Available online at www.sciencedirect.com

SCIENCE DIRECT

Genomics 85 (2005) 462–471

GENOMICS

www.elsevier.com/locate/ygeno

Identification and characterization of multiple isoforms of a murine and human tumor suppressor, *patched*, having distinct first exons☆

Kazuaki Nagao[a], Masashi Toyoda[a], Kaori Takeuchi-Inoue[a], Katsunori Fujii[b], Masao Yamada[a], Toshiyuki Miyashita[a,*]

[a]*Department of Genetics, National Research Institute for Child Health and Development, 2-10-1 Ohkura, Setagaya-ku, Tokyo 157-8535, Japan*
[b]*Department of Pediatrics, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan*

Received 3 September 2004; accepted 23 November 2004
Available online 11 January 2005

Abstract

Mutations in mouse and human *patched* (*PTCH*) genes are associated with birth defects and cancer. PTCH, a 12-pass transmembrane protein, is a receptor for Sonic hedgehog (Shh) signaling proteins. Shh proteins activate transcription of target genes, including *PTCH,* via GLI transcription factors. Here we identified seven and five isoforms of human and mouse *PTCH* mRNA, respectively, which are generated by the complex alternative use of five exons as the first exon (exons 1a to 1e in the 5′-to-3′ order). Although expression profiles of these isoforms were highly variable among human tissues, three of them, *PTCHa, PTCHb,* and *PTCHd,* were predominantly expressed in most tissues, *PTCHd* being most ubiquitous. In contrast, *PTCHb* was always predominant and reached a maximum at E10.5 during mouse development. These three mRNA isoforms encode three PTCH proteins with distinct N-termini, $PTCH_L$, $PTCH_M$, and $PTCH_S$. The expression of these three isoforms was regulated by GLI transcription factors, and at least two functional GLI-binding sequences were identified, one in exon 1a and the other between exon 1a and exon 1b. $PTCH_L$ and $PTCH_M$ were equally active in terms of suppressing GLI-mediated transcription and inducing apoptosis. $PTCH_S$ protein (encoded by *PTCHd*), lacking the first transmembrane domain, was more unstable than the other two, resulting in a reduced activity. This study may shed light on the mechanism whereby a single *PTCH* gene plays a role in both tumor cell growth and embryonic development.

Keywords: Patched; Sonic hedgehog; Basal cell carcinoma; Medulloblastoma; Alternative splicing

The Sonic hedgehog (Shh) signaling cascade is pivotal to embryonic development, because holoprosencephaly (HPE), characterized by a failure of the forebrain to separate completely into hemispheres, and HPE-like abnormalities are associated with a loss of Shh function in humans and in mice [1–3]. The role of the Shh pathway in tumorigenesis was also established with the discovery that inactivating mutations in the *Patched* (*PTCH*) gene, which encodes one component of the Shh receptor, are responsible for the inherited cancer predisposition disorder known as Gorlin's or nevoid basal cell carcinoma syndrome (NBCCS) [4,5], as well as sporadic basal cell carcinomas (BCCs) and medulloblastomas [6–8]. NBCCS is an autosomal dominant neurocutaneous disorder characterized by developmental abnormalities such as palmar and plantar pits, jaw cysts, calcification of the falx cerebri, and skeletal anomalies and also by a predisposition to cancers such as BCC and medulloblastoma [9]. Familial and sporadic BCCs display loss of heterozygosity in this region, consistent with *PTCH* being a tumor suppressor gene [6,10]. In addition, activating mutations in *Smoothened* (*Smo*), also encoding another component of the Shh receptor, have been detected in BCCs [11], further emphasizing the importance of this pathway in tumor development. More importantly, the recent finding that this pathway is essential for growth of a wide range of tumor types not associated with NBCCS, such as lung

☆ Sequence data from this article have been deposited with the GenBank Library under Accession Nos. AB164615, AB164616, and AB189436–AB189442.

* Corresponding author. Fax: +81 3 5494 7035.
E-mail address: tmiyashita@nch.go.jp (T. Miyashita).

0888-7543/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2004.11.014

cancers or digestive tract tumors, sheds light on potential new diagnostic and therapeutic approaches [12–14].

PTCH, a 12-pass transmembrane protein, is the ligand-binding component of the Shh receptor complex. In the absence of Shh binding, PTCH is thought to hold Smo, a 7-pass transmembrane protein, in an inactive state and thus inhibit signaling to downstream genes. Upon the binding of Shh, the inhibition of Smo is released and signaling is transduced, leading to the activation of target genes by the Gli family of transcription factors [15]. The transcription of *PTCH* itself is induced by Shh pathway activity [16], thus generating a negative feedback loop, which may play an important role in tumor suppression by inhibiting a sustained activation of the pathway.

Hahn et al. predicted that there are three different forms of the PTCH protein present in humans: the ancestral form and two human-specific forms [4]. Recently, a detailed characterization of three alternative first exons was reported [17]. However, our study using the 5′ rapid amplification of cDNA ends (5′RACE) technique revealed the existence of an additional first exon and unexpectedly complex splicing between the first and the second exons that is evolutionarily conserved across species. Therefore, the characterization of several potential forms of the PTCH protein may reveal the mechanism whereby a single *PTCH* gene could play a role in different pathways, and the determination of the regulation of different splice forms of *PTCH* mRNA may shed light on the apparent role of the gene in tumor cell growth as well as embryonic development. Here we characterize multiple isoforms of *PTCH* in humans and mice and discuss the functions of their products, expression profiles, and transcriptional regulation.

Results

Isolation of isoforms of human and mouse PTCH

PTCH is a multiexon gene comprising 23 exons distributed over a region of ~70 kb. To date, three cDNA sequences encoding the human *PTCH* gene's first exon have been reported and named exons 1, 1A, and 1B [17], and another exon has recently been deposited with GenBank (exon 1a described below, GenBank Accession No. BC043542). In contrast, only a single mRNA species of *PTCH* has been reported in mice [18] (GenBank Accession No. U46155). Due to the use of alternative exons, several mRNA isoforms are generated. On the basis of this background we performed a comprehensive analysis of the 5′ structure of mRNA species derived from the human *PTCH* gene employing the 5′RACE technique. Sequencing of 31 RACE clones revealed an additional alternative first exon (exon 1c described below, submitted to GenBank as Accession No. AB189438) and complex splicing between the first and the second exon. Using a genomic sequence containing the *PTCH* gene (GenBank Accession No. AL161729), the precise genomic organization of the human *PTCH* gene was determined as shown in Fig. 1. For the sake

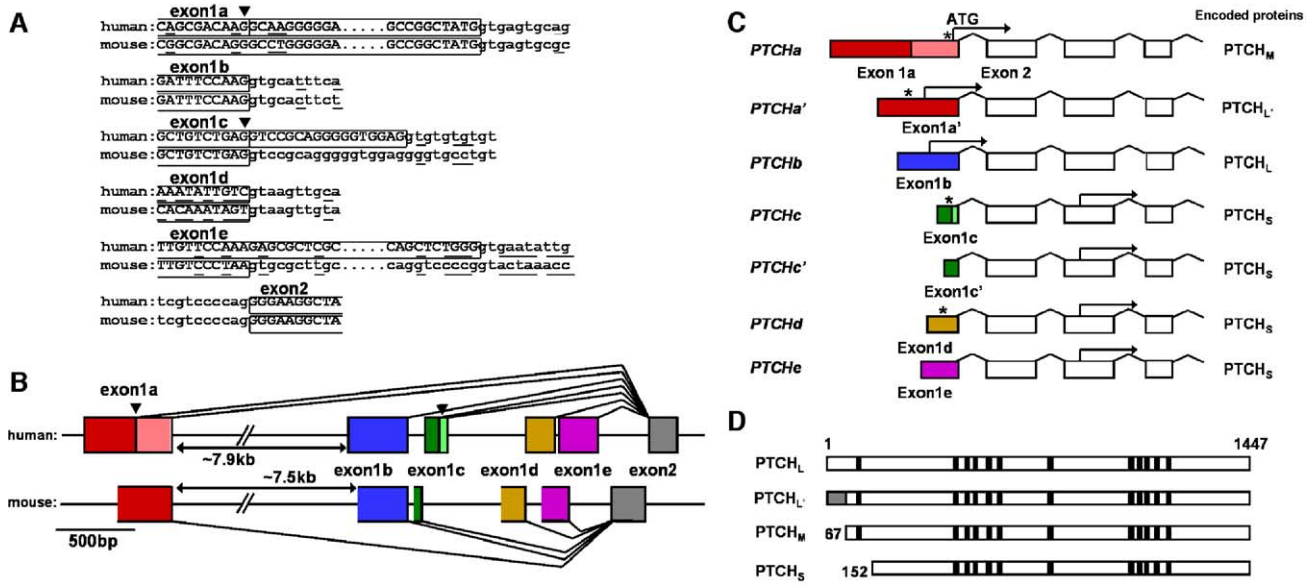

Fig. 1. Identification of human and mouse *PTCH* isoforms. (A) Comparison of human and mouse exon–intron boundaries. Upper- and lowercase letters indicate exon and intron sequences, respectively. Nucleotides not conserved between the two species are underlined. Alternative splice donor sites are indicated by arrowheads. (B) 5′ region of human and mouse *PTCH* gene structure. The 5′ ends of the mouse first exons have not been determined. (C) 5′ structure of *PTCH* isoforms. The positions of the first methionine codons and in-frame stop codons are indicated by arrows and asterisks, respectively. In four of seven mRNAs, in-frame stop codons were identified. The first in-frame methionine codon could be determined in the other three transcripts since the 5′RACE system we employed amplifies only full-length transcripts [47]. (D) PTCH protein isoforms encoded by mRNA species described in (C). Numbers refer to amino acid positions relative to the first methionine of PTCH$_L$. The positions of the 12 transmembrane regions are indicated by filled boxes. PTCH$_{L'}$ has 65 unique amino acid residues at the N-terminus depicted with a shaded box.