IN VITRO SECRETING PROFILE OF HUMAN MESENCHYMAL STEM CELLS

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Original Research Report

In Vitro Secreting Profile of Human Mesenchymal Stem Cells

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ABSTRACT

In addition to their multilineage potential, mesenchymal stem cells (MSCs) also have a wide range of functionality. Not only can MSCs reconstruct a tissue, but they also have the ability to control or cure other cells and can reconstruct a coordinating function. The opportunity to control other cells depends on MSCs being able to secrete factors like cytokines and chemokines. Therefore, we focused on asking, Which factors can be secreted by human MSCs? To answer this question, we analyzed the secreting profile of in vitro-expanded MSCs by using cytokine arrays. The media concentrations of 44 of the 120 analyzed cytokines were significantly increased by MSCs. Conversely, concentrations of 40 cytokines given with the sera were significantly decreased. The data presented here provide an overview about a large range of factors that were secreted by MSCs under cell culture conditions. These data indicate that MSCs demonstrate all previously described functions in cellular interactions without an external stimulus. The MSCs secreted angiogenic, immunosuppressive, anti-apoptotic, and proliferation-stimulating factors.

INTRODUCTION

Mesenchymal stem cells (MSCs) are progenitors of all connective tissue cells. In the adults of multiple vertebrate species, MSCs have been isolated from bone marrow (BM) and other tissues, expanded in culture, and differentiated into several tissue-forming cells such as bone, cartilage, fat, muscle, tendon, liver, kidney, heart, and even brain cells. [1,2]. Comparative studies have demonstrated that MSCs from different sources show comparable characteristics, even though the expression profile differs for a large scale of genes [3]. The most commonly used source for MSCs is the bone marrow.

MSCs seem to be a useful tool for cellular therapy in cases of injured tissues. Different types of tissue engineering have been examined in the last years using MSCs. Besides their capability to differentiate into various kinds of cells, MSCs are able to rebuild, e.g., bone tissue [4,5], cartilage tissue [6], and vessels [7]. The reconstruction of vessels, or neovascularization, is of prime importance in a wide range of diseases. One focus of interest is the cure of cardiac infarct [8–14].

MSCs are not only able to reconstruct a tissue; they also have the ability to control or cure for other cells and are able to reconstruct a coordinating function. Le Blanc and colleges demonstrated the potent immunosuppressive effect of MSCs in humans [15]. Other groups have also shown the immunosuppressive activity in vitro and in vivo [16]. This activity may be based on the capability of MSCs to reconstitute a functional hematopoietic microenvironment in vivo [17].

The opportunity to control other cells depends on the capability to secrete many factors, like cytokines and chemokines. In addition to the fact that MSCs are able to control neovascularization, we would expect that MSCs are able to secrete a large number of angiogenic factors. The capability to engraft hematopoiesis depends on the care of hematopoietic stem and progenitor cells. Immunosuppressive activity is also coupled with secre-

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tion of cytokines, which also acts in an immunosuppressive manner. mRNA expression studies have indicated that MSCs may have the possibility of producing different kinds of soluble factors [18–20]. Therefore, we focused on the question: Which factors can be secreted byMSCs?

MATERIALS AND METHODS

Cell culture and manipulation

MSCs of human origin were obtained from bone marrow aspirates as well as bone marrow from femur and hip head as described before [21–24]. Before preparing Ficoll-Paque™ PLUS density gradient centrifugation (Amersham Pharmacia Biotech, Uppsala, Sweden), bone marrow was filtered (mesh 70 μm). The first change of medium, consisting of αminimum essential medium (α-MEM), 20% (vol/vol) fetal calf serum (FCS), 200 μM L-glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin, was prepared 2 days after culturing at 95% humidity and 5% CO_2. Cells were used until passage 3. For every passage or experiment, cells were plated at 2,000 cells/cm^2. Medium was changed twice a week.

Hematopoietic stem cells (HSCs) were obtained from unused clinical surplus peripheral blood of stem cell-mobilized donors. The HSCs were isolated using the magnetic cell separation system (MACS) from Miltenyi Biotech (Bergisch Gladbach, Germany) and anti-CD34 according to the manufacturer's guidelines. HSCs were always used fresh for experiments. Experimental cultivation of HSCs was done using the complete medium StemPro-34 SFM (Invitrogen, CA), which consists of STEMPRO-34 SFM and STEMPRO-34 Nutrient Supplement. For proliferation, stem cell factor (SCF), thrombopoietin (TPO), and granulocyte colony-stimulating factor (G-CSF; Sigma-Aldrich, MO) were added to a final concentration of 100 ng/ml.

This study was approved by the local ethics committee and conforms to the Declaration of Helsinki.

Serum analysis of cytokines and chemokines

A cytokine/chemokine array kit (Ray Biotech Inc., Norcross, GA) was used to detect a panel of 120 secreted cytokines and chemokines. The manufacturer's recommended protocol was used.

For quantitative analysis of vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), and tumor necrosis factor (TNF), the BD Cytometric Bead Array Flex system (CBA-Flex system) from BD Biosciences (San Diego, CA) was used. The manufacturer's recommended protocol was used.

Densitometry

For densitometry analysis, the gray scale values were measured using the computer-based system ImageJ Version 1.33 from the National Institutes of Health (NIH) [25]. All values were recalculated as relative intensity in relation to the range between positive and negative control.

RESULTS

MSCs from independent donors were cultured for 3 days. As a control, HSCs as well as the medium used for MSCs in absence of cells were cultured using same conditions. The supernatant of the stem cell culture as well as the medium control were analyzed using the RayBiotech cytokine array for analyzing 120 different cytokines and chemokines. By using computer-based densitometry, the relative intensity in relation to the range between positive and negative control was calculated (Fig. 1).

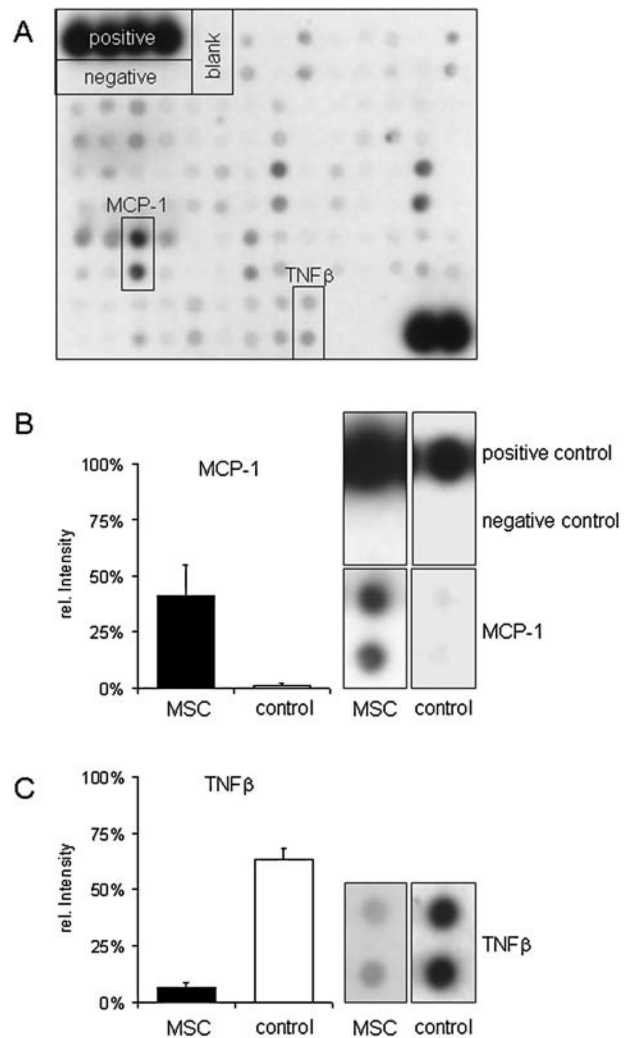


FIG. 1. (**A**) A typical secretion profile of MSCs using the cytokine array membrane. The positive and negative controls as well as blank positions are placed at two opposite corners. For calculation of the relative intensity, the gray scale value between negative and positive controls was used as the given range. The MSC supernatant showed an increased content of MCP-1 compared to the control (**B**), whereas the concentration of TNF-β is reduced in the supernatant of MSCs (**C**).

By comparing MSC samples with medium controls and also the supernatant of HSCs, a high number of cytokines were secreted specifically by MSCs. For example, TIMP-2, which shows a relative intensity of 1%, was not significantly influenced by HSCs (10%), but it was increased in the supernatant of MSCs up to a mean value of 62%. The factor angiopoetin-2 increased in the supernatant of MSCs up to a mean value of 47%, whereas the relative concentration in HSCs (1%) compared to control (2%) remained unchanged (Fig. 2A). In contrast, other factors were consumed by the cells and therefore decreased in the supernatant. An overview of the 10 factors that underwent the highest decrease in the supernatant is shown in Fig. 2B.

By comparing all MSC samples with the controls, 44 of the 120 cytokines were secreted in a significant manner based on a significance level of $p < 0.05$. In contrast, 40 of the tested cytokines showed a significant decrease in the supernatant of MSCs. The secreted cytokines can be pooled in different groups. First of all, different factors that are able to prevent the extrinsic apoptosis were secreted by MSCs. Factors such as soluble TRAIL R3,

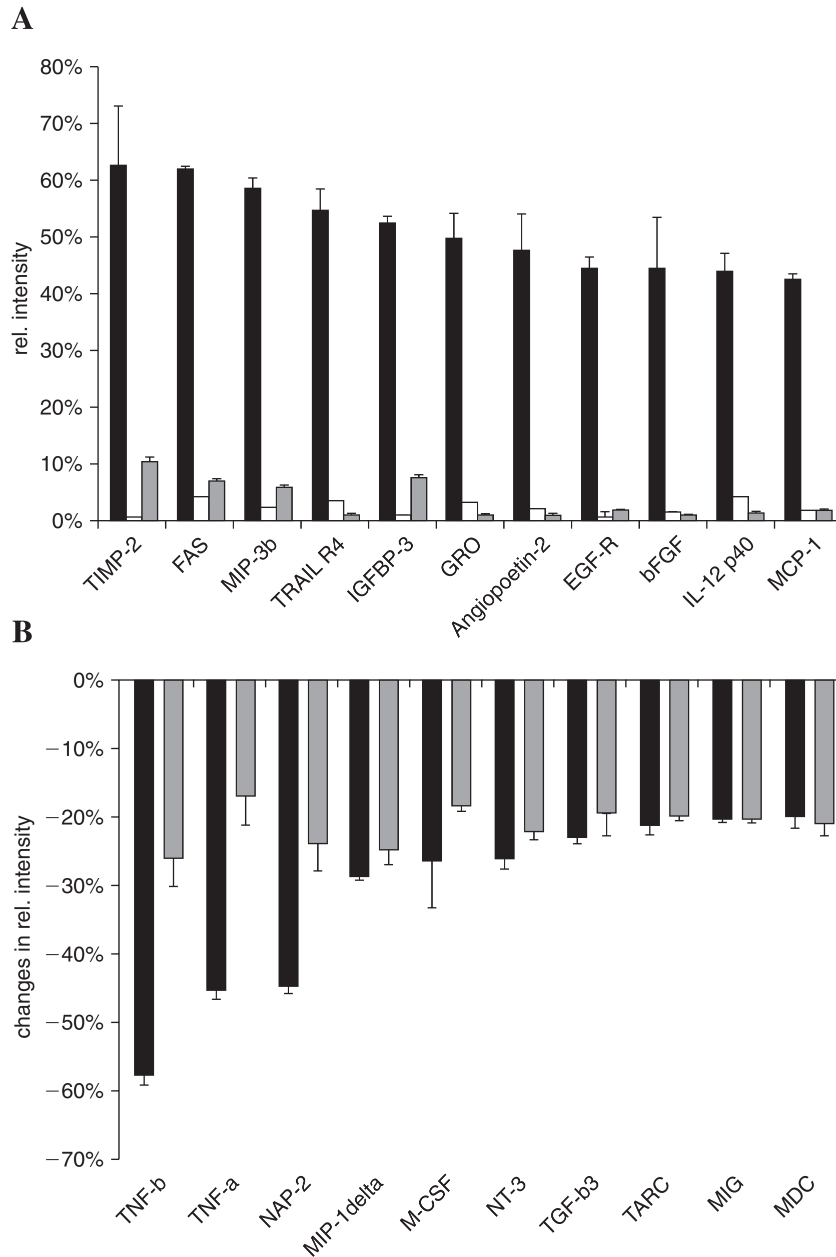


FIG. 2. (**A**) Overview of the 10 most increased cytokines in the supernatant of MSCs (black bars) compared to medium control (white bars) and HSC supernatant (gray bars). (**B**) Comparison of the changes in cytokine concentrations in the supernatant of MSCs (black bars) and HSCs (gray bars) of the 10 most decreased factors.

TRAIL R4, or FAS receptor are examples of these. This effect was supported by consuming pro-apoptotic factors like tumor necrosis factor-α (TNF-α or TNF-β. The second group contained factors that are immunosuppressive or factors for which changes result in an immunosuppressive effect. These are factors like interleukin-12 p40 (IL-12 p40), Acrp30, or the soluble IL-2 receptor α. This effect was also supported by decreasing inflammatory factors like IL-1α/β, IL-2, or RANTES. A third group included factors that are able to induce cell proliferation, especially in hematopoietic cells. These are factors like G-CSF, fibroblast growth factor-4 (FGF-4), or FGF-9. Finally, factors that are able to interfere with angiogenesis were secreted by MSCs. These were factors like angiopoietin-2, bFGF, TIMP-1, TIMP-2, or VEGF (Table 1). In contrast, the HSCs demonstrated only four factors that had shown a significant increase in the cell supernatant. Those significantly increased were IL-16, FGF-7, Dtk, and CTACK.

To verify the results of the cytokine array, we also performed quantitative analysis of selected factors on an enlarged group of MSC samples. Using the CBA-Flex system, we analyzed the concentration of VEGF, IL-8, and TNF in 10 independent MSC samples. After 3 days of cell culture, the MSCs produced a mean value of 5,330pg/ml of VEGF. The production of IL-8 was a mean value of 345pg/ml, whereas TNF was not produced by all of the tested samples (Fig. 3).

DISCUSSION

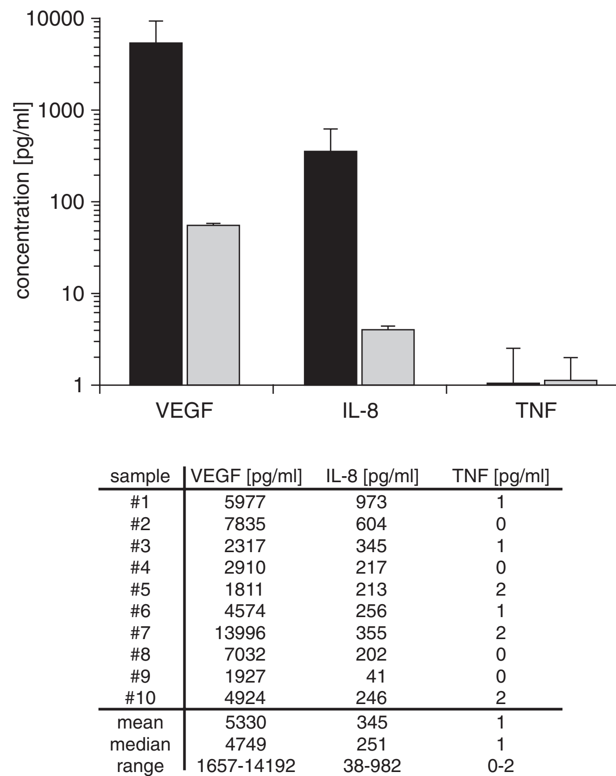
The results presented here are the first large-scale description of factors that are secreted by human (h) bone marrow-derived MSCs. These were obtained with hMSCs that were isolated as described by Pittenger et al. [1]. Pittenger described that MSCs expressed, e.g., IL-6R and TNF-RII on their cell surfaces [1]. We found the soluble form of both receptors in the supernatant of our cultures. Former studies had also analyzed the secreting potential of hMSCs by quantifying the mRNA [18–20]. These studies had described that the mRNA of IL-11 is expressed in hMSCs, and we also detected in this mRNA supernatant. Data were not in agreement in the case of G-CSF [19,20], which we also found in the supernatant. Kim and colleges did not detect the mRNA for IL-7, which also conforms with our secreting data [20].

Liu and colleges described a large-scale expression profile of hMSCs derived from human cord blood [26]. He described 30 factors that were also tested by us. Twelve of these factors were secreted in cord blood MSCs as well as in our hMSCs (e.g., FGF-4, IL-8, TIMP-1, TIMP-2, VEGF). Seven factors were not secreted in both studies (e.g., IL-4, IL-7, TNF-α, TNF-β). FGF-7 was secreted in cord blood-derived MSCs but not in bone marrow-derived MSCs. Liu described a secretion of 10 factors that did not agree with our data (e.g., IL-6, GM-CSF, IGFBP-1). For all of these 10 factors, we also ob-

TABLE 1. OVERVIEW OF ALL ANALYZED CYTOKINES

Secreted	*Consumed*	*Non-significant*
Acrp30, AgRP, Angiopoietin-2, bFGF, BTC, EGF-R, FAS, FGF-4, FGF-9, G-CSF, GITR, GITR-Ligand, GRO, HGF, ICAM-3, IGF-1SR, IGFBP3, IGFBP6, IL-2Rα, IL-6R, IL-8, IL-11, IL-12p40, IL-17, Lymphotaktin, MCP-1, MIF, MIP-1α, MIP-1β, MIP-3β, MSPα, NT-4, Oncostatin M, Osteoprotegerin, PIGF, sgp130, sTNF RII, TIMP-1, TIMP-2, TRAIL R3, TRAIL R4, uPAR, VEGF, VEGF-D	BLC, BMP-6, CKβ8-1, EGF, Eotaxin, FracTalkine, GCP-2, GM-CSF, I-309, IGFBP1, IGFBP2, IGFBP4, IFNγ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-16, MCP-3, MCP-4, M-CSF, MDC, MIG, MIP-1δ, MIP-3α, NAP-2, NT-3, PARC, PDGF-BB, RANTES, SCF, SDF-1, TARC, TGF-β1 TGF-β3, TNF-α, TNF-β	Amphiregulin, Angiogenin, Axl, BDNF, BMP-4, βNGF, CCL-28, CNTF, CTACK, Dtk, ENA-78, Eotaxin02, Eotaxin-3, FGF-6, FGF-7, Flt-3 Ligand, GDNF, GROα, HCC-4, ICAM-1, IGF-1, IL-1R4/ST2, IL-1Rα, IL-1RI, IL-6, IL-10, IL-12p70, IL-13, IL-15, I-TAC, Leptin, LIGHT, MCP-2, sTNF-RI, TECK, Thrombopoetin

Cytokines that showed a significantly increased concentration within the supernatant of MSCs were grouped as secreted. Cytokines that showed a significantly decreased concentration were grouped as consumed. All cytokines that had not shown a significant change in concentration were sorted into the nonsignificant group.



sample	VEGF [pg/ml]	IL-8 [pg/ml]	TNF [pg/ml]
#1	5977	973	1
#2	7835	604	0
#3	2317	345	1
#4	2910	217	0
#5	1811	213	2
#6	4574	256	1
#7	13996	355	2
#8	7032	202	0
#9	1927	41	0
#10	4924	246	2
mean	5330	345	1
median	4749	251	1
range	1657-14192	38-982	0-2

FIG. 3. Quantitative analyses of VEGF, IL-8, and TNF in the supernatant of MSCs compared to control. The MSC supernatant of 10 different donors was analyzed.

tained a positive signal in the RayBiotech cytokine array. But in all of these cases, the signal in the medium control was equal or higher. We postulate that the signal results from a cross-reactivity with the fetal cytokines contained in the medium. In case of TNF-α and TNF-β, we obtained a strong signal in the medium control that was significantly decreased in the hMSC culture. By analyzing the supernatant as well as the medium control with a non-cross-reactive CBA-system for TNF, all samples were negative.

Functional grouping of the secreted and consumed factors indicated four independent units. The hMSCs are acting by soluble factors as anti-apoptotic, immunosuppressive, proliferation enhancing, and modulator for angiogenesis. Six factors from the TNF family were changed by hMSCs. Osteoprotegerin, TNF-RII, TRAIL R3, and TRAIL R4 were secreted, whereas TNF-α and TNF-β were consumed by the hMSCs. In contrast to the TRAIL receptors TRAIL-R1 and TRAIL-R2, which contain a cytoplasmic death domain and signal apoptosis, the two secreted receptors TRAIL-R3 and TRAIL-R4 only act as decoy receptors. They lack a functional death domain and do not mediate apoptosis [27]. Osteoprotegerin is also a member of the TNF receptor superfamily and is known to be produced by bone marrow stromal cells. Besides its normal function, osteoprotegerin also protects breast cancer cells from TRAIL-induced apoptosis [28]. It can also act as a soluble factor in the regulation of bone mass [29].

Zappia and colleges have shown that MSCs are able to reduce the serum level of interferon-γ (IFN-γ and TNF-α produced by activated T cells [30]. Both factors were also reduced by the tested MSCs. The TNF-α reduction can be explained by secretion of soluble TNF receptor II, which acts as an antagonist for TNF-α and TNF-β [31]. IL-12 p40, but not the subunit IL-12 p70, were secreted by the MSCs. Homodimers of IL-12 p40 act antagonistically to IL-12-mediated immune response. It is known that IL-12 p40 reduces the activity of T helper cells [32]. MSCs inhibited IL-2-induced lymphocytic proliferation [33]. This can be explained by the observed secretion of soluble IL-2 receptor α. In contrast, the secreted sIL-6 receptor promotes the growth of hematopoietic progenitor cells [34]. Different factors, including the secreted IGFBP3, were postulated, with which stromal cells were able to support proliferation of hematopoietic progenitor cells in a cell contact-free co-cultivation [35].

There is last, but not least, a large number of factors that are associated with angiogenesis and were secreted by MSCs. These were the classical angiogenic factors like angiopoietin-2, bFGF, FGF-4, FGF-9, TIMP-1, TIMP-2, VEGF, or VEGF-D. But factors like G-CSF are also able to accelerate neovascularization [36]. These angiogenic factors also have impacts on hematopoietic cell differentiation and proliferation. For example, FGF-9 is able to stimulate thrombopoiesis in vivo [37], or FGF-4 induces cell proliferation in megacaryocytes (38).

MSCs are one of the two most common stem cells found in the bone marrow. Therefore, we also compared the results with HSCs. Whereas the MSCs had shown the described broad range of secreted factors, in the case of HSCs, it was only possible to identify four significant secreted factors. These findings underlined the outstanding importance of MSCs for the bone marrow environment.

In summary, the presented data provide an overview about a large range of factors that were secreted by MSCs under cell culture conditions. These data indicate that MSCs demonstrate all previously described functions in cellular interactions without an external stimulus. The cells secreted angiogenic, immunosuppressive, antiapoptotic, and proliferation-stimulating factors.

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REFERENCES

1. Pittenger MF, AM Mackay, SC Beck, RK Jaiswal, R Douglas, JD Mosca, MA Moorman, DW Simonetti, S Craig and DR Marshak. (1999). Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147.
2. Alhadlaq A and JJ Mao. (2004). Mesenchymal stem cells: isolation and therapeutics. Stem Cells Dev 13:436–448.
3. Wagner W, F Wein, A Seckinger, M Frankhauser, U Wirkner, U Krause, J Blake, C Schwager, V Eckstein, W Ansorge and AD Ho. (2005). Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 33:1402–1416.
4. Meinel L, V Karageorgiou, R Fajardo, B Snyder, V Shinde-Patil, L Zichner, D Kaplan, R Langer and G Vunjak-Novakovic. (2004). Bone tissue engineering using human mesenchymal stem cells: effects of scaffold material and medium flow. Ann Biomed Eng 32:112–122.
5. Meinel L, V Karageorgiou, S Hofmann, R Fajardo, B Snyder, C Li, L Zichner, R Langer, G Vunjak-Novakovic and DL Kaplan. (2004). Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds. J Biomed Materials Res 71:25–34.
6. Li WJ, R Tuli, C Okafor, A Derfoul, KG Danielson, DJ Hall and RS Tuan. (2004). A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. Biomaterials 26:599–609.
7. Koike N, D Fukumura, O Gralla, P Au, JS Schechner and RK Jain. (2004). Tissue engineering: creation of long-lasting blood vessels. Nature 428:138–139.
8. Orlic D, J Kajstura, S Chimenti, DM Bodine, A Leri and P Anversa. (2003). Bone marrow stem cells regenerate infarcted myocardium. Pediatr Transplant 7 (Suppl 3):86–88.
9. Wang JS, D Shum-Tim, E Chedrawy and RC Chiu. (2001). The coronary delivery of marrow stromal cells for myocardial regeneration: pathophysiologic and therapeutic implications. J Thorac Cardiovasc Surg 122:699–705.
10. Poss KD, LG Wilson and MT Keating. (2002). Heart regeneration in zebrafish. Science 298:2188–2190.
11. Leferovich JM, K Bedelbaeva, S Samulewicz, XM Zhang, D Zwas, EB Lankford and E Heber-Katz. (2001). Heart regeneration in adult MRL mice. Proc Natl Acad Sci USA 98:9830–9835.
12. Orlic D, J Kajstura, S Chimenti, F Limana, I Jakoniuk, F Quaini, B Nadal-Ginard, DM Bodine, A Leri and P Anversa. (2001). Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 98:10344–10349.
13. Jackson KA, SM Majka, H Wang, J Pocius, CJ Hartley, MW Majesky, ML Entman, LH Michael, KK Hirschi and MA Goodell. (2001). Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 107:1395–1402.
14. Strauer BE, M Brehm, T Zeus, M Kostering, A Hernandez, RV Sorg, G Kogler and P Wernet. (2002). Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 106:1913–1918.
15. Le Blanc K, I Rasmusson, B Sundberg, C Gotherstrom, M Hassan, M Uzunel and O Ringden. (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363:1439–1441.
16. Bacigalupo A. (2004). Mesenchymal stem cells and haematopoietic stem cell transplantation. Best Pract Res Clin Haematol 17:387–399.
17. Muguruma Y, T Yahata, H Miyatake, T Sato, T Uno, J Itoh, S Kato, M Ito, T Hotta and K Ando. (2006). Reconstitution of the functional human hematopoietic microenvironment derived from human mesenchymal stem cells in the murine bone marrow compartment. Blood 107:1878–1887.
18. Silva WA, Jr., DT Covas, RA Panepucci, R Proto-Siqueira, JL Siufi, DL Zanette, AR Santos and MA Zago. (2003). The profile of gene expression of human marrow mesenchymal stem cells. Stem Cells 21:661–669.
19. Majumdar MK, MA Thiede, SE Haynesworth, SP Bruder and SL Gerson. (2000). Human marrow-derived mesenchymal stem cells (MSCs) express hematopoietic cytokines and support long-term hematopoiesis when differentiated toward stromal and osteogenic lineages. J Hematother Stem Cell Res 9:841–848.
20. Kim DH, KH Yoo, KS Choi, J Choi, SY Choi, SE Yang, YS Yang, HJ Im, KH Kim, HL Jung, KW Sung and HH Koo. (2005). Gene expression profile of cytokine and growth factor during differentiation of bone marrow-derived mesenchymal stem cell. Cytokine 31:119–126.
21. Schmidt A, D Ladage, T Schinkothe, U Klausmann, C Ulrichs, FJ Klinz, K Brixius, S Arnhold, B Desai, U Mehlhorn, RH Schwinger, P Staib, K Addicks and W Bloch. (2006). Basic fibroblast growth factor controls migration in human mesenchymal stem cells. Stem Cells 24:1750–1758.
22. Schmidt A, D Ladage, C Steingen, K Brixius, T Schinkothe, FJ Klinz, RH Schwinger, U Mehlhorn and W Bloch. (2006). Mesenchymal stem cells transmigrate over the endothelial barrier. Eur J Cell Biol 85:1179–1188.
23. Arnhold S, H Klein, FJ Klinz, Y Absenger, A Schmidt, T Schinkothe, K Brixius, J Kozlowski, B Desai, W Bloch and K Addicks. (2006). Human bone marrow stroma cells display certain neural characteristics and integrate in the subventricular compartment after injection into the liquor system. Eur J Cell Biol 85:551–565.
24. Klinz FJ, A Schmidt, T Schinkothe, S Arnhold, B Desai, F Popken, K Brixius, R Schwinger, U Mehlhorn, P Staib, K Addicks and W Bloch. (2005). Phospho-eNOS Ser-114 in human mesenchymal stem cells: constitutive phosphorylation, nuclear localization and upregulation during mitosis. Eur J Cell Biol 84:809–818.
25. Rasband W. (2004). ImageJ, http://rsb.info.nih.gov/ij/. National Institutes of Health, Bethesda, MD.
26. Liu CH and SM Hwang. (2005). Cytokine interactions in mesenchymal stem cells from cord blood. Cytokine 32: 270–279.
27. Baetu TM and J Hiscott. (2002). On the TRAIL to apoptosis. Cytokine Growth Factor Rev 13:199–207.
28. Neville-Webbe HL, NA Cross, CL Eaton, R Nyambo, CA Evans, RE Coleman and I Holen. (2004). Osteoprotegerin (OPG) produced by bone marrow stromal cells protects breast cancer cells from TRAIL-induced apoptosis. Breast Cancer Res Treat 86:269–279.

29. Simonet WS, DL Lacey, CR Dunstan, M Kelley, MS Chang, R Luthy, HQ Nguyen, S Wooden, L Bennett, T Boone, G Shimamoto, M DeRose, R Elliott, A Colombero, HL Tan, G Trail, J Sullivan, E Davy, N Bucay, L Renshaw-Gegg, TM Hughes, D Hill, W Pattison, P Campbell, S Sander, G Van, J Tarpley, P Derby, R Lee and WJ Boyle. (1997). Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319.
30. Zappia E, S Casazza, E Pedemonte, F Benvenuto, I Bonanni, E Gerdoni, D Giunti, A Ceravolo, F Cazzanti, F Frassoni, G Mancardi and A Uccelli. (2005). Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 106:1755–1761.
31. Diez-Ruiz A, GP Tilz, R Zangerle, G Baier-Bitterlich, H Wachter and D Fuchs. (1995). Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. Eur J Haematol 54:1–8.
32. Yoshimoto T, CR Wang, T Yoneto, S Waki, S Sunaga, Y Komagata, M Mitsuyama, J Miyazaki and H Nariuchi. (1998). Reduced T helper 1 responses in IL-12 p40 transgenic mice. J Immunol 160:588–594.
33. Rasmusson I, O Ringden, B Sundberg and K Le Blanc. (2005). Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. Exp Cell Res 305:33–41.
34. Jones SA, S Horiuchi, N Topley, N Yamamoto and GM Fuller. (2001). The soluble interleukin 6 receptor: mechanisms of production and implications in disease. FASEB J 15:43–58.
35. Oostendorp RA, C Robin, C Steinhoff, S Marz, R Brauer, UA Nuber, EA Dzierzak and C Peschel. (2005). Long-term maintenance of hematopoietic stem cells does not require contact with embryo-derived stromal cells in cocultures. Stem Cells 23:842–851.
36. Minamino K, Y Adachi, M Okigaki, H Ito, Y Togawa, K Fujita, M Tomita, Y Suzuki, Y Zhang, M Iwasaki, K Nakano, Y Koike, H Matsubara, T Iwasaka, M Matsumura and S Ikehara. (2005). Macrophage colony-stimulating factor (M-CSF), as well as granulocyte colony-stimulating factor (G-CSF), accelerates neovascularization. Stem Cells 23:347–354.
37. Poderoso C, C Paz, A Gorostizaga, FC Maciel, CF Mendez and EJ Podesta. (2002). Protein serine/threonine phosphatase 2A activity is inhibited by cAMP in MA-10 cells. Endocr Res 28:319–323.
38. Avecilla ST, K Hattori, B Heissig, R Tejada, F Liao, K Shido, DK Jin, S Dias, F Zhang, TE Hartman, NR Hackett, RG Crystal, L Witte, DJ Hicklin, P Bohlen, D Eaton, D Lyden, F de Sauvage and S Rafii. (2004). Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. Nature Med 10:64–71.

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