Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2006 March ; 26(3): e23–e24. doi:10.1161/01.ATV.0000202661.61837.93.

Generation of an Adult Smooth Muscle Cell–Targeted Cre Recombinase Mouse Model

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To the Editor:

The smooth muscle cell (SMC)–targeted Cre recombination mice are critical tools for in vivo analysis of gene function in the vasculature and for establishing animal models for cardiovascular diseases. Therefore, there is a continuing effort to generate SMC-targeted Cre recombinase mice for in vivo loss-of-gene function studies. Currently, several genetically engineered mice express the Cre-recombinase under the control of SMC-specific promoters

such as SM22α (also known as transgelin, a 22-kDa protein that is abundantly and exclusively expressed in SMCs of adult animals) promoters and smooth muscle myosin heavy chain promoters.[1–6] However, there are potential limitations in their uses for knockout studies; some of them show relatively low excision efficiency and potential embryonic lethality, which prevent subsequent in vivo analyses in adult SMCs. In our effort to generate an SMC-targeted Cre recombination mouse line that effectively excises loxP-flanked target gene, we obtained a valuable Cre recombinase mouse (SM22α-CreKI) that unexpectedly does not express Cre recombinase in embryonic SMCs and cardiac myocytes but highly expresses the Cre in adult SMCs and cardiac myocytes.

This SM22α-CreKI mouse line was generated by knocking in the Cre-recombinase coding sequence into the endogenous SM22α gene locus via homologous recombination of embryonic stem cells (supplemental Figure S1, available online at http://atvb.ahajournals.org). Consistent with previous reports of SM22α knockout mice, our SM22α-CreKI heterozygous and homozygous mice were fertile and appeared phenotypically normal.

To determine the temporospatial patterns of Cre-mediated recombination in SM22α-CreKI mice, we crossed the homozygous SM22α-CreKI mice with the reporter mice *Gt(Rosa)26Sor* (The Jackson Laboratory, Bar Harbor, Me; cat. 003309). As shown in the Figure, A, we found that the Cre expression in a heterozygous mouse is sufficient to induce homologous recombination at loxP sites and thus to remove the loxP-flanked STOP signal between the lacZ gene and the *Gt(Rosa)26Sor* promoter, which leads to β-galactosidase (β-gal) activities. We observed β-gal–positive staining in almost all adult SMCs that comprise the medial layer of all large and small arteries and veins, including the arterial circle of Willis, aorta, femoral arteries and veins, the pulmonary artery, small arteries in skeletal muscles, and coronary arteries (Figure, A). The Cre-mediated recombination in vascular SMCs occurs in all of the arteries and veins we examined (Figure, A; online supplement S2). Consistent with the expression pattern of endogenous SM22α, β-gal–positive staining in the visceral SMCs, including the bladder and gastrointestinal tract in adult mice is observed at high efficiency (Figure, A; online supplement S2). In addition, β-gal activity is also found in cardiomyocytes (Figure, A). However, we found no β-gal–positive staining in other tissues such as brain, liver, and skeletal muscle cells.

Distinct from the endogenous SM22α expression, the SM22α-CreKI mice failed to exhibit specific β-gal activities in SMCs at all embryonic stages examined, ranging from embryonic day 9 to embryonic day 16.5 (online supplement S3). We detected β-gal activities in SMCs and the cardiac myocytes in newborn pups right after birth (day 1).

We also bred SM22α-CreKI mice with another reporter mouse model, *Gt(Rosa)26Sor*–green fluorescent protein (GFP; The Jackson Laboratory, Cat#: 004077) that contains an enhanced GFP (eGFP) gene inserted into the *Gt(Rosa)26Sor* locus. Consistent with the β-gal staining pattern, eGFP is expressed higher in the aorta, lower in the heart, but not in skeletal muscle and the brain by Western blot analyses (Figure 1B).

These results show that we generated an SM22α-CreKI mouse line that exhibits SMC-targeted Cre recombinase activities in all arteries, veins, visceral organs, and in cardiac myocytes in adults. This mouse line will be a useful addition to the diverse tools used to study the complicated smooth muscle and cardiac gene function in vivo in adult mice and to establish animal models for cardiovascular diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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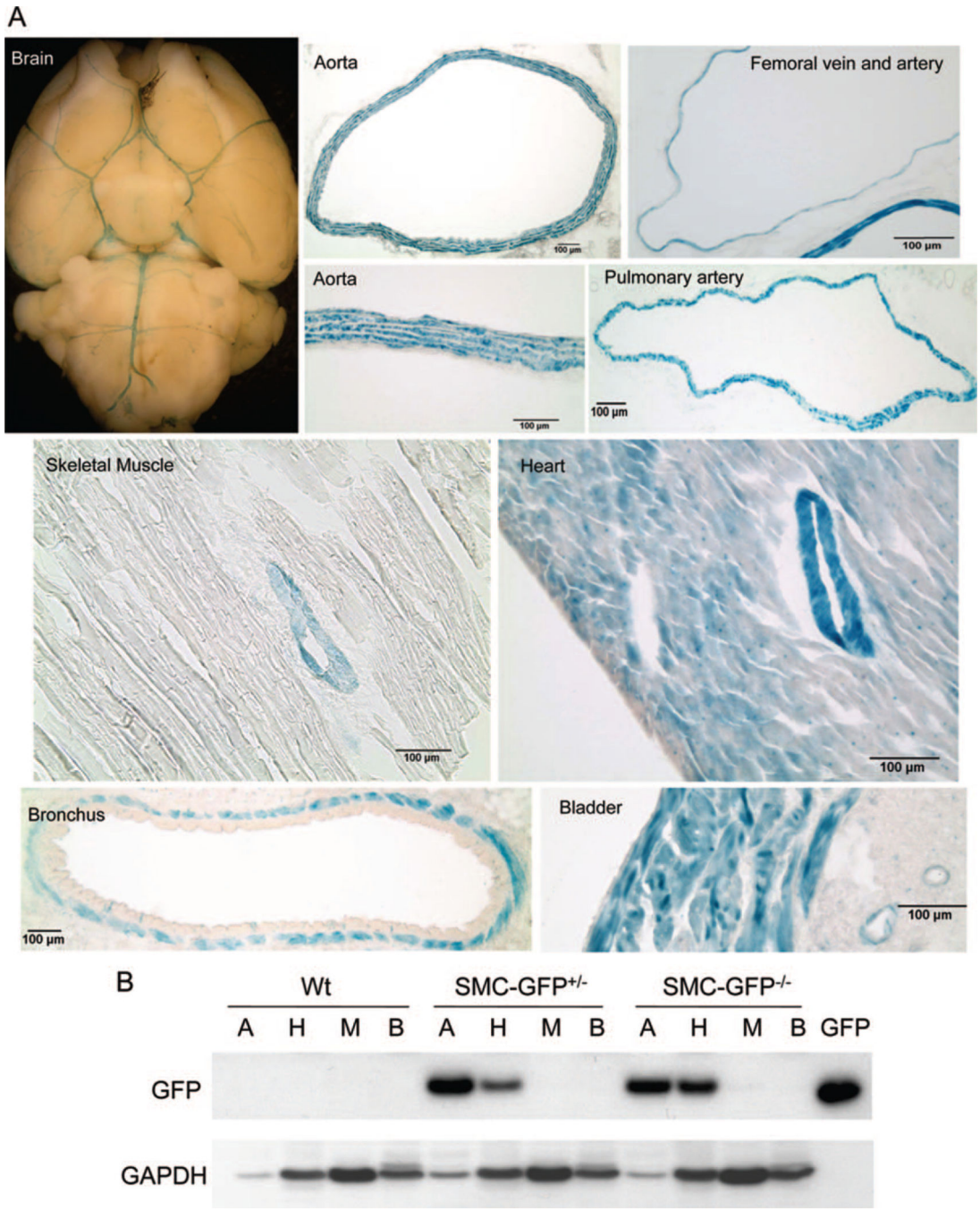

Figure.

Characterization of SM22α-CreKI mice. A, Cellular expression of Cre-recombinase in SM22α-CreKI mice. X-Gal–stained organs and sections were prepared from 8-week-old SM22α-CreKI and *Gt(Rosa)26Sor* double heterozygous mice or wild-type littermates. X-Gal staining shows that Cre-recombinase is expressed in SMCs and cardiomyocytes in adult. B, Western blot analyses show GFP protein expressed in the aorta and the heart of 8-week-old SM22α-CreKI and *Gtrosa26* double heterozygous or double homozygous mice and the

control wild-type littermates. GFP protein is the positive control. Wt indicates wild-type; A, aorta; B, brain; H, heart; M, skeletal muscle. Bar = 100 μm.