Epigenetic upregulation of VEGF-A decreases infarct size in mouse myocardium: MR Cine imaging of left ventricle

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Introduction

Gene delivery of angiogenic growth factors, such as vascular endothelial growth factor A (VEGF-A), have been studied for the treatment of ischemic myocardium [1-4]. Previously, VEGF-A gene therapy response was demonstrated in swine model of reperfused myocardial infarction using adenoviral gene delivery and therapeutic response was imaged by using Gadolinium enhanced MRI [5]. In this study, endogenous VEGF-A expression was upregulated by lentiviral delivery of shRNA molecules that are complementary to the promoter region of VEGF-A gene. This leads to epigenetic modifications of histone marks in transduced cells (decreased methylation and increased acetylation) which increase VEGF-A expression [5]. Mismatch shRNA was used as a control. The effects of Epigenetherapy response were followed up using cine MRI.

Materials and methods

A left anterior descending artery (LAD) was ligated in 16 male c57BL/6J mice as described earlier in [6] resulting 21±7% infarction size. During surgery, mice were injected intramyocardially (3x5 µl) with lentiviruses expressing either promoter targeted shRNA (n=9) or mismatch shRNA control (n=7). Short axis cine MRI was performed at 9.4 T Varian scanner after 4th, 7th and 14th days after LAD ligation/injection. For cine imaging, a gradient echo sequences (TR=6.1 ms, TE=2.7 ms, flip angle=20°, matrix size 256x256, FOV 30x30 mm^2, slice thickness 1.5 mm, 6 slices) was used. The left ventricle (LV) volumes at end diastolic (EDV) and end systolic (ESV) phases, ejection fraction (EF), cardiac output (CO), infarct size, and infarct thickness (Inf. thk) were calculated from cine images. The transduction efficiency was measured using Immunohistological staining of co-expressed GFP marker gene. VEGF-A protein levels were analyzed using ELISA assay.

Results and Discussion

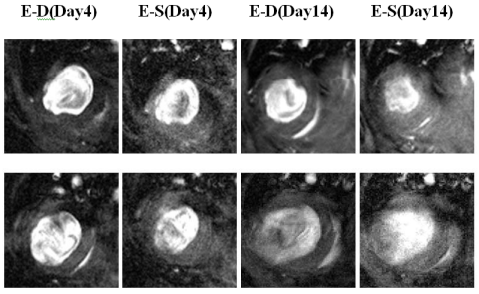

Fig. 1 Cine images at day 4 and day 14 of VEGF injected mouse (upper row) , ShRNA injected mouse (middle row) and shRNA control mouse (lower row) in end diastolic (E-D) & end systolic (E-S) phases

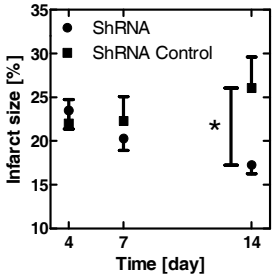

Fig. 2 Time curve of Infarction size in ShRNA and ShRNA control groups in myocardium. * p < 0.05 Two-way ANOVA.

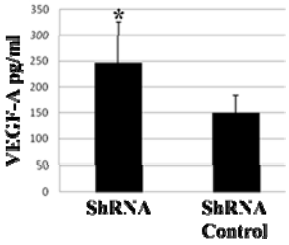

Fig. 3 ELISA analysis of VEGF-A protein in myocardium at day 14. * p<0.05 Student's t-test.

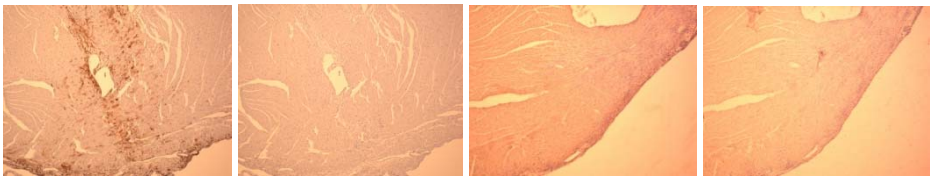

Fig. 4 Immunohistological staining of GFP marker gene and VEGF-A in myocardium. The expression is highest near injection tract. Same section with primary antibody omitted is used as negative control.

An example of cine images of shRNA and shRNA control hearts in end diastole and end systole are shown in **Fig. 1**. The infarct size was found to decrease in shRNA group compared to shRNA control group (**Fig. 2**). Significantly increased VEGF-A levels were measured in myocardium treated with promoter targeted shRNA compared to control group using ELISA (**Fig. 3**) and the representative immunohistology images are shown in **Fig. 4** . ELISA findings support the decreased infarct size in shRNA group. No significant difference during time span of the experiments was found in infarct thickness measured in the middle of the infarct. At day 4, EF was found low compared to normal heart [7] and showed no significant improvement up to 14 days, also no changes in other left ventricle parameters derived from cine images were found (**Tab. 1**). The cine MRI results indicate the therapeutic response of shRNA VEGF-A as decreased infarct size at time point of 14 days. As conclusion, epigenetic upregulation of endogenous VEGF-A expression by promoter targeted shRNA, where therapeutic response is detected by MRI, is potential way to decrease infarct size and may find clinical applications in the future. **References:** 1.) Ylä-Herttuala S, TCM Vol. 14, No. 8, 2004. 2.) Rutanen J et al., Circulation 2004;109(8):1029–1035. 3.) Ferrarini M et al., Circ Res 2006;98(7):954–961. 4.) Pearlman JD et al., Nat Med 1995;1(10):1085–1089. 5.) Turunen M et al. Circ Res105: 604-609, 2009, 6.) Gao E et al., Circ Res. 107 1445- 2010 7.) Musthafa HSN et al. 2012 MRM publ. online. **Acknowledgements**: Academy of Finland, Sigrid Juselius Foundation.

Tab. 1 Mean LV Parameters

Group	shRNA			ShRNA Control		
	Day 4	Day 7	Day 14	Day 4	Day 7	Day 14
EDV(µl)	77.7±23	72.8±29.7	79.9±33.8	65.1±26.2	78.5±37.9	85.7±42.5
ESV(µl)	44.7±17.6	32.1±16.2	51±39	39.3±21	41.7±26.7	54.8±28.6
SV(µl)	40.7±15.2	40.7±15.2	28.9±16.3	25.8±6.5	36.8±16.4	36.7±14.3
EF (%)	43.5±10.3	57.8±10.1	41.4±23.8	43.2±11.8	49.9±14.9	46.8±12.8
CO(ml/min)	18.2±5.5	10.4±3.6	9.7±4.6	14.4±3.8	14.4±3.6	13.5±3.4
Inf. thk (mm)	0.6±0.1	0.6±0.2	0.7±0.2	0.6±0.2	0.7±0.3	0.7±0.3