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Difference in gene expression between human fetal liver and adult bone marrow mesenchymal stem cells

Cecilia Götherström
Anne West
Johan Liden
Mehmet Uzunel
Riitta Lahesmaa
Katarina Le Blanc

Background and Objectives. Mesenchymal stem cells (MSC) are progenitor cells that are capable of differentiating into mesenchymal tissues. Fetal and adult MSC have similar morphology but differ in proliferative, differentiating and immunosuppressive properties. Further exploring their differences could help in choosing the right source for cellular therapy.

Design and Methods. The gene expression profiles of undifferentiated MSC derived from first trimester fetal liver and adult bone marrow were compared by serial analysis of gene expression, and validated by either reverse transcription polymerase chain reaction or immunoblotting of selected genes. The immunophenotype was compared by flow cytometry and cell ELISA.

Results. Seventy genes were differentially induced two-fold or more in fetal MSC compared to adult MSC. These involved transcripts regulating germ plasm and limb patterning, brain and early muscle development. Transcripts implicated in cell cycle promotion, chromatin regulation and DNA repair were also more abundant in fetal MSC. Ninety-seven genes were decreased two-fold or more in fetal MSC, including transcripts involved in smooth muscle and keratinocyte differentiation and transcripts for immunological genes. Although phenotypically largely similar, fetal MSC had a higher expression of ICAM1 and contained intracellular deposits of HLA-G while expression of HLA class I and II molecules and VCAM1 was increased in adult MSC.

Interpretation and Conclusions. This study reports the first extensive investigation of the differences in gene expression profiles between fetal and adult MSC. The results suggest that fetal MSC have higher proliferative capacity and are less lineage committed than adult MSC.

Key words: mesenchymal stem cells, fetal stem cells, hematopoiesis, gene expression and development.

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From the Division of Clinical Immunology (CG, MU, KLB); Center of Allogeneic Stem Cell Transplantation, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden (KLB); Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland (AW, RL); Department of Biosciences, Novum, Karolinska Institutet, Stockholm, Sweden (JL).

Correspondence:
Cecilia Götherström, PhD, Division of Clinical Immunology, F79, Karolinska University Hospital Huddinge, SE-141 86 Stockholm Sweden. E-mail: cecilia.gotherstrom@labmed.ki.se

Mesenchymal stem cells (MSC) are multipotent non-hematopoietic cells capable of differentiating *in vitro* and *in vivo* into different mesenchymal lineages, including adipose, bone, cartilage and bone marrow stroma.[1-4] MSC were initially identified in bone marrow and later in muscle, adipose and connective tissue of human adults.[5-8] Supporting their stem cell nature is the fact that single-cell colonies of MSC express endothelial, epithelial and neuronal surface markers, indicating that they can give rise to a broad range of cells.[9] However, because the frequency and differentiating capacity of MSC decrease with age,[10] alternative sources of MSC have been sought. MSC have been identified in human amniotic fluid, placenta, umbilical cord blood and veins[11-13] as well as in several fetal tissues including bone marrow, liver, blood, lung and spleen.[14-16] Fetal and adult MSC share several characteristics, including their morphology and the expression of many surface molecules. Both types of MSC may also give rise to the stroma that constitutes the hematopoietic microenvironment. Fetal MSC were recently shown to localize within hematopoietic sites throughout ontogeny, raising the possibility of a parallel and coordinate development of both hematopoietic and mesenchymal systems.[17] Adult and fetal MSC have the capacity to maintain and expand lineage-specific colony-forming units from $CD34^+$ cells in long-term cultures *in vitro*.[14,18,19] Similarly, in experimental animal models, both cell types promote engraftment of unrelated hematopoietic stem cells in NOD/SCID mice and fetal sheep.[20-24] The enhancing effect is most prominent when the HSC dose is limiting and involves cells of the myeloid, lymphoid and megakaryocytic lineages. Furthermore, adult MSC appear to be immunosuppressive as they reduce alloreactivity and the formation of

cytotoxic lymphocytes *in vitro*.[24-27] We have recently shown that adult MSC may also be immunosuppressive *in vivo* as infusion of haploidentical MSC reversed grade IV acute graft-versus-host-disease of the gut and liver.[28] The immunosuppressive properties of first trimester fetal MSC are less pronounced, but inducible with interferon (INF)γ.[15,29]

Adult and fetal MSC differ in their expression of alloantigens.[29,30] Both types express HLA class I. Adult MSC contain intracellular deposits of HLA class II and cell surface expression can be induced by treatment of the cells with INFγ. Fetal MSC have no HLA class II intracellularly and require seven days of INFγ exposure for *de novo* synthesis and cell surface expression. However, neither fetal nor adult MSC appear to be inherently immunogenic as they escape recognition by lymphocytes.[15,25,26,31] Persistence of adult MSC after infusion *in utero* to fetal sheep indicates that adult MSC also have immunoprivileged properties *in vivo*.[4] Similarly, when fully mismatched allogeneic fetal MSC were transplanted into an immunocompetent fetus in the third trimester of gestation, the recipient, diagnosed with severe osteogenesis imperfecta, showed long-term engraftment of transplanted MSC in bone.[32] Alloreactivity against donor MSC was not detected either before or after the transplant, indicating that MSC can be tolerated when transplanted across MHC barriers in humans.

Besides differing in alloantigen expression and their immunosuppressive properties, other differences exist. Fetal MSC have a shorter cell doubling time and their differentiation potential may differ.[14,15]

Potential uses of MSC in the future include the regeneration of various damaged tissues, enhancement of hematopoietic recovery after hematopoietic stem cell transplantation and the manipulation of immune responses. However, the optimal source of MSC for these applications remains to be established. Largely because of the ability of MSC to differentiate into multiple lineages, and hence their heterogeneous gene expression, a specific molecular profile has been difficult to establish, leaving MSC poorly characterized at both the molecular and cellular level. Using restriction fragment differential display polymerase chain reaction (PCR), a number of genes have been suggested to be up-regulated in undifferentiated MSC compared to in MSC that have differentiated into osteoblasts.[33] The transcriptome of human adult undifferentiated and differentiated MSC has been analyzed,[9,34-38] which further facilitates the search for genes that are enriched in MSC. Investigating the gene expression of bone marrow- and umbilical cord-derived MSC revealed the shared expression of about a thousand transcripts between the two lineages.[13] Yet, there has been no analysis of the difference in expression profiles of fetal and adult MSC. If one speculates that fetal stem cells would be less committed than adult stem cells, this study could provide a unique genetic signature for both stem cells and the mesenchymal stem cell lineage. The present study is the first attempt to segregate the divergence between fetal and adult MSC by means of oligonucleotide arrays scanning 22,284 human transcripts and approximately 14,500 genes. The dissimilarities may imply that fetal and adult MSC should be used for different indications in cellular therapy.

Design and Methods

Isolation, culture and characterization of human fetal and adult MSC

To isolate human fetal MSC, liver samples from three male fetuses (gestational ages 7, 10 and 10 weeks) aborted in the first trimester were collected where the women had volunteered to donate fetal tissue. Human adult MSC were isolated from three bone marrow aspirates from the iliac crest of normal male donors (ages 25, 27 and 34 years). Both studies were approved by the Ethics Committee at Karolinska University Hospital and written consent was obtained from all donors.

Human fetal and adult MSC were prepared as described previously.[15,25] Briefly, mononuclear cells were collected by gradient centrifugation and suspended in DMEM-LG (Invitrogen, Paisley, Scotland) supplemented with 10% FBS (Sigma, St. Louis, Mo., USA) specially selected for maximum proliferation and differentiation. Cells were plated at 1.6×10^5 cells/cm^2 and after three days the non-adherent cells were discarded. The medium was changed every three to four days thereafter. At near confluence, the cells were replated at a density of 4×10^3 cells/cm^2.

Osteogenic, chondrogenic and adipogenic differentiation and surface expression of ITGB1 (CD29), CD44, NT5E (CD73), THY1 (CD90), CD105, ALCAM (CD166), CD14, CD34, PTPRC (CD45), HLA class I and HLA class II by fetal and adult MSC were analyzed as previously described.[15]

Microarray analysis

Three individual collections of fetal and adult MSC were cultured as described above to 70% confluence. The cells were carefully washed three times with phosphate-buffered saline (PBS), after which TRIzol Reagent (Invitrogen, Cat No 15596-018) was added to the culture flasks to obtain total RNA. Extracted RNA was treated with DNase and purified through columns (Qiagen, Hilden, Germany). The quality of the RNA samples was assessed spectrophotometrically and by gel analysis. Two micrograms of total RNA were amplified and labeled according to a standard protocol (Affymetrix, Santa Cruz, CA, USA). The product was run on a gel to ensure the success of the synthesis reac-

tion. The labeled RNA was hybridized to Affymetrix HG U133A arrays, probed and scanned according to Affymetrix standard procedures. The Gene Chip Operative System 1.2 program (GCOS 1.2, Affymetrix) was used to obtain signal intensities. The data were analyzed as follows: (i) Signals from the Affymetrix statistical alogorithm were tested using the software Significance Analysis of Microarrays (SAM) developed at Stanford University laboratories.[39] Settings used were two-fold changes and a delta value of 1.0; (ii) pair wise comparisons (in total 9) were performed in GCOS 1.2 and further analysed using the Affymetrix Data Mining Tool (DMT) 3.1 program. Probe sets not showing a two-fold difference in all possible comparisons were excluded; (iii) only genes with a unique probe set according to Affymetrix nomenclature were further analyzed and genes that scored as absent in both fetal and adult MSC were eliminated; (iv) *p*-values were calculated for the differences of the remaining probe sets using unpaired t tests and (v) significantly increased and decreased transcripts were grouped into different functional categories: cell cycle regulation, chromatin regulation, DNA repair, cytoskeleton, adhesion/extracellular, development, immune response, signaling, metabolism, mitochondrial proteins, RNA processing, translation, transcriptional regulation, protein folding, protein modification, protein degradation, transport, stress, other and unknown.

In addition, the data were browsed for specified genes to discover whether transcripts were present in fetal and adult MSC. The expression of selected molecules was validated by reverse transcriptase-polymerase chain reaction (RT-PCR), immunoblotting, flow cytometry and cell enzyme-linked immunosorbet assay (ELISA).

Surface protein expression (cell ELISA and flow cytometry)

To analyze the presence of surface molecules on fetal and adult MSC, the cells were cultured in flat-bottomed 96-well plates, as described above. The cells were washed with PBS, fixed in 1% paraformaldehyde and residual binding sites were blocked with PBS- 5% low-fat milk powder, after which the cells were probed with the monoclonal antibodies specified below. TMB (Sigma) was added to visualize staining and reactions were halted with 2 M sulfuric acid. Staining was measured at 450 nm in a spectrophotometer. The antibodies HLA A, B, C (Harlan Sera-Lab, Belton, UK), HLA-DR, VWF (DAKO, Glostrup, Denmark), HLA E (kindly donated by Dr D. Geraghty, Fred Hutchinson Cancer Center, Seattle, Washington, USA), HLA G (Abcam, Cambridge, UK), ICAM1 (CD54), VCAM1 (CD106), SELE (CD62E), CD105 (Pharmingen, San Diego, CA, USA), CXCL12 (SDF1), CXCR4 (R & D, Abingdon, UK), negative control mouse IgG (DAKO) horseradish peroxidase-conjugated goat-anti-mouse IgG secondary antibody (Biorad), FL1 (VEGFR), PDGFRβ (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and horseradish peroxidase-conjugated IgG swine-anti-rabbit secondary antibody (DAKO). MSC were also cultured and harvested for flow cytometric analysis with corresponding monoclonal antibodies as previously described.[15]

Immunoblotting

Immunoblotting against HLA-E and -G (1 μg/mL, Abcam) antigens was performed on 0.5×10^6 cultured fetal and adult MSC, peripheral blood lymphocytes and term placental cells as described previously.[29] Fetal and adult MSC were stimulated with 100 U/mL IFNγ (Sigma) in culture for two days when specified.

Reverse transcriptase polymerase chain reaction

Genes showing a discrepancy in gene expression and protein analysis were further analyzed with RT-PCR. These genes were ICAM1, CD105 and CXCR4. The methodology of RNA extraction, cDNA synthesis and realtime quantitative RT-PCR has been described previously.[40] Primer and probe sequences for the transcripts were: CD105-F: CTG TGG CAT GCA GGT GTC A, CD105-R: ATG TTG AGG CAG TGC ACC TTT, CD105-probe: FAM-AG GCG GTG GTC AAT ATC CTG TCG AGC T -TAMRA, ICAM1-F: GCA GTC AAC AGC TAA AAC CTT CCT, ICAM1-R: GCA GCG TAG GGT AAG GTT CTT G, ICAM1-probe: FAM-CG TGT ACT GGA CTC CAG AAC GGG TGG-TAMRA. The PCR reaction was performed and analyzed on the ABI 7000 Sequence Detection System with the following PCR conditions: 50°C for 2 min, 95°C for 10 min followed by 40 PCR amplification cycles with 95°C for 15 s and 58°C for 1 min. ABL gene expression was used as reference.

Results

Isolation, culture and characterization of human fetal and adult MSC

Cultured MSC from both sources were strongly positive for the β1-intregrin ITGB1 (CD29), CD44, NT5E (CD73), THY1 (CD90), the endoglin receptor CD105 and ALCAM (CD166) in flow cytometric analyses. MSC were negative for the lipopolysaccharide CD14, the leukocyte common antigen PTPRC (CD45) and the hematopoietic stem cell marker CD34. Staining of phosphate minerals with von Kossa stain showed osteogenic differentiation in induced cultures. When plated on adipogenic media, fetal and adult MSC produced lipid-containing vacuoles that could be detected with Oil-Red-O staining. Formed pellets in chondrogenic differentiation stained positive for glycosaminoglycans with Alcian green.[15, 25]

Table 1. Antigen expression of fetal and adult MSCs analyzed by immunoblotting, flow cytometry, Cell ELISA and searching the gene array data.

Antigens	*Gene array*		*Cell surface*		*Immuno-blotting*	
Surface molecules	*Fetal*	*Adult*	*Fetal*	*Adult*	*Fetal*	*Adult*
HLA-A, B, C	P	P	++	+++	++	+++
HLA-DP, DQ, DM, DR	A	P	–	–	–	++
HLA-E	P	P	–	–	++	++
HLA-G	P	P	–	–	+	–
CD14	A	A	–	–		
CD34	A	A	–	–		
NT5E	P	P	+++	+++		
PTPRC	A	A	–	–		
VWF	P*	P*	++	++		
Adhesion molecules	*Fetal*	*Adult*	*Fetal*	*Adult*		
ALCAM	P	P	+++	+++		
CD44	P	P	+++	+++		
CD58	P	M	na	na		
CD105	P*	P	+++	+++		
ICAM1	P*	P*	++	+		
ICAM2	A	P	na	na		
ICAM3	P	A	na	na		
ITGA4	P	A	na	na		
ITGAL	A	A	na	na		
ITGβ1	P	P	+++	+++		
SELE	A	A	–	–		
THY1	P	P	+++	+++		
VCAM1	P	P	+	++		
ESC markers	*Fetal*	*Adult*				
CJA1	P	P				
CJA7	P	A				
GDF3	A	A				
LIN28	A	A				
NANOG	A	A				
POU5F1	A	A				
SOX2	A	A				
TERT	A	A				
TDGF1	A	A				
UTF1	A	A				

Antigens	*Gene array*		*Cell surface*	
Cytokines and their receptors	*Fetal*	*Adult*	*Fetal*	*Adult*
CXCL12	P	P	–	–
CXCR4	P*	P*	+	+
FLT1	A	A	–	–
PDGFR,	P	P	d+	d+
IL2	A	A		
IL2Rα,β,γ	A	A		
IL4	A	A		
IL4R	A	A		
IL10	A	A		
IL10R	A	A		
LIF	P	P		
LIFR	A	A		
TGFα	A	A		
TGFβ1	A	A		
TGFβ2	A	A		
TGFβ3	A	A		
TGFβR1	M	A		
TGFβR2	P	P		
TGFβR3	P	P		
Cell differentiation markers	*Fetal*	*Adult*		
ACTC	P	P		
GATA2	P	A		
GATA4	A	A		
IPF1	A	A		
KRT8	P	A		
KRT18	P	P		
MYF5	A	A		
MYOD1	A	A		
NES	A	A		
SOX1	A	A		
TNNT1	A	A		

*ESC: embryonic stem cell; P: present; M: moderate; A: absent in gene array; na: not analyzed; *present after reanalysis of signal intensities with Bioconductor software packages and validated with RT PCR (Figure 3). – negative, + positive, d+ dim positive when investigated by protein analysis.*

Microarray analysis

Data were treated as follows; (i) signals from the Affymetrix statistical algorithm were tested using the SAM software. Two-fold changes in fetal versus adult cells yielded 3,459 significantly changed probe sets and a median number of false significance of 337, in other words a false discovery rate of 10%, (ii) pair wise comparisons (in total 9) were performed in GCOS 1.2 and further analyzed using DMT 3.1, resulting in 616 genes that showed ≥ two-fold higher expression and 285 genes that were decreased two-fold or more in all possible comparisons in fetal compared to adult MSC; (iii) only probe sets directed to a unique transcript were kept and those that scored as absent in both fetal and adult MSC were eliminated, yielding 70 increased and 97 decreased transcripts; (iv) the resulting data were analyzed for statistical significance using unpaired t-tests. All transcripts were in the 95% confidence interval; and (v) increased and decreased transcripts were grouped into the specified functional categories (Figures 1 A,B, *see Table 1, Supplementary data*). A dendrogram, which displays true multi-object data, was created using Euclidean distances (Figure 2). The dendrogram is a branching, tree-like diagram that illustrates the hierarchical relationships among items in a dataset in which the distance from the root to a cluster indicates the similarity of the clusters. Highly similar clusters have joining points that are farther from the root. The dendrogram in Figure 2 corroborates an overall difference in gene expression between fetal and adult MSC since they clearly cluster in two separate groups. In total, fetal MSC expressed a higher number of genes than did

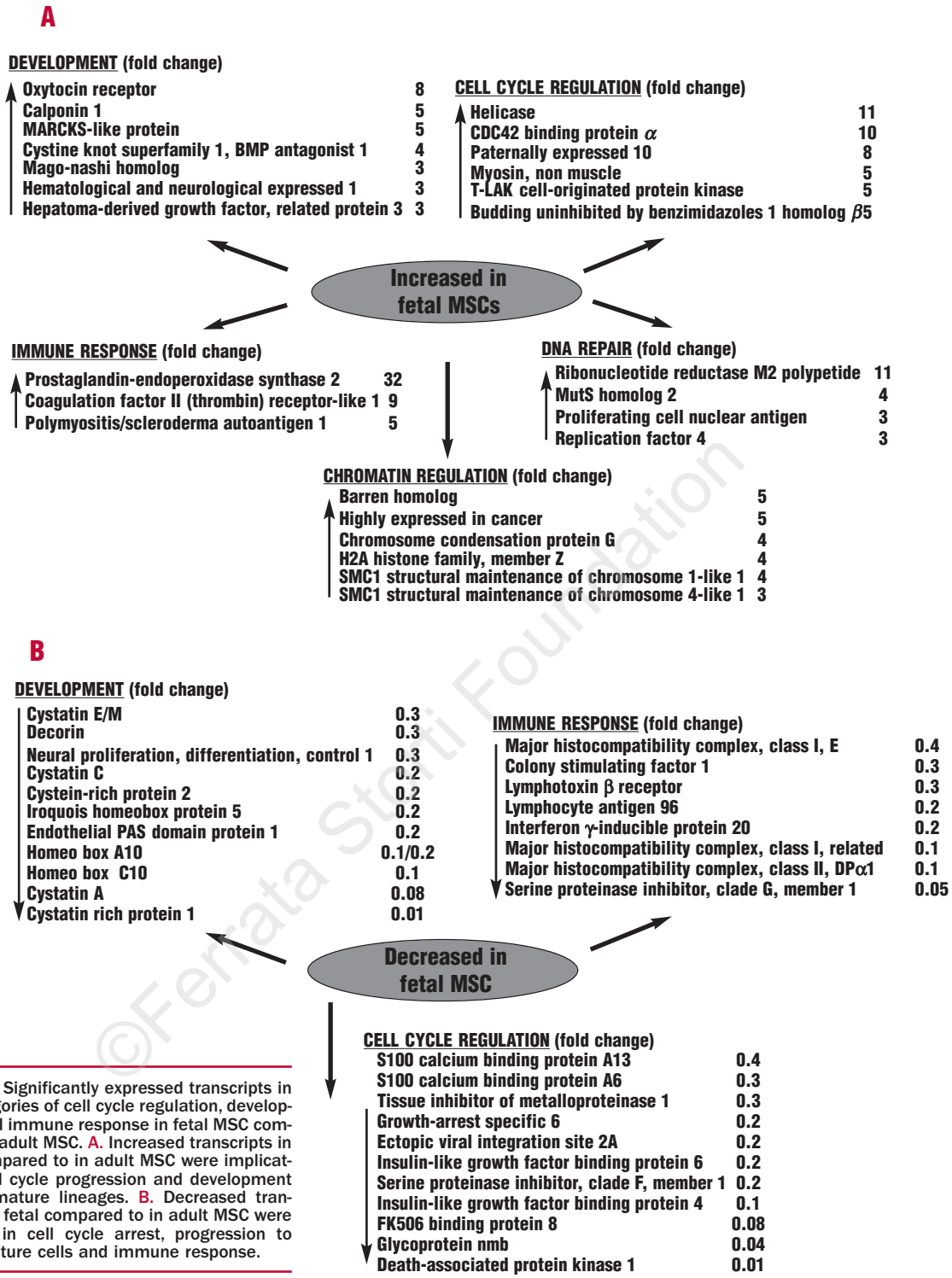

Figure 1. Significantly expressed transcripts in the categories of cell cycle regulation, development and immune response in fetal MSC compared to adult MSC. A. Increased transcripts in fetal compared to in adult MSC were implicated in cell cycle progression and development to less mature lineages. B. Decreased transcripts in fetal compared to in adult MSC were involved in cell cycle arrest, progression to more mature cells and immune response.

adult MSC. However, the transcripts for embryonic markers were not detected in either fetal or adult MSC (Table 1). Transcripts involved in cell cycle regulation, DNA repair and especially chromatin regulation were more abundant in fetal MSC (Figure 1A). In the developmental category, expression of transcripts involved in differentiation to more mature cells, e.g., keratinocytes, smooth muscle and bone, were decreased in fetal MSC (Figure 1B). Fetal MSC had an increased expression of transcripts regulating limb and germ plasm as well as brain and early smooth muscle development. Other differences were the immune response

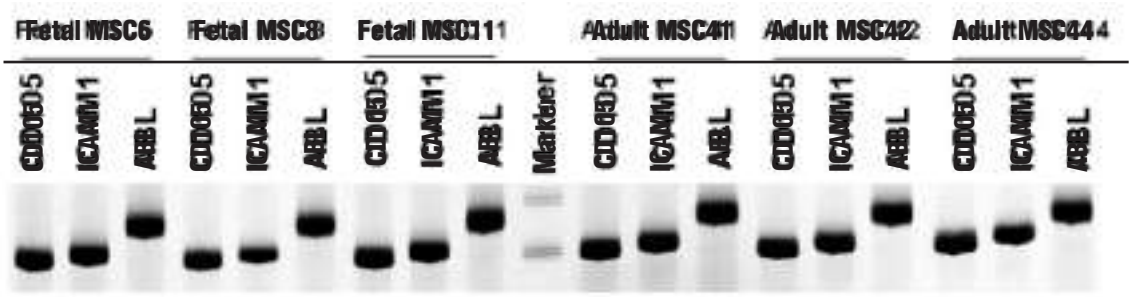

Figure 3. Reverse transcriptase PCR validation of genes with dissimilar expression in gene expression and protein analysis. The three collections of fetal (MSC5, MSC8, MSC11) and adult (MSC41, MSC42, MSC44) MSC were analyzed for expression of CD105 and ICAM1. The ABL gene was used as reference.

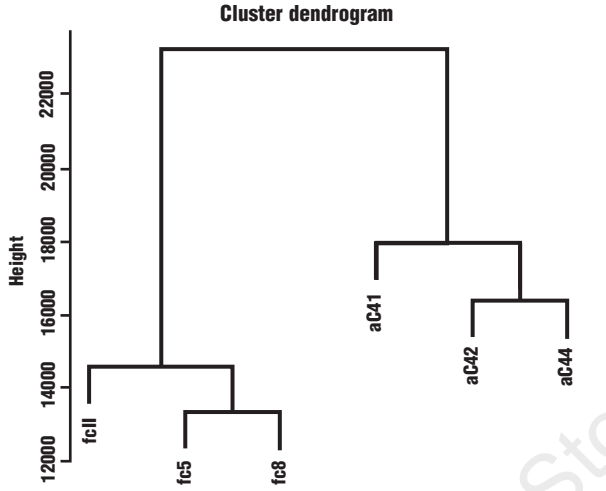

Figure 2. Fetal (fC5, fC8, fC11) and adult (aC41, aC42, aC44) MSC cluster into separate groups in a dendrogram showing multidimensional relationships using Euclidean distances. Highly similar clusters have joining points that are farther from the root.

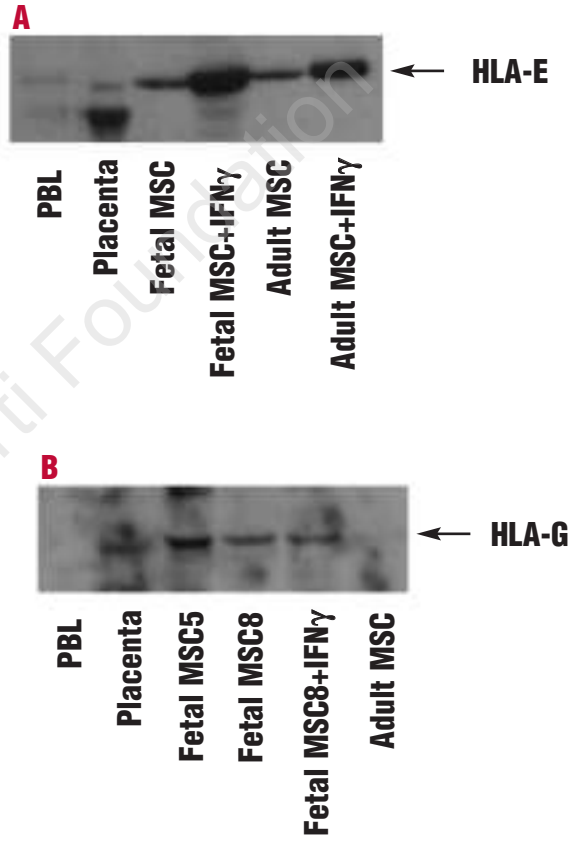

Figure 4. HLA E and -G expression by fetal and adult MSC analyzed by immunoblotting. Fetal and adult MSC, peripheral blood lymphocytes (PBL) and term placental cells were analyzed. (A) HLA E (42 kDa), as indicated by the arrow, was present in fetal and adult MSC and weakly expressed in PBL and placental cells. When fetal and adult MSC were stimulated with IFNγ for two days, increased expression of HLA E was observed. The lower band in the placental cells shows a second allelic variant of HLA E. (B) HLA G (35 kDa), as indicated by the arrow, was detected in fetal MSC and placental cells but not in adult MSC or PBL. IFNγ stimulation for two days did not increase the expression of HLA G in fetal MSC.

transcripts, which were decreased in fetal MSC. Signaling molecules were decreased in fetal compared to adult MSC; only one was increased.

The majority of the mRNA and protein analyses coincided, validating all methods used. Transcripts for CD105, ICAM1, CXCR4 and VWF could not be detected by the parameters used by Affymetrics algorithms. When the raw data for these transcripts were analyzed with the Bioconductor software packages,[41] it was clear that the mismatched probes (negative controls) gave high signal intensities compared to perfectly matched probes. This results in Affymetrics algorithms scoring the transcripts as absent when the transcript is, in reality, expressed. Indeed, when validated with RT PCR, the expression of these genes was confirmed (Figure 3). Consequently, these transcripts were scored as present.

Antigen expression by MSC

The expression of different proteins was analyzed by RT-PCR, immunoblotting, flow cytometry and cell ELISA and compared to the gene array data. The data

are summarized in Table 1. The results confirmed our previous findings of a lower expression of alloantigens in fetal MSC. HLA class I molecules were present on both cell-types but the expression was higher on the adult MSC. Fetal MSC express no HLA class II antigens. Although not detected on the cell-surface, immunoblotting on cell lysates of unstimulated adult MSC showed that the cells contained intracellular deposits of class II alloantigens. We further investigated expression of non-classical class I antigens. HLA-E was present intracellularly in both cell types as well as in peripheral blood lymphocytes and placental cells, but not expressed on the cell surface (Figure 4A). IFNγ treatment increased its synthesis (Figure 4A) but not the cell surface expression (*data not shown*). Transcripts for HLA-G were detected in fetal and adult MSC, whereas its protein could only be identified intracellularly in fetal MSC and placental cells (Figure 4B). The levels were unchanged by IFNγ stimulation. Fetal and adult cells shared the common MSC adhesion molecule markers but adult MSC had an increased expression of VCAM1 (CD106) whereas the fetal MSC expressed more ICAM1 (CD54).

Discussion

In this study, we have further explored the differences between human fetal and adult MSC by analyzing their expression profiles with gene arrays detecting 22,248 transcripts and approximately 14,500 genes. Fetal liver and adult bone marrow-derived MSC are similar in many respects, but differ in several ways, e.g., in their proliferative and differentiating capacity and in some of their immunological properties. Their dissimilarity is supported by the dendrogram in which fetal and adult MSC clearly cluster in separate groups. In the search for a molecular stem cell marker, the presence of recognized and suggested embryonic stem cell (ESC) markers[42,43] was analyzed by browsing the data. The majority of ESC suggested genes were not expressed by either fetal or adult MSC. Transcripts were slightly more common in fetal MSC, which may be explained by the cells diverse origins: fetal vs. adult tissue. The specificity of the connexin genes (CJA1 and CJA7) as ESC markers could either be questioned or the genetic profile added to the stem cell signature of MSC, since they were expressed by both fetal and adult cells.

When investigating the expression of developmental genes, the data suggest that fetal MSC are not as lineage committed as their adult counterpart. Developmental genes more abundant in fetal MSC include HN1, involved in embryo development[44] and MAGOH, a gene highly conserved during evolution. In *Drosophila*, MAGOH is essential for normal germ plasm development,[45] the specialized cytoplasm where components necessary for the determination of the primordial germ cells localize. Similarly, the expression of CKTSF1B1, another highly conserved gene important for early limb development and neural crest differentiation was increased.[46] Additional highly expressed transcripts involved in brain development and axonal outgrowth include HDGFRP3 and MLP,[47,48] as well as CNN1, important for early muscle differentiation during embryonic and post-natal life.[49] Fetal MSC expressed fewer transcripts involved in terminal tissue differentiation. Transcripts for smooth muscle (CRIP1 and CRIP2),[50] keratinocyte (CSTA),[51] epidermal (CST6)[52] and terminal neural cell differentiation (NPDC1)[53] were decreased, corroborating previous findings of higher expression of NPDC1 in adult brain than in fetal brain. The decreased HOXA10 and HOXC10 transcripts are necessary for embryo implantation and are expressed in uterine endometrium.[54]

The gene array data confirm observations made by ourselves and others[14,15] indicating an increased proliferative capacity of fetal MSC as transcripts involved in cell proliferation, chromatin regulation and DNA repair were more abundantly expressed in fetal MSC. Only transcripts promoting cell cycling were increased. For example, TOPK, increased in normal fetal and rapidly proliferating malignant tissues[55] and HELLS, highly expressed in proliferating organs and absent from non-proliferating tissues[56] was expressed in higher amounts. Other genes associated with cell cycle progression are PEGS10, BUB1B, MLYH10 and CDC42BPA,[57-59] and all showed increased expression in fetal MSC. The proliferative state of fetal MSC is also demonstrated by the chromatin regulation genes, all of which were increased. This group of genes is associated with transcription, replication and DNA repair and influences genomic and nucleosome stability (HEC, H2AFZ, SMC1L1)[60-62] as well as the condensing complex during mitosis (SMC4L1, BBRN1, HCAP-G).[62,63] Concurrently with the higher proliferation rate of fetal MSC, there is increased expression of DNA repair transcripts. The expression of transcripts with a negative effect on cell proliferation was reduced. For example, SERPINF1/EPC 1 is reported to be linked to the replicative age of cells and is found in lower levels in fetal skin fibroblasts than in adult ones.[64] Similarly, CREG, induced early during differentiation of pluripotent cells, inhibits cell growth.[65] The decreased transcripts IGFBP4 and IGFBP6 induce growth arrest[66] and DAPK1 promotes apoptosis.[67] On the other hand, the anti-apoptotic transcripts GAS6 and FKBP8/38[68,69] were decreased in fetal MSC.

Although both fetal and adult MSC express HLA class I antigens, the expression is lower in fetal MSC.

Adult MSC contain intracellular deposits of class II alloantigens and cell surface expression is induced by INFγ. Fetal MSC have no HLA class II. The immunological inertness of fetal MSC is further illustrated by the gene array analysis. We could only detect three immunological transcripts that were abundantly expressed in fetal MSC, whereas the expression was reduced for eight transcripts. In spite of their alloantigen expression, MSC are not inherently immunogenic. Preliminary data suggest that both fetal and adult MSC may be transplantable across MHC barriers in humans.[28,32] HLA-G and -E are ligands for inhibitory receptors on natural killers cells and lymphocytes. Soluble HLA-G isoforms may act as specific immunosuppressors during pregnancy and control HLA-E expression.[70,71] HLA-G was only expressed intracellularly by fetal MSC and the levels were unaffected by INFγ treatment. Although lower in fetal MSC than in adult ones, mRNA for HLA-E scored present in the gene array. Intracellular deposits that increased with INFγ treatment were detected but no protein was expressed on the cell surface. Thus, expression of non-classical alloantigens cannot explain why MSC are not immunostimulatory but their expression pattern appears to be similar to that of HLA class II.[29] Overall, fetal and adult MSC exhibit a similar pattern of surface protein expression. However, expression of VCAM1 was higher in the adult MSC while fetal MSC have increased expression of ICAM1. An expression pattern similar to that of fetal MSC, with lower expression of VCAM1 and higher expression of ICAM1, has been observed in MSC derived from adipose tissue.[72] The differentially expressed genes may reflect differences related to the cells' origin: bone marrow vs. other tissues. ICAM1 is a key molecule in immune and inflammatory processes, mediating the adhesion of lymphocytes to other cells facilitating the migration of circulating cells into tissues.[73] The expression of α4 integrin, which associates with CD29 to form VLA4/CD49d, was increased in MSC derived from liver compared to adult bone marrow-derived MSC (*data not shown*). As for ICAM1, α4 integrin is also a key molecule in leukocyte interactions.[73,74] VCAM1 is another molecule important for cellular interactions, particularly between stromal and hematopoietic precursors.[75] MSC are found in sites just before hematopoiesis begins and may recruit hematopoietic precursor cells, in part by expression of VCAM1.[17] CXCL12 (stromal derived factor, SDF 1) and its receptor CXCR4 are also important for homing of hematopoietic cells to the bone marrow.[76] Transcripts for CXCL12 were detected but not its protein on the cell surface. However, CXCL12 produced by MSC is not expressed on the cell surface but rather secreted into the culture supernatant (*Le Blanc et al., unpublished observation*). Consistent with our data, a small subset of adult MSC has been shown to express CXCR4.[77]

Cytokines and growth factors are important in maintaining cell growth and survival. The expression of certain cytokines and their receptors by MSC was analyzed by browsing the gene array data. Transcripts for transforming growth factor (TGF) β1, β2 and β3 were not detected, while their corresponding receptors were present in both fetal and adult MSC. The TGF-β family are immunoregulatory cytokines and contribute to the function and generation of regulatory T cells. Neither TGF-α, interleukin-2, -4, -10, nor their corresponding receptors were present in fetal or adult MSC. The mechanism by which MSC act to suppress proliferation of lymphocytes appears to be complex, affecting interleukin-2 and interleukin-10 signaling.[78]

We conclude that differences exist in the genetic profiles of fetal and adult MSC. In fetal MSC the increased expression of transcripts promoting cell proliferation and the reduced expression of transcripts involved in terminal cell differentiation and immune recognition suggest that fetal MSC have an increased proliferative and differentiating capacity and immunological inertness. Whether these findings translate into superior persistence of mismatched cells after transplantation and a greater regenerative capacity of fetal MSC used to enhance hematopoietic recovery after allogeneic stem cell transplantation as well as in tissue engineering awaits future *in vivo* studies.

CG designed the study, isolated and cultured the cells, analyzed the gene array data and the protein expression. The complementary data and manuscript draft were completed by CG. RL's laboratory was responsible for the microarray gene expression analysis. AW is a post-doctoral fellow in RL's laboratory and had primary responsibility for conducting the Affymetrix experiments. JL conducted the normalization, filtering, statistical tests and bioinformatics. MU performed the RT-PCR analyses. KLB was in charge of the design of the study, the analysis of the results and the preparation of the manuscript. All the co-authors actively participated in the preparation of the manuscript. The authors declare that they have no potential conflicts of interest.

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