Biochemical and Biophysical Research Communications 446 (2014) 1102–1107

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Adipose-derived stromal cells inhibit prostate cancer cell proliferation inducing apoptosis

Kiyoshi Takahara [a], Masaaki Ii [b,*], Teruo Inamoto [a], Kazumasa Komura [a], Naokazu Ibuki [a], Koichiro Minami [a], Hirofumi Uehara [a], Hajime Hirano [a], Hayahito Nomi [a], Satoshi Kiyama [a], Michio Asahi [b], Haruhito Azuma [a]

[a] Department of Urology, Faculty of Medicine, Osaka Medical College, Osaka, Japan
[b] Department of Pharmacology, Faculty of Medicine, Osaka Medical College, Osaka, Japan

ARTICLE INFO

Article history:
Received 16 March 2014
Available online 26 March 2014

Keywords:
AdSCs
PCa
Stem cell
Prostate cancer
Apoptosis

ABSTRACT

Mesenchymal stem cells (MSCs) have generated a great deal of interest in the field of regenerative medicine. Adipose-derived stromal cells (AdSCs) are known to exhibit extensive proliferation potential and can undergo multilineage differentiation, sharing similar characteristics to bone marrow-derived MSCs. However, as the effect of AdSCs on tumor growth has not been studied sufficiently, we assessed the degree to which AdSCs affect the proliferation of prostate cancer (PCa) cell. Human AdSCs exerted an inhibitory effect on the proliferation of androgen-responsive (LNCaP) and androgen-nonresponsive (PC3) human PCa cells, while normal human dermal fibroblasts (NHDFs) did not, and in fact promoted PCa cell proliferation to a degree. Moreover, AdSCs induced apoptosis of LNCaP cells and PC3 cells, activating the caspase3/7 signaling pathway. cDNA microarray analysis suggested that AdSC-induced apoptosis in both LNCaP and PC3 cells was related to the TGF-β signaling pathway. Consistent with our in vitro observations, local transplantation of AdSCs delayed the growth of tumors derived from both LNCaP- and PC3-xenografts in immunodeficient mice. This is the first preclinical study to have directly demonstrated that AdSC-induced PCa cell apoptosis may occur via the TGF-β signaling pathway, irrespective of androgen-responsiveness. Since autologous AdSCs can be easily isolated from adipose tissue without any ethical concerns, we suggest that therapy with these cells could be a novel approach for patients with PCa.

1. Introduction

Recent studies in the field of stem cell biology have suggested that somatic stem cells, i.e. mesenchymal stem cells (MSCs) such as bone marrow and adipose stromal progenitor cells, could have therapeutic applications for regenerative medicine. Since MSCs have the potential for multiple passages in culture and differentiation into various cell types [1], they offer considerable promise for tissue regeneration, and clinical studies of MSC therapy for several diseases are now ongoing. Although adipose-derived stromal cells (AdSCs) share a number of characteristics with bone marrow MSCs [2], certain intriguing differences between AdSCs and bone marrow MSCs have been reported [3] specifically in relation to malignant diseases. Some studies have indicated that AdSCs have a promotional effect on the proliferation of glioma cells [4] and the growth of both breast cancer [5] and prostate cancer [6], while Cousin et al. have reported that co-culture with AdSCs has a suppressive effect on the proliferation of pancreatic cancer cells [7]. Thus, the influence of AdSCs on tumor cell proliferation is still controversial.

Prostate cancer (PCa) is now a major and escalating international health problem in men, and is one of the most common malignant solid tumors in Western countries [8]. Androgen deprivation therapy (ADT) is the primary disease management option for patients with advanced PCa, but most patients invariably develop treatment resistance at some stage, developing a castration-resistant (CR) status with an eventual fatal outcome even after potentially curative treatment. Therefore, there is an urgent need for novel therapeutic strategy that can overcome the emergence of CR PCa. To clarify the effect of AdSCs on PCa cell proliferation and PCa cell-derived tumor growth, we carried out in vitro and in vivo studies using androgen-responsive (LNCaP) and androgen-nonresponsive (PC3) human PCa cells with human AdSCs under direct co-culture and indirect separate culture conditions. We also attempted to gain a mechanistic insight into the effect of AdSCs on PCa cell proliferation/apoptosis using cDNA microarray

* Corresponding author. Address: Department of Pharmacology, Faculty of Medicine, Osaka Medical College, 2-7 Daigaku-machi, Takatsuki, Osaka 569-8686, Japan. Fax: +81 72 684 6518.
E-mail address: masaii@art.osaka-med.ac.jp (M. Ii).

http://dx.doi.org/10.1016/j.bbrc.2014.03.080
0006-291X/

analysis. Here we provide the first preclinical proof-of-principle data to indicate that AdSCs influence proliferation/apoptosis, suggesting that further investigations aimed at the development of novel AdSC therapy for PCa patients would be justified. Our data regarding not only the anti-proliferative and pro-apoptotic effects of AdSCs on PCa cells but also their inhibitory effect on PCa cell-derived tumor growth appear to have important implications for future research on PCa.

2. Materials and methods

2.1. Cell lines

LNCaP and PC3 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Detailed information on the characteristics of these cells and the culture conditions employed is given in Supplementary data 2.

2.2. Cell proliferation assay

LNCaP cell or PC-3 cell proliferation activity was evaluated using both co-culture and separated culture of AdSCs versus NHDFs. The protocols are described briefly in Supplementary data 2.

2.3. Apoptosis assay

AdSC- or NHDF-induced apoptosis in LNCaP cells and PC-3 cells was assessed using an APO Percentage™ apoptosis assay kit (Biocolor Ltd., Belfast, UK). The protocol is described briefly in Supplementary data 2.

2.4. Assessment of activated caspase-3/7

Caspase-3/7 activation assay was performed using a Caspase-Glo™ 3/7 assay kit (Promega, Madison, WI) in accordance with the manufacturer's instructions. The protocol is described briefly in Supplementary data 2.

2.5. Assessment of tumor growth

All animal procedures were performed according to the guidelines of the Osaka Medical College Animal Care and Use Committee with the approval protocol number 24089. LNCaP cell- or PC-3 cell-derived subcutaneous tumors were induced in athymic nude mice, and the size of each tumor was measured weekly following local injection of AdSCs versus NHDFs at sites of tumor development. Details of the procedure are described in Supplementary data 2.

2.6. Human genome cDNA microarray analysis

Total RNA of AdSCs or NHDFs was isolated with TRIzol reagent (Life Technologies, Carlsbad, CA) and analyzed using a Whole Human Genome 4 × 44 k microarray (Agilent Technologies, Polo Alto, CA) at Alliance Biosystems, Inc. (Osaka, Japan) in accordance with the manufacturer's instructions. The protocol used for the analysis is described briefly in Supplementary data 2.

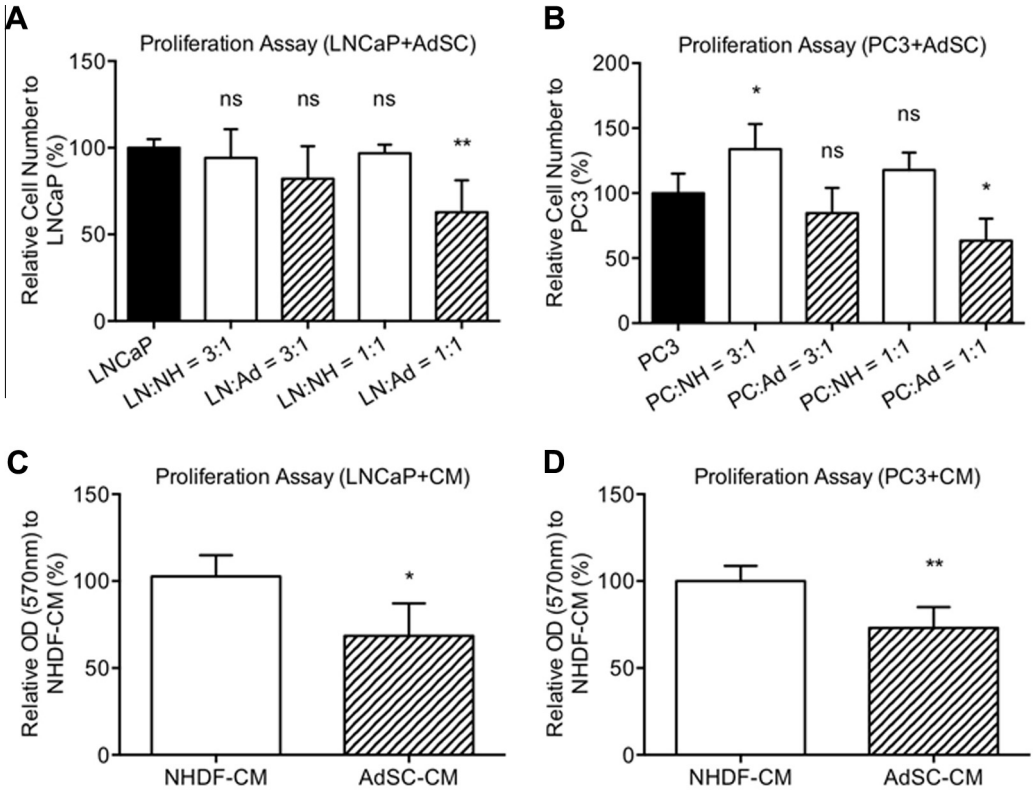

Fig. 1. Effect of AdSCs on proliferation activity in LNCaP cells and PC3 cells. DiI-LNCaP cells (LN) (A) or DiI-PC3 cells (PC) (B) were co-cultured with NHDFs or AdSCs at ratios of 1:1 and 3:1. The proliferations of LN and PC were assessed by counting DiI positive cell number in each experimental group, and the percentage of cell number to LNCaP or PC3 in each group were expressed as relative proliferation activities to LNCaP cell/PC3 cell alone. ns, not significant and $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. LNCaP or PC3. LNCaP cells (C) or PC3 cells (D) cells were cultured in the presence of ADSC-or NHDF-conditioned medium (CM) under a separate culture condition. Cell viability/proliferation was expressed as values at optical density (OD) of 570 nm wavelength. ns, not significant and $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. NHDF-CM.

2.7. Statistical analysis

All values in vitro are presented as mean ± SD, and those in vivo are presented as mean ± SEM. Statistical comparisons between 2 groups were performed by Mann–Whitney test. Multiple groups were analyzed by one-way ANOVA followed by appropriate post hoc test (Bonferroni procedure) or two-way ANOVA with a repeated measurement test to determine statistical significance. All in vitro experiments were repeated at least in triplicate and analyzed.

3. Results

3.1. AdSCs inhibit proliferation of LNCaP and PC3 cells

LNCaP or PC3 cells were co-cultured with AdSCs or NHDFs at different ratios for 48–72 h. AdSCs exhibited a significant inhibitory effect on the proliferation of both cell types. Although there was a tendency for a greater reduction of LNCaP cell number at a 3:1 ratio with AdSCs in comparison with NHDFs at the same ratio, a significant reduction was also observed at a 1:1 ratio with AdSCs, whereas the reduction was not significant with NHDFs (Fig. 1A). On the other hand, a significant dose-dependent reduction of PC3 cell number was observed at both 3:1 and 1:1 ratios with AdSCs, while PC3 cell number was not reduced when co-cultured with NHDFs (Fig. 1B). Next, we examined the proliferation of LNCap and PC3 cells in the presence of CM derived from AdSCs or NHDFs using a Transwell 24 well plate under direct cell-to-cell contact-free conditions. As shown in Fig. 1C and D, AdSC-derived CM significantly inhibited the proliferation/viability of both LNCaP and PC3 cells compared with NHDF-derived CM. These co-culture experiments revealed that not only direct cell-to-cell contact but also certain paracrine factors derived from AdSCs inhibited the proliferation of both LNCaP and PC3 cells.

3.2. AdSCs induce apoptosis in LNCaP cells and PC3 cells

To assess PCa cell apoptosis as well as inhibition of proliferation by AdSCs, an apoptosis assay was performed using LNCaP cells and PC3 cells with AdSC- or NHDF-derived CM. Apoptosis was observed more frequently in both LNCaP cells and PC3 cells with AdSC-CM than in cells with NHDF-derived CM (Fig. 2A). Quantification of PCa cell apoptosis indicated that the ratio of apoptotic cells (%) was significantly higher for AdSCs than for NHDFs in both LNCaP and PC3 cells (Fig. 2B).

We then examined caspase 3/7 expression, which is activated when cells undergo apoptosis, in LNCaP cells and PC3 cells cultured with AdSC- or NHDF-derived CM. Caspase 3/7 expression in the presence of AdSCs-derived CM was significantly increased in both LNCaP cells and PC3 cells compared with that in the presence of NHDF-derived CM (Fig. 2C and D).

3.3. AdSCs inhibit the growth of tumors derived from LNCaP and PC3 cells

We next evaluated the effects of AdSCs on PCa cell-derived tumor growth in vivo. Male athymic mice bearing LNCaP tumors were castrated and simultaneously subjected to subcutaneous injection of AdSCs or NHDFs to determine whether AdSCs inhibited androgen-independent LNCaP tumor growth. Mice bearing PC3 tumors were also randomly treated with AdSC or NHDF by subcutaneous injection. The mean tumor volume was similar in all groups before each treatment. In LNCaP xenografts, AdSCs inhibited tumor

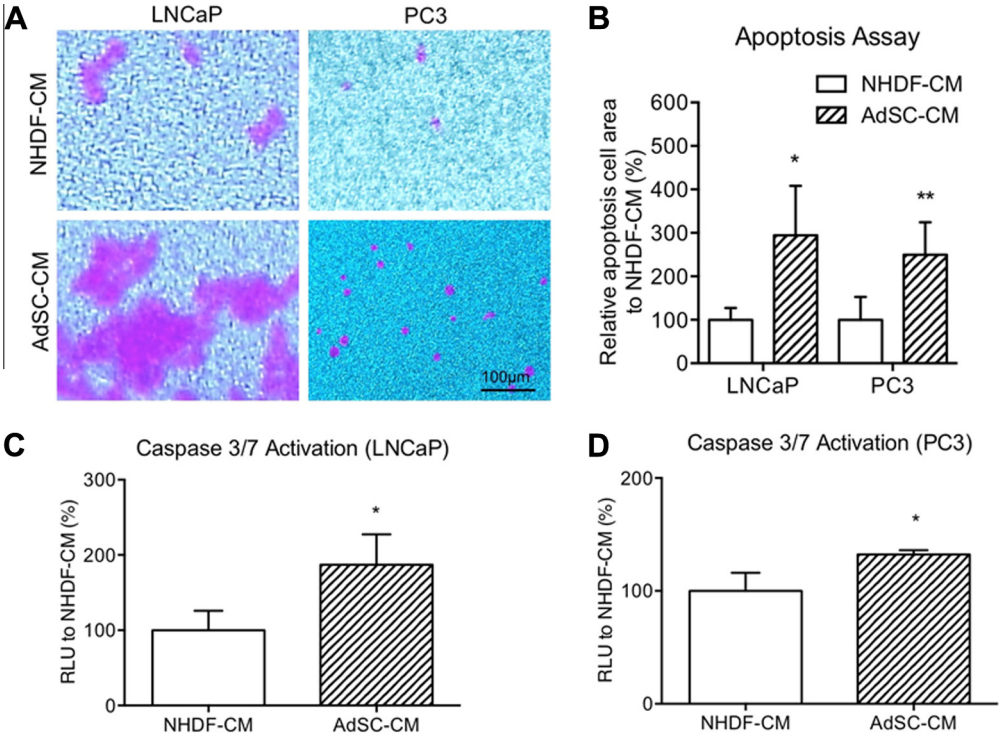

Fig. 2. AdSC-induced apoptosis in LNCaP cells and PC3 cells. (A) Photomicrographs representing purple stained apoptotic cells in LNCaP cells or PC3 cells with AdSC- or NHDF-derived -conditioned medium (CM). (B) Percent of apoptotic cell area of LNCaP cells or PC3 cells with AdSC (filled bar)- or NHDF (open bar)-derived CM in high power field (200X) were quantified. The relative apoptosis area of LNCaP cells or PC3 cells in NHDF-CM to that in AdSC-CM is expressed as pro-apoptotic activity. Caspase 3/7 was measured in LNCaP cells (C) or PC3 (D) cells with AdSC- or NHDF-derived CM and relative luminescence (RLU) of the cells with AdSC-CM to those with NHDF-CM was expressed as Caspase 3/7 activity. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. NHDF-CM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

growth after castration (Fig. 3A). The mean tumor volume of LNCaP xenografts with AdSCs was significantly lower than that with NHDFs at 3 and 4 weeks after castration. In androgen-independent PC3 xenografts, AdSCs significantly reduced the rate of tumor growth as compared with those treated with NHDFs from 3 weeks after the treatment (Fig. 3B). At 6 weeks, the mean tumor volume of PC3 xenografts with AdSCs was significantly lower than that with NHDFs. These findings indicate that single local transplantation of AdSCs significantly inhibits the growth of androgen-independent PC3 xenografts and CR progression of LNCaP androgen-responsive xenografts when combined with castration.

3.4. High expression of the TGF-β1 gene in AdSCs

To further elucidate how AdSCs negatively influence PCa cell proliferation/viability, we next performed cDNA microarray analysis with AdSCs and NHDFs (Supplementary data 1). We focused on apoptosis-related genes (>450) whose expression levels were high and more than 3-fold higher in AdSCs than in NHDFs (Table 1). Among them, the TGF-β1 gene was more highly expressed in AdSCs than in NHDFs (401330 vs. 41480) and the ratio of AdSCs expressing TGF-β1 relative to NHDFs was more than 9-fold (9.68). Up-regulation of TGF-β1, which is released as a protein, in AdSCs might be a key factor involved in induction of apoptosis in PCa cells, inhibiting their proliferation via the TGF-β signaling pathway. In addition, members of the tumor necrosis factor superfamily, CD70 and TNFSF9, were also up-regulated in AdSCs (5.5-fold and 4.6-fold, respectively) compared with those in NHDFs. Both CD70 and TNFSF9 are also secreted ligands that can activate/generate cytotoxic T cells, which may induce cancer cell death. Furthermore, the apoptosis-inducible transcription factors ATF5 and GADD45G were up-regulated in AdSCs (9.1-fold and 3.4-fold, respectively) to a greater degree than in NHDFs. These AdSC-derived transcription factors may play a role in cancer cell proliferation/apoptosis when AdSCs and cancer cells come into contact with each other. These findings suggest that AdSCs negatively regulate PCa cell viability indirectly through secretion of cytokines/ligands and also by direct contact.

4. Discussion

We have examined the anti-proliferative and pro-apoptotic effects of AdSCs on PCa cells, and the data we obtained from in vitro co-culture experiments indicated that AdSCs inhibited the growth of the human PCa cell lines, LNCaP and PC3, regardless of their androgen-responsiveness. Moreover, the inhibitory effect of AdSCs on PCa cell proliferation was observed not only under cell-to-cell contact conditions but also when the cells were cultured separately, suggesting that AdSCs regulate PCa cell proliferation/apoptosis both directly and remotely.

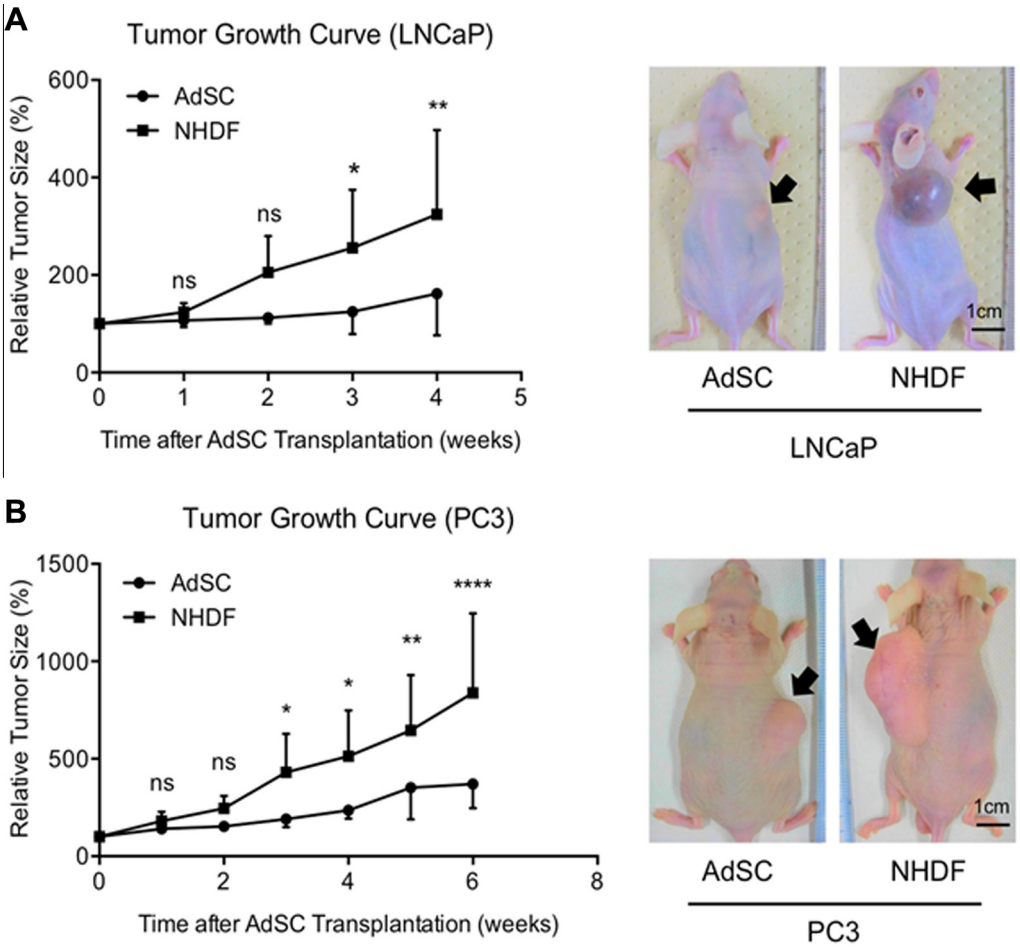

Fig. 3. Effect of AdSCs on LNCaP cell- and PC3 cell-derived tumor growth. (A) LNCaP cells were inoculated in mice subcutaneously, and when tumor volume reached 200–500 mm^3 (right panels) mice were castrated and randomly assigned in either AdSC or NHDF subcutaneous local injection to sites of tumor. (B) PC3 cells were inoculated in mice subcutaneously and when tumors reached to be palpable (right panels), mice were randomly selected for treatment with the same protocol as LNCaP. Each time point represents the relative mean size of LNCaP-derived tumor (A, left panel) and PC3-derived tumor (B, left panel) (%) in each group. Arrows indicate sites of tumor. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{****}p < 0.0001$ vs. AdSC.

Table 1
Apoptosis-related gene expressions in human AdSC and NHDF.

Expression ratio (AdSC/NHDF)	Gene expression normalized to GAPDH		Gene symbol	Genebank ID	Gene name
	AdSC	NHDF			
9.7	401330	41480	TGFB1	NM_000358	Transforming growth factor, beta-induced, 68 kDa
9.1	5044	557	ATF5	NM_012068	Activating transcription factor 5
5.5	589	106	CD70	NM_001252	CD70 molecule (tumor necrosis factor superfamily member 7, TNFSF7)
4.6	1477	319	CD137	NM_001561	CD137 molecule (tumor necrosis factor superfamily member 9, TNFSF9)
3.4	457	134	GADD45G	NM_006705	Growth arrest and DNA-damage-inducible, gamma

With regard to the remote/paracrine effect of AdSCs on PCa cells, the data obtained by cDNA microarray with AdSCs versus NHDFs allowed us to select genes whose expression was up-regulated more than 3-fold (Table 1). These included secreted cytokines/ligands and transcription factors as candidate molecules playing a role in the regulation of proliferation/apoptosis. Among the genes up-regulated in AdSCs, we focused on TGF-β1 as a key secretory factor. Members of the TGF-β family regulate physiological responses such as wound healing as well as pathophysiological responses, and also play essential roles in the regulation of cell migration, survival, proliferation, and differentiation [9]. Apart from these functions, TGF-β1 is known to be a potent inhibitor of cell cycle progression in a variety of cell types including epithelial, endothelial, hematopoietic, and mesenchymal cells [10]. However, any definitive role of TGF-β1 in cancer biology is highly controversial. For instance, various members of the TGF-β family have been found to play an important role in PCa progression [11]. Indeed, clinical studies have shown that up-regulation of TGF-β1 in PCa tissues, and high urinary/serum levels of TGF-β1, are associated with enhanced tumor angiogenesis and tumor metastasis, resulting in a poor clinical outcome [12], whereas there is no direct promotional effect of TGF-β1 on PCa progression. In contrast, a very recent study has clearly demonstrated that TGF-β1 suppresses both androgen-sensitive (LNCaP) and castration-resistant (C4–2) cell growth through activation of Smad2/3 signaling [13], thus strongly supporting our present findings. We assume that these observed discrepancies with regard to the effect of AdSCs on PCa cells/tumors are likely attributable to complex variations in endogenous TGF-β1 production and indirect effects of TGF-β1 signaling in the stroma surrounding the tumor, whereas in our present experimental model exogenous TGF-β1 was supplied simply from AdSCs. We therefore propose that AdSCs could be employed as a TGF-β1 donor and that AdSC transplantation would have sufficient capacity to reduce PCa tumor growth through apoptosis.

Another possible mechanism for the indirect suppressive effect of AdSCs on tumor growth might be mediated by transformation of T lymphocytes into a cytotoxic phenotype in vivo. Our cDNA microarray data showed that members of the TNF superfamily, CD70 (TNFSF7) and CD137 (TNFSF9), were also more upregulated in AdSCs than in NHDFs (5.5-fold and 4.6-fold, respectively) (Table 1). The ligand CD70 has been shown to stimulate cytotoxicity and cytokine production in human T cell clones [14]. The ligand CD137 and its receptor (4-1BB) are involved in the antigen presentation process and the generation of cytotoxic T cells [15]. Thus, cytotoxic T cells activated by transplanted AdSC-derived CD70 or CD137 might inhibit the progression of PCa cells through apoptosis.

Another possible mechanism for the inhibitory effect of AdSCs on PCa cell proliferation may be cell-to-cell contact, perhaps involving transfer of transcription factors such as ATF5 and GADD45G, which can induce cell cycle arrest/apoptosis (Table 1) following contact with PCa cells. Although we did not obtain any direct evidence of cell fusion in the present series of experiments, some previous reports have indicated that mitochondria of AdSCs can be transferred to cardiomyocytes, resulting in cellular reprogramming [16], and that fusion of bone marrow-derived MSCs with alveolar epithelial cells can make the latter resistant to acute lung injury through transfer of MSC mitochondria [17].

In conclusion, we have provided the first evidence to suggest that AdSCs can directly inhibit the proliferation of two different types of PCa cells, androgen-responsive (LNCaP) and androgen–nonresponsive (PC3) cells, through apoptosis, and that AdSCs have an inhibitory effect on the growth of PCa cell-derived tumors in vivo. The anti-proliferative effect of AdSCs on PCa cells appears to be mediated, at least in part, by TGF-β1 secretion and signaling. Since AdSCs are easily obtainable as a stem cell source without any ethical concerns, and can be quickly expanded to the desired volume in culture, autologous AdSC transplantation appears to have promise as a novel therapeutic strategy for PCa.

Acknowledgments

The authors disclose no potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.03.080.

References

[1] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[2] J.M. Gimble, A.J. Katz, B.A. Bunnell, Adipose-derived stem cells for regenerative medicine, Circ. Res. 100 (2007) 1249–1260.
[3] D. Noel, D. Caton, S. Roche, C. Bony, S. Lehmann, L. Casteilla, C. Jorgensen, B. Cousin, Cell specific differences between human adipose-derived and mesenchymal–stromal cells despite similar differentiation potentials, Exp. Cell Res. 314 (2008) 1575–1584.
[4] J.M. Yu, E.S. Jun, Y.C. Bae, J.S. Jung, Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo, Stem Cells Dev. 17 (2008) 463–473.
[5] F.L. Muehlberg, Y.H. Song, A. Krohn, S.P. Pinilla, L.H. Droll, X. Leng, M. Seidensticker, J. Ricke, A.M. Altman, E. Devarajan, W. Liu, R.B. Arlinghaus, E.U. Alt, Tissue-resident stem cells promote breast cancer growth and metastasis, Carcinogenesis 30 (2009) 589–597.
[6] L. Prantl, F. Muehlberg, N.M. Navone, Y.H. Song, J. Vykoukal, C.J. Logothetis, E.U. Alt, Adipose tissue-derived stem cells promote prostate tumor growth, Prostate 70 (2010) 1709–1715.
[7] B. Cousin, E. Ravet, S. Poglio, F. De Toni, M. Bertuzzi, H. Lulka, I. Touil, M. Andre, J.L. Grolleau, J.M. Peron, J.P. Chavoin, P. Bourin, L. Penicaud, L. Casteilla, L. Buscail, P. Cordelier, Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo, PLoS One 4 (2009) e6278.
[8] A. Jemal, T. Murray, E. Ward, A. Samuels, R.C. Tiwari, A. Ghafoor, E.J. Feuer, M.J. Thun, Cancer statistics, CA Cancer J. Clin. 55 (2005) (2005) 10–30.
[9] J. Massague, TGFbeta in cancer, Cell 134 (2008) 215–230.
[10] G.J. Hannon, D. Beach, P15INK4B is a potential effector of TGF-beta-induced cell cycle arrest, Nature 371 (1994) 257–261.
[11] H.L. Adler, M.A. McCurdy, M.W. Kattan, T.L. Timme, P.T. Scardino, T.C. Thompson, Elevated levels of circulating interleukin-6 and transforming

growth factor-beta1 in patients with metastatic prostatic carcinoma, J. Urol. 161 (1999) 182–187.
[12] R. Weissert, A. Melms, H. Link, Altered tumor growth factor beta mRNA expression is associated with thymectomy-related clinical remission in myasthenia gravis, J. Neurol. Sci. 151 (1997) 49–55.
[13] F.L. Miles, N.S. Tung, A.A. Aguiar, S. Kurtoglu, R.A. Sikes, Increased TGF-beta1-mediated suppression of growth and motility in castrate-resistant prostate cancer cells is consistent with Smad2/3 signaling, Prostate 72 (2012) 1339–1350.
[14] C. Cormary, R. Gonzalez, J.C. Faye, G. Favre, A.F. Tilkin-Mariame, Induction of T-cell antitumor immunity and protection against tumor growth by secretion of soluble human CD70 molecules, Cancer Gene Ther. 11 (2004) 497–507.
[15] C. Gullo, L.K. Koh, W.L. Pang, K.T. Ho, S.H. Tan, H. Schwarz, Inhibition of proliferation and induction of apoptosis in multiple myeloma cell lines by CD137 ligand signaling, PLoS One 5 (2010) e10845.
[16] A. Acquistapace, T. Bru, P.F. Lesault, F. Figeac, A.E. Coudert, O. le Coz, C. Christov, X. Baudin, F. Auber, R. Yiou, J.L. Dubois-Rande, A.M. Rodriguez, Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer, Stem Cells 29 (2011) 812–824.
[17] M.N. Islam, S.R. Das, M.T. Emin, M. Wei, L. Sun, K. Westphalen, D.J. Rowlands, S.K. Quadri, S. Bhattacharya, J. Bhattacharya, Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury, Nat. Med. 18 (2012) 759–765.