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Smooth muscle protein 22 alpha-Cre is expressed in myeloid cells in mice

Zhuxia Shen [a,1], Chao Li [a,1], Ryan A. Frieler [b,c,1], Alena S. Gerasimova [b], Soo Jung Lee [d], Jing Wu [e], Michael M. Wang [b,d], Carey N. Lumeng [d,f], Frank C. Brosius III [b,g], Sheng Zhong Duan [a,*], Richard M. Mortensen [b,c,h,*]

[a] Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Clinical Research Center of Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
[b] Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI, USA
[c] Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI, USA
[d] Department of Neurology, University of Michigan Medical School, Ann Arbor, MI, USA
[e] Department of Orthodontics, Beijing Stomatological Hospital & School of Stomatology, Capital Medical University, Beijing, China
[f] Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, MI, USA
[g] Department of Internal Medicine, Nephrology Division, University of Michigan Medical School, Ann Arbor, MI, USA
[h] Department of Internal Medicine, Metabolism Endocrinology and Diabetes Division, University of Michigan Medical School, Ann Arbor, MI, USA

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ABSTRACT

Background: Experiments using Cre recombinase to study smooth muscle specific functions rely on strict specificity of Cre transgene expression. Therefore, accurate determination of Cre activity is critical to the interpretation of experiments using smooth muscle specific Cre. *Methods and results:* Two lines of smooth muscle protein 22 α-Cre (SM22α-Cre) mice were bred to floxed mice in order to define Cre transgene expression. Southern blotting demonstrated that SM22α-Cre was expressed not only in tissues abundant of smooth muscle, but also in spleen, which consists largely of immune cells including myeloid and lymphoid cells. PCR detected SM22α-Cre expression in peripheral blood and peritoneal macrophages. Analysis of SM22α-Cre mice crossed with a recombination detector GFP mouse revealed GFP expression, and hence recombination, in circulating neutrophils and monocytes by flow cytometry. *Conclusions:* SM22α-Cre mediates recombination not only in smooth muscle cells, but also in myeloid cells including neutrophils, monocytes, and macrophages. Given the known contributions of myeloid cells to cardiovascular phenotypes, caution should be taken when interpreting data using SM22α-Cre mice to investigate smooth muscle specific functions. Strategies such as bone marrow transplantation may be necessary when SM22α-Cre is used to differentiate the contribution of smooth muscle cells versus myeloid cells to observed phenotypes.

1. Introduction

Vascular smooth muscle cells play essential roles in development and progression of cardiovascular diseases such as atherosclerosis and hypertension. In atherosclerosis, smooth muscle cells (SMCs) proliferate and switch from a contractile phenotype to a synthetic phenotype characterized by production of extracellular matrix, adhesion molecules, and cytokines [1,2]. They also contribute to the formation of neointima, foam cells, and plaques at later stage of atherosclerosis. SMCs contribute to the vascular reactivity and remodeling changes that contribute to the pathophysiology of hypertension and its complications [3].

Myeloid cells are also important in these diseases. Inflammation is a critical factor in the progression of atherosclerosis [4]. Monocytes, macrophages, polymorphonuclear leukocytes, dendritic cells, and mast cells have been implicated in all stages of atherosclerosis, from initiation to progression to late complications [5,6]. Monocytes and macrophages are also involved in vascular inflammation and remodeling in hypertensive animal models and reduced vascular inflammation is associated with improved blood pressure [3,7–9].

In order to study functions of genes in SMCs specifically, several Cre recombinase mouse lines have been created [10–16]. These

* Corresponding authors. Addresses: Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Rd., Shanghai 200031, China. Fax: +86 21 5492 0981 (S.Z. Duan); Department of Molecular and Integrative Physiology, University of Michigan Medical School, 7641 Med. Sci. II, 1150 W. Med. Ctr. Dr., Ann Arbor, MI 48109-5622, USA. Fax: +1 734 936 8813 (R.M. Mortensen).
E-mail addresses: szduan@sibs.ac.cn (S.Z. Duan), rmort@umich.edu (R.M. Mortensen).
[1] These authors contributed equally to this work.

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mice have been extensively used to study functions of a variety of genes in cardiovascular diseases. The specificities of these lines were tested against different cell types and Cre recombinase was found to be expressed mostly in SMCs with different degrees of expression in cardiomyocytes in different lines. However, it has not been reported whether Cre recombinase is expressed in myeloid cells in these mouse lines. Given the importance of both SMCs and myeloid cells in vascular inflammation, it is critical to clarify this in order to determine whether SMCs are the only important target of certain gene functions in atherosclerosis or hypertension. We hypothesized that SM22α-Cre expression may not be strictly limited to SMCs. To test this, we have analyzed Cre expression in two commonly used SM22α-Cre strains available from the Jackson. In these two lines, Cre recombinase was either controlled by promoter of smooth muscle protein 22 alpha (SM22α) or inserted into the SM22α locus [12,16].

2. Materials and methods

2.1. Animals

SM22α-Cre mice (4746 and 6878) were obtained from the Jackson Laboratory (Bar Harbor, Maine). SM22α-Cre(4746) mice were bred to floxed peroxisome-proliferator-activated-receptor-γ (PPAR-γ) mice [21] to produce smooth muscle specific PPAR-γ knockout (SM-PGKO; PPAR-$\gamma^{flox/flox}$:Cre^{+}) mice, littermate floxed control (LFC; PPAR-$\gamma^{flox/flox}$:Cre^{wt}) mice, and littermate Cre control (LCC; PPAR-$\gamma^{wt/wt}$:Cre^{+}) mice.

Both SM22α-Cre(4746) and SM22α-Cre(6878) mice were bred to floxed dominant-negative-mastermind-like-green-fluorescent-protein (DNMAML-GFP) mice [18] to produce smooth muscle specific DNMAML-GFP (DNMAML-$GFP^{flox/wt}$:Cre^{+}) mice, and their LFC (DNMAML-$GFP^{flox/wt}$:Cre^{wt}) and LCC (DNMAML-$GFP^{wt/wt}$:Cre^{+}) mice. Smooth muscle specific DNMAML-GFP mice produced using SM22α-Cre(6878) mice were designated as DNMAML1 and those using SM22α-Cre(4746) mice as DNMAML2. All animal protocols were approved by the University Committee on Use and Care of Animals of the University of Michigan (Animal Welfare Assurance Number A3114-01).

2.2. Southern blotting

Genomic DNA was extracted from a variety of tissues and digested with BamH1, separated by electrophoresis, transferred to nylon membrane, and hybridized with a ^{32}P-labled DNA probe as described before [22,23]. The results were captured using a PhosphorImager screen (Bio-Rad Laboratories, Hercules, California).

2.3. Genotyping PCR

Tail DNA was extracted and traditional PCR was used to detect floxed or recombined allele.

2.4. Flow cytometry analysis of peripheral blood

Tail vein blood samples were collected in heparinized capillary tubes. After erythrocyte lysis, blood cells were blocked with FcBlock (BD Biosciences, San Jose, California) and stained with antibodies for flow cytometry (APC Anti-CD115, APC-Alexa750 Anti-Gr-1, and PE Anti-CD8 all from Ebioscience, San Diego, California). Cells were analyzed on a FACSCanto II system (BD Bioscience).

3. Results

3.1. SM22α-Cre is expressed in spleen, peripheral blood, and peritoneal macrophages

We first evaluated the specificity of SM22α-Cre in both SM-PGKO and DNMAML1 mice using Southern blotting and/or genotyping PCR. Southern blotting results showed that in SM-PGKO mice, the recombined allele was present in smooth muscle-enriched tissues such as aorta, bladder, heart, mesenteric tissue, stomach, uterus, and lungs as expected (Fig. 1A). Surprisingly, the recombined allele was also present in high percentage in the spleen, demonstrating the expression of SM22α-Cre(4746). Although the spleen does not contain large numbers of SMCs, it is capable of contraction and smooth muscle myosin has been seen in other splenic cells in addition to vascular SMCs [17]. Still, because the majority of cells in the spleen are of immune origin and high degree of recombination was detected, we investigated whether immune cells also demonstrated recombination. Genotyping PCR detected the recombined allele not only in aorta but also in the peripheral blood and peritoneal macrophages of both SM-PGKO and DNMAML1 mice, demonstrating the expression of SM22α-Cre(4746 and 6878) in these tissues/cells (Fig. 1B, C).

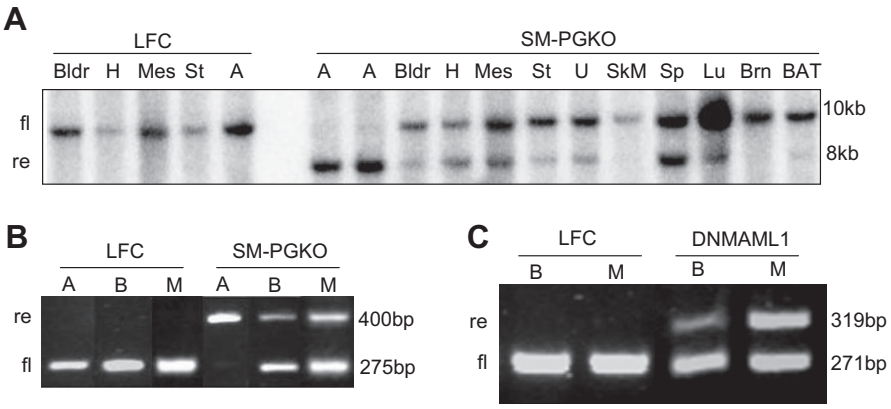

Fig. 1. SM22α-Cre is expressed in spleen, peripheral blood, and peritoneal macrophages. (A) Southern blotting results of a variety of tissues from LFC and SM-PGKO mice produced by crossing SM22α-Cre(4746) mice with floxed PPAR-γ mice. LFC: littermate floxed control; SM-PGKO: smooth muscle specific PPAR-γ knockout; fl: floxed allele; re: recombined allele. A: aorta; BAT: brown adipose tissue; Bldr: bladder; Brn: brain; H: heart; Lu: lung; Mes: mesenteric tissue; SkM: skeletal muscle; Sp: spleen; St: stomach; U: uterus. (B) PCR genotyping results of tissues from LFC and SM-PGKO mice. A: aorta; B: blood; M: macrophage (peritoneal). (C) PCR genotyping results of tissues from LFC and DNMAML1 mice produced by crossing SM22α-Cre(6878) mice with floxed DNMAML-GFP mice. For all experiments similar results were obtained from 2–3 independent mice and representative analysis is shown. The results of littermate Cre control (LCC) were similar to those of LFC.

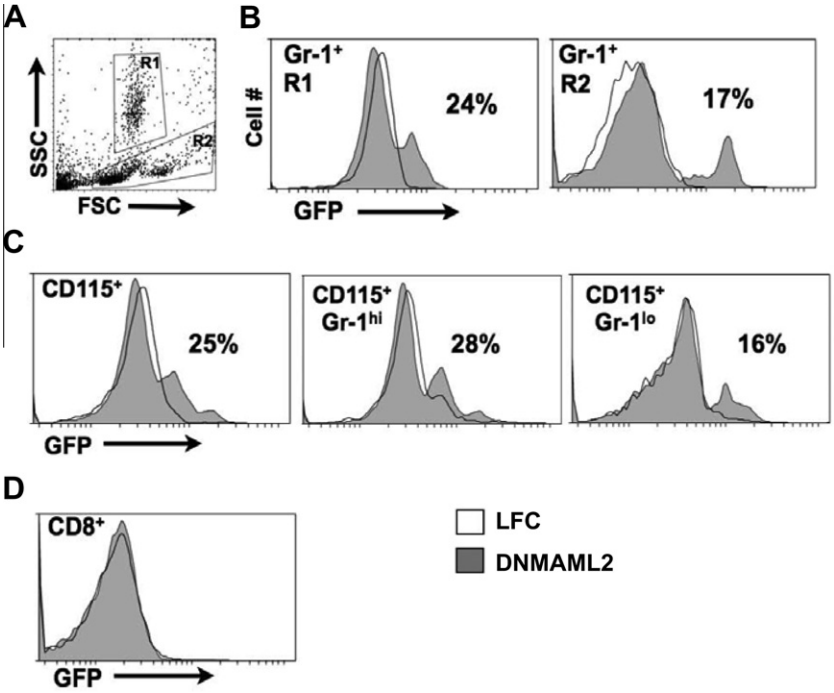

Fig. 2. SM22α-Cre is expressed in neutrophils and monocytes in peripheral blood. (A) Forward (FSC) and side scatter (SSC) plots from peripheral blood with gates used for neutrophils and monocytes/lymphocytes. (B) GFP expression in Gr-1$^+$ cells in neutrophils (R1) or monocytes/lymphocytes (R2) gates from blood of LFC and DNMAML2 mice produced by crossing SM22α-Cre(4746) mice with floxed DNMAML-GFP mice. Percentage of GFP$^+$ cells in DNMAML2 mice is shown. LFC: littermate floxed control. (C) GFP expression in CD115$^+$ cells demonstrates SM22α-Cre expression in circulating monocytes. CD115$^+$ cells were further assessed for Gr-1 expression delineating monocyte subtypes. hi: high; lo: low. (D) GFP is not expressed in CD8$^+$ lymphocytes. For all experiments similar results were obtained from 2–3 independent mice and representative analysis is shown. The results of littermate Cre control (LCC) were similar to those of LFC.

3.2. SM22α-Cre is expressed in neutrophils and monocytes in peripheral blood

To identify which populations of peripheral blood cells express SM22α-Cre(4746), we used flow cytometry to examine the expression of GFP in blood samples from DNMAML2 and their control mice. The floxed DNMAML-GFP is ubiquitously expressed because Rosa 26 locus was used to generate the floxed mice [18,19]. Therefore, any cell type that expresses the Cre will have GFP expression to show recombination. Initial analysis showed that a portion of Gr-1$^+$ neutrophils and monocytes/lymphocytes in the circulation were GFP$^+$ in DNMAML2 mice (Fig. 2A and B). LFC and LCC mice did not show GFP expression in either of these cell populations. To determine whether SM22α-Cre(4746) is expressed in peripheral monocytes or lymphocytes, we used CD115 to label monocytes specifically and detected GFP expression in CD115$^+$ cells, demonstrating the expression of SM22α-Cre in this subpopulation (Fig. 2C). Further analysis showed that SM22α-Cre(4746) was activated in a subset of Gr-1^{hi} and Gr-1^{lo} monocytes in DNMAML2 mice. However, GFP was not expressed in CD8$^+$ lymphocytes from DNMAML2 mice, indicating that SM22α-Cre(4746) was not expressed in this population (Fig. 2D).

4. Discussion

Smooth muscle specific Cre is an important tool to study gene functions in smooth muscle cells [10–16]. However, its specificity has not been fully investigated, although expression in cardiomyocytes and epithelial cells had been noticed [11,15,16,20]. In this study, we have demonstrated that SM22α-Cre, a frequently used smooth muscle specific Cre, is expressed in a portion of myeloid cells including neutrophils, monocytes, and macrophages. Such expression will lead to genetic changes in these cells, as well as in SMCs, when SM22α-Cre is used in combination with floxed mice. In this circumstance, therefore, it will be impossible to unequivocally attribute the resulting phenotypes to changes in SMCs, myeloid cells or both. Given the importance of both cell types in vascular development and cardiovascular disease, this is a critical distinction. In order to circumvent this potential problem and restrict genetic changes to SMCs, bone marrow transplantation may be useful to replace the recombined circulating myeloid cells to determine if they contribute to the resultant phenotypes.

Disclosures

None.

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