Human Smooth Muscle α-Actin Promoter Drives Cre Recombinase Expression in the Cranial Suture in Addition to Smooth Muscle Cell

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Received January 19, 2007; Accepted February 6, 2007; Online Publication, April 7, 2007
[doi:10.1271/bbb.70043]

Tissue-specific gene deletion by the Cre-loxP system is a powerful tool to investigate the roles of specific genes. To determine the specificity and efficiency of the Cre-mediated recombination under the control of the human smooth muscle α-actin promoter, we mated SMαA-Cre mice and R26R reporter mice. Cre-mediated recombination was observed in visceral and vascular smooth muscle cells. Partial recombination was also found in cranial sutures. Hence, we propose that SMαA-Cre mice are good tool for conditionally deleting gene function in the cranial suture in addition to smooth muscle cells.

Key words: smooth muscle α-actin; Cre recombinase; Cre/loxP system

Conditional recombination of specific genes in mice has been achieved using the Cre-loxP system. Expression of Cre recombinase under the control of tissue-specific promoters results in recombination between pairs of 34-bp loxP sites. Tissue-specific expression of Cre recombinase can circumvent embryonic lethality associated with homozygous germline knockout of essential genes. We used a 4.7-kb fragment of the human smooth muscle α-actin (SMαA) promoters, from −891 to +3,828 bp, to control Cre-expression.1

SMαA is one of six actin isoforms in mammals.2–6 It is known to be expressed in vascular and visceral smooth muscle cells.3) However, SMαA expression has also been reported in osteoblasts and several musculoskeletal connective tissues, including articular chondrocytes, meniscus, and anterior cruciate ligaments.7–13

Our selected smooth muscle α actin promoter from −891 to +3,828 was sufficient to drive expression in vascular and visceral smooth muscle cells in vivo.14 Because Cre-mediated excision is an irreversible act, the deletion will persist once Cre recombinase expression has occurred even if the expression is extinguished. Our purpose in this study was to characterize the spatial pattern of Cre-mediated recombination in smooth muscle cells and non-smooth muscle cells.

To monitor Cre-mediated recombination, we generated SMαA-Cre+/−;R26R+/− mice. R26R conditional reporter mice have been described previously.15 In the presence of active Cre recombinase, the stop signal is excised and β-galactosidase expression can be detected by X-gal staining.

In SMαA-Cre+/−;R26R+/− adult mice, β-galactosidase activity was detected in smooth muscle tissues, including the stomach, bladder, intestine, esophagus, and aorta (Fig. 1A). Histological sections showed X-gal staining in the visceral smooth muscle layer of the bladder, large intestine, small intestine, stomach, and esophagus, but not in the epithelial layer of these organs (Fig. 1B–F). X-gal staining was also observed in the vascular smooth muscle layer of the aorta (Fig. 1G).

Partial staining was also found in the heart (Fig. 1A). Histological section of the heart showed partial staining in cardiac myocytes (Fig. 1H). This was probably due to transient expression of SMαA-Cre in the embryo, because SMαA-Cre expression in the heart was observed at E11.5.16 Endogenous SMαA has been reported to express transiently at the early stage of differentiation.16–18

SMαA-Cre+/−;R26R+/− mice also showed β-galactosidase expression in musculoskeletal connective tissues and osseous tissues. At postnatal day 2 (P2), β-galactosidase activity was detected in the joints of the shoulder, elbow, wrist, knee and ankle (Fig. 2A). The calvaria and ribs also showed X-gal staining (Fig. 2A, C). A histological section of the knee showed partial X-gal staining in chondrocytes of articular cartilage of the femur (Fig. 2B). Staining in articular chondrocytes is consistent with endogenous SMαA expression.9,10 β-galactosidase activity in the head was clearly...
detected in the osteogenic fronts of parietal and frontal bones at P2 (Fig. 2C). A histological section of the sagittal suture showed β-galactosidase activity in smooth muscle cell-containing tissues (A). Whole-mount X-gal staining of tissues demonstrated β-galactosidase activity in smooth muscle cell-containing tissues (A). A histological section showed β-galactosidase in the visceral SMC layer of the bladder (B), large intestine (C), small intestine (D), stomach (E), and esophagus (F). LacZ staining was observed in the vascular SMC layer of the aorta (G). Partial staining was found in cardiac myocytes throughout the left (lv) and right (rv) ventricles (H). Genotypes of the mice were determined by PCR. Genomic DNA was isolated from the tail. The 5’ and 3’ primers used for detecting SmxA-Cre gene were primer 1 (5’-agtgcacagctctagc-3’) and primer 2 (5’-tcgacagtttagttaccc-3’). At 4°C, tissues were fixed with 4% paraformaldehyde in PBS solution for 30 min, then stained at 37°C in X-gal stain buffer (0.5 mg/ml X-gal, 2 mM MgCl₂, 0.02% NP-40, 4 mM K₃Fe(CN)₆, and 4 mM K₄Fe(CN)₆ in PBS). For X-gal staining of tissue sections, frozen sections were made by cryostat at 7 μm thickness, and then X-gal staining was carried out as described above.

Fig. 1. Cre-Mediated β-Galactosidase Expression in Adult SMαA-Cre+/−:R26R+/− Mice.

Cre-mediated β-galactosidase expression in visceral and vascular smooth muscle cells (SMCs) in adult SMαA-Cre+/−:R26R+/− mice (4 months old). Whole-mount X-gal staining of tissues demonstrated β-galactosidase activity in smooth muscle cell-containing tissues (A). A histological section showed β-galactosidase in the visceral SMC layer of the bladder (B), large intestine (C), small intestine (D), stomach (E), and esophagus (F). LacZ staining was observed in the vascular SMC layer of the aorta (G). Partial staining was found in cardiac myocytes throughout the left (lv) and right (rv) ventricles (H). Genotypes of the mice were determined by PCR. Genomic DNA was isolated from the tail. The 5’ and 3’ primers used for detecting SmxA-Cre gene were primer 1 (5’-agtgcacagctctagc-3’) and primer 2 (5’-tcgacagtttagttaccc-3’). Three oligonucleotides were used to genotype R26R transgenic animals as previously reported. For whole-mount X-gal staining, tissues were fixed with 4% paraformaldehyde in PBS solution for 30 min at 4°C, then stained at 37°C in X-gal stain buffer (0.5 mg/ml X-gal, 2 mM MgCl₂, 0.02% NP-40, 4 mM K₃Fe(CN)₆, and 4 mM K₄Fe(CN)₆ in PBS). For X-gal staining of tissue sections, frozen sections were made by cryostat at 7 μm thickness, and then X-gal staining was carried out as described above.

result in abnormal skull shapes. The staining pattern in the head suggested that mice can be a valuable tool for studying the formation and maintenance of sutures.

As stated above, Cre-mediated recombination was found in smooth muscle cells, cardiac myocytes, and chondrocytes of the joints, ribs, and calvarial suture in SMαA-Cre mice. In particular, a high rate of Cre-mediated recombination was detected in visceral and vascular smooth muscle cells and cranial sutures. SMαA is a contractile protein that comprises 40% of total smooth muscle cell protein. The high rate of Cre-mediated recombination in smooth muscle cells was consistent with expression of the endogenous SMαA gene. On the other hand, no SMαA expression in cranial
The X-gal staining at the osteogenic front in osteoblast cells has been described in several reports. The X-gal staining of SMaA-Cre+/−;R26R+/− mice at P2 (A) and R26R+/− mice (B, D). β-galactosidase activity was detected in the osteogenic front in SMaA-Cre+/−;R26R+/− mice at P2 (A). β-galactosidase activity was found in osteocytes, osteoblasts, and mesenchymal cells in SMaA-Cre+/−;R26R+/− mice at P14 (C).

suture has been reported. In contrast, SMaA expression in osteoblast cells has been described in several reports. The X-gal staining at the osteogenic front in P2 mice was probably due to SMaA expression of active osteoblasts. The high rate of Cre-mediated recombination in the cranial suture in P14 mice might reflect SMaA expression induced by mechanical tension in the cranial suture. Numerous studies have found that mechanical tension induces expression of SMaA. Given those observations, in addition to being a valuable resource for studying smooth muscle cells, SMaA-Cre mice are a novel tool for studying the mechanism of the development and maintenance of cranial sutures.

References


