmental anomalies (1, 2). PTCH codes for a membrane receptor of the autokatally cleaved (protein-spliced), amnion-terinal domain of SHH (3, 4). In the non-signaling state, PTCH is thought to inhibit the constitutive signaling of another membrane protein, SMO, however, binding of SHH to PTCH relieves this inhibition (5). This cascade of signaling events, best characterized in Drosophila, also involves a number of intracellular components including fused (a serine threonine kinase), suppressor of fused, costal cutter, also involves a number of intracellular components including fused (a serine threonine kinase), suppressor of fused, costal cutter, and the colon, which contained sequences similar to, but distinct from, the mouse Ptch2 sequence. Characterization of the PTCH2 gene was performed essentially as described before (10), using the Marathon kit (Promega). The primer sequences used for RACE are available on request. A BAC clone encompassing the PTCH2 gene was isolated using primers corresponding to the 3' untranslated region of the cDNA. The genomic organization was obtained by a combination of direct sequencing of the BAC and by sequencing of PCR products generated with primers originating from adjacent exons. The same 3' end primers used to isolate the BAC clone were also used to screen the GENEBRIDGE radiation hybrid panel.

Tumor Samples. A total of 11 formalin-fixed paraffin-embedded BCCs derived from seven patients were obtained from the Department of Dermatology, Karolinska Hospital (Stockholm, Sweden). Five tumors were from NBCCS patients, two tumors were from a young patient with multiple BCCs (not classified as NBCCS), and four tumors were sporadic BCCs. All histological subtypes of BCCs were represented. In addition, samples from two TEs were included. Both were sporadic tumors. The histological diagnoses were confirmed by an experienced dermatopathologist.

In Situ Hybridization. Preparation of PTCH2-mRNA probes and in situ hybridization were performed as described previously (9). Briefly, two human PTCH2 cDNA fragments corresponding to positions 218–437 and 838–920, cloned into pGEM5, were used. Tumor sections were treated with proteinase K and washed in 0.1 M triethanolamine buffer containing 0.25% acetic anhydride. Sections were hybridized with 2.5 × 10\(^6\) cpm of each of the two labeled antisense or sense probes at 55°C. Autoradiography was performed for 2 weeks.

Immunohistochemistry. A monoclonal mouse antibody directed against human Ki-67 was used (Immunotech). Sections from each tumor sample were incubated with the antibody for 1 h and processed using a standard avidin-biotin immunoperoxidase/diaminobenzidine detection system.

Results and Discussion

To identify additional components of the PTCH/SHH cascade of signaling events, the Incyte LifeSeqTM database was searched using PTCH sequences. In addition to clones representing the PTCH cDNA, two nearly identical cDNAs were identified from the parotid gland and the colon, which contained sequences similar to, but distinct from, the 3' end of PTCH. By 5' RACE analysis using fetal brain cDNAs, additional sequence information from these transcripts (termed PTCH2) and, corresponding to a full-length cDNA, was obtained (Fig. 1A). PTCH2 is 57% identical to PTCH1, with a significantly variable region present between the transmembrane domains 6 and 7, and 91% identical to the recently published mouse Pch2 sequence (11). In similarity with the mouse gene, PTCH2 lacks the COOH-terminal extension present in human, mouse, and chicken PTCH1 (12, 13). However, the human PTCH2 cDNA terminates 36 amino acids earlier than the mouse Pch2 sequence. Characterization of the PTCH2 gene revealed 22 coding exons and conservation of the intron-exon junctions relative to PTCH1, except for the last intron (Fig. 1B). Moreover, when 3' RACE was performed from fetal brain, an alternate...

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1 Supported by the Swedish Cancer Fund, the Welander-Finsen Foundation, the Swedish Radiation Protection Institute, the Swedish Children Cancer Fund, and the Swedish Children Cancer Fund, the Welander-Finsen Foundation, the Swedish Radiation Protection Institute, the Swedish Children Cancer Fund, and the Swedish Children Cancer Fund, the Welander-Finsen Foundation, the Swedish Radiation Protection Institute, the Swedish Children Cancer Fund, and the Swedish Children Cancer Fund, the Welander-Finsen Foundation, the Swedish Radiation Protection Institute, the Swedish Children Cancer Fund, and the Swedish Children Cancer Fund, the Welander-Finsen Foundation, the Swedish Radiation Protection Institute, the Swedish Children Cancer Fund, and the Swedish Children Cancer Fund, the Welander-Finsen Foundation, the Swedish Radiation Protection Institute, the Swedish Children Cancer Fund, and the Swedish Children Cancer Fund, the Welander-Finsen Foundation, the Swedish Radiation Protection 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A NOVEL PTCH GENE

Fig. 1. A, amino acid sequence comparison of the human PTCH2 (top lines) and PTCH1 sequences. Vertical lines, identical amino acids; dots, similar amino acids. The PTCH2 sequence presented is composed of the original cDNA clones and of the products of the 5′ RACE analysis.

B

Table 1

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*Fig. 1 A NOVEL PTCH GENE on July 21, 2017. © 1999 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from*
Fig. 1. C, D, Continued.
COOH-terminal region was identified. This had a high structural similarity with the mouse Ptch2 COOH-terminal sequence and originates from the genomic region that links the last two exons of PTCH2 (Fig. 1C). Therefore, in these alternatively spliced transcripts, the penultimate exon with a segment of the contiguous 3' intron serves as the terminal exon. Furthermore, the human and mouse transcripts differed in the position of the termination signals (the human sequence is 21 amino acids longer), suggesting a nonconserved, species-specific function of this alternate COOH-terminal domain. The finding of two possible COOH-terminal regions for PTCH2 is intriguing and implies a role of this phenomenon in modulating signaling. Additional alternatively spliced transcripts were also identified by the RACE analysis (Fig. 1D). Transcript A lacks the sequence that corresponds to exons 9 and 10 of PTCH1, with the open reading frame being retained at the exon 8 to exon 11 junction. Exons 9 and 10 code for the last part of the first extracellular loop and for transmembrane domains 2 and 3 in the putative structure of the PTCH1 protein. Furthermore, this transcript also lacks a 5' segment of the canonical exon 2, due to the use of an alternative 3' splice site present in this exon, with the open reading frame being maintained. The functional consequence of this alternative splicing is not yet known, but it is interesting to note that the extracellular loops in PTCH1 are presumed to be involved in binding of the ligand SHH (3, 4) and that insertion of

Fig. 2. A, a dark-field photomicrograph of a BCC tumor hybridized with 35S-labeled antisense probe showing abundant signal for PTCH1 mRNA (light grains) in all BCC tumor cells. B, PTCH2 mRNA overexpression in BCC is in contrast mainly expressed in the basaloid cells in the periphery of the tumor nests (arrow). C, another BCC showing a strong PTCH2 mRNA signal in the periphery of the tumor nest (Tu), whereas no signal is detected in epidermis (Ep). D, sections of the same tumor (C) hybridized with the PTCH2 sense probe showed no signal. E, immunoreactivity for Ki-67 (brown precipitate) is seen in the periphery in the cells that showed strong up-regulation of PTCH2 mRNA. F, tumor nests under high power magnification demonstrate abundant PTCH2 mRNA signal (black grains) in the dark basaloid tumor cells and lower signal in the center (arrow). Bars (A-E), 24 mm and 6 mm (F).
a neo-cassette in intron 9 of the mouse PTCH1 gene is associated with a severe phenotype (14). Furthermore, exons 9 and 10 encode part of a putative sterol sensing domain (15), also found in PTCH1, and which has recently been implicated in mediating the potent modulating effect of cholesterol on SHH/PTCH signaling (16). Thus, if PTCH2 also serves as a receptor for SHH and/or related factors, the receptor form lacking exons 9 and 10 may show altered signaling properties. Transcript B contains additional sequences between canonical exons 1 and 2 that originate from the 5′ end of intron 1. The open reading frame that includes the initiator methionine of exon 1 is not maintained in this transcript, suggesting that, if this transcript is functional, either the methionine in exon 2 or nonmethionine codons are used to produce a protein product, in similarity to what has been proposed for the alternative spliced products of human PTCH1 (1).

By radiation hybrid mapping, the PTCH2 gene was localized to the chromosome 1p33-1p34 region, flanked by microsatellite markers D1S197 and D1S443. Interestingly, several genetic diseases have been mapped in this 2cM chromosomal segment, including paroxysmal choreoathetosis (17) and a form of hereditary congenital ptosis (18). The presence of PTCH2 transcripts in several ocular tissues (19) provides additional evidence that this gene might be a good candidate for the above mentioned hereditary eye disorder. In addition, PTCH2 is also located within the putative candidate region for the cancer predisposition locus hMOMI (20), and this region is, furthermore, often lost in several tumor types including neuroblastomas and colorectal tumors (21–23). Whether or not PTCH2, in similarity to PTCH1, is a tumor suppressor gene is presently under investigation.

The mouse and zebrafish homologues of PTCH2 have been reported to be expressed in a partly overlapping pattern with PTCH1 during embryonic development and to be induced by SHH (11, 24), implicating a role in this signaling pathway. We were, with this background, interested to analyze the expression of PTCH2 in BCCs and TEs, which show consistent upregulation of PTCH1 in all tumor cells (9, 25). By in situ hybridization analysis, a positive signal for PTCH2 mRNA was seen in all the 11 BCCs and the 2 TEs examined. The signal was seen exclusively in the tumor cells in both tumor types and was consistently stronger in the palisading peripheral cells of the tumor nests (Fig. 2). These cells also showed a positive immunostaining for the cell proliferation marker, Ki-67. Moreover, no or only weak signal could be detected in normal epidermis or in hair follicles. It is interesting to note that the prominent PTCH2 expression was observed both in familial (NBCCS, n = 5) and sporadic (n = 6) tumors. One of the BCCs analyzed was from a NBCCS patient (Fig. 2, C-F), with a known 4-bp germline deletion in exon 3 in the PTCH1 gene, which results in a premature stop and a truncated protein. Furthermore, one of the TEs was earlier shown to contain a large deletion in exon 20 that removes the COOH-terminal part of the 12th transmembrane domain of the PTCH1 gene (25).

The finding that in BCCs having frequent mutations in the PTCH1 gene the expression of the PTCH2 mRNAs is up-regulated tightly links PTCH2 with the PTCH/SHH cascade of signaling events. It is, therefore, likely that PTCH2 represents a target gene of this pathway, which is under the negative regulation of PTCH1, precisely as PTCH1 itself. Moreover, this observation strongly suggests that PTCH2 has functions distinct from PTCH1 since up-regulation of PTCH2 expression seems unable to compensate for inactive PTCH1 protein. This conclusion is also supported by the early embryonic lethality seen in PTCH1 (−/−) mice (5, 14) and the lack of genetic heterogeneity in Gorlin’s syndrome. However, whether PTCH2 may block the constitutive signaling of SMO or could act as an additional SHH receptor, possibly dependent on alternative splicing, remains as the subject of further experimentation.

Acknowledgments

We thank Karima Raza and Lotta Malmqvist for assistance in the sequencing PTCH2 and Bengt Sandstedt, MD, Ph.D., for help with the Ki-67 immunostaining.

References


6 Unpublished observations.


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*Cancer Res* 1999;59:787-792.

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