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Short communication

Expression of the *Sox10* gene during mouse inner ear development

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Abstract

Mutations in the *SOX10* gene, encoding a cell-lineage specific transcription factor, are associated with congenital deafness. We analyzed the expression of *Sox10* mRNA in developing mouse inner ear by in situ hybridization. *Sox10* mRNA is expressed in the entire epithelia of the otic vesicle at embryonic day 11.5 (E11.5) and in the developing cochlea and vestibule at E13.5. In postnatal day 8 and adult cochleas, *Sox10* expression is restricted to the supporting cells of the organ of Corti. These expression profiles suggest that Sox10 is important for development of the cochlea.

Theme: Development and regeneration

Topic: Sensory systems

Keywords: Sox10; Inner ear; Cochlea; Organ of Corti; Supporting cell; Otic vesicle

The *Sox* genes encode transcription factors with a high-mobility group box as a DNA-binding motif [17]. They are highly conserved through evolution and show diverse patterns of expression throughout development. Recently, a new member of this family, Sox10, was identified [5,9,18]. In mice, the *Sox10* gene is selectively expressed in neural crest cells during early stages of development, and in glia cells of the peripheral and central nervous systems later in development and in adulthood [5,9,12]. A truncation mutation of the *Sox10* gene is associated with the mouse mutant *Dominant megacolon*, a model for human congenital megacolon, Hirschsprung disease [3,12]. Its human homologue SOX10 is defective in some cases of Shah–Waardenburg syndrome, also known as Waardenburg–Hirschsprung disease [8]. Shah–Waardenburg syndrome is characterized by the aganglionic colon as well as the sensorineural deafness and pigmentation abnormalities, in which the two types of neural crest-derived cells, melanocytes and intestinal ganglia cells, are affected [8,11]. Moreover, a single heterozygous mutation of SOX10 (S135T) was found in a patient affected with a mild form of the Yemenite deaf–blind hypopigmentation syndrome [2]. No vestibular dysfunction has been reported in these auditory pigmentary syndromes. It is therefore conceivable that SOX10 plays an important role in the development of the auditory system.

Mammalian inner ear consists of the cochlear and vestibular components, both of which are derived from the otic vesicle, a transient embryonic structure of ectodermal origin. It has been reported by other investigators that the *SOX10* gene is expressed in the human otic vesicle [1]. In addition, *Sox10* is expressed in the otic vesicle of mouse embryos (embryonic day 9.5–12.5), as judged by whole mount in situ hybridization [5,9,12]. However, the spatial and temporal expression pattern of *Sox10* during inner ear development has not been reported. In this study, to assess the role of Sox10 in the developing inner ear, we investigated the expression pattern of *Sox10* mRNA in the otic vesicle and in the developing cochlea by in situ hybridization.

BALB/c albino mice at embryonic day 11.5 (E11.5), E13.5 and E16.5 and at postnatal day 0 (P0), P8 and >P60 (adult) were examined. Series of embryos were collected from pregnant mice, anesthetized with diethylether, and

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quickly fixed with 4% paraformaldehyde (PFA) in 0.01 M phosphate-buffered saline (PBS). Postnatal mice were decapitated, and temporal bones were fixed with PFA in PBS, followed by 8% EDTA in PBS for decalcification. The specimens were immersed in graded series of sucrose in PBS, embedded in OCT compound (Miles, Elkhart, IN, USA) and frozen. Sections were cut at 15 μm thickness with a cryostat and mounted on silane-coated slides.

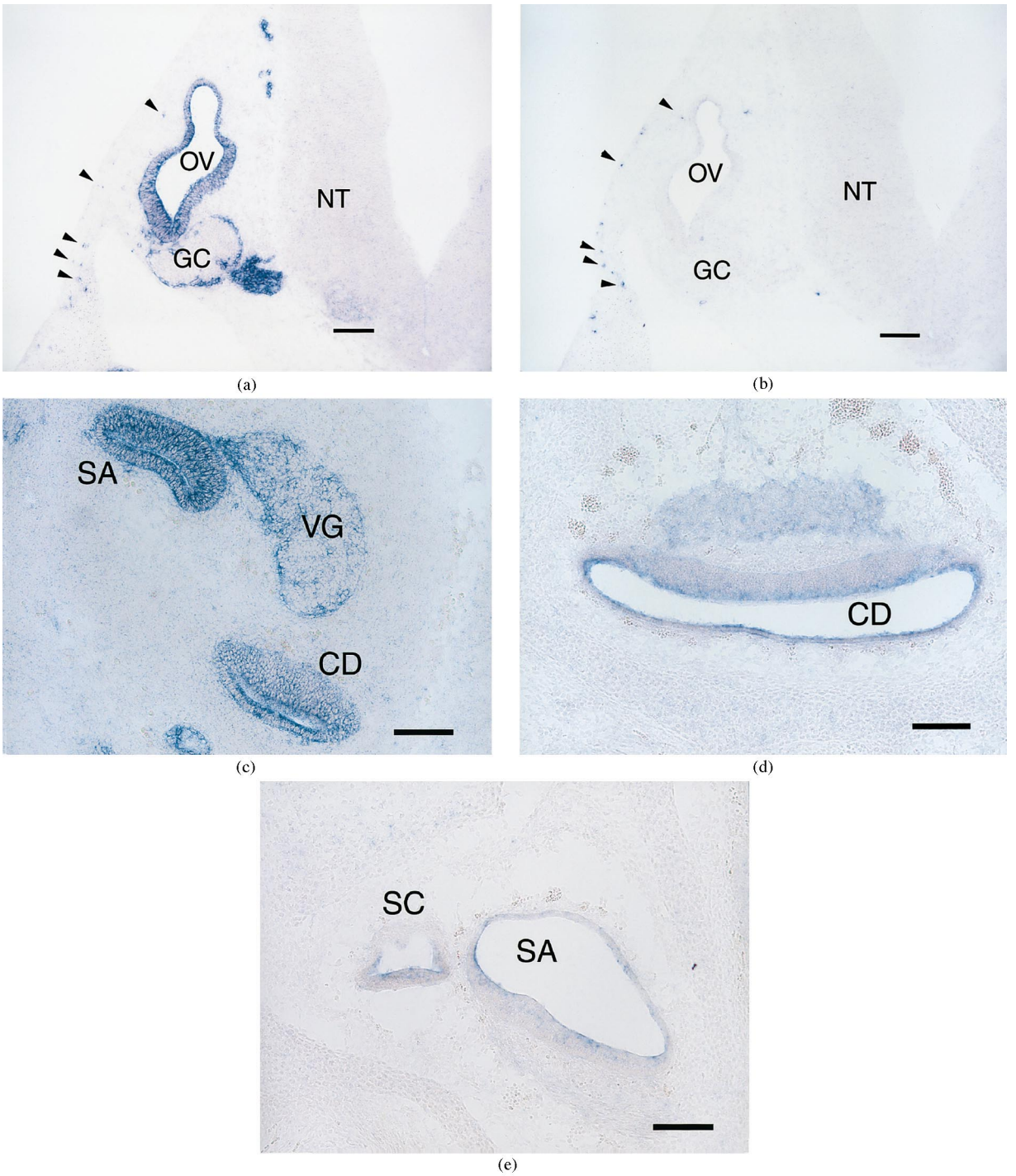

Fig. 1. *Sox10* mRNA expression in the otic vesicle and in the developing inner ear as detected by in situ hybridization. (a) E11.5. *Sox10* signals are prominent in entire epithelia of otic vesicle (OV), lateral to the neural tube (NT), and the facio-acoustic ganglion complex (GC). Scattered single cells (arrowheads) in the dorso-lateral pathway, presumptive melanoblast precursors, also express *Sox10.* (b) E11.5. The expression of *Mitf* mRNA, a melanoblast marker, is restricted to the migrating melanoblasts. (c) E13.5. *Sox10* expression over the epithelia of presumptive cochlear duct (CD), saccule (SA), and vestibulocochlear ganglion (VG). (d) E16.5. *Sox10* expression in cochlear duct. (e) E16.5. *Sox10* expression in saccule and semicircular canal (SC). Scale bar=100 μm (a–e).

The template for the *Sox10* RNA probe was generated from mouse cochlea cDNA by PCR with the following primers: 5′-TGTGTGCCCTGCTCCTCATCAG-3′ and 5′-GGCAGCGATGTGTTACATGTGG-3′, both of which are located at the 3′-untranslated region of *Sox10* mRNA [3]. The PCR product of 880 bp was subcloned into pGEM-T vector (Promega) and sequenced. The determined nucleotide sequence was identical to that of the mouse *Sox10* cDNA [3], indicating the expression of *Sox10* mRNA in adult mouse cochlea. In vitro transcription was performed using SP6 and T7 RNA polymerases and a digoxigenin RNA labeling kit (Boehringer Mannheim, IN, USA) to synthesize the antisense and sense RNA probe, respectively. The RNA probe for *microphthalmia-associated transcription factor* (*Mitf*) mRNA, an early marker for melanoblasts of neural crest origin [7,14], corresponds to the positions 151–719 of the mouse cDNA [4]. The reason for using the melanoblast specific probe is that pigmentation abnormalities are associated with SOX10/Sox10 mutations [3,8,12]. In this context, mutations in the *MITF* gene, the human counterpart of *Mitf*, are also associated with certain types of auditory–pigmentary syndromes, such as Waardenburg syndrome type 2 [15]. Non-radioactive in situ hybridization was performed according to the standard procedures [10].

At E11.5, *Sox10* expression was detected with the antisense RNA probe in the entire epithelium of the otic vesicle as well as in the facio-auditory ganglion complex (Fig. 1a), but no hybridization signals were detected with a sense *Sox10* RNA probe (data not shown). It is noteworthy that *Sox10* is expressed in the medial–ventral and the lateral–dorsal regions of the otic vesicle, which mainly give rise to the cochlea and the vestibular apparatus, respectively [16]. Prominent expression of *Sox10* mRNA in the entire otic vesicle suggests its important role in the differentiation and development of the inner ear. *Sox10*

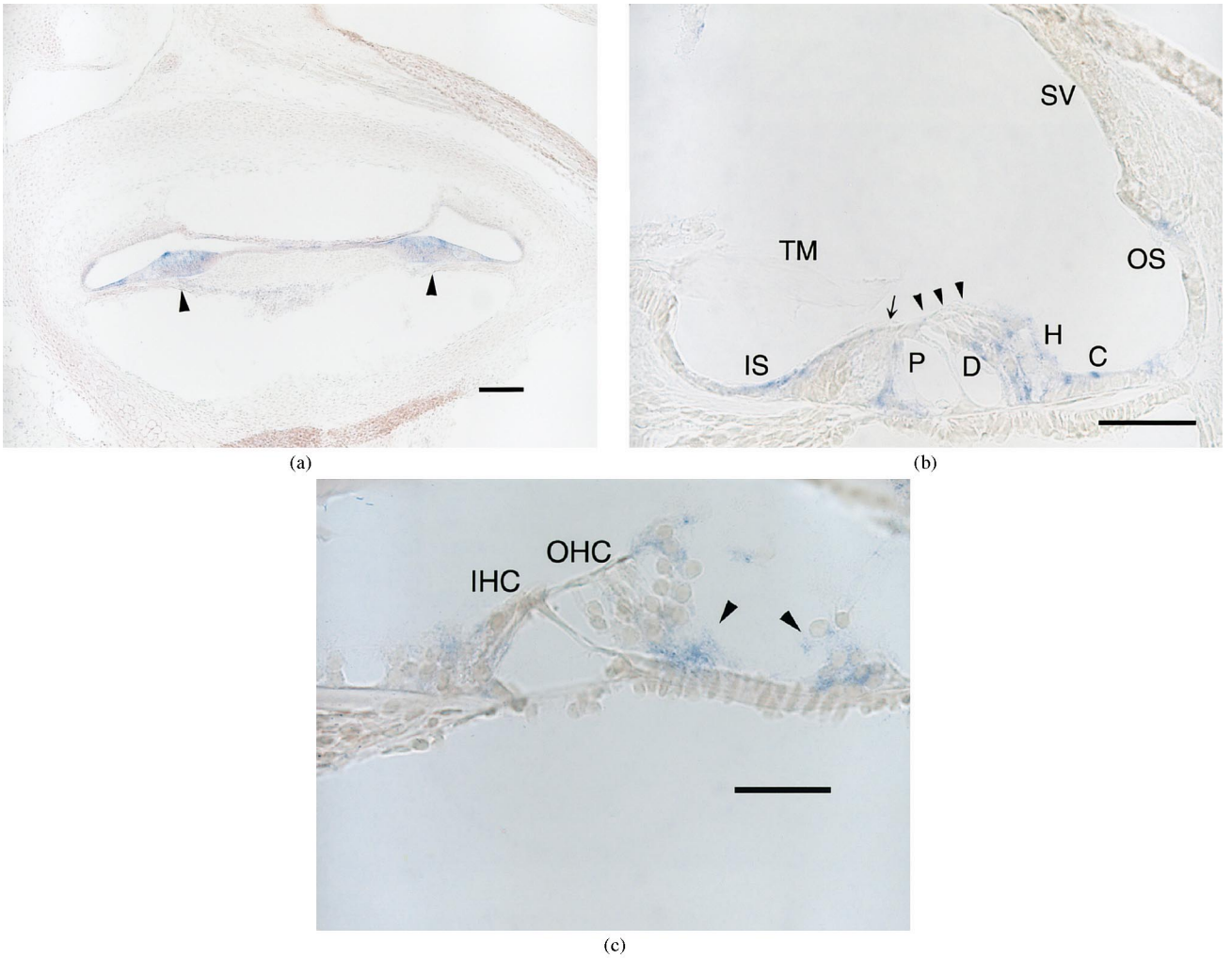

Fig. 2. *Sox10* expression in the postnatal and adult cochleas as detected by in situ hybridization. (a) P0 cochlea. *Sox10* expression in the presumptive organ of Corti (arrowheads). (b) P8 cochlea. *Sox10* expression in the supporting cells of the organ of Corti, such as inner sulcus cells (IS), inner pillar cells (P), Deiters' cells (D), Hensen's cells (H), Claudius' cells (C) and outer sulcus cells (OS). Note that inner hair cells (arrow) and outer hair cells (arrowheads) are negative. SV, stria vascularis; TM, tectorial membrane. (c) Adult organ of Corti. *Sox10* expression confined to the supporting cells (arrowheads) lying under and lateral to the outer hair cells (OHC). IHC, inner hair cells. Scale bars: a=100 μm; b=50 μm; c=20 μm.

expression was also detected in scattered migrating neural crest-derived melanoblasts among mesenchyme (Fig. 1a), because these cells also express *Mitf* mRNA, as shown in the adjacent tissue section (Fig. 1b). Unlike *Sox10* mRNA, *Mitf* mRNA was not detected in the otic vesicle (Fig. 1b). By E13.5, *Sox10* expression became undetectable in migrating melanoblasts, which however continued to express *Mitf* mRNA (data not shown). During further development, some of these melanoblasts migrate to the lateral wall of the cochlear duct and are incorporated into the stria vascularis as intermediate cells. On the other hand, at E13.5, *Sox10* mRNA was detected in the primordial epithelia of the cochlea and the saccule (Fig. 1c). At E16.5, *Sox10* mRNA was detected in the epithelium of the cochlea (Fig. 1d) and of the vestibular system, consisting of the saccule, utricle and semicircular canals (Fig. 1e). The expression levels of *Sox10* mRNA appeared to decrease with embryonic age.

The organ of Corti is a sensory apparatus in the cochlear duct and is composed of sensory cells, namely inner hair cells and outer hair cells, and various types of supporting cells. The organ of Corti transduces the sound stimulation to the cochlear nerve via the spiral ganglion. In P0 cochlea, *Sox10* is specifically expressed in the presumptive organ of Corti (Fig. 2a). By P8, *Sox10* mRNA was detected in supporting cells of the organ of Corti (Fig. 2b), but not in sensory cells and in intermediate cells of the stria vascularis. *Sox10* expression was also detected in Schwann cells of the spiral ganglion (data not shown), which is consistent with the expression of *Sox10* in glia cells [5,9,12]. In adult cochlea, *Sox10* expression is restricted to the supporting cells lying under and lateral to outer hair cells in the organ of Corti, but not in inner and outer hair cells (Fig. 2c). These supporting cells include Deiters' cells, which hold base and apex of the outer hair cells. Such cell–cell interactions are important for the function of the organ of Corti which senses the force produced by sound-induced motion of the outer hair cells. Deiters' cells were also reported to be involved in the maintenance of high K^+ concentration in endolymph [13]. These notions suggest the functional significance of the supporting cells in hearing.

The expression profiles of *Sox10* mRNA suggest that Sox10 plays an important role in differentiation and development of the cochlea. Especially, we show for the first time that the *Sox10* gene is expressed in the supporting cells of the organ of Corti. Sox10 may function as a transcriptional modulator by interacting with other transcription factors [5,6], thereby regulating certain target genes in the supporting cells. In this context, distinct *SOX10* gene mutations are associated with dominantly inherited auditory–pigmentary syndromes [2,8,11], indicating that the reduced expression levels (haploinsufficiency) of SOX10 in developing inner ear are responsible for congenital deafness. However, the molecular basis of deafness in these patients is unknown. The present study raises the possibility that reduced SOX10 levels may affect the correct arrangement of sensory cells and supporting cells in the organ of Corti, which could lead to congenital hearing impairment.

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