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Potential role of vascular smooth muscle cell-like progenitor cell therapy in the suppression of experimental abdominal aortic aneurysms

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ABSTRACT

Abdominal aortic aneurysms (AAA) are a growing problem worldwide, yet there is no known medical therapy. The pathogenesis involves degradation of the elastic lamina by two combined mechanisms: increased degradation of elastin by matrix metalloproteinases (MMP) and decreased formation of elastin due to apoptosis of vascular smooth muscle cells (VSMC). In this study, we set out to examine the potential role of stem cells in the attenuation of AAA formation by inhibition of these pathogenetic mechanisms. Muscle-derived stem cells from murine skeletal muscles were isolated and stimulated with PDGF-BB in vitro for differentiation to VSMC-like progenitor cells (VSMC-PC). These cells were implanted in to elastase-induced AAAs in rats. The cell therapy group had decreased rate of aneurysm formation compared to control, and MMP expression at the genetic, protein and enzymatic level were also significantly decreased. Furthermore, direct implantation of VSMC-PCs in the intima of harvested aortas was visualized under immunofluorescent staining, suggesting that these cells were responsible for the inhibition of MMPs and consequent attenuation of AAA formation. These results show a promising role of stem cell therapy for the treatment of AAAs, and with further studies, may be able to reach clinical significance.

1. Introduction

Abdominal aortic aneurysm (AAA) is a chronic degenerative disease primarily affecting the elderly males, with an incidence of up to 12.5% in men aged 75 or higher [1]. This incidence is increasing as the population life-span increases, and ruptured AAA is currently the 13th leading cause of death in the US. The mainstay of therapy is endovascular or surgical treatment of the aneurysm when the diameter is more than 5 cm. Unfortunately there is no proven medical therapy to treat or halt the progression of AAA, and for aneurysms that do not meet the treatment criteria, watchful waiting is the only option. Many studies have reported possible medical therapies or targets to retard AAA growth, however none of them have reached widespread clinical use [2,3].

The pathogenesis of AAA is very complex, but there is increasing evidence that the degradation of the media layer by means of a proteolytic process is the basic underlying patho physiologic mechanism. During this process, there is a decrease in elastin content with expansion of the aortic wall, leading to a compensatory increase in collagen synthesis and subsequent remodeling of the AAA wall [4]. The main proteolytic enzyme involved is metalloproteinase (MMP), which is directly involved in the degradation of elastin in the aortic wall [5,6]. Many studies have also shown that the decrease in elastin can be attributed to senescence and apoptosis of vascular smooth muscle cells (VSMC), which are involved in the production of elastin [7,8].

In this study, muscle-derived stem cells (MDSC) were differentiated into VSMC-like progenitor cells (VSMC-PC) in vitro and were incubated in elastase-induced abdominal aortic aneurysms in rats in an attempt to replenish the elastin content of the aortic wall and stop the progression of AAA formation.

2. Materials and methods

2.1. Isolation and differentiation of MDSCs

MDSCs were isolated from the gastrocnemius muscle of 4 wk old male Spraque-Dawley (S-D) rats using the same methodology as previously published by our group [9]. Isolated MDSCs were cultured with antibodies for Sca-1, CD34, desmin and CD45 (as previously published) to verify their nature. These cells were then transfected with lentivirus (Microgen, Korea) tagged with green

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fluorescent protein (GFP), by adding 1 ml of lentivirus into 6-well plates containing MDSCs (at 70–90% confluence) and 4–8 μg of polybrene (Sigma, USA). The whole mixture was incubated overnight in a 37 °C and 5% CO_2 atmosphere and the cells were moved to a new culture medium after 24 h. These cells were differentiated by stimulation with 20 ng/ml of PDGF-BB (Upstate, USA) for 2–3 d and changed to new culture media. PDGF-BB was added every time the culture medium was changed and cells were allowed to differentiate for 7–10 d.

2.2. Abdominal aortic aneurysm model

An abdominal aortic aneurysm model was created in S-D rats after induction of anesthesia with isoflurane. A midline incision was made, the infrarenal aorta was exposed and cross-clamping was performed proximally and distally. An aortotomy was made just above the distal clamp site and a silastic tube connected to a syringe was inserted in the cranial direction. The aorta was clamped just above the aortotomy site and 0.1 cc of type I elastase was injected for incubation during 30 min. In the cell therapy group, 1×10^7 VSMC-like progenitor cells were incubated for another 30 min, while in the control group, the same amount of saline was used. After incubation, the aortotomy site was closed and clamps were removed for restoration of flow in the aorta. A total of 12 rats were used in our study, 6 in each group. This whole study was performed under the approval of the institutional animal care and use committee (IACUC) at our institution.

2.3. Immunohistochemical staining, zymography, Western blot and RT-PCR

The aortas were harvested at 6 wk and the diameter of the aneurysms and the adjacent normal aorta were measured. Positive aneurysm formation was defined as an enlargement of the aorta by more than 2 times compared to the adjacent normal aorta, in order to compensate for possible measurement errors when using the traditional 1.5 times criteria. The harvested tissues were tested for metalloproteinases (MMP-2 and MMP-9).

Immunohistochemical staining was performed by immediate fixation of AAA specimens with 4% formalin for 24 h and subsequent embedding in paraffin. Sections were stained by incubation of primary antibodies MMP-2 and MMP-9 (1:100, Abcam, UK) overnight, and their respective secondary antibodies for 30 min. Immunoreactivity was visualized by incubation with 3,3-diaminobenzidine (DAB) substrate, counterstained with hematoxylin, mounted and visualized under microscopy.

Gel zymography was performed by homogenizing aortic tissues with lysis buffer consisting of 0.25% Triton X-100 and 0.1 M Tris–HCl (pH 8.1), and 15 μl of the supernatant together with Tris–Glycine SDS sample buffer 2X (Sigma–Aldrich, USA) were loaded on to 10% Zymogram gelatin gel (Invitrogen, USA). Electrophoresis was performed according to the manufacturer's instructions and densitometric analysis of the bands was performed with a computer-assisted bioimage analyzer (EXT-20MX, Vilber Lourmat, France).

Western blotting was performed by homogenizing aortic tissues with PRO-PREP Protein Extraction kit (Intron Biotechnology, USA). The protein concentration was determined using the Bradford method. A Laemmli 2× sample buffer (Sigma–Aldrich, USA) was mixed with the same amount of protein and heated for 5 min, after which SDS–PAGE electrophoresis was performed. Proteins were transferred to a nitrocellulose membrane filter at 100 V for 1 h, blocked with 5% nonfat dry milk in TBS, incubated overnight with primary antibodies MMP-2 and MMP-9 (1:1000, Abcam, UK) and incubated for 1 h with anti-rabbit IgG, HRP-linked antibody. For analysis of the bands, the enhanced chemiluminescence kit (Amersham, USA) was used and densitometric analysis was performed with the Chemiluminescent Image Analysis System (Bio-rad, USA).

RT-PCR was performed by extracting total RNA using the RNeasy Mini kit (Qiagen, USA) according to the manufacturer's instructions. Quantification of the extracted RNA was done at 260 nm and 280 nm wavelengths using the Smartspec Plus Spectrophotometer (Bio-rad, USA). Reverse transcription was performed by sequentially mixing 2 μg of total RNA with an oligonucleotide deoxythymidine primer, 5X reaction buffer, 1 mM dNTP (Bioline, UK), 20U of RNase inhibitor and reverse transcriptase Bioscript (Bioline, UK). The resulting cDNA was mixed with forward and reverse primers and 10X PCR buffer (MangoMix, Bioline, UK). The sequences of primers were as follows: MMP-2 forward 5′-AGCTCCCGGAAAAGATTGAT-3′, reverse 5′-TCCAGTTAAA GGCAGCGTCT-3′, MMP-9 forward 5′-TTCGACGCTGACAAGTG-3′, reverse 5′-AGGGGAGTCCTCGTGGTAGT-3′, GAPDH forward 5′-TGCC ACTCAGAAGACTGT-3′, reverse 5′-GCATGTCAGATCCACAATGG-3′. PCR amplification was performed with PTC-200 PCR Thermo cycler (MJ Research, USA) using the following cycling conditions: 94 °C for 2 min (initial denaturation); 94 °C for 30 s, 53–55 °C for 45 s, 72 °C for 30 s (35cycles); and 72 °C for 10 min (final extension). PCR products were subjected to electrophoresis in 1–2% agarose gel containing ethidium bromide and images were analyzed using a computer-assisted bioimage analyzer (EXT-20MX, Vilber Lourmat, France). Band intensities were normalized against GAPDH.

2.4. Statistical analysis

All data are presented as mean and standard error of the mean. Comparisons between the 2 groups were performed using the independent variables Student's t test. SPSS release 18.0 (SPSS Inc, USA) was used for analysis and differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Characteristics of VSMC-PCs

MDSCs isolated from S-D rats showed positive expression of surface markers Sca-1 and CD34, while there was little or no expression of desmin or CD45 (Fig. 1A). When these cells were tagged with GFP, a characteristic green color was seen under ICC staining. When GFP-tagged MDSCs were cultured with PDGF-BB, the resulting cells showed a characteristic yellow color under ICC stain, which is a result of merging of the green GFP with a red color representing α-SMA, a characteristic marker for smooth muscle cells (Fig. 1B).

3.2. Aneurysm formation in S-D rats

Aneurysms created in rats with elastase showed characteristic enlargement of the aorta (Fig. 2A). IHC staining with MMP-9 showed an overall higher expression of MMP-9 in the aneurysmal wall compared to control (Fig. 2B–D).

Aneurysm formation was found in 5 out of 6 rats (83%) in the control group, while only 3 rats out of 6 (50%) had aneurysm formation in the cell therapy group. In the cell therapy group, harvested aneurysms showed positive expression of GFP, which suggests that GFP tagged VSMC-PCs may have had an effect in the overall smaller rate of aneurysm formation in this group (Fig. 2E and F).

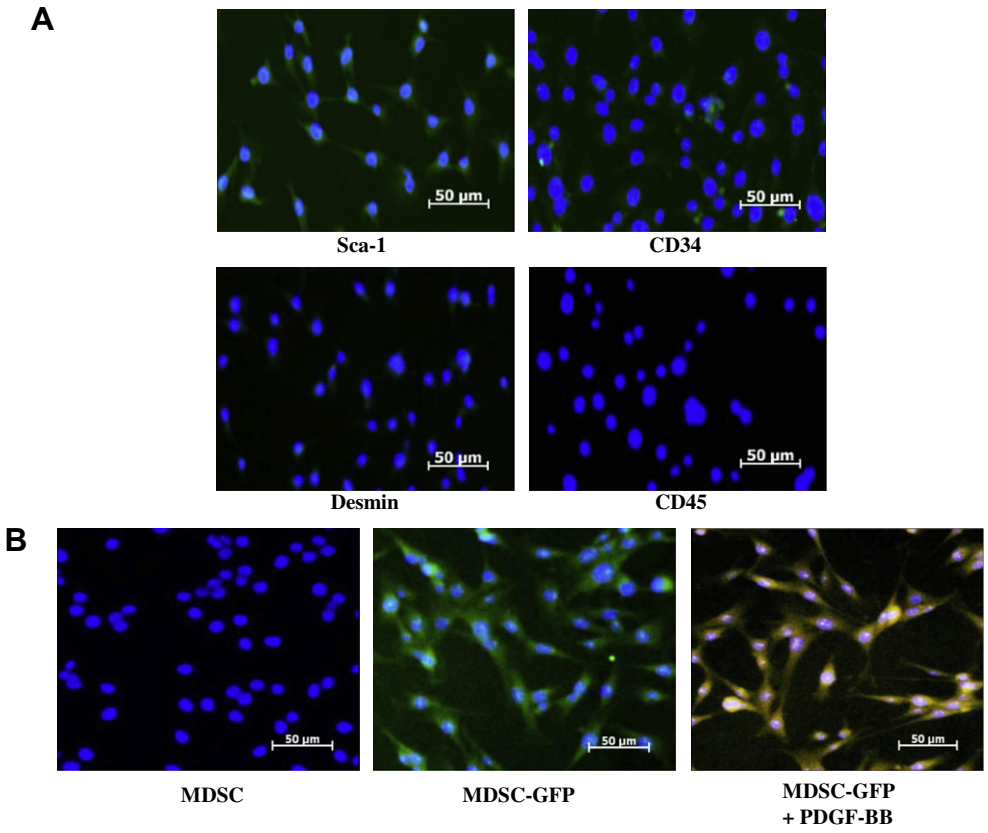

Fig. 1. Characteristics of MDSC, (A) Immunocytochemical staining of MDSCs showing positive expression of Sca-1 and CD34, and negative expression of des min and CD45 (400× magnification). (B) Immunofluorescent staining of MDSCs tagged with GFP (green) and after stimulation with PDGF-BB to show expression of α-smooth muscle actin (red) (100× magnification). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. MMP activity in the aneurysmal wall

Harvested tissues from both cell therapy and control groups were used to perform zymography, RT-PCR and Western blot. Zymography of both groups showed characteristic bands for the pro and active forms of MMP-2 and MMP-9 (Fig. 3A). The detection of active forms of MMP on zymography demonstrates the activity of MMPs since MMPs are usually secreted in an inactive pro-MMP form and are activated only during certain processes such as cell migration or invasion. The cell therapy group has a significantly lower activity of pro and active MMP-9 compared to control (Fig. 3B), and there was a tendency for lower activity of pro and active MMP-2, but without statistical significance (Fig. 3C).

RT-PCR for MMP-2 and MMP-9 mRNA expression was done for both groups and the relative intensities of the bands were represented graphically (Fig. 4A and B). The results show that there was a significantly lower expression of MMP-2 in the cell therapy group compared to control ($p < 0.05$). There was also a tendency for lower expression of MMP-9 in the cell therapy group, without statistical significance.

The expression of MMP-2 and MMP-9 were also investigated at the protein level by Western blot (Fig. 4C and D). Both MMP-2 and MMP-9 protein content was lower in the cell therapy group compared to control, which was statistically significant ($p < 0.05$).

Overall, these results demonstrate that MMP activities were present in both control and cell therapy groups, however there was a decreased amount of expression of MMPs at the gene and protein level after implementation of VSMC-PCs, which correlates with a lower rate of aneurysm formation.

4. Discussion

This study shows that MDSCs exhibited VSMC-like properties when stimulated with PDGF-BB for differentiation in vitro. These VSMC-PCs significantly decreased expression of MMPs and were able to attenuate formation of elastase-induced murine AAA, with successful implantation of these cells being observed in the specimens. Both MMP9 and MMP2 were significantly decreased in the cell therapy group under Western blot, while only MMP9 was significantly lower in zymography and MMP2 in RT-PCR. MMP9 is known to be more specific in the pathogenesis of AAA, but studies have demonstrated that both MMP2 and MMP9 are needed for AAA formation [10,11]. MMP2 is thought to be involved in the initiation of aneurysm formation, while MMP9 is involved in the propagation of growth and thus found more abundantly in larger and ruptured aneurysms.[12] However, the patterns of MMPs observed in our study are probably due to the small numbers in each group rather than the different stages of AAA formation.

Recently, a few studies have reported the possible use of stem cell therapy for the attenuation of AAA in animal models [13,14]. Mesenchymal stem cells (MSC) from the bone marrow were used in these studies, with successful attenuation of murine AAA formation through elastin preservation, associated suppression of MMPs and inhibition of inflammatory cytokines, especially IL-17. In another study, the successful implantation of MSCs was demonstrated in a porcine AAA model [15]. In our study we used MDSCs, which are a population of multipotent somatic stem cells derived from skeletal muscles. MDSCs are known for their high regeneration capacity, their ability to differentiate to multilineage

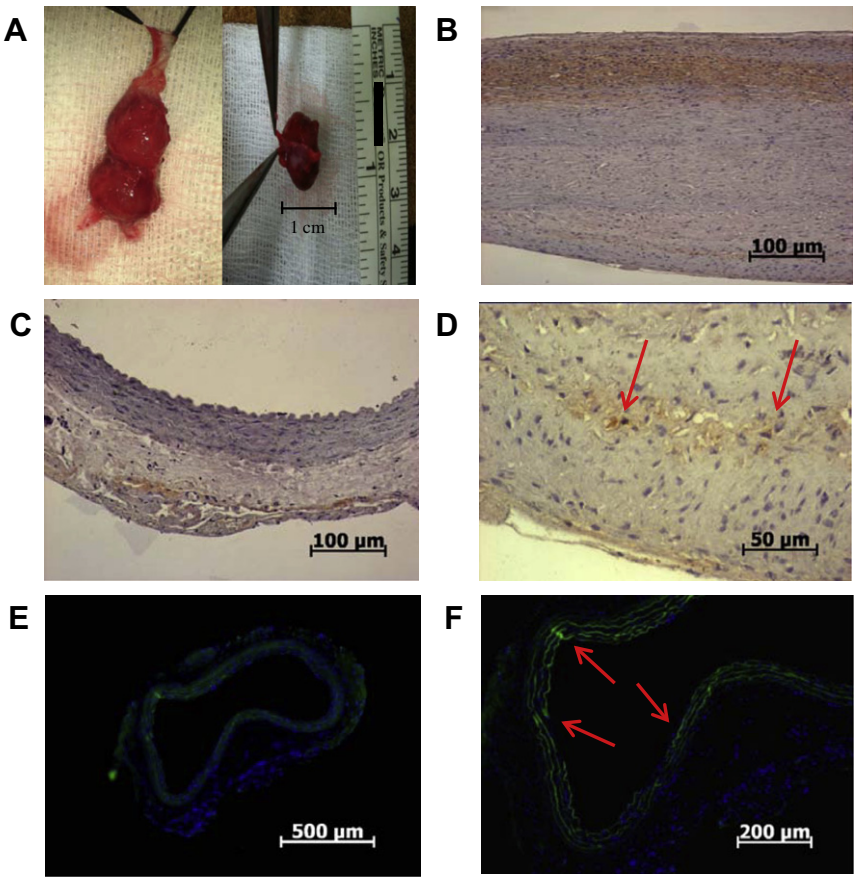

Fig. 2. In vivo AAA formation, (A) Representative infrarenal AAAs from elastase perfused rats, showing increased diameter compared to adjacent aorta and common iliac arteries (left) and an axial diameter of around 0.7 cm (right). (B) Immunohistochemical staining for MMP-9 in aneurysmal tissue and (C) adjacent normal aorta (200× magnification). (D) Higher amplification of aneurysmal tissue reveals positive expression of MMP-9 stained cells (arrows) (400× magnification). (E) Immunofluorescent staining of aneurysmal tissue shows implantation of GFP-tagged VSMC-PC in the intima layer (50× magnification) and (F) higher magnification shows characteristic green stained areas (arrows) demonstrating direct VSMC-PC implantation (100× magnification).

cells, and their self-renewal capabilities [16]. It has been demonstrated that MDSCs can be differentiated in to myogenic, osteogenic chondrogenic, adipogenic, endothelial, neuronal and hematopoietic lineages [17–22]. MDSCs also maintain their stem cell characteristics in vitro and in vivo even after 200 population doublings, demonstrating a high regeneration and self-renewal capacity [23]. Above all, muscle tissue is the most abundant tissue in the body, which can be easily and safely obtained, making it an attractive source compared to MSCs [24].

We have previously been able to differentiate MDSCs in vitro into endothelial lineage cells using shear stress and VEGF, which were able to increase angiogenesis in murine ischemic hindlimbs, and also attenuate intimal hyperplasia in murine carotid injury models [9]. We used a similar strategy in this study, where we stimulated MDSCs in vitro with PDGF-BB. PDGF is known to have effects on VSMCs by inducing proliferation, migration and contraction [25,26] and stem cells have been previously differentiated to VSMCs by stimulation with PDGF [27–29]. Such differentiated MDSCs showed positive α-SMA expression, a specific marker for SMCs. Therefore PDGF-BB stimulation of MDSCS was able to drive these multipotent cells to differentiate towards the SMC lineage, thus showing features of VSMC progenitors (VSMC-PC).

Many studies have tried to investigate the role of VSMC progenitors in vascular diseases, especially atherosclerosis, and there is increasing evidence that these cells have a contributory effect rather than protective effect in vascular diseases [30]. The underlying mechanism involves differentiation of stem cells and VSMC progenitor cells into SMCs in the intima layer leading to neointimal hyperplasia, a cause of atherosclerosis [31,32]. In our study, in vivo studies of VSMC-PCs demonstrated attenuation of AAA formation with suppression of MMP expression. The mechanism of these findings is unknown, but we were able to show implantation of exogenously administered VSMC-PCs in the intima layer. Implanted VSMC-PCs probably had some influence, but cannot solely explain for the observed findings. We suggest that administered VSMC-PCs exerted their effects in a paracrine manner via multiple factors such as cytokines or growth factors, depending on the microenvironmental condition. This microenvironment together with the associated immunomodulatory and homing functions by paracrine factors are known to be fundamental for differentiation of stem cells to desired lineages for specific functions [33,34]. In our study, this microenvironment leads to the repair of injured aorta, probably by replenishment of apoptotic VSMCs and suppression of MMPs, in other cases it can lead to formation of intimal hyperplasia, as is the case with atherosclerosis.

Our study has the advantage of stimulating MDSCs in vitro, since we provided the drive for differentiation in a controlled ex vivo environment, which may be more suited to survive the initial environment and contribute to the regeneration process [35]. Additionally it is one of the few studies showing the potential role of stem cell based therapies in AAA, and the first to use MDSCs, which is an attractive source. However further studies with larger numbers are needed to investigate the underlying mechanism and the factors involved in this process.

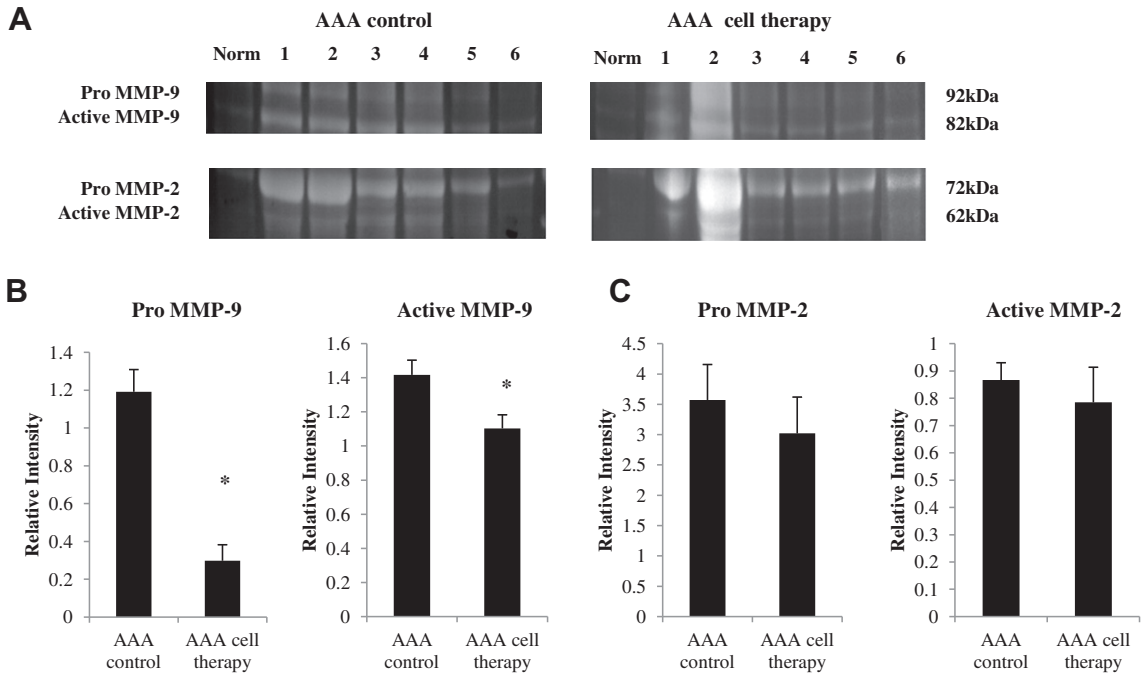

Fig. 3. MMP enzymatic activity of cell treated and control AAA, (A) Gel zymography shows representative bands for MMP-2 and MMP-9 in both cell therapy and control groups. Norm represents normal aorta. (B) Pro and active MMP-9 activity from cell therapy and control groups, normalized against GAPDH. Cell therapy group had significantly lower expression of both pro and active MMP-9 compared to control. (C) Pro and active MMP-2 activity from cell therapy and control groups, normalized against GAPDH. $^{*}p < 0.05$.

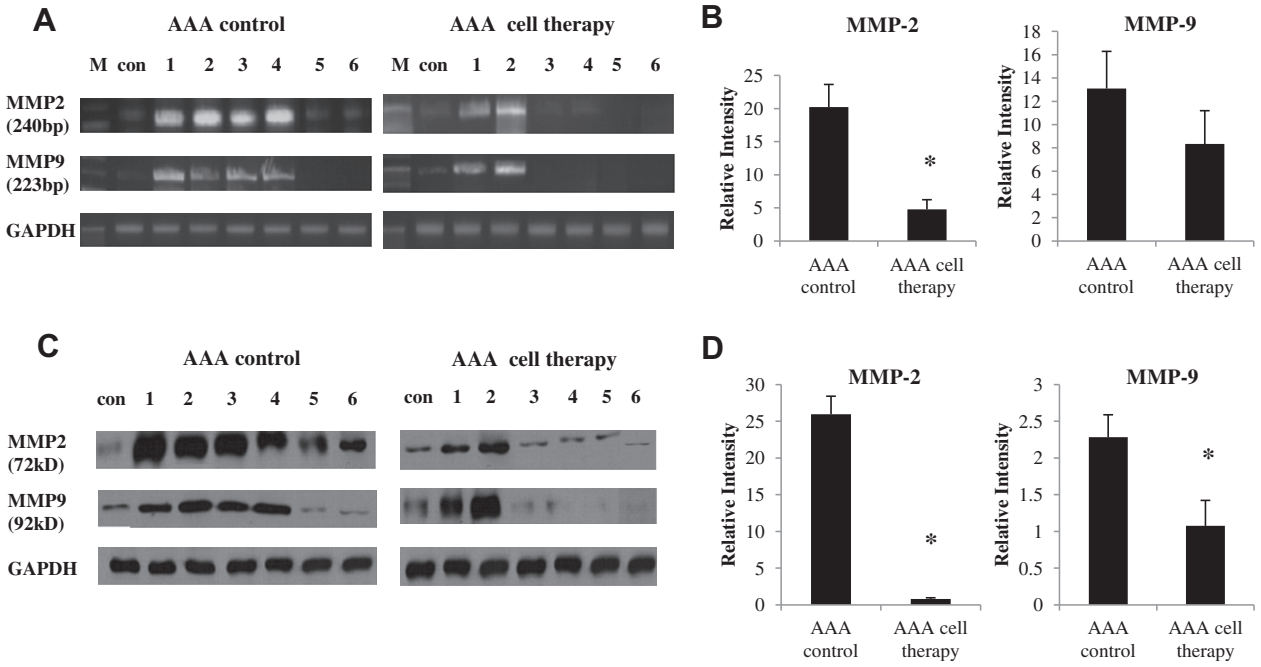

Fig. 4. MMP gene expression and protein analyses of cell treated and control AAA, (A) RT-PCR analysis for MMP-2 and MMP-9 in cell therapy and control groups. (B) Quantification of gene expression normalized against GAPDH for MMP-2 and MMP-9. Cell therapy group had significantly lower expression of MMP-2 compared to control. (C) Western blot of MMP-2 and MMP-9 for cell therapy and control groups. (D) Quantification of protein content normalized against GAPDH shows significantly lower expression on both MMP-2 and MMP-9 in the cell therapy group compared to control. $^{*}p < 0.05$.

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References

[1] D. Lloyd-Jones, R.J. Adams, T.M. Brown, M. Carnethon, S. Dai, G. De Simone, T.B. Ferguson, E. Ford, K. Furie, C. Gillespie, A. Go, K. Greenlund, N. Haase, S. Hailpern, P.M. Ho, V. Howard, B. Kissela, S. Kittner, D. Lackland, L. Lisabeth, A. Marelli, M.M. McDermott, J. Meigs, D. Mozaffarian, M. Mussolino, G. Nichol, V.L. Roger, W. Rosamond, R. Sacco, P. Sorlie, T. Thom, S. Wasserthiel-Smoller,

N.D. Wong, J. Wylie-Rosett, Heart disease and stroke statistics-2010 update: a report from the American Heart Association, Circulation 121 (2010) e46–e215.
[2] K. Yoshimura, H. Aoki, Y. Ikeda, K. Fujii, N. Akiyama, A. Furutani, Y. Hoshii, N. Tanaka, R. Ricci, T. Ishihara, K. Esato, K. Hamano, M. Matsuzaki, Regression of abdominal aortic aneurysm by inhibition of c-Jun N-terminal kinase, Nat. Med. 11 (2005) 1330–1338.
[3] J. Liu, W. Xiong, L. Baca-Regen, H. Nagase, B.T. Baxter, Mechanism of inhibition of matrix metalloproteinase-2 expression by doxycycline in human aortic smooth muscle cells, J. Vasc. Surg. 38 (2003) 1376–1383.
[4] E. Choke, G. Cockerill, W.R. Wilson, S. Sayed, J. Dawson, I. Loftus, M.M. Thompson, A review of biological factors implicated in abdominal aortic aneurysm rupture, Eur. J. Vasc. Endovasc. Surg. 30 (2005) 227–244.
[5] Z.S. Galis, J.J. Khatri, Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly, Circ. Res. 90 (2002) 251–262.
[6] N.A. Tamarina, W.D. McMillan, V.P. Shively, W.H. Pearce, Expression of matrix metalloproteinases and their inhibitors in aneurysms and normal aorta, Surgery 122 (1997) 264–271;
discussion 271–262
[7] V.L. Rowe, S.L. Stevens, T.T. Reddick, M.B. Freeman, R. Donnell, R.C. Carroll, M.H. Goldman, Vascular smooth muscle cell apoptosis in aneurysmal, occlusive, and normal human aortas, J. Vasc. Surg. 31 (2000) 567–576.
[8] E. Allaire, B. Muscatelli-Groux, C. Mandet, A.M. Guinault, P. Bruneval, P. Desgranges, A. Clowes, D. Melliere, J.P. Becquemin, Paracrine effect of vascular smooth muscle cells in the prevention of aortic aneurysm formation, J. Vasc. Surg. 36 (2002) 1018–1026.
[9] H.S. Park, S. Hahn, G.H. Choi, Y.S. Yoo, J.Y. Lee, T. Lee, Muscle-derived stem cells promote angiogenesis and attenuate intimal hyperplasia in different murine vascular disease models, Stem Cells Dev. (2012). [Epub ahead of print].
[10] R. Pyo, J.K. Lee, J.M. Shipley, J.A. Curci, D. Mao, S.J. Ziporin, T.L. Ennis, S.D. Shapiro, R.M. Senior, R.W. Thompson, Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms, J. Clin. Invest. 105 (2000) 1641–1649.
[11] G.M. Longo, W. Xiong, T.C. Greiner, Y. Zhao, N. Fiotti, B.T. Baxter, Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms, J. Clin. Invest. 110 (2002) 625–632.
[12] W.B. Keeling, P.A. Armstrong, P.A. Stone, D.F. Bandyk, M.L. Shames, An overview of matrix metalloproteinases in the pathogenesis and treatment of abdominal aortic aneurysms, Vasc. Endovascular Surg. 39 (2005) 457–464.
[13] R. Hashizume, A. Yamawaki-Ogata, Y. Ueda, W.R. Wagner, Y. Narita, Mesenchymal stem cells attenuate angiotensin II-induced aortic aneurysm growth in apolipoprotein E-deficient mice, J. Vasc. Surg. 54 (2011) 1743–1752.
[14] A.K. Sharma, G. Lu, A. Jester, W.F. Johnston, Y. Zhao, V.A. Hajzus, M.R. Saadatzadeh, G. Su, C.M. Bhamidipati, G.S. Mehta, I.L. Kron, V.E. Laubach, M.P. Murphy, G. Ailawadi, G.R. Upchurch Jr., Experimental abdominal aortic Aneurysm formation is mediated by IL-17 and attenuated by mesenchymal stem cell treatment, Circulation 126 (2012) S38–S45.
[15] I.C. Turnbull, L. Hadri, K. Rapti, M. Sadek, L. Liang, H.J. Shin, K.D. Costa, M.L. Marin, R.J. Hajjar, P.L. Faries, Aortic implantation of mesenchymal stem cells after aneurysm injury in a porcine model, J. Surg. Res. 170 (2011) e179–e188.
[16] Z. Qu-Petersen, B. Deasy, R. Jankowski, M. Ikezawa, J. Cummins, R. Pruchnic, J. Mytinger, B. Cao, C. Gates, A. Wernig, J. Huard, Identification of a novel population of muscle stem cells in mice. Potential for muscle regeneration, J. Cell Biol. 157 (2002) 851–864.
[17] T. Tamaki, A. Akatsuka, K. Ando, Y. Nakamura, H. Matsuzawa, T. Hotta, R.R. Roy, V.R. Edgerton, Identification of myogenic-endothelial progenitor cells in the interstitial spaces of skeletal muscle, J. Cell Biol. 157 (2002) 571–577.
[18] P. Bosch, D. Musgrave, S. Ghivizzani, C. Latterman, C.S. Day, J. Huard, The efficiency of muscle-derived cell-mediated bone formation, Cell Transplant. 9 (2000) 463–470.
[19] S.H. Lu, A.H. Yang, C.F. Wei, H.S. Chiang, M.B. Chancellor, Multi-potent differentiation of human purified muscle-derived cells: potential for tissue regeneration, BJU Int. 105 (2010) 1174–1180.
[20] L. De Angelis, L. Berghella, M. Coletta, L. Lattanzi, M. Zanchi, M.G. Cusella-De Angelis, C. Ponzetto, G. Cossu, Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration, J. Cell Biol. 147 (1999) 869–878.
[21] G. Alessandri, S. Pagano, A. Bez, A. Benetti, S. Pozzi, G. Iannolo, M. Baronio, G. Invernici, A. Caruso, C. Muneretto, G. Bisleri, E. Parati, Isolation and culture of human muscle-derived stem cells able to differentiate into myogenic and neurogenic cell lineages, Lancet 364 (2004) 1872–1883.
[22] K.A. Jackson, T. Mi, M.A. Goodell, Hematopoietic potential of stem cells isolated from murine skeletal muscle, Proc. Natl. Acad. Sci. USA 96 (1999) 14482–14486.
[23] B.M. Deasy, B.M. Gharaibeh, J.B. Pollett, M.M. Jones, M.A. Lucas, Y. Kanda, J. Huard, Long-term self-renewal of postnatal muscle-derived stem cells, Mol. Biol. Cell 16 (2005) 3323–3333.
[24] X. Wu, S. Wang, B. Chen, X. An, Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy, Cell Tissue Res. 340 (2010) 549–567.
[25] A.D. Hughes, G.F. Clunn, J. Refson, C. Demoliou-Mason, Platelet-derived growth factor (PDGF): actions and mechanisms in vascular smooth muscle, Gen. Pharmacol. 27 (1996) 1079–1089.
[26] H. Inui, Y. Kitami, M. Tani, T. Kondo, T. Inagami, Differences in signal transduction between platelet-derived growth factor (PDGF) alpha and beta receptors in vascular smooth muscle cells. PDGF-BB is a potent mitogen, but PDGF-AA promotes only protein synthesis without activation of DNA synthesis, J. Biol. Chem. 269 (1994) 30546–30552.
[27] Y. Hu, Z. Zhang, E. Torsney, A.R. Afzal, F. Davison, B. Metzler, Q. Xu, Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in ApoE-deficient mice, J. Clin. Invest. 113 (2004) 1258–1265.
[28] A. Margariti, Q. Xiao, A. Zampetaki, Z. Zhang, H. Li, D. Martin, Y. Hu, L. Zeng, Q. Xu, Splicing of HDAC7 modulates the SRF-myocardin complex during stem-cell differentiation towards smooth muscle cells, J. Cell Sci. 122 (2009) 460–470.
[29] T. Miyata, H. Iizasa, Y. Sai, J. Fujii, T. Terasaki, E. Nakashima, Platelet-derived growth factor-BB (PDGF-BB) induces differentiation of bone marrow endothelial progenitor cell-derived cell line TR-BME2 into mural cells, and changes the phenotype, J. Cell Physiol. 204 (2005) 948–955.
[30] A. Margariti, L. Zeng, Q. Xu, Stem cells, vascular smooth muscle cells and atherosclerosis, Histol. Histopathol. 21 (2006) 979–985.
[31] C.I. Han, G.R. Campbell, J.H. Campbell, Circulating bone marrow cells can contribute to neointimal formation, J. Vasc. Res. 38 (2001) 113–119.
[32] J.L. Hillebrands, F.A. Klatter, B.M. van den Hurk, E.R. Popa, P. Nieuwenhuis, J. Rozing, Origin of neointimal endothelium and alpha-actin-positive smooth muscle cells in transplant arteriosclerosis, J. Clin. Invest. 107 (2001) 1411–1422.
[33] B. Gharaibeh, M. Lavasani, J.H. Cummins, J. Huard, Terminal differentiation is not a major determinant for the success of stem cell therapy - cross-talk between muscle-derived stem cells and host cells, Stem Cell Res. Ther. 2 (2011) 31.
[34] N.K. Satija, V.K. Singh, Y.K. Verma, P. Gupta, S. Sharma, F. Afrin, M. Sharma, P. Sharma, R.P. Tripathi, G.U. Gurudutta, Mesenchymal stem cell-based therapy: a new paradigm in regenerative medicine, J. Cell. Mol. Med. 13 (2009) 4385–4402.
[35] B.M. Deasy, R.J. Jankowski, J. Huard, Muscle-derived stem cells: characterization and potential for cell-mediated therapy, Blood Cells Mol. Dis. 27 (2001) 924–933.