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Mesenchymal stem cell like (MSCl) cells generated from human embryonic stem cells support pluripotent cell growth

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ABSTRACT

Mesenchymal stem cell like (MSCl) cells were generated from human embryonic stem cells (hESC) through embryoid body formation, and isolated by adherence to plastic surface. MSCl cell lines could be propagated without changes in morphological or functional characteristics for more than 15 passages. These cells, as well as their fluorescent protein expressing stable derivatives, efficiently supported the growth of undifferentiated human embryonic stem cells as feeder cells. The MSCl cells did not express the embryonic (Oct4, Nanog, ABCG2, PODXL, or SSEA4), or hematopoietic (CD34, CD45, CD14, CD133, HLA-DR) stem cell markers, while were positive for the characteristic cell surface markers of MSCs (CD44, CD73, CD90, CD105). MSCl cells could be differentiated toward osteogenic, chondrogenic or adipogenic directions and exhibited significant inhibition of mitogen-activated lymphocyte proliferation, and thus presented immunosuppressive features. We suggest that cultured MSCl cells can properly model human MSCs and be applied as efficient feeders in hESC cultures.

1. Introduction

Mesenchymal stem (stromal) cells (MSCs), originally isolated from bone marrow [1], provide a supportive microenvironment for hematopoietic stem cells. Moreover, MSCs show multipotent stem cell characteristics [2] and special immunological features [3]. MSCs can also be obtained from various tissues including peripheral blood, umbilical cord, placenta, vein wall, muscle, adipose and connective tissues [4]. MSCs can be differentiated into canonical mesodermal tissues, e.g. bone, fat and cartilage, but may also retain a wider differentiation capacity [5]. MSCs play a role in tissue repair due to their differentiation potential and immunosuppressive activities [6], as well as by trophic effects mediated by growth factors and cytokines they produce [7].

These features initiated the use of MSCs in various cell-based therapies and by now more than 50 clinical trials related to this cell type have been reported (see http://www.clinicaltrials.gov) [8]. However, there are several limitations of large scale, reproducible, and well characterized production of human MSCs. The aspiration of MSCs from donors is an invasive procedure and several reports have demonstrated donor- and tissue-dependent variability of human bone-marrow-derived MSCs [4,9–13]. Isolation and enrichment of tissue-derived MSCs usually results in heterogeneous cell populations, and a long term *ex vivo* expansion of human MSCs has been shown to reduce replicative capacity, impair differentiation potential, alter gene expression profiles, and lead to karyotype instability [9,10].

In contrast to MSCs, pluripotent human embryonic stem cell (hESCs) lines have the capacity of unlimited growth and self-renewal [14] and can differentiate into all cell types of the human body [15]. There are numerous studies investigating the differentiation of hESCs, especially to yield the clinically most relevant cell types, including cardiomyocytes, hemopoietic cells, neuronal or pancreatic cells (for a recent review see [16]).

Recent publications have also reported the derivation of MSC-like cells from hESC lines [17–20], by using various protocols and producing MSCs with different characteristics. In the present study we have established a method to derive MSCl cell lines from hESCs with well defined gene and protein expression patterns and immunological features, examined their differentiation potential, supportive role for culturing hESCs, and produced genetically modified MSCl cell clones stably expressing fluorescent marker proteins. This work may significantly help our understanding of human MSC characteristics and the use of these cells in biotechnology applications.

Abbreviations: hESC, human embryonic stem cell; MSC, mesenchymal stem (stomal) cells; bmMSC, bone marrow derived MSC.

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2. Materials and methods

2.1. HuES, MSCl cells, HFF-1 and bmMSC cultures

The human embryonic stem cell lines (HUES9 and HUES1 originally provided by Dr. Douglas Melton, Harvard University) were cultured on mitotically inactivated mouse embryonic fibroblasts (MEF) [21]. Spontaneous differentiation of the hESCs were performed via embryoid body (EB) formation as described previously [21]. The cells were trypsinized at day 80 and were further cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Invitrogen) on a gelatinized 10-cm plate. The cells were trypsinized when confluent and split to 1:3 ratio. After two passages most of the cultured cells presented fibroblast-like morphology. The hESC-derived fibroblast-like cells were designated as hESC-derived mesenchymal stem cell like (MSCl) cells. All MSCl cells used in this study were of polyclonal origin.

HFF-1 cells were obtained from ATCC and were cultured according to the manufacturer's instructions (http://www.stemcells.atcc.org).

Collection of bone marrow samples from patients with hematological diseases was approved by the Regional and Institutional ethical review board of the Medical and Health Science Center of the University of Debrecen (protocol No.: UD-MHSC REC/IEC 2754-2008). Bone marrow samples were harvested from the iliac crest under medical examination. bmMSC isolated and cultured as described earlier [22]. The cells were used for phenotypic and functional analysis after P5.

2.2. Characterization of MSCl, HFF-1 and bmMSC cells

Cell surface expression of MSC marker proteins on the surface of MSCl, HFF-1 and bmMSC cells was analyzed by three-color flow cytometry using FITC-, APC- or PE-conjugated monoclonal antibodies (mAb) with isotype-matched control mAbs; specific against hematopoietic-, MSC related-, endothelial markers, cell adhesion molecules and integrins as well (see Supplementary Table I for full list). Fluorescence intensities were measured by FACS Calibur flow cytometer and the data were analyzed by using the WinMDI freeware (Joseph Trotter, La Jolla, CA). Results were expressed as means of positive cells (%) ± SD. Pluripotency markers of MSCs were tested by immunostaining (Oct4, SSEA4, PODXL) and by real time PCR (Nanog, ABCG2) performed as described previously [21]. The differentiation potential of MSCs was performed by using the Gibco's StemPro® Adipogenesis, Osteogenesis and Chondrogenesis Differentiation Kits according to the manufacturer's guide.

2.3. Lentiviral transduction of MSCl cells

For viral-based gene delivery, a third generation lentiviral vector system was used, as described in [23]. Determination of virus titers and the transduction procedures were performed as described previously [24]. The MSCl-2 cells were transduced by a MOI of 2–5, with an eGFP encoding lentiviral vector and further handling was the same as for the parental cell line.

2.4. Mitogen-induced cell proliferation and cytokine secretion

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient centrifugation (Amersham Biosciences, Uppsala, Sweden). Mitogen-activated T lymphocyte proliferation was induced by concanavalin A (ConA) or phytohaemagglutinin (PHA) used at a final concentration of 10 μg/ml (Amersham Pharmacia Biotech) and 1 μg/ml (Sigma–Aldrich), respectively added to 1×10^6 PBMCs. MSCs were added to 1×10^6 PBMCs at 10^3 and 10^4 cell numbers and co-cultured for 3 days. On day three proliferation was detected by the BrDU colorimetric assay directly in the culture plate according to the manufacturer's instructions (Roche). IL-6, IL-10 and IFN-γ cytokine secretion was quantitated by enzyme linked immunosorbent (ELISA) assay (OptEIA, BD Pharmingen) following the supplier's instruction. All experiments were performed in triplicates.

2.5. Statistical analysis

Each experiment was performed at least three times and each sample was tested in triplicates. Data are expressed as mean + SD. Statistically significant differences were determined by two-way ANOVA or paired student-*t* tests. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3. Results and discussion

3.1. Generation of HUES9-derived MSCl cells – application as feeder cells

Pluripotent HUES9 cells were spontaneously differentiated via EB formation (6 days), and then the differentiation process was

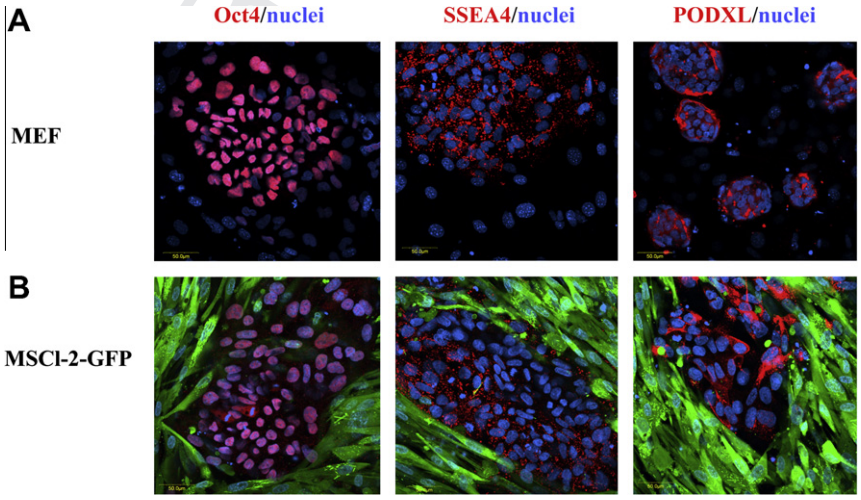

Fig. 1. Pluripotency markers of HUES9 cells cultured on different feeder layers, HUES9 cells were grown on MEF (**A**) or MSCl-2-GFP (**B**) feeder cells for two days in eight-well chambers for confocal microscopy. Co-culture of HUES and feeder cells were fixed and stained with the antibodies recognizing the following proteins: Oct4, SSEA4, PODXL (red). Antibodies specific for undifferentiated cell markers stained only the HUES9 clumps, while the MEF or MSCl-2-GFP cells did not show any staining with these markers. Nuclei were counterstained with DAPI (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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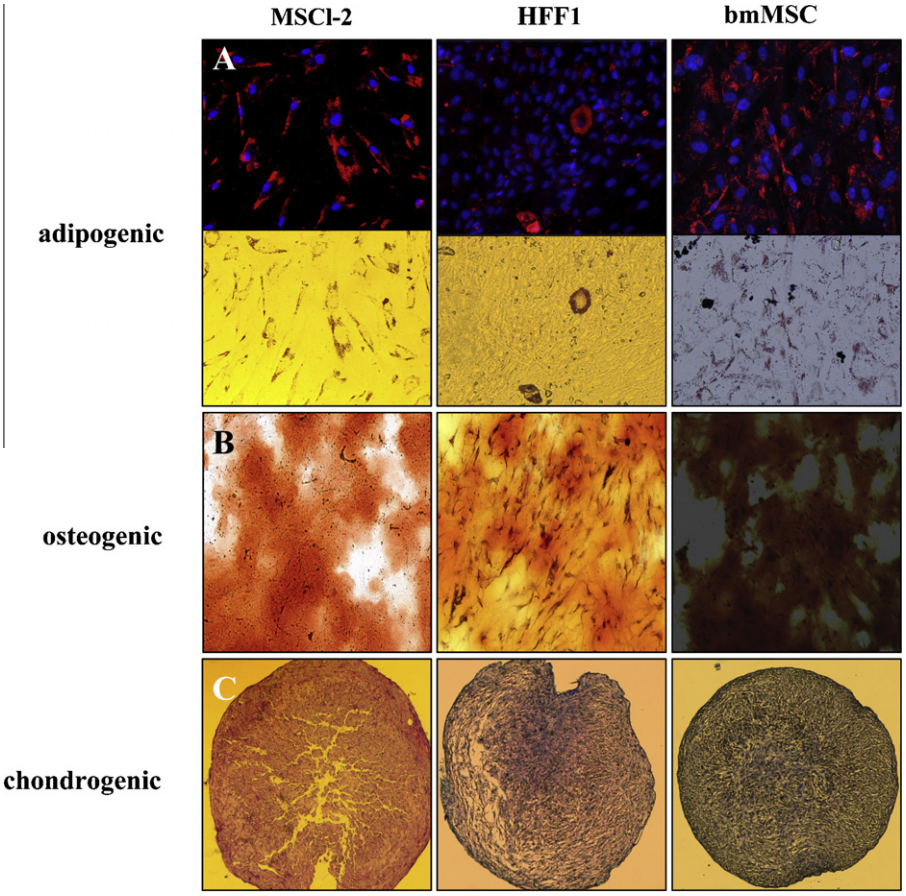

Fig. 2. Differentiation of MSCl-2, HFF-1 and bmMSC cells. Adipogenic differentiation is demonstrated by intracellular lipid vacuoles stained in red by Oil Red O (red) and with nuclei counterstained with DAPI (blue) (A). Osteogenic differentiation of MSCs is demonstrated by the formation of calcium-hydroxyapatite-positive areas stained in red by Alizarin Red (B). High density cultures showed the development of chondrogenic phenotype when cultured in micromass; pink extracellular matrix staining marks proteoglycans stained with toluidine blue (C). Magnification: 200×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

completed by further cell culturing on adherent surface in the presence of 10% FBS for 80 days. The differentiated fibroblast-like cells were separated from the other cell types by short trypsinization. After 2–3 passages on adherent surface the cells achieved uniform, fibroblast like morphology, and these cells (termed MSCl cells) could be propagated at least for 15 passages without morphological or kariotypical changes (Supplementary Fig. 1). By using the same protocol, more than five polyclonal MSCl cultures were independently generated from the HUES9 cell line.

In the following experiments we tested the capacity of the MSCl cells as autogenic feeder cells for maintaining pluripotency of hESCs. When undifferentiated HUES1 or HUES9 cells were passaged to mitomycin treated MSCl cultures, each MSCl cell line supported the growth of these pluripotent hESCs without any change in the expression of pluripotency markers or the rate of cell growth, as compared to those seen with cells growing on MEF (Supplementary Fig. 2). Recent publications have already described various methods for the establishment of autogenic feeder cells from hESC [25–27]. Our present method provides means to create applicable feeders for hESC without cell sorting or mechanical separation, and the derived feeder cells demonstrate the capacity to promote hESC expansion.

In order to allow a fast separation of hESCs from the human MSCl cells, we have generated eGFP expressing MSCl cells by using a lentiviral gene delivery method. The MSCl-GFP cells were sorted for eGFP positivity, treated with mitomycin and used as feeder for hESCs. As documented in Fig. 1, the MSCl-GFP cells promoted the growth of HUES9 cells in an undifferentiated state. This Figure also documents that the pluripotency markers Oct4, SSEA4 and PODXL were expressed in the pluripotent HUES9 cells, while these markers were absent in the MSCl-GFP feeder cells.

We also extended these studies with RT-PCR measurements of pluripotency markers expression in the undifferentiated hESC and in the MSCl cells. The transcription factors Oct4 and Nanog and the ABCG2 cell surface protein showed high levels of gene expression in the HUES9 cells, while they were close to detection levels in the MSCl cells (data not shown).

3.2. Multi-lineage differentiation potential

MSCs should fit the functional and phenotypic criteria defined by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy [28]. We first tested whether the MSCl cells could be differentiated toward the canonical mesodermal (osteoblastic, chondrogenic, adipogenic) directions. We found that all four established MSCl cell lines exhibited the potential to differentiate to at least one direction, but one of them (MSCl-2) showed robust differentiation potential toward all three directions (Supplementary Fig. 3). To complete a more detailed characterization we next compared the differentiation capacity of MSCl cells with the commercially available human foreskin fibroblast cells (HFF-1) widely used as a feeder cell line for hESC cultures, and that of MSCs isolated from human bone marrow. All these MSCs of different origin could be differentiated *in vitro* to adipogenic, oesteogenic and chondrogenic directions. Followed by a three-week adipogenic induction period, a large number of

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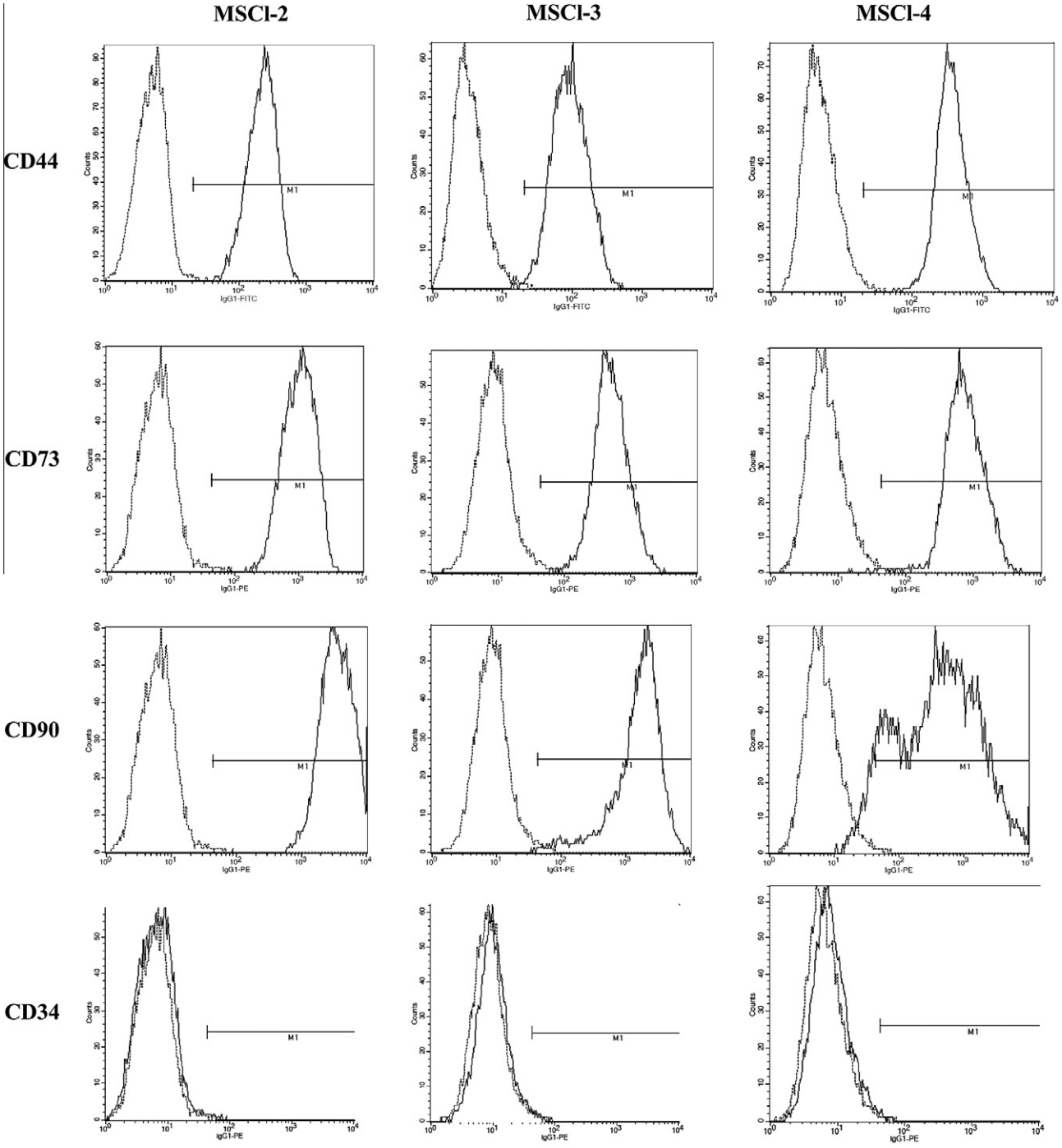

Fig. 3. Expression of common MSC markers in different MSCl cell lines. Flow cytometry measurements of cell surface marker expression of HUES9-derived MSCl cells. Single cell suspensions from MSCl-2, MSCl-3 and MSCl-4 cells were obtained by gentle trypsinization. Non-viable cells were excluded by Topro3 or 7AAD staining. Monoclonal antibodies specific for CD44 (conjugated to FITC), CD73 and CD90 (conjugated to PE) were used to detect MSC markers. Anti CD34-PE was used to demonstrate the absence of hematopoietic cells in the MSCl cultures. Dashed lines show staining with the relevant isotype-matched control mAbs.

MSCl-2 and bmMSC cells showed oil red positive staining, characteristic for adipocytes (Fig. 2A), while only a small number of HFF-1 cells became oil red positive. Similarly, when differentiation was induced by osteogenic induction medium for 2–3 weeks, MSCl-2 and bmMSC cells showed dense calcium deposits stained with alizarin red, while the HFF-1 cells exhibited weak osteogenic potential (Fig. 2B). After 3 weeks of differentiation sections made from chondrogenic mass cultures showed metachromazy upon toluidin-blue staining indicating chondrocyte formation from all three cell types (Fig. 2C). Based on these data we suggest that MSCl-2 cells generated from HUES9 cells retained their multilineage differentiation potential, similar to that found in bmMSCs [4,29], whereas HFF-1 cells possessed reduced differentiation capacity.

3.2.1. Cell surface markers of MSCl cells

In the first set of experiments summarized in Fig. 3 we compared the phenotypic characteristics of three different HUES9-derived polyclonal MSCl cell lines: MSCl-2, MSCl-3 and MSCl-4, which could be cultured for a long period of time, for the expression of the key cell surface molecules of MSCs. As shown by flow

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Table 1
Expression of common phenotypic markers by MSCs of various origins.

	MSCl-2	HFF-1	bmMSC
CD44/H-CAM	96.97 ± 2.81	93.23 ± 9.44	89.69 ± 10.04
CD105/Endoglin	89.61 ± 3.95	95.73 ± 6.19	86.08 ± 8.85
CD73/NT5E	99.51 ± 0.25	99.21 ± 0.22	93.57 ± 6.44
CD90/Thy-1	96.24 ± 2.41	87.38 ± 7.87	89.78 ± 7.67
CD34	0.00	1.24 ± 2.49	0.00
CD14	1.04 ± 1.88	85.27 ± 10.47	0.09 ± 0.19
CD45/Protein tyrosine phosphatase receptor C	0.00	0.00	0.00
CD117/c-kit	0.00	0.00	0.00
CD133/Prominin 1	0.00	0.00	0.00
HLA-DR	0.00	0.00	0.00

cytometry, the selected cell lines expressed similar high levels of CD44, CD73, and CD90 with some variability in the magnitude of CD90 expression and none of these cells expressed CD34, an early hematopoietic stem cell marker (Fig. 3).

For a detailed characterization, we have compared the expression of the cell surface markers in bmMSC, HFF-1 and MSCl-2 cells, by using flow cytometry analysis. As documented in Table 1, all the three cell types showed a uniformly high expression for all well known MSC markers, e.g. CD44, CD73, CD90, and CD105. Because none of these markers are considered as MSC specific, we further analyzed the expression of integrins, cell adhesion molecules and endothel-related surface proteins on MSCl-2 cells as compared to that of the other cell types. No difference in the expression pattern of these cell surface markers could be shown (Supplementary Table II). Importantly, none of the MSCs showed measurable expression of the hematopoietic markers CD14, CD34, CD45, CD117, CD133 or HLA-R. The unique property of HFF-1 cells was their high cell surface expression of CD14, which was undetectable in bmMSC or MSCl cells (Table 1). Thus in line with previous publications [17,20,30], the detailed phenotypic analysis of HUES9-derived MSCl-2 and adult bmMSCs cells revealed close phenotypic similarity.

3.2.2. Immunosuppressive effects of MSCl-2, HFF-1 and bmMSCs

Due to their clinical utility, the immunosuppressive properties of MSCs have extensively been studied and MSCs were shown to suppress immune responses both *in vitro* and *in vivo* mediated by multiple mechanisms [31–33] validating MSC as a therapeutically relevant cell type. Numerous studies have demonstrated that human MSCs decrease alloreactive responses, interfere with dendritic cell (DC) and T-cell functions, and are able to generate an immunosuppressive microenvironment. Under *in vitro* conditions bmMSCs are capable to suppress various T-cell effector functions [33,34] and inhibit mitogen-stimulated lymphocyte proliferation [35,36].

In the present study mitogen-induced T-cell proliferation was used to compare the immunosuppressive properties of MSCl-2, HFF-1 and bmMSC cells. Human PBMC of healthy donors was used as responder cells, and ConA or PHA as mitogenic activators. We found that all cell types responded to mitogenic activation and had an effect on lymphocyte-proliferation induced by ConA or PHA (Fig. 4). As shown in Fig. 4A, the presence of MSCls suppressed the mitogenic response of lymphocytes in a dose-dependent manner and the addition of both 10^3 and 10^4 MSCl-2 cells to 1×10^6 PBL efficiently reduced ConA-induced lymphocyte proliferation, whereas the PHA-induced mitogenic response was significant in the presence of 10^4 MSCl-2 cells, only (Fig. 4A). A similar effect could be detected when using bmMSCs as suppressors (Fig. 4C). Proliferation of lymphocytes could also be suppressed by HFF-1 cells when ConA but not PHA was used for stimulation (Fig. 4B).

In order to elicit their immunosuppressive function, MSCs should acquire increased production of certain cytokines such as IL-6, IL-10 and IFNγ [37,38]. IL-6 was shown to increases PGE_2 production and thus plays a key role in the inhibition of DC maturation and T-cell proliferation [31,39–42]. Dependent on local IFNγ levels MSC-mediated immunosuppression can be enhanced [43], and bmMSCs can drive DC differentiation to IL-10 secreting cells [33], which are potent inhibitors of T lymphocyte proliferation [44].

In the next set of experiments we sought to measure the concentration of secreted IL-6, IL-10 and IFNγ cytokines in the supernatant of mitogen-activated PBMCs co-cultured with MSCl-2, HFF-1 and bmMSC cells (Supplementary Fig. 4). Our results revealed that bmMSCs induced significant increases in IL-6 (Supplementary Fig. 4C), IFNγ (Supplementary Fig. 4F) and IL-10 (Supplementary Fig. 4I) cytokine levels, whereas MSCl-2 cells were unable to do so even though they efficiently inhibited T lymphocyte proliferation (Fig. 4A, D, and G). The presence of HFF-1 cells induced a slight increase in IL-6 levels (Supplementary Fig. 4B), but the concentration of secreted IFNγ and IL-10 did not change (Supplemantary Fig. 4E and H). These results indicate that the production of the functionally relevant cytokines is not an absolute requirement for exerting the immunosuppressive effects of MSCs, and the MSCl-2 cells may use different means to inhibit T-lymphocyte associated effector functions.

Although the detailed immunological characterization of HUES9-derived MSCl-2 needs further investigations, our present results indicate a strong immunosuppressive effect by the HUES9-derived MSCl-2 cells. Our current experiments reinforce previous observations [20,30,45] demonstrating that MSCs derived from human embryonic stem cells have the capacity to suppress peripheral blood T lymphocyte proliferation and also extend them by demonstrating the lack of MSCs-induced enhancement of cytokine secretion upon their immunosuppressive action.

As a summary, we have prepared and characterized human MSCl cells obtained from the human embryonic stem cell line, HUES9. In previous studies various protocols have been applied to obtain MSCs from hESC, including co-culture with mouse OP9 cells [46], embryoid body differentiation [47], spontaneous differentiation in monolayer [20], selection of cell populations by FACS [17], using Rho-associated kinase inhibitor [30], over expression of HOXB4 gene [18] and mechanical separation [19]. In the present study we have developed a novel, simplified method to obtain phenotypically and functionally homogeneous hESC-derived MSC populations. Our derivation protocol required neither the use of xenogenic feeder cell, nor selection (manual or FACS), nor chemical inducers. These MSCl cells showed long term stability and could be maintained in culture over 30 passages without detectable phenotypic or functional changes. Cell surface markers, multipotent differentiation potential and immunosuppressive effects of MSCl-2 proved to be similar to bmMSCs but was different from that of HFF-1. Our data suggest that MSCl-2 cells may be successfully applied as a model system for studying human MSC features and may be used in biotechnology applications.

4. Disclosures

The authors indicate no potential conflicts of interest.

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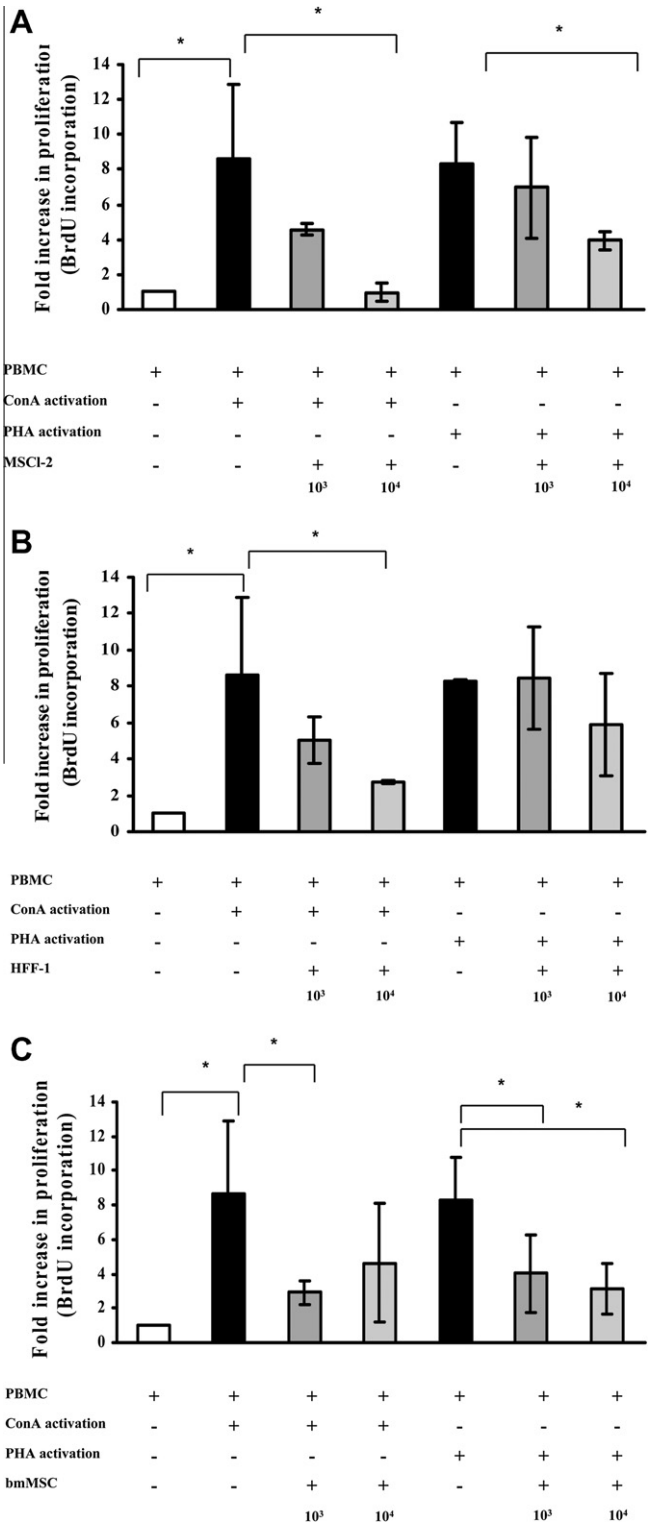

Fig. 4. Effect of MSCl-2, HFF-1 and bmMSC cells on mitogenic lymphocyte stimulation. Mitogenic stimulation of PBMCs were measured in the presence of the indicated number of HUES9-derived MSCl-2 (A), HFF-1 fibroblast (B) or human adult bmMSC (C). Stimulation of lymphocytes was induced by ConA or PHA and the cells were co-cultured with the indicated numbers of MSCs. Mean ± SD of relative increase of cell proliferation measured in 4 independent experiments are shown.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.09.089.

References

[1] A.J. Friedenstein, R.K. Chailakhyan, N.V. Latsinik, et al., Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues cloning in vitro and retransplantation in vivo, Transplantation 17 (1974) 331–340.
[2] M.F. Pittenger, A.M. Mackay, S.C. Beck, et al., Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[3] E. Klyushnenkova, J.D. Mosca, V. Zernetkina, et al., T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression, J. Biomed. Sci. 12 (2005) 47–57.
[4] S. Kern, H. Eichler, J. Stoeve, et al., Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue, Stem Cells 24 (2006) 1294–1301.
[5] J.E. Grove, E. Bruscia, D.S. Krause, Plasticity of bone marrow-derived stem cells, Stem Cells 22 (2004) 487–500.
[6] O. Ringden, M. Uzunel, I. Rasmusson, et al., Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease, Transplantation 81 (2006) 1390–1397.
[7] A.I. Caplan, J.E. Dennis, Mesenchymal stem cells as trophic mediators, J. Cell Biochem. 98 (2006) 1076–1084.
[8] B. Philippe, S. Luc, P.B. Valerie, et al., Culture and use of mesenchymal stromal cells in phase I and II clinical trials, Stem Cells Int. (2010) 503–593.
[9] G.M. de Peppo, P. Sjovall, M. Lenneras, et al., Osteogenic potential of human mesenchymal stem cells and human embryonic stem cell-derived mesodermal progenitors: a tissue engineering perspective, Tissue Eng. Part A 16, 3413–3426.
[10] R. Siddappa, R. Licht, C. van Blitterswijk, et al., Donor variation and loss of multipotency during in vitro expansion of human mesenchymal stem cells for bone tissue engineering, J. Orthop. Res. 25 (2007) 1029–1041.
[11] L. Wang, I. Tran, K. Seshareddy, et al., A comparison of human bone marrow-derived mesenchymal stem cells and human umbilical cord-derived mesenchymal stromal cells for cartilage tissue engineering, Tissue Eng. Part A 15 (2009) 2259–2266.
[12] Y. Zhu, T. Liu, K. Song, et al., Adipose-derived stem cell: a better stem cell than BMSC, Cell Biochem. Funct. 26 (2008) 664–675.
[13] L.F. Cooper, Y. Zhou, J. Takebe, et al., Fluoride modification effects on osteoblast behavior and bone formation at TiO2 grit-blasted c.p. titanium endosseous implants, Biomaterials 27 (2006) 926–936.
[14] J.A. Thomson, J. Itskovitz-Eldor, S.S. Shapiro, et al., Embryonic stem cell lines derived from human blastocysts, Science 282 (1998) 1145–1147.
[15] J. Itskovitz-Eldor, M. Schuldiner, D. Karsenti, et al., Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers, Mol. Med. 6 (2000) 88–95.
[16] C.E. Murry, G. Keller, Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development, Cell 132 (2008) 661–680.
[17] Q. Lian, E. Lye, K. Suan Yeo, et al., Derivation of clinically compliant MSCs from CD105+, CD24− differentiated human ESCs, Stem Cells 25 (2007) 425–436.
[18] Y.P. Liu, P. Hematti, Generation of mesenchymal stromal cells from HOXB4-expressing human embryonic stem cells, Cytotherapy 11 (2009) 716–725.
[19] E.N. Olivier, A.C. Rybicki, E.E. Bouhassira, Differentiation of human embryonic stem cells into bipotent mesenchymal stem cells, Stem Cells 24 (2006) 1914–1922.
[20] P. Trivedi, P. Hematti, Derivation and immunological characterization of mesenchymal stromal cells from human embryonic stem cells, Exp. Hematol. 36 (2008) 350–359.
[21] A. Apati, T.I. Orban, N. Varga, et al., High level functional expression of the ABCG2 multidrug transporter in undifferentiated human embryonic stem cells, Biochim. Biophys. Acta 1778 (2008) 2700–2709.
[22] M.F. Pittenger, Mesenchymal stem cells from adult bone marrow, Methods Mol. Biol. 449 (2008) 27–44.
[23] A. Schambach, M. Galla, T. Maetzig, et al., Improving transcriptional termination of self-inactivating gamma-retroviral and lentiviral vectors, Mol. Ther. 15 (2007) 1167–1173.
[24] O. Ujhelly, C. Ozvegy, G. Varady, et al., Application of a human multidrug transporter (ABCG2) variant as selectable marker in gene transfer to progenitor cells, Hum. Gene. Ther. 14 (2003) 403–412.
[25] P. Stojkovic, M. Lako, R. Stewart, et al., An autogeneic feeder cell system that efficiently supports growth of undifferentiated human embryonic stem cells, Stem Cells 23 (2005) 306–314.

[26] Q. Wang, Z.F. Fang, F. Jin, et al., Derivation and growing human embryonic stem cells on feeders derived from themselves, Stem Cells 23 (2005) 1221–1227.
[27] C. Xu, J. Jiang, V. Sottile, et al., Immortalized fibroblast-like cells derived from human embryonic stem cells support undifferentiated cell growth, Stem Cells 22 (2004) 972–980.
[28] M. Dominici, K. Le Blanc, I. Mueller, et al., Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement, Cytotherapy 8 (2006) 315–317.
[29] J.M. Gimble, F. Guilak, M.E. Nuttall, et al., In vitro differentiation potential of mesenchymal stem cells, Transfus. Med. Hemother. 35 (2008) 228–238.
[30] Z. Tan, Z.Y. Su, R.R. Wu, et al., Immunomodulative effects of mesenchymal stem cells derived from human embryonic stem cells in vivo and in vitro, J. Zhejiang Univ. Sci. B, 12, 18–27.
[31] M.J. Hoogduijn, F. Popp, R. Verbeek, et al., The immunomodulatory properties of mesenchymal stem cells and their use for immunotherapy. Int. Immunopharmacol., 10, 1496–500.
[32] K. Le Blanc, L. Tammik, B. Sundberg, et al., Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex, Scand. J. Immunol. 57 (2003) 11–20.
[33] H. Yagi, A. Soto-Gutierrez, B. Parekkadan, et al., Mesenchymal stem cells: Mechanisms of immunomodulation and homing, Cell Transplant., 19, 667–79.
[34] M. Krampera, S. Glennie, J. Dyson, et al., Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide, Blood 101 (2003) 3722–3729.
[35] M. Di Nicola, C. Carlo-Stella, M. Magni, et al., Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli, Blood 99 (2002) 3838–3843.
[36] I. Rasmusson, O. Ringden, B. Sundberg, et al., Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms, Exp. Cell Res. 305 (2005) 33–41.
[37] E.J. Bassi, C.A. Aita, N.O. Camara, Immune regulatory properties of multipotent mesenchymal stromal cells: Where do we stand?, World J. Stem. Cells, 3, 1–8.
[38] A.J. Nauta, W.E. Fibbe, Immunomodulatory properties of mesenchymal stromal cells, Blood 110 (2007) 3499–3506.
[39] C. Bouffi, C. Bony, G. Courties, et al., IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis, PLoS One, 5, e14247.
[40] F. Djouad, L.M. Charbonnier, C. Bouffi, et al., Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism, Stem Cells 25 (2007) 2025–2032.
[41] L. Jarvinen, L. Badri, S. Wettlaufer, et al., Lung resident mesenchymal stem cells isolated from human lung allografts inhibit T cell proliferation via a soluble mediator, J. Immunol. 181 (2008) 4389–4396.
[42] M. Najar, R. Rouas, G. Raicevic, et al., Mesenchymal stromal cells promote or suppress the proliferation of T lymphocytes from cord blood and peripheral blood: the importance of low cell ratio and role of interleukin-6, Cytotherapy 11 (2009) 570–583.
[43] J.M. Ryan, F. Barry, J.M. Murphy, et al., but promotes the immunosuppressive capacity of adult human mesenchymal stem cells, Clin. Exp. Immunol. 149 (2007) 353–363.
[44] A. Nasef, A. Chapel, C. Mazurier, et al., Identification of IL-10 and TGF-beta transcripts involved in the inhibition of T-lymphocyte proliferation during cell contact with human mesenchymal stem cells, Gene. Expr. 13 (2007) 217–226.
[45] B.L. Yen, C.J. Chang, K.J. Liu, et al., Brief report-human embryonic stem cell-derived mesenchymal progenitors possess strong immunosuppressive effects toward natural killer cells as well as T lymphocytes, Stem Cells 27 (2009) 451–456.
[46] T. Barberi, L.M. Willis, N.D. Socci, et al., Derivation of multipotent mesenchymal precursors from human embryonic stem cells, PLoS Med. 2 (2005) e161.
[47] N.S. Hwang, S. Varghese, H.J. Lee, et al., In vivo commitment and functional tissue regeneration using human embryonic stem cell-derived mesenchymal cells, Proc. Natl. Acad. Sci. USA 105 (2008) 20641–20646.

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