SM22α Modulates Vascular Smooth Muscle Cell Phenotype During Atherogenesis

Susanne Feil, Franz Hofmann, Robert Feil

The function of cytoskeletal proteins in the modulation of vascular smooth muscle cell (SMC) phenotype during vascular disease is poorly understood. In this report, we used a combination of gene targeting and Cre/lox-mediated cell fate mapping in mice to investigate the role of SM22α, an SMC-specific cytoskeletal protein of unknown function, in the development of atherosclerosis. In hypercholesterolemic ApoE-deficient mice, genetic ablation of SM22α resulted in increased atherosclerotic lesion area and a higher proportion of proliferating SMC-derived plaque cells. These results identify a role for SM22α in the regulation of SMC phenotype during atherogenesis.

SM22α (also known as transgelin, WS3-10, or p27) is a 22-kDa protein that is considered a marker of contractile smooth muscle cells (SMCs) and is exclusively and abundantly expressed in SMCs of adult animals. It is structurally related to the actin-binding protein calponin and has been localized within the cytoskeletal apparatus (see review1). The analysis of SM22α-deficient mice showed that the protein is not required for the development and basal homeostatic functions of SMCs, suggesting that its function can be compensated by homologous proteins like calponin and SM22β.1 The role of SM22α under pathophysiological settings such as atherosclerosis was not studied yet. A critical step in atherogenesis is the phenotypic modulation of vascular SMCs from contractile to synthetic/proliferative cells,6 a process which may involve the cytoskeleton.6 In this study, we have investigated the function of SM22α in a mouse model of atherosclerosis by using gene targeting technology combined with temporally controlled Cre/lox-mediated activation of a reporter gene to visualize the fate of SMCs during plaque development.6,7

Materials and Methods

The Materials and Methods section is available in the online data supplement at http://circres.ahajournals.org.

Results and Discussion

The generation of mice carrying a SM22α knock-in allele, also designated SM-CreERT2(ki), has been described.8 The structures of the SM22α wild-type (+) and knock-in (−) allele are depicted in Figure 1A. The knock-in allele expresses the tamoxifen-activated CreERT2 recombinase instead of the endogenous SM22α gene. Western blot analysis demonstrated that the SM22α protein was expressed in SMC-containing tissues of SM22α+/- and SM22α−/− mice, but not in SM22α−/− (Figure 1B). Confirming previous results with another SM22α-deficient mouse line,2 the SM22α−/− mice generated in the present study were viable and fertile, and exhibited no obvious phenotype of SMC-containing tissues under basal conditions (data not shown).

As measured by the tail-cuff method, heart rate and mean blood pressure of SM22α−/− mice (644±19 bpm and 87±5 mm Hg) was not different from wild-type mice (620±24 bpm and 88±5 mm Hg).

To study the role of SM22α in the modulation of SMC phenotype during atherogenesis, control (CTR) and SM22α knockout (KO) mice were generated on an ApoE-deficient9 C57BL/6 genetic background (genotype: SM22α+/-; ApoE−/- and SM22α−/-; and ApoE−/-, respectively) and fed an atherogenic diet (20% fat, 1.5% cholesterol) for 8 or 16 weeks. The presence of the knock-in allele in both CTR and KO mice allowed us to control for potential unspecific effects related to CreERT2 expression as well as to label SMCs during atherogenesis (see later). SM22α was expressed in aortic SMCs of CTR mice and appeared to be downregulated in atherosclerotic lesions (Figure 1C), in agreement with published data.10 Body weight and plasma lipid levels were not significantly different between CTR and KO mice (online Table 1 in the online data supplement).

The mean atherosclerotic lesion area, as determined by Oil Red O staining of the aorta, was significantly elevated in KO mice compared with CTR mice (Figures 2A through 2D). In KO mice, the lesion area in the aortic arch (Figures 2A and 2C) after 8 and 16 weeks on the atherogenic diet, respectively, was increased by ~39% and ~27% in female animals, and by ~60% and ~28% in male animals. Similarly, the lesion area of KO mice in the brachiocephalic artery, left carotid artery, and left subclavian artery (Figures 2B and 2D) after 8 and 16 weeks on the atherogenic diet, respectively, was increased by ~82% and ~48% in female animals, and by ~65% and ~23% in male animals. These results indicated that SM22α inhibits plaque growth at various stages and sites of atherogenesis in both female and male mice. The relative increase in lesion area of KO versus CTR mice was higher after 8 weeks than 16 weeks, suggesting a role for SM22α in the initiation of SMC phenotypic modulation.

To determine the effect of SM22α deficiency on the properties of plaque SMCs in vivo, the fate of SMCs during the development of atherosclerotic lesions was followed in CTR and KO mice that carried the ROSA26 Cre reporter (R26R) allele11 (genotype: SM22α+/-; ApoE−/-; R26R−/− and SM22α−/-; ApoE−/-; and R26R−/−, respectively). The R26R allele encodes a nonfunctional lacZ gene, which is activated...
A targeted disruption and expression of the SM22α gene. A, Diagram of the SM22α wild-type (+) and knock-in (−) allele. Filled boxes denote exons and the open boxes represent the CreER T2 encoding sequence, a simian virus 40 polyadenylation signal (pA), and a neomycin-resistance gene (neo). B, Western blot analysis of SM22α protein expression in various tissues of adult SM22α−/−, SM22α+/−, and SM22α−/− mice. Blots were stained with a rabbit polyclonal antiserum to SM22α (bottom) or, to control loading of gels, to cGMP-dependent protein kinase type I (cGKI, top). Positions of molecular weight markers are indicated to the right. C, Immunohistochemical detection of SM22α on aortic sections (10 μm) of SM22α−/−; ApoE−/− mice. Photomicrographs show regions without (left) and with (right) an atherosclerotic plaque (bars=100 μm). No staining was detected in aortic sections of SM22α−/−; ApoE−/− mice (data not shown).

Acknowledgments
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References
Figure 2. Analysis of atherosclerosis and SMC fate in SM22α-deficient mice. Control (ctr) and SM22α knockout (ko) mice on an ApoE-deficient background were fed an atherogenic diet (20% fat, 1.5% cholesterol) beginning at 5 weeks of age. A, Representative photomicrographs of Oil Red O-stained atherosclerotic lesions in the aortic arch after 16 weeks on the atherogenic diet (bars=1 mm). B, Hematoxylin/eosin-stained sections (10 μm) of the brachiocephalic artery, left carotid artery, and left subclavian artery after 16 weeks on the atherogenic diet (bars=1 mm). C and D, Oil Red O-positive surface area in the aortic arch (bars=500 μm). E, SMC fate mapping by tamoxifen-induced recombination of the R26R allele and subsequent activation of lacZ expression. Four-week-old mice were injected with tamoxifen (1 mg tamoxifen IP for 5 consecutive days), the atherogenic diet was started at 5 weeks of age, and aortas were stained with X-Gal after 16 weeks on the diet. Panels show aortic sections (10 μm) of ctr (a’ through e’) and ko (f’ through j’) mice at equivalent positions in the ascending aorta. Serial sections of the boxed regions in a’ and f’ are shown in b’ through e’ and g’ through j’, respectively. Sections were stained with X-Gal (blue) for SMC-derived cells (a’ through d’; f’ through i’) and with a rabbit polyclonal antiserum to PCNA (brown) to detect proliferating cells (c’ and h’) or a rat monoclonal antibody to MAC-2 (brown) to detect macrophage-like cells (d’ and i’). Cell nuclei were visualized with Hoechst 33258 (e’ and j’). Bars=100 μm.


**Key Words:** mouse | vascular remodeling | phenotypic modulation | Cre recombinase | fate mapping
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Materials and Methods

LacZ activity was detected by staining aortas with X-Gal.\textsuperscript{1} Western blot analysis, immunohistochemistry, measurement of plasma lipids, Oil Red O-staining, and quantification of atherosclerotic lesion area was performed as described.\textsuperscript{2} Immunostaining was performed with rabbit polyclonal antisera to SM22\(\alpha\) (a gift from Mario Gimona) or proliferating cell nuclear antigen (PCNA, Santa Cruz Biotechnology), and with a rat monoclonal antibody to the macrophage marker, MAC-2 (Cedarlane). For immunodetection of SM22\(\alpha\) on aortic sections, antigen retrieval was accomplished by microwaving slides in 0.2 mol/L Na\textsubscript{2}HPO\textsubscript{4}, 0.1 mol/L Na\textsubscript{3}Citrate, pH 4.5 for 40 sec Data are presented as mean \(\pm\) SEM. Statistical analyses were performed by t-test or ANOVA, followed by Newman-Keuls post-hoc test. Significance was accepted if \(P < 0.05\).

References

Table 1. Physiological parameters after eight weeks on the atherogenic diet.

<table>
<thead>
<tr>
<th></th>
<th>female mice</th>
<th>male mice</th>
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<tbody>
<tr>
<td></td>
<td>ctr</td>
<td>ko</td>
</tr>
<tr>
<td>Body weight [g]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=22)</td>
<td>(n=15)</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
<td>23.1±0.6</td>
<td>23.7±0.3</td>
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<td></td>
<td>(n=7)</td>
<td>(n=3)</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
<td>512±88</td>
<td>552±63</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>63±20</td>
<td>46±9</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>101±13</td>
<td>107±11</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>398±72</td>
<td>437±50</td>
</tr>
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</table>

Table 2. Quantitative analysis of lesional cells.*

<table>
<thead>
<tr>
<th></th>
<th>ctr</th>
<th>ko</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-Gal positive cells / total cells</td>
<td>0.18±0.02</td>
<td>0.39±0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PCNA positive cells / total cells</td>
<td>0.19±0.02</td>
<td>0.30±0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(X-Gal positive + PCNA positive cells) / total cells</td>
<td>0.06±0.01</td>
<td>0.15±0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* Serial sections (n = 7-9) of plaques at equivalent positions in the ascending aorta were analysed. X-Gal positive (blue), PCNA positive (brown) and X-Gal/PCNA double positive cells were counted. The total number of cells in each section was determined by staining nuclei with Hoechst 33258.