Int J Physiol Pathophysiol Pharmacol 2010;2(1):12-19
www.ijppp.org /IJPPP911003

Original Article

Sm22α transcription occurs at the early onset of the cardiovascular system and the intron 1 is dispensable for its transcription in smooth muscle cells during mouse development

Maozhou Yang[1, 4], Hong Jiang[1], and Li Li[1, 2, 3]

[1]Dept of Internal Medicine, Wayne State University, Detroit MI 48201 ;[2]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI 48201, USA; [3]Cardiovascular Research Institute, Wayne State University, Detroit, MI 48201, USA; [4]Current address: Bone and Joint Center, Henry Ford Hospital, 2799 West Grand Blvd., Detroit, MI 48202, USA

Received November 11, 2009; accepted November 18, 2009; available online November 22, 2009

Abstract: SM22α, also known as SM22, has been widely used as a smooth muscle cell (SMC) marker and is known to be expressed in the embryonic heart. The intron 1 of *Sm22* contains multiple important and evolutionarily conserved regulatory elements. To determine the role of the intron 1 in *Sm22* transcriptional regulation and the function of SM22 during development, we generated a *Sm22* knockout mouse by replacing the intron 1 and the translation initiation with a nuclear localized *LacZ* (*nLacZ*) reporter. The resulting *Sm22* knockout mice (*Sm22*$^{-/-}$) were viable and fertile without any apparent developmental defects. Using X-gal staining assay, we found that *Sm22* transcription was detectable in the chorion formation region and in the heart field before formation of the heart tube at E7.5, namely much earlier than the looped heart stage where it had been previously reported. The expression of lacZ progressively expanded throughout the heart tube by E8.5. LacZ was transiently expressed in the heart and somites and then became restricted to the vascular and visceral SMC organs. These results indicate that SM22 is not required for mouse basal homeostatic function and that the intron 1 is dispensable for *Sm22* transcription during development. Given the importance of vasculature in organogenesis and in diseases, this mouse line may be a valuable tool to trace the development and pathology of the cardiovascular system.

Key words: SM22α, Transgelin, smooth muscle cells, intron 1, knockout mouse, cardiac crescent

Introduction

SM22α, also known as transgelin or simply as SM22 , is a 22 KD protein highly expressed in vascular and visceral smooth muscle cell (VSMC) tissues and its expression is sensitive to cell shape changes [1-3]. SM22 is a member of the calponin family, containing a calponin homology domain conserved from yeast to human [4, 5]. SM22 directly binds to the actin cytoskeleton and induces actin bundling [2, 4-6]. However, little is known about the function of SM22 in SMC development.

The transcription of *Sm22* is highly expressed in the cardiovascular system during embryogenesis [7-11]. Specifically, the *Sm22* promoter is highly expressed in the heart tube and selectively expressed in a subset of arterial SMCs, but not in venous or visceral SMCs. However, it has not been known whether *Sm22* transcription is expressed in the heart fields before formation of the heart tube.

Several regulatory elements that regulate *Sm22* transcription have been characterized in transgenic mice. The CArG boxes (the SRF binding site), especially the proximal CArG box, play a central role in controlling the expression of the *Sm22* promoter in arterial SMCs [12, 13]. The TCE (TGFB Control Element) and the SBE site (a Smad Binding Site)

are found to be important for *Sm22* transcription during embryogenesis in transgenic mice [14, 15]. Interestingly, a G/C-rich element (a SP1 binding site) in the *Sm22* promoter is dispensable for *Sm22* transcription in arterial SMCs but is required for the down regulation of *Sm22* transcription in response to vascular injury [16]. Given the complexity of vascular development and pathogenesis of vascular diseases, much remains to be uncovered about the regulatory network that controls *Sm22* transcription.

In an ongoing effort to identify transcriptional regulatory elements for *Sm22* expression, we performed bioinformatics sequence analyses of *Sm22* and found that the intron 1 of *Sm22* contained multiple important evolutionarily conserved regulatory elements. The intron 1 of several SMC marker genes such as *SM α-actin, SM-MHC,* and *Calponin* contains critical regulatory elements for their transcription in SMCs *in vivo* [17-19]. To determine the role of the intron 1 of *Sm22* in transcriptional regulation in development, we generated *Sm22* knockout mice in which a nuclear localized *LacZ* reporter gene was knocked into the first intron of the *Sm22*. Consistent with previous reports [9, 20], SM22 deficiency did not affect mouse development; the knockout mice were viable and fertile. We analyzed the temporospatial patterns of LacZ activities in *Sm22* knockout mice and found that the expression of the *LacZ* reporter was detectable in the chorion formation region and in the heart field at E7.5. LacZ activities were transiently detected in the heart tube and somites during embryogenesis. The expression in the vascular and visceral tissues continuously increased throughout embryogenesis into adulthood. These results demonstrate that the regulatory elements in the intron 1 of *Sm22* are not essential for *Sm22* transcription during development. Given the importance of vasculature in organogenesis and in diseases, this mouse line may be a valuable tool to trace the development and pathology of the cardiovascular system.

Materials and methods

Generation of Sm22 mutant mice

A *Sm22* targeting vector was designed to replace the intron 1 and the translation initiation region of *Sm22* in exon2 with a nuclear localized *LacZ* and *pGKneo* cassette using a modified pKO-lacZ vector (a generous gift from L Gan, Rochester, NY) [21], in which a nuclear localization signal was inserted into the *LacZ/pGK-neo-TK* cassette. Genomic DNA fragments flanking the intron 1 and exon2 of the *Sm22* were PCR-amplified using the genomic DNA from a SV129 mouse as the template. The left arm fragment contained 5kb 5' upstream sequence and the entire exon 1; the right arm fragment contained the 4.5 kb *Sm22* genomic sequence starting at 63 nucleotides downstream of the SM22 translation initiation codon in exon 2. The left and right arms were inserted into the targeting vector pKO-nLacZ. Through homologous recombination, the intron 1 was substituted by the nLacZ-pGK-neo cassette, placing the expression of *LacZ* under the control of the endogenous *Sm22* promoter without the intron 1.

The targeting vector was linearized at the NotI site and was injected into SV129 derived ES cells. G418-resistant ES colonies with correct homologous recombination were identified by PCR genotyping and Southern blot using a probe 3' to *Sm22*. The $Sm22^{+/-}$ mice were backcrossed into B6 and SV129 genetic background for at least 4 generations. The $Sm22^{+/-}$ mice were maintained in mixed genetic background for phenotype analyses. The targeted ES cells and *Sm22* knockout chimera mice were generated in Dr. Beverly Koller's lab at the University of North Carolina.

The wild type (WT), $Sm22^{+/-}$ and $Sm22^{-/-}$ mice were identified by PCR using allele-specific primers: "a" (5'CCCAGCCCAGACACCGAAGCTA C 3' in exon 1), "b"(5' TCCCTTGGCCTCATTTGT CACCTC 3' in intron 1), and "c" (5' TACCACA GCGGATGGTTCGG 3' in *lacZ* gene). "d" (GTGGA AGGCCTGCTTAGCACAAAT in intron 1) "e" ACTC ACCACACCATTCTTCAGCCA in exon2). The PCR products were 1.35kb (for the targeted allele), 313bp and 303bp (for the WT allele) using primers a/c, a/b and d/e respectively. The PCR amplification was performed in 30 cycles by denaturation at 95°C for 15", annealing at 60°C for 30", and elongation at 72°C for 1.5 min.

All animal experimentation was performed according to the National Institutes of Health guidelines and approved by the *Institutional Animal Care and Use Committee* (*IACUC)* at Wayne State University.

Western blot

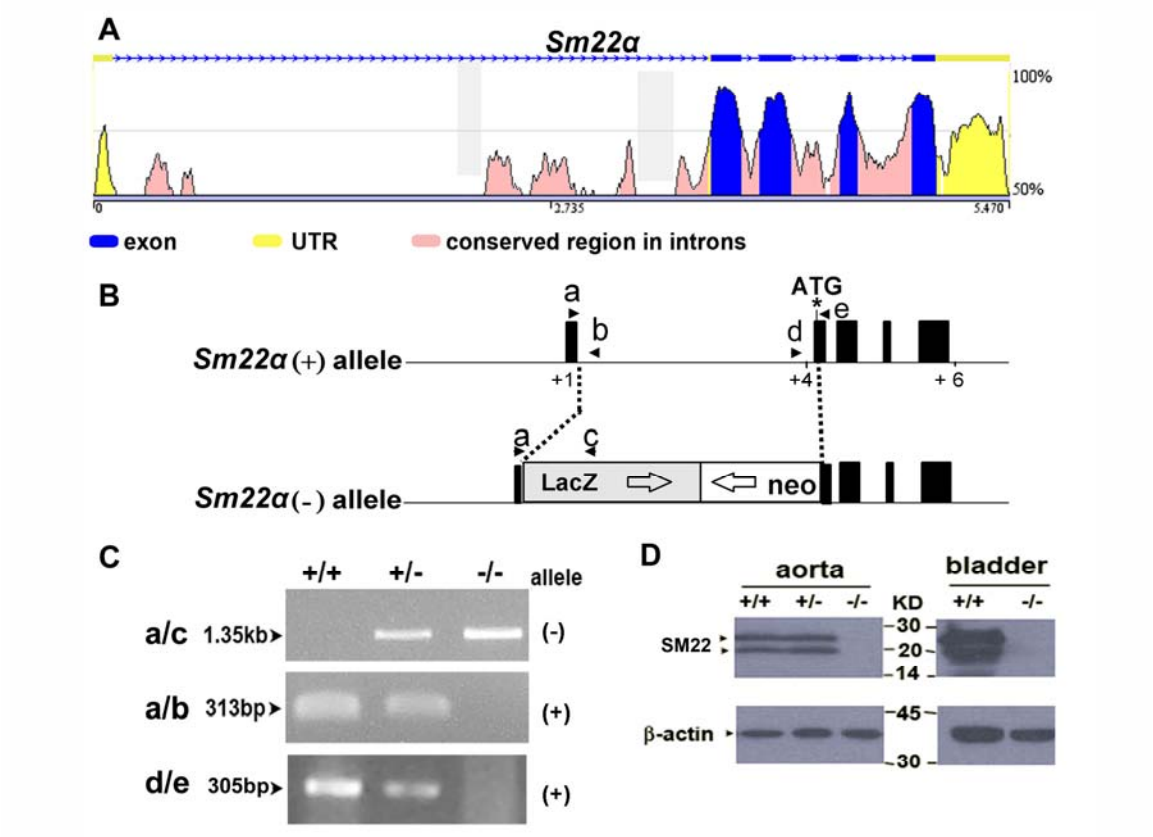

Figure 1. Generation of *Sm22α* knockout mice. (A) VISTA genome browser output for sequence comparison of the mouse *Sm22* with the human *SM22*. The exons (in blue) including the UTRs (in yellow) are highly conserved between mouse and human. The intron 1 contains several evolutionarily conserved regions (in pink). (B) The wild type and the targeted mouse *Sm22α* allele are shown. Homologous recombination resulted in the deletion of the intron 1 and *Sm22* translation initiation region in the *Sm22α* and its replacement with a lacZ-pGKneo cassette. The symbol * denotes the translation initiation site ATG for SM22. (C) The *Sm22* knockout (*Sm22*$^{-/-}$) mice can be identified by PCR genotyping using primer pairs (a/b, d/e, and a/c) specific to the wild type and targeted alleles. (D) Western blot analyses showed the expression of SM22 in the aorta and bladder from the WT (+/+) and the heterozygous (+/-) but not from the homozygous (-/-) *Sm22* mice. The expression of β-actin was used as the internal control.

SMC tissues were isolated from WT, *Sm22*$^{+/-}$, and *Sm22*$^{-/-}$ mice. Equal amount (20 μg) lysates were used for SDS-polyacrylamide gel electrophoresis (PAGE) analyses. The SDS-PAGE gel was transferred to Immun-Blot PVDF Membrane (Bio-Rad) and subsequently probed with anti-SM22α goat polyclonal antibody (Santa Cruz, sc-18513) and anti β-actin monoclonal antibodies (Sigma, A5316). Primary antibodies were detected using NEN Chemiluminescence Western Blotting substrates (NEN, NEL102).

X-gal staining

The expression of *LacZ* in *Sm22*$^{+/-}$ *mice* was detected by X-gal (β-glactosidase) staining. Mouse *Sm22*$^{+/-}$ embryos at different developmental stages and tissues from *Sm22*$^{+/-}$ mice were collected by breeding CD1 female with *Sm22*$^{-/-}$ male. X-gal staining was performed as previously described [8]. After X-gal staining, the tissues were fixed in 4% paraformaldehyde. The paraffin sections were then counterstained with H&E before being photographed.

Results and discussion

Targeting strategy for generating the Sm22 knockout mouse

Sequence analyses of *Sm22* revealed that its intron 1 contained several evolutionarily con-

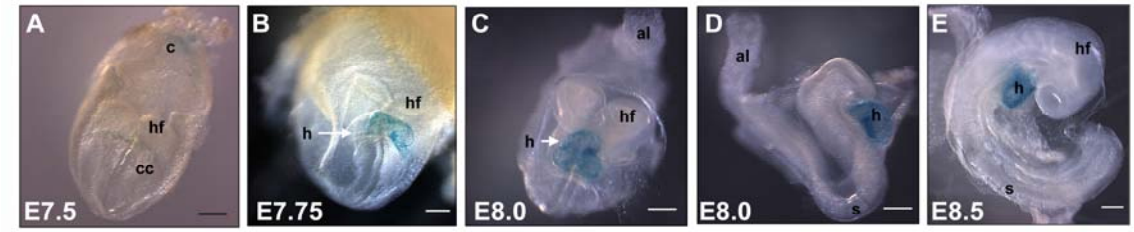

Figure 2. Transcriptional activation of *LacZ* in the early cardiovascular system. The *Sm22*$^{+/-}$ embryos at E7.5-E8.5 were harvested for whole-mount X-gal staining. (A) At E7.5, X-gal staining was detected in the chorion (c) formation area and in cells lying medial and immediately cranial to the cardiac crescent (cc). (B) At E7.75, LacZ activities became high in the rapidly growing heart tube (h) caudal to the head fold (hf). (C-D) were two views of the same embryo at E8.0. The heart tube had been looped to form distinct heart chambers. The allantois (al) was growing towards the chorion region. (E) At E8.5, the LacZ activities were not detectable in the aorta and somites (s). Scale bar: 200 μm.

served regions (**Figure 1A**). We were interested in characterizing the regulatory network for *Sm22* transcription and in determining the function of SM22 in cardiovascular development. Therefore, our strategy of generating a *Sm22* knockout mouse was to replace the intron 1 and the translation initiation of *Sm22* with a nuclear localized *LacZ* reporter (**Figure 1B**). Homologous recombination resulted in the deletion of intron 1 in the *Sm22* and placed the LacZ-pGKneo cassette under the control of the endogenous *Sm22* promoter without the intron 1. We anticipated that the expression of *Sm22* would be abolished in *Sm22*$^{-/-}$ mice. We also expected that analyses of the phenotypes of *Sm22*$^{-/-}$ mice would reveal the functional role of SM22 in development. The role of intron 1 in *Sm22* transcriptional regulation could be determined by the expression of LacZ using X-gal staining.

The wild type, *Sm22*$^{+/-}$, and *Sm22*$^{-/-}$ mice were identified by allele-specific PCR genotyping (**Figure 1C**). The loss of SM22 in SMC tissues such as the aorta and the bladder from the *Sm22*$^{-/-}$ were confirmed by Western blot

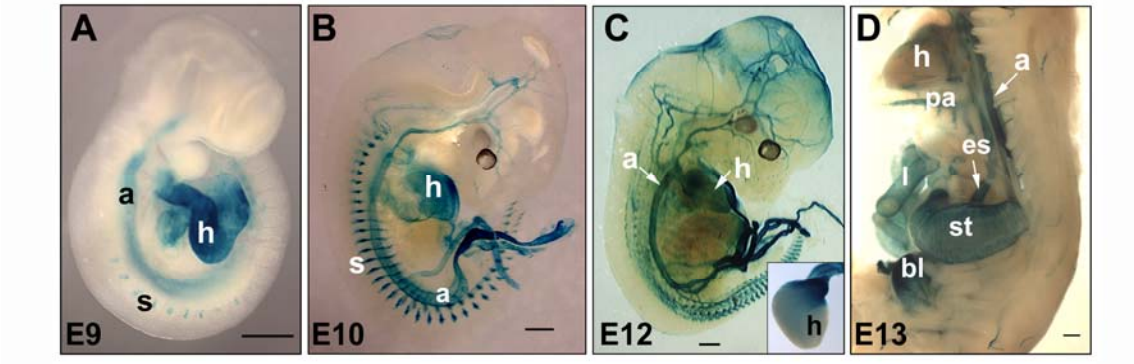

Figure 3. The LacZ temporospatial expression patterns in *Sm22*$^{+/-}$ mice from E9 to E13. The *Sm22*$^{+/-}$ embryos at E9-E13 were harvested for whole-mount X-gal staining. (A) The LacZ activities began to be detectable in the heart (h), aorta (a) and somites (s) at E9. (B) The embryo at E10 was stained in X-gal solution followed by dehydration and clearing in benzoate/benzyl alcohol (BBA) solution. The expression of LacZ increased in the somites (s), aorta (a) and its arteries. (C) At E12, the expression of LacZ in the heart (h) and the somites (s) was diminishing. The insert showed the heart with the aorta isolated from the same embryo. The LacZ expression was clearly seen in the arteries extended into the brain. (D) At E13, the expression of LacZ disappeared from the heart and somites, and became restricted to arterial and visceral SMC tissues such as the aorta (a), pulmomary arteries in the lung (pa), the esophagus (es) into the stomach (st), the intestine (l) and the bladder (bl). Scale bar: 500 μm.

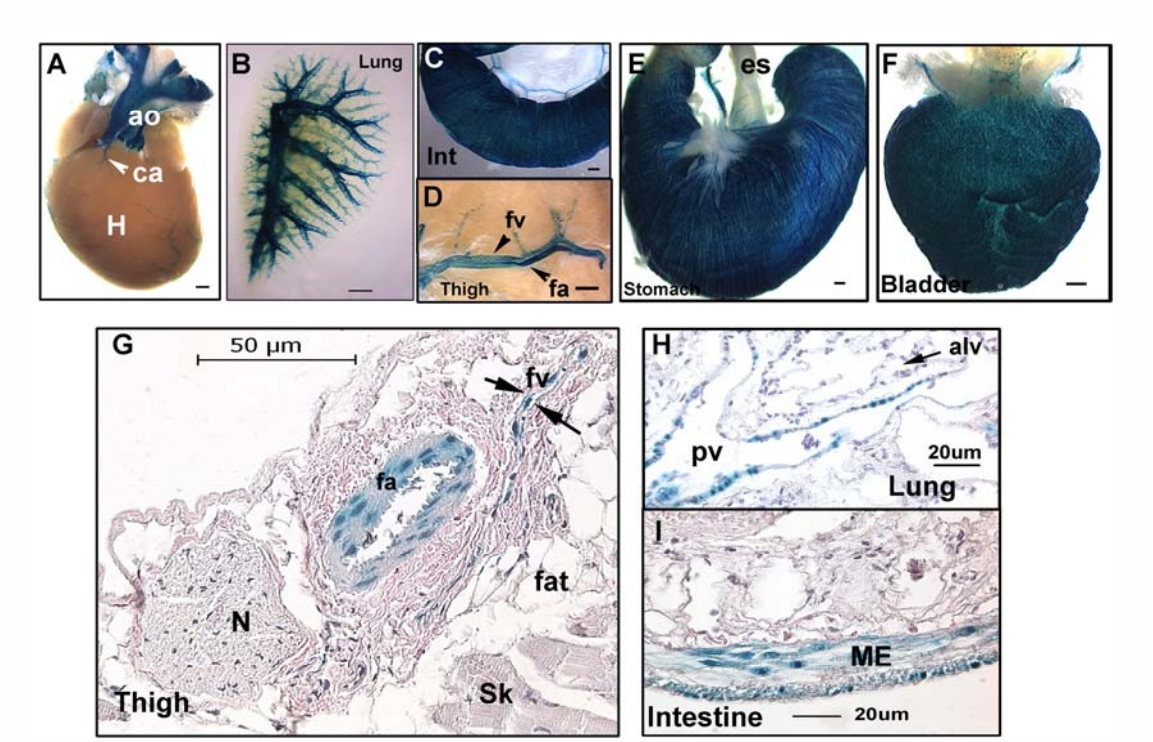

Figure 4. LacZ expression in adult *Sm22*$^{-/-}$ mice. A variety of tissues were harvested from the adult *Sm22*$^{+/-}$ mice. X-gal staining assay showed that LacZ expression was restricted to SMC tissues in the heart (A), lung (B), intestine (C), thigh (D), stomach (E) and the bladder (F). H&E of sections from the thigh (G), lung (H) and intestine (I) were shown. Abbreviations: alveoli (alv), aorta (ao), coronary artery (ca), esophagus (es), femoral artery (fa), femoral vein (fv), heart (H), intestine (I), muscularis externa (ME), nerve fiber (N), pulmonary vein (pv), and skeletal muscle (sk). Scale Bar (in A-F): 500 μm.

(**Figure 1D**). In agreement with previous studies [9, 20], *Sm22*$^{-/-}$ mice were viable and fertile with no obvious phenotypes during normal development. These results suggest that SM22 is not required for development and for the basal homeostatic functions of SMCs.

Temporospatial expression patterns of LacZ in the early cardiovascular system

Previous studies showed that the transcription of *Sm22* is high in the heart at E8.0 [7-9]. The cardiogenesis arises from progenitors in the cardiac crescent region and the secondary heart field at E7.5 [22-24]. To determine whether *Sm22* transcription occurs in early cardiogenesis, we examined the LacZ expression in a series of timed *Sm22*$^{+/-}$ embryos at E6.0 to E8.5.

At E7.5, LacZ activities were detectable in the chorion formation area and in the cells lying medial and immediate cranial to the cardiac crescent (**Figure 2A**). The precise fate of these cells remains to be determined. Consistent with the highly dynamic nature of cardiogenesis at this stage, the expression of *LacZ* expanded rapidly and progressively towards the arterial pole as the heart tube forms (**Figure 2B-D**). By E8.5, LacZ activities were highly expressed in the heart (**Figure 2E**).

Transcriptional expression patterns of the Sm22 during embryogenesis and in the adult

The *LacZ* reporter was highly expressed in the looped heart at E9.0 (**Figure 3A**). In addition, LacZ activities in the aorta and somites became evident at this stage. LacZ activities continued increasing in the aorta and somites at E10 (**Figure 3B**). The expression of *LacZ* in the heart and somites diminished while the expression in the aorta and in the branches of arteries throughout the embryos became

prominent at E12 (**Figure 3C**). At E13, a noticeable high level of LacZ expression was observed in the aorta and arteries as well as in the newly formed visceral organs such as esophagus, stomach, intestine and bladder (**Figure 3D**).

LacZ expression increased continuously in vascular and visceral SMC organs throughout embryogenesis and into adulthood. In adults, X-gal staining showed LacZ activities in the aorta and coronary arteries but not in the myocardium (**Figure 4A**). In the lung, LacZ expression was high in the pulmonary arteries and pulmonary veins but not in the simple squamous epithelium of alveoli (**Figure 4B**, **4G**). LacZ activities were high in visceral SMC organs such as the stomach and intestine, but not the esophagus in the digestion system (**Figure 4C, 4E**). LacZ expression was also high in the bladder (**Figure 4F**). Histology analyses showed that the LacZ activities were specifically expressed in arterial, venous and visceral SMCs. In the thigh, LacZ expression was observed in the femoral artery, femoral vein, but not the nerve fiber, fat and skeletal muscles (**Figure 4D, 4G**). In the intestine, LacZ expression was detected in the muscularis externa consisting of both circular and longitudinal smooth muscle layers (**Figure 4I**).

Taken together, the temporospatial expression patterns of *LacZ* reporter recapitulate those observed in transgenic and knockout mice in which the *LacZ* is under the control of *Sm22* promoters and the endogenous *Sm22* promoter [8-11, 25]. These results suggest that the intron 1 is not required for *Sm22* transcription during SMC development.

In summary, this study demonstrated that the transcription of *Sm22* occurred at the onset of cardiogenesis and vasculogenesis during development, at a stage much earlier than previously reported. Although the intron 1 contained multiple evolutionarily conserved regulatory elements, it appeared that these elements were not essential for *Sm22* transcription under normal development. Consistent with this notion, the *Sm22* promoters without or with the intron 1 have shown similar temporospatial expression patterns during development [8, 11, 12, 16, 25, 26]. Given the complexity of gene regulation in SMC phenotypic modulation [27], it is quite possible that some of these regulatory elements may participate in *Sm22* transcriptional regulation under pathological conditions. Several SMC regulatory elements such as the SP1 in the *Sm22* promoter and the CArGs in the *SM α-actin* promoter have been shown to be dispensable for the promoter activities in arteries but are required for the down regulation of the promoter activities in response to vascular injuries [16, 28]. Therefore, it is worthwhile to explore whether those evolutionarily conserved elements within the intron 1 are required for the down regulation of *Sm22* transcription in response to vascular injury.

Increasing evidence supports the notion that the actin cytoskeleton plays important roles in SMC phenotypic modulation [29, 30]. SM22 is an actin binding protein highly expressed in SMCs and in the early cardiovascular system. In view of this, it is rather unexpected that SM22 is not required for mouse basal homeostatic function. Given the importance of the SM22 associated actin cytoskeleton in cell function, it is possible that the function of SM22 can be compensated through molecular redundancy. Indeed, SM22α and its homologue SM22β colocalize with the cytoskeletal actin filaments; it is thus likely that SM22β can compensate for the loss of SM22α [31, 32]. However, the defects of this compensation system may be revealed under pathological conditions. The analyses of *Sm22*$^{-/-}$ mice showed that ablation of SM22 reduces SMC contractility [32, 33] and increases atherosclerotic plaques in the *ApoE*$^{-/-}$ mice [20].

Although SM22 is not required for SMC homeostatic functions, SM22 may participate in SMC phenotypic modulation. Consistent with this notion, *Sm22* expression is sensitive to cell shape change and is down regulated in vascular diseases and a variety of cancers [2, 34, 35]. Understanding the molecular mechanisms whereby SM22 mediates cytoskeleton remodeling may reveal that SM22 plays an active role in biological processes involved in the pathogenesis of vascular diseases and cancer.

Conclusion

This study demonstrates that the transcription of *Sm22* occurs at the early onset of cardiogenesis and vasculogenesis. In spite of its high level of expression in the early heart, deficiency of SM22 in mouse does not perturb development. The evolutionarily conserved elements in the intron 1 of *Sm22* are not required

for SM22 expression in smooth muscle cells during development.

Acknowledgments

We are grateful to Alex Gow for sharing the dissection microscope camera and Giuseppe Rossi for editorial assistance. We are thankful to Jianbin Shen, Donghong Ju, Jianpu Zheng, Da-zhi Wang, and Randy Armant for valuable discussions. The work in Li's Laboratory was supported by grants from the NIH (HL058916 and HL087014 to L.L) and from American Heart Association (0555680Z to L.L.).

Please address correspondence to: 421 E. Canfield Ave. Room #1107, Detroit MI 48201, USA. Tel: 313-577-8749, FAX: 313-577-8615; E-mail: lili@med.wayne.edu.

References

[1] Lees-Miller JP, Heeley DH and Smillie LB. An abundant and novel protein of 22 kDa (SM22) is widely distributed in smooth muscles. Purification from bovine aorta. Biochemical Journal 1987; 244: 705-709.

[2] Shapland C, Hsuan JJ, Totty NF and Lawson D. Purification and properties of transgelin: a transformation and shape change sensitive actin-gelling protein. J Cell Biol 1993; 121: 1065-1073.

[3] Shapland C, Lowings P and Lawson D. Identification of new actin-associated polypeptides that are modified by viral transformation and changes in cell shape. J Cell Biol 1988; 107: 153-161.

[4] Fu Y, Liu HW, Forsythe SM, Kogut P, McConville JF, Halayko AJ, Camoretti-Mercado B and Solway J. Mutagenesis analysis of human SM22: characterization of actin binding. J Appl Physiol 2000; 89: 1985-1990.

[5] Winder SJ, Jess T and Ayscough KR. SCP1 encodes an actin-bundling protein in yeast. Biochem J 2003; 375: 287-295.

[6] Gimona M, Kaverina I, Resch GP, Vignal E and Burgstaller G. Calponin repeats regulate actin filament stability and formation of podosomes in smooth muscle cells. Mol Biol Cell 2003; 14: 2482-2491.

[7] Li L, Miano JM, Cserjesi P and Olson EN. SM22 alpha, a marker of adult smooth muscle, is expressed in multiple myogenic lineages during embryogenesis. Circulation Research 1996; 78: 188-195.

[8] Li L, Miano JM, Mercer B and Olson EN. Expression of the SM22alpha promoter in transgenic mice provides evidence for distinct transcriptional regulatory programs in vascular and visceral smooth muscle cells. J Cell Biol 1996; 132: 849-859.

[9] Zhang JC, Kim S, Helmke BP, Yu WW, Du KL, Lu MM, Strobeck M, Yu Q and Parmacek MS. Analysis of SM22alpha-deficient mice reveals unanticipated insights into smooth muscle cell differentiation and function. Mol Cell Biol 2001; 21: 1336-1344.

[10] Moessler H, Mericskay M, Li Z, Nagl S, Paulin D and Small JV. The SM 22 promoter directs tissue-specific expression in arterial but not in venous or visceral smooth muscle cells in transgenic mice. Development 1996; 122: 2415-2425.

[11] Kim S, IP HS, Lu MM, Clendenin C and Parmacek MS. A serum response factor-dependent transcriptional regulatory program identifies distinct smooth muscle cell sublineages. Molecular and Cellular Biology 1997; 17: 2266-2278.

[12] Li L, Liu Z, Mercer B, Overbeek P and Olson EN. Evidence for serum response factor-mediated regulatory networks governing SM22alpha transcription in smooth, skeletal, and cardiac muscle cells. Developmental Biology 1997; 187: 311-321.

[13] Strobeck M, Kim S, Zhang JC, Clendenin C, Du KL and Parmacek MS. Binding of serum response factor to carg box sequences is necessary but not sufficient to restrict gene expression to arterial smooth muscle cells. J Biol Chem 2001; 276: 16418-16424.

[14] Adam PJ, Regan CP, Hautmann MB and Owens GK. Positive- and negative-acting Kruppel-like transcription factors bind a transforming growth factor beta control element required for expression of the smooth muscle cell differentiation marker SM22alpha in vivo. J Biol Chem 2000; 275: 37798-37806.

[15] Qiu P, Ritchie RP, Fu Z, Cao D, Cumming J, Miano JM, Wang DZ, Li HJ and Li L. Myocardin enhances Smad3-mediated transforming growth factor-beta1 signaling in a CArG box-independent manner: Smad-binding element is an important cis element for SM22alpha transcription in vivo. Circ Res 2005; 97: 983-991.

[16] Regan CP, Adam PJ, Madsen CS and Owens GK. Molecular mechanisms of decreased smooth muscle differentiation marker expression after vascular injury. J Clin Invest 2000; 106: 1139-1147.

[17] Mack CP and Owens GK. Regulation of smooth muscle alpha-actin expression in vivo is dependent on CArG elements within the 5' and first intron promoter regions. Circ Res 1999; 84: 852-861.

[18] Manabe I and Owens GK. CArG elements control smooth muscle subtype-specific expression of smooth muscle myosin in vivo. J Clin Invest 2001; 107: 823-834.

[19] Miano JM, Carlson MJ, Spencer JA and Misra RP. Serum response factor-dependent regulation of the smooth muscle calponin gene. J Biol Chem 2000; 275: 9814-9822.

[20] Feil S, Hofmann F and Feil R. SM22alpha mod-

ulates vascular smooth muscle cell phenotype during atherogenesis. Circ Res 2004; 94: 863-865.
[21] Gan L, Wang SW, Huang Z and Klein WH. POU domain factor Brn-3b is essential for retinal ganglion cell differentiation and survival but not for initial cell fate specification. Dev Biol 1999; 210: 469-480.
[22] Harvey RP. Patterning the vertebrate heart. Nat Rev Genet 2002; 3: 544-556.
[23] Harvey RP, Meilhac SM and Buckingham ME. Landmarks and lineages in the developing heart. Circ Res 2009; 104: 1235-1237.
[24] Buckingham M, Meilhac S and Zaffran S. Building the mammalian heart from two sources of myocardial cells. Nat Rev Genet 2005; 6: 826-835.
[25] Xu R, Ho YS, Ritchie RP and Li L. Human SM22 alpha BAC encompasses regulatory sequences for expression in vascular and visceral smooth muscles at fetal and adult stages. Am J Physiol Heart Circ Physiol 2003; 284: H1398-1407.
[26] Kuhbandner S, Brummer S, Metzger D, Chambon P, Hofmann F and Feil R. Temporally controlled somatic mutagenesis in smooth muscle. Genesis 2000; 28: 15-22.
[27] Kawai-Kowase K and Owens GK. Multiple repressor pathways contribute to phenotypic switching of vascular smooth muscle cells. Am J Physiol Cell Physiol 2007; 292: C59-69.
[28] Hendrix JA, Wamhoff BR, McDonald OG, Sinha S, Yoshida T and Owens GK. 5' CArG degeneracy in smooth muscle alpha-actin is required for injury-induced gene suppression in vivo. J Clin Invest 2005; 115: 418-427.
[29] Gunst SJ and Zhang W. Actin cytoskeletal dynamics in smooth muscle: a new paradigm for the regulation of smooth muscle contraction. Am J Physiol Cell Physiol 2008; 295: C576-587.
[30] Tang DD and Anfinogenova Y. Physiologic properties and regulation of the actin cytoskeleton in vascular smooth muscle. J Cardiovasc Pharmacol Ther 2008; 13: 130-140.
[31] Zhang JC, Helmke BP, Shum A, Du K, Yu WW, Lu MM, Davies PF and Parmacek MS. SM22beta encodes a lineage-restricted cytoskeletal protein with a unique developmentally regulated pattern of expression. Mech Dev 2002; 115: 161-166.
[32] Je HD and Sohn UD. SM22alpha is required for agonist-induced regulation of contractility: evidence from SM22alpha knockout mice. Mol Cells 2007; 23: 175-181.
[33] Zeidan A, Sward K, Nordstrom I, Ekblad E, Zhang JC, Parmacek MS and Hellstrand P. Ablation of SM22alpha decreases contractility and actin contents of mouse vascular smooth muscle. FEBS Lett 2004; 562: 141-146.
[34] Assinder SJ, Stanton JA and Prasad PD. Transgelin: an actin-binding protein and tumour suppressor. Int J Biochem Cell Biol 2009; 41: 482-486.
[35] Shanahan CM, Cary NR, Metcalfe JC and Weissberg PL. High expression of genes for calcification-regulating proteins in human atherosclerotic plaques. J Clin Invest 1994; 93: 2393-2402.