Supplementary Material

Materials & Methods

Animal

The strategy for generation of systemic *KLF15*$^{-/-}$ mouse was previously described [1]. Briefly, the targeting vector for KLF15 was constructed by inserting a nuclear lacZ reporter into the targeting vector which replaced exon 2 of the endogenous mouse KLF15 locus.

Rat and mouse models of vascular injury

Mouse femoral artery wire injury to the mouse common femoral artery was performed as previously described[2, 3, 4] in wildtype (n=17) and *KLF15*$^{-/-}$ mice (n=17).

Primary rat and mouse smooth muscle culture

Rat aortic smooth muscle cells (RASMC) and mouse aortic smooth muscle cells (MASMC) were obtained from 4-6 weeks old rats or mice by aorta explants culture method. Briefly, the aortas were removed and rinsed several times in PBS. The adventitia was gently removed; aorta was opened and endothelial cells were denuded. Aorta were cut into small pieces and incubated in SmBm (Clonetics) + 5%FBS at 37°C. Smooth muscle cells (SMC) were passaged when cells reached 80% confluence. SMC purity was detected by SMC α-actin staining. At least 95% of cultured SMC were SMC α-actin positive at passage 5.

Experiments were performed at cell passage 6-11. Same passage of wild type and *KLF15*$^{-/-}$ SMCs was used for each set of experiment. Cells were growth arrested at 80% confluence for 48h with growth factors depleted medium containing 0.1% fetal bovine serum. Experiments shown are representative of results from three independent cultures from each group of mice.

Histology and immunohistochemical analysis

β-galactosidase staining was performed as previously described[1].

Two weeks after wire injury, femoral arteries were harvested for histological and morphometric analyses. Femoral artery sections were stained for elastin (Sigma), 5-bromo-2-deoxyuridine (Brdu)(Dako)[5, 6], SMA α- actin(Sigma), and CD45(BD Biosciences) staining. 1.5mg BrdU was injected into mice 1 day before euthanasia and another dose was given 1 hour before euthanasia. Femoral arteries were prepared and sectioned. Morphometric analysis and measurement were conducted by using Image-pro analyzer software. Every vessel was calculated as individual object when calculating intimal/medial area ratio.

Boyden chamber assay

VSMCs migration was examined as previously described[7].

DNA Synthesis Measurement

DNA synthetic rate in RASMC's was determined by [^{3}H]-thymidine incorporation as previously described[8]. RASMC's were infected with adenovirus for 12h at 50 MOI in DMEM after 12h serum starvation (>90% infection efficiency was

achieved at this time point). After 24 h serum starvation, cells were treated with fresh medium containing PDGFBB at 20ng/ml for 12h. ^{3}H-thymidine was added to a final concentration at 1μCi/ml during the final 2 hours of incubation.

RNA Isolation/Northern Analysis/Real-time PCR

Total RNA was isolated from flash-frozen aortic tissue obtained from sham and vascular injury animals using TRIzol (Invitrogen) per manufacturer's instruction. 10mg of RNA was subjected to gel electrophoresis, blotted onto a nylon membrane, and hybridized for KLF15 and 18S as previously described[9]. Real-time PCR is performed as previously described[10].

Statistical Analysis

Data is expressed as mean ± SD. Differences between experimental groups were evaluated for statistical significance by using the Student's t test for unpaired data. P values <0.05 were considered statistically significant.

Supplemental Table I

REFERENCES

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Supplemental Table I.

	WT	KLF15-/-	P value
intimal area(μm^2)	9385±6186	15779±8314	0.019
medial area(μm^2)	13438±1567	15219±2614	0.016
intimal area/medial Area	0.65±0.41	0.98±0.47	0.011
EEL area	42616±6739	50367±8073	0.018
% of intimal BrdU positive cell	12.0±7.1	20.0±9.8	0.045

EEL : external elastic lamina