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A 4.3 kb Smad7 promoter is able to specify gene expression during mouse development

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Abstract

Members of transforming growth factor- β (TGF- β) superfamily play important roles in diverse biological functions including early development. These extracellular factors exert their effects by interacting with membrane receptors followed by signal transduction by a group of Smad proteins. Smad7 is an inhibitory Smad protein that specifically antagonizes TGF- β and activin signaling. To characterize the developmental role of Smad7, a transgenic mouse model was generated using a 4.3 kb mouse Smad7 promoter driving β -galactosidase expression. In these mice, the Smad7 promoter defined a restrictive expression pattern of β -galactosidase in a tightly regulated temporal and spatial manner. The β -galactosidase gene was transiently expressed in the cardiovascular structures including heart cushion tissues and the endothelium of major arteries at E11.5 to E12.5. Through E12.5 to E17.5, β -galactosidase expression was prominently detected in the epithelium of developing cochlea and nasolacrimal duct. In addition, it was temporally expressed in trigeminal ganglion, the skeletal muscles surrounding the major joints, primordium of the jaws, as well as genital tubercle. These studies indicated that the 4.3 kb Smad7 promoter contains sufficient regulatory elements to define controlled gene expression during mouse development.

Keywords

TGF- β ; activin; Smad7; heart; aorta; cochlea; nasolacrimal duct; development; transgenic

Members of TGF- β superfamily including TGF- β s, activins and bone morphogenetic protein (BMPs) are pleiotropic factors involved in various biological processes including early development, regulation of cell growth and differentiation, hormone secretion, bone formation, and immune modulation [1,2]. Smad7 is an inhibitory Smad protein that is able to antagonize signaling by TGF- β family members [3–5]. This is mediated by direct binding of Smad7 with TGF- β and activin type I receptors to prevent activation of pathway-specific Smad2 and Smad3 upon ligand binding [3,4,6]. Previous studies have indicated that Smad7 expression is regulated

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by TGF- β signaling [7]. Smad7 promoter contains a Smad binding element (SBE) which is directly controlled by Smad2 and Smad3 upon TGF- β signaling in collaboration with AP1, Sp1 and TFE3 [7–9]. The upregulation of Smad7 by TGF- β signaling is hypothesized to form a negative feedback loop to timely terminate TGF- β action. To further analyze the potential role of Smad7 in early mouse development, we generated a transgenic mouse that expresses a β -galactosidase gene driven by a 4.3 kb mouse Smad7 promoter as previously characterized by our group [7].

The transgenic construct was made by cloning a β -galactosidase gene with the poly(A) signal (Tropix) under a 4.3 kb mouse Smad7 promoter (–4.2kb to +112 relative the transcription initiation site) [7]. The plasmid was injected into inbred mouse zygotes (C3HeB/FeJ strain) using standard methodologies (Transgenic Facility, Indiana University School of Medicine). Pups from the micro-injected embryos were screened for the presence of the transgene using PCR with primers specific for the transgene. The embryos at various developmental stages of the F2 generation were used in X-gal staining followed by section and eosin staining. Two independent lines of transgenic mouse were used and both of them had similar tissue distribution patterns of β -galactosidase.

Mouse embryos at various developmental stages were used to stain for β -galactosidase to analyze the regulation of gene expression by the 4.3 kb Smad7 promoter as previously characterized by us [7]. In whole mount staining, β -galactosidase was prominently expressed in the ear area, the nasolacrimal ducts, the primordium of upper and lower jaws, as well as the skeletal muscles surrounding the primordium of major joints (Fig. 1).

Using this mouse model, we found profound expression of Smad7 in cardiovascular structures at E11.5 and E12.5. As shown in Fig. 2, β -galactosidase staining was clearly seen in the endocardial cushion tissue lining the atrio-ventricular canal. In addition, it was highly expressed in the endothelium of the aortic sac, dorsal aorta, thoracic aorta and umbilical artery. The cardiovascular expression profile of β -galactosidase in the transgenic mouse is largely consistent with the results by *in situ* hybridization reported by other groups [10,11], indicating that the 4.3 kb promoter is able to specify the correct expression pattern of the endogenous Smad7 gene in these structures. Interestingly, the expression of β -galactosidase in cardiovascular system is very transient and only occurs through E11.5 to E12.5. By E13.5 and onwards, its expression was hardly detected in any vascular structures. Smad7 was originally isolated from vascular endothelium as one of the genes upregulated by shear stress [5]. The predominant localization of Smad7 in endothelium suggests a function of Smad7 for the development and functioning of vascular system.

Through E12.5 to E17.5, β -galactosidase was highly expressed in the epithelium of cochlea and nasolacrimal duct (Fig. 3). The β -galactosidase staining was consistently detected in the cochlea epithelium in both the early stage of cochlea formation at the otic vesicle and during the condensation of the underlying mesenchyme of the developing spiral limbus. The expression of β -galactosidase in cochlea epithelium partly overlaps with that of TGF- β 2 and TGF- β 3 in mouse [12,13]. However, detailed examination indicated that β -galactosidase staining was predominantly found in the anterior and posterior corners of the cochlea epithelium (Fig. 3, A), while TGF- β 2 is uniformly expressed in the whole epithelium and TGF- β 3 mRNA is mainly present in the mesenchymal tissues of the cochlea surrounding the otic epithelium [12,13]. At these stages of mouse development, β -galactosidase was also predominantly expressed in the epithelium of nasolacrimal duct (Fig. 3, D to F). These findings suggest a potential function of Smad7 in the development of these specialized organs.

In addition to its expression in the cochlea and nasolacrimal duct, β -galactosidase expression could be detected in various other structures in a tightly regulated manner (Fig. 4). At E12.5,

β -galactosidase staining could be detected in the trigeminal ganglion as well as its projection toward the primordium of upper jaw (Fig. 4, C). The expression of β -galactosidase was also detected in the inferior ganglion of the vagus nerve (Fig. 4, D). The prominent blue staining in the nose region as revealed in Fig. 1 appeared to be caused by β -galactosidase expression in the primordium of the upper and lower jaws (Fig. 4, E and F), as well as in the epithelium lining the interior of the nasal cavity (Fig. 4, A and B). In addition, β -galactosidase expression could be detected in the developing skeletal muscles in the tongue (Fig. 4, A) and those around the primordium of the shoulder and elbow joints (Fig. 4, G to J), as well as the hip and knee joints (not shown). The genital tubercle also manifested significant β -galactosidase signals predominantly at E16.5 (Fig. 4, K and L).

The spatial expression pattern of Smad7 mRNA during mouse development has been previously reported [10,11]. By careful comparison of our data with the reported results, we found that the β -galactosidase-positive tissues in our study is very consistent with the Smad7 expression pattern revealed by *in situ* hybridization [10,11], such as in cardiovascular structures and cochlea. This indicated that the regulatory region contained in the 4.3 kb promoter does have sufficient elements to regulate the temporal and spatial expression pattern of Smad7 during development. In addition, these data suggest that Smad7 may play an important role for early development and organogenesis in mouse. Smad7 is an inhibitory Smad that is able to abolish TGF- β and activin signaling. The highly restrictive expression pattern of Smad7 at various stages of development would indicate that the blocking of TGF- β /activin signaling is crucial for the development of certain tissues and organs. As TGF- β family members play important roles in a large variety of biological processes, this transgenic mouse would be able to serve as a valuable model for future studies to delineate the *in vivo* regulation of Smad7 expression.

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Fig. 1. Whole animal staining of the transgenic mice at E13.5. A. front view; B. lateral view. Scale bar: 250 μ m.

Fig. 2. Prominent expression of β -galactosidase in cardiovascular structures at E11.5 and E12.5. Note that a strong β -galactosidase expression was seen in the heart cushion regions and major arteries. Abbreviation: as, aortic sac; da, dorsal aorta; ec, endocardial cushion tissue; fbb, mandibular component of the first branchial branch; sbb, second branchial branch; st, stomach; ta, thoracic aorta; ua, umbilical artery. Scale: 100 μ m. All sections are sagittal with the dorsal to the right and the anterior to the top.

Fig. 3.

Expression of β -galactosidase in cochlea and nasolacrimal duct through E12.5 to E17.5. Sagittal sections at various developmental stages were shown. (A to C) expression in developing cochlea; (D to F) expression in tear ducts. Abbreviation: c, cochlea; iv, inferior ganglion of vagus nerve; le, lens; nd, nasolacrimal duct; tg, trigeminal ganglion. Scale: 100 μ m. All sections are sagittal with the dorsal to the right and the anterior to the top.

Fig. 4.

Restrictive expression of β -galactosidase in various organs and tissues. (A–B) Expression of β -galactosidase in the epithelium of nasal cavity and the skeletal muscles of the tongue; (C) in the trigeminal ganglion, (D) in the inferior ganglion of the vagus nerve; (E, F) in the primordium of upper and lower jaws; (G–J) in the skeletal muscles surrounding the primordium of shoulder and elbow joints; (K, L) in the genital tubercle. F, H, J and L are high magnification of E, G, I and K respectively. Abbreviation: iv, inferior ganglion of vagus nerve; nv, nasal cavity; tg, trigeminal ganglion; tn, tongue. Scale: 500 μ m for A, B, E, G, I and K; 100 μ m for C, D, F, H, J and L. All sections are sagittal.