REGULAR ARTICLE

Gene and microRNA expression signatures of human mesenchymal stromal cells in comparison to fibroblasts

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Abstract Human mesenchymal stromal cells (MSCs) offer great hope for the treatment of tissue degenerative and immune diseases, but their phenotypic similarity to dermal fibroblasts may hinder robust cell identification and isolation from diverse tissue harvests. To identify genetic elements that can reliably discriminate MSCs from fibroblasts, we performed comparative gene and microRNA expression profiling analyses with genome-wide oligonucleotide microarrays. When taken globally, both gene and microRNA expression profiles of MSCs were highly similar to those of fibroblasts, accounting well for their extensive phenotypic and functional overlaps. Scattered expression differences were pooled to yield an MSC-specific molecular signature, consisting of 64 genes and 21 microRNAs whose expressions were at least 10-fold and two-fold higher, respectively, in MSCs compared with fibroblasts. Genes either encoding transmembrane proteins or associated with tumors were relatively abundant in this signature. These data should provide the molecular basis not only for the discovery of novel diagnostic markers discriminating MSCs from fibroblasts, but also for further studies on MSC-specific signaling mechanisms.

Keywords Gene expression profile · MicroRNA profile · Mesenchymal stromal cells · Fibroblasts

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Introduction

Mesenchymal stromal cells (MSCs), also called mesenchymal stem cells, possess long-term self-renewal and multi-lineage differentiation potential, thus offering great hope for the treatment of a variety of degenerative diseases (Tocci and Forte 2003; Le Blanc and Pittenger 2005; Keating 2006; Dazzi and Horwood 2007). Human MSCs, although widely recognized as a unique cellular entity, are observed intriguingly to be similar to dermal fibroblasts with respect to cell size, morphology, growth property, cell surface phenotype, and immunomodulatory function (Lysy et al. 2007; Sabatini et al. 2005; Haniffa et al. 2007). Traditionally, mesodermal differentiation potential has been used to distinguish between MSCs and fibroblasts (Javazon et al. 2004), but this line of demarcation has recently lost its clarity since fibroblasts, like MSCs, have been found to differentiate into osteocytes (Hee et al. 2006; Phillips et al. 2006), chondrocytes (Rutherford et al. 2003), adipocytes (Feldon et al. 2006), and even hepatocytes (Lysy et al. 2007).

These broad structural and functional overlaps between the two cell types pose a potential hurdle to the development

of clinical grade MSC products for cell therapy and regenerative medicine. Fibroblasts are widely distributed throughout the body (Jelaska et al. 1999; Chang et al. 2002; Rinn et al. 2006) and are thus likely to remain as an undesirable contaminant in tissue harvests as they propagate together with MSCs during in vitro culture expansion. To ensure that isolated MSC cultures are free from fibroblasts, therefore, molecular markers should be used that can reliably discriminate the two cell types. Whether such diagnostic markers exist is currently unknown but, in theory, they can be rapidly assessed through comparative genomics and/or proteomics approaches. Two different research groups have previously performed comparative gene expression profiling studies between the two cell types (Ishii et al. 2005; Brendel et al. 2005), but their uses of subgenomic DNA microarray platforms implementing fewer than 10,000 gene probes render the analyses incomplete and suboptimal. Hence, our present study has been designed primarily to compare the gene expression profiles of these two cell types at the whole-genome scale by using genome-wide oligonucleotide microarrays and to identify any molecular messages that can readily distinguish MSCs from fibroblasts.

In parallel, we have endeavored to define and compare microRNA expression profiles of the two cell types. These small non-coding RNA molecules are known to occur in a cell-type-specific manner (Kim and Nam 2006) and modulate gene expression and/or protein synthesis (Bushati and Cohen 2007). They have been demonstrated to play key regulatory roles in diverse biological processes, but little is known about their global expression patterns in either MSCs or fibroblasts. Therefore, this microRNA expression data, when combined with genome-wide gene expression profiles, might provide an in-depth assessment of transcriptional networks that are differentially operated between MSCs and fibroblasts

Materials and methods

Cell preparation and differentiation

Cryopreserved human bone marrow (BM)-derived MSCs and dermal fibroblast samples were purchased from Lonza (Allendale, N.J.). The samples were quickly thawed, centrifuged, and re-suspended in culture medium consisting of low-glucose Dulbecco's modified Eagle medium (LG-DMEM; Invitrogen, Grand Island, N.Y.), 15% fetal bovine serum (FBS; JRH Biosciences, Lenexa, Kan.), 2 mM L-glutamine, 1 mM sodium pyruvate, and 1% antibiotics/antimycotics (Life Technologies, Gaithersburg, Md.). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2, and culture medium was changed

every 5 days. For serial passage, cells at approximately 60% confluence were detached with 0.1% trypsin-EDTA and replated at a density of 2×10^3 cells per cm^2. Cells at the fifth passage were collected for the microarray experiment. For mesodermal lineage cell differentiation, the cells were incubated with osteogenic, adipogenic, and chondrogenic media (Lonza) for 2–3 weeks and then assessed with Von Kossa, Oil red S, and Alcian blue stains, respectively.

Flow cytometry

All analyses were performed by Guava PCA-96 flow cytometry (Guava Technologies, Hayward, Calif.) with murine monoclonal antibodies against CD10, CD59, CD73, CD90, CD105, CD109, and caveolin 1 (Santa Cruz Biotechnology, Santa Cruz, Calif.), with Alexa-488-conjugated goat anti-mouse IgG (Invitrogen, Carlsbad, CA) as the secondary antibody. All measurements were analyzed with Guava Express software and De Novo software (Thornhill, ON, Canada).

Comparative gene expression profiling

Whole Human Genome 44K Microarrays (Agilent Technologies, Santa Clara, Calif.) were used for this comparative gene expression profiling study between MSCs and fibroblasts. After total RNA had been extracted from 1×10^7 cells by using Trizol (Invitrogen), the synthesis and labeling of target cRNA probes were performed with the Agilent's Low RNA Input Linear Amplification kit (Agilent Technologies). The labeled cRNA target was purified on a cRNA Cleanup Module and quantified by using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Del.). Then, cRNAs were fragmented and hybridized according to manufacturer's standard protocols. The hybridized image was scanned by using the Agilent DNA microarray scanner and quantified with Feature Extraction Software (Agilent Technologies). All data normalization and selection of fold-changed genes were performed by using GeneSpringGX 7.3. Intensity-dependent normalization was performed, where the ratio was reduced to the residual of the Lowess fit of the intensity versus ratio curve. The averages of normalized ratios were calculated by dividing the average of normalized signal channel intensity by the average of normalized control channel intensity.

MicroRNA expression profiling

MicroRNA expression profiling was performed with Human miRNA Microarrays probing for 470 human and 64 human viral microRNAs (Agilent Technologies). Total RNAs were extracted, dephosphorylated, labeled, and

hybridized according to the manufacturer's instructions. Images were scanned, quantified as described above, and presented without further data manipulation.

Analysis by reverse transcription with polymerase chain reaction

To assess the differentiation potential of the cells, the incubated cells were stimulated to differentiate. To verify the microarray-generated MSC-specific molecular signature, the top ten genes with the highest MSC-to-fibroblast intensity ratios and *pre-mir-335* were subjected to reverse transcription with polymerase chain reaction (RT-PCR) analysis with β-actin mRNA as an internal control. The sense and antisense primers used for these analyses were as presented in Table 1.

Results

Immunophenotypic comparison between MSCs and fibroblasts

Before undertaking comparative gene and microRNA expression profiling analyses, we sought to examine prospectively how similar or different our MSC and fibroblast samples were in readily accessible terms. First, we performed an immunophenotypic analysis, in which the

Table 1 Primers and annealing temperatures used for analysis by reverse transcription with polymerase chain reaction (*F* forward, *R* reverse, *ACTB* β-actin control)

Gene	Sequence	Product size (bp)	Annealing temperature (°C)
ALP	F 5′-CTG GTA GGC GAT GTC CTT A-3′	475	50
	R 5′-ACG TGG CTA AGA ATG TCA TC-3′		
BSP	F 5′-GCA CGC CTA CTT CTA TCC TC-3′	185	56
	R 5′-CGG CCT CGG AGT CTT-3′		
aP2	F 5′-GGC CAA ACC CAA CCT GA-3′	167	60
	R 5′-GGG CGC CTC CAT CTA AG-3′		
PPAR-γ2	F 5′-GCT GTT ATG GGT GAA ACT CTG-3′	350	62
	R 5′-ATA AGG TGG AGA TGC AGG TTC-3′		
COLX	F 5′-GCC CAA GAG GTG CCC CTG GAA TAC-3′	703	57
	R 5′-CCT GAG AAA GAG GAG TGG ACA TAC-3′		
SOX9	F 5′-AGA CAG CCC CCT ATC GAC TTC-3′	230	63
	R 5′-TGC TGC TTG GAC ATC CAC AC-3′		
FLJ37824	F 5′- CCC CCA AGA CGA CCA GAA AAT -3′	110	57
	R 5′- AAA CCA AGA CAC TAG GCA AACTT -3′		
PLXDC1	F 5′- CCT GGG CAT GTG TCA GAG C -3′	230	57
	R 5′- GGT GTT GGA GAG TAT TGT GTG G -3′		
SLC22A3	F 5′- GAA AGG GGA CGT GGA TGA CTT -3′	169	52
	R 5′- CGT GAT GGC TAA CTG GAT TCC T -3′		
SRGN	F 5′-GAG CCA GGT ACC AAT GGG-3′	290	50
	R 5′-ACC CAA GTC CTG GCT GTC-3′		
OTUB2	F 5′- TTC TTC GGG ACC ATC CTG AAA -3′	561	58
	R 5′- TGG TTC AGG GCG GTA TCC AT-3′		
GALNT3	F 5′- CAG CAG AAT TGA AGC CTG TCC -3′	252	61
	R 5′- TGC AAA GCC TGG TGA TAA AGA A -3′		
ANKRD1	F 5′-GCT GTT ATG GGT GAA ACT CTG-3′	350	62
	R 5′-ATA AGG TGG AGA TGC AGG TTC-3′		
SHOX	F 5′-GCG CGA GGA CGT GAA GTC -3′	420	57
	R 5′-ATC AGG TAG GGC GCG TGC -3′		
CHI3L1	F 5′-TGA TGT GAC GCT CTA CGG -3′	230	63
	R 5′-AAT GGC GGT ACT GAC TTG ATG -3′		
TPD52L1	F 5′-CCC TGA GTC ACG CAG GGC -3′	590	65
	R 5′-GCT CTG GGC ACT GGC ATG -3′		
pre-mir-335	F 5′-TTG AGC GGG GGT CAA GAG -3′	89	56
	R 5′-GAA TAT AGC AAA TGA GAG GAG -3′		
ACTB	F 5′- AGA AAA TCT GGC ACC ACA CC-3′	435	57
	R 5′-CCA TCT CTT GCT CGA AGT CC-3′		

two cell samples were individually labeled with mouse antibodies against membrane metallo-endopeptidase (CD10), protectin (CD59), ectonucleotidase (CD73), Thy-1 (CD90), endoglin (CD105), CD109, and caveolin 1. Flow cytometric analyses of the labeled cells showed that MSCs were strongly positive for CD73, CD90, and CD105 (Fig. 1), all being known as MSC-associated antigens and officially recommended for their concurrent use to define unambiguously an MSC type (Dominici et al. 2006). Enigmatically, however, these surface proteins were found at quantitatively similar levels in fibroblasts, indicating that the CD73$^+$ CD90$^+$ CD105$^+$ immunophenotype might be not exclusive to MSCs but common to both cell types. Furthermore, the two cell types were also observed to express a high CD59 level and weak CD109 and CAV1 levels in common, although they featured a notable difference with respect to CD10 levels. These observations suggest that MSCs and fibroblasts share a highly similar, but not perfectly matching, cell surface phenotype.

Comparison of differentiation potentials

Next, we separately incubated the cells under osteogenic, adipogenic, and chondrogenic differentiation conditions and assessed their differentiation potential by cytochemical staining and RT-PCR analyses of various differentiation marker genes. When incubated with osteogenic medium, MSCs differentiated readily along an osteogenic lineage, as evidenced by positive staining with von Kossa dye (Fig. 2d) and the gene expression of alkaline phosphatase (ALP) and bone sialoprotein (BSP; Fig. 2g). Fibroblasts incubated under the same conditions, on the other hand, showed no signs of

staining (Fig. 2a) or marker gene expression (Fig. 2g). When exposed to adipogenic medium, MSCs, but not fibroblasts, differentiated into adipocytes, as demonstrated by Oil red O staining (Fig. 2b,e) and the gene expressions of adipocyte fatty-acid-binding protein (aP2) and peroxisome proliferator-activated receptor γ2 (PPAR-γ2; Fig. 2h). Likewise, the preferential chondrogenic differentiation of MSCs over fibroblasts induced by micromass pellet culture in the presence of chondrogenic medium was demonstrated by Alcian blue staining (Fig. 2c,f) and the increased expression of SOX9 and COLX genes (Fig. 2i). The results indicated that MSC cultures could readily differentiate into osteocytes, adipocytes, and chondrocytes, whereas fibroblast cultures failed to differentiate along any of these lineages. Our observations suggest that these mesengenic differentiation assays can be used reliably to discriminate MSCs from fibroblasts and also argue that pro-mesengenic molecular mechanisms and underlying factors are actively engaged in MSCs, but absent in fibroblasts.

Comparative genome-wide gene expression profiles

To identify genetic elements that can reliably discriminate MSCs from fibroblasts, we performed a comparative gene expression profiling analysis between MSCs and fibroblasts by using genome-wide oligonucleotide microarrays. The median standard deviation of 264 gene probes across 10 replicates was calculated to be about 0.065, indicating that the experiment had been performed with satisfactory intra-array reproducibility. The pair-wise comparison of the two gene expression profiles generated a collinear distribution of normalized intensity with a correlation coefficient of

Fig. 1 Comparative flow cytometric analyses of MSCs and fibroblasts. The fluorescence intensity of MSCs (shaded profiles) and fibroblasts (open profiles) stained only with the secondary antibody (a, control) or antibodies against CD10 (b), CD59 (c), CD73 (d), CD90 (e), CD105 (f), CD109 (g), or caveolin 1 (h) were measured. Note that the two cell types shared similar levels of CD59, CD73, CD90, CD105, CD109, and CAV1 but that, exceptionally, CD10 was up-regulated in fibroblasts compared with MSCs
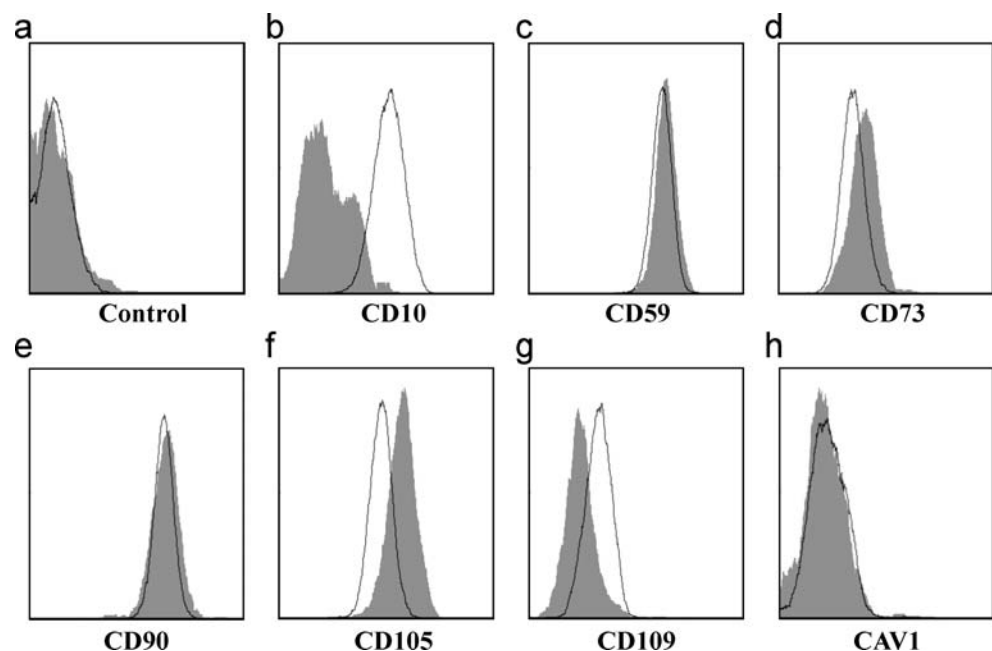
Fig. 2 Comparative analysis of mesenchymal differentiation potential of MSCs and fibroblasts. Both fibroblast and MSC samples were separately incubated under osteogenic (a, d), adipogenic (b, e), or chondrogenic (c, f) conditions and assessed for their differentiation potential by Von Kossa, Oil red O, or Alizarin red stains, respectively. Note that MSCs (d–f), but not fibroblasts (a–c), were strongly stained by all these dyes. Bars 100 µm. RT-PCR analyses were performed on differentiated MSCs and fibroblasts with primers specific for the bone-related ALP and BSP genes (g), the fat-related aP2 and PPAR-γ2 genes (h), and the cartilage-related SOX9 and COLX genes (i) for the assessment of osteogenic, adipogenic, and chondrogenic differentiation, respectively. Note that these marker genes were expressed at much higher levels in differentiated MSCs compared with fibroblast counterparts (ACTB β-actin control)
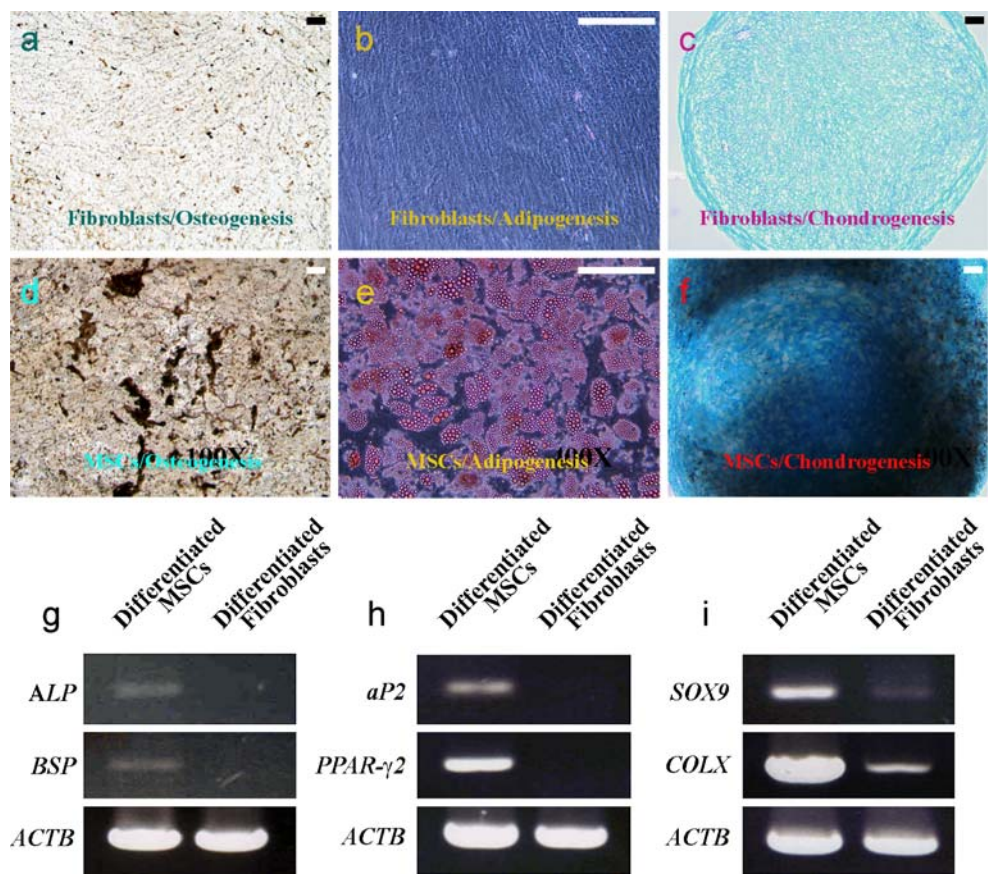

about 0.93 (Fig. 3a). This finding indicates that the two cell types share a strikingly similar gene expression pattern, thus accounting for their extensive phenotypic and functional overlaps. However, the expression intensities of a number of genes deviated from linearity and were scattered over the entire intensity range. These genes were considered, as a whole or in part, to represent either MSC- or fibroblast-specific molecular signatures.

To obtain an MSC-specific signature, we sorted the genes by the intensity ratios in MSCs compared with fibroblasts and selected 1446 unique genes with two-fold or higher intensity ratios. We then extracted a distinct molecular signature consisting of 64 unique genes by applying a cutoff ratio of 10 and eliminating gender-related genes. This distinct signature contained 56 known and 8 uncharacterized genes and could be further classified into four groups based on the primary subcellular location of their products (Supplementary Table 1).

In quantity, approximately one third of known genes were found to encode integral membrane proteins

Fig. 3 Comparison of genome-wide gene and microRNA expression profiles between MSCs and fibroblasts. Intensity scatter plots and calculated correlation coefficients indicate that MSCs and fibroblasts share highly similar gene (a) and microRNA (b) expression profiles
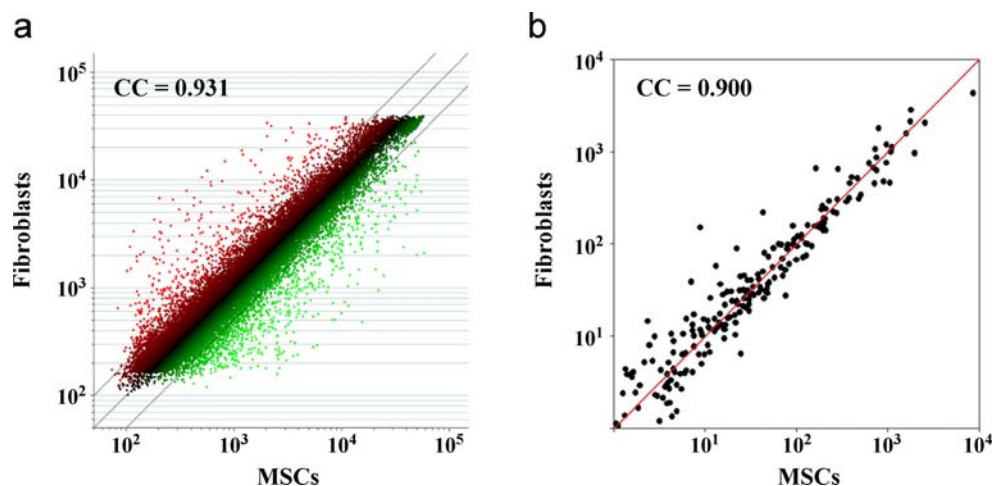
(*PLXDC1*, *SLC22A3*, *GALNT3*, *VCAM1*, *EPHA3*, *CDH6*, *TM4SF1*, *TMEM16A*, *TMEM46*, *MEST*, *GPR177*, *ABI3BP*, *CHGN*, *CNTNAP3*, *FGFR2*, and *CD200*) and membrane-associated proteins (*EPB41L4B*, *SPTLC3*, and *KCTD16*). Furthermore, our bioinformatic analyses of *FLJ41687* and *FLJ38080* revealed that their products probably belonged to transmembrane proteins, SLC14A1 and MPZL3, respectively. This abundancy of membrane genes implies that the cell membrane is the divergent subcellular region between MSCs and fibroblasts.

The signature was also found to contain a large number of genes previously associated with tumors and cancers, including *PLXDC1*, *GALNT3*, *CHI3L1*, *TPD52L1*, *EPHA3*, *PRDM16*, *POSTN*, *TM4SF1*, *TMEM16A*, *PNMA2*, *TIAM1*, *FNDC1*, *ABI3BP*, and *JAG1*. Of particular interest among them were the metastasis-associated genes, such as *PLXDC1* (also known as *TEM7*; Fuchs et al. 2007), *GALNT3* (Inoue et al. 2007), *CHI3L1* (also known as *YKL-40*; Johansen et al. 2007), and *POSTN* (Bao et al. 2004).

Comparison of microRNA expression profiles

In parallel to our gene expression profiling efforts, we attempted to obtain comparative microRNA expression profiles by using the same MSC and fibroblast samples. The correlation coefficient between the two microRNA profiles was measured to be around 0.9 (Fig. 3b), indicating that MSCs and fibroblasts share a similar microRNA repertoire. Nevertheless, we tried to sort these microRNAs with respect to their hybridization

intensity ratio (Supplementary Table 2) and to select 21 microRNAs with a two-fold or higher intensity ratio (Table 2). We found that *mir-335* was the most differentially expressed gene in MSCs over fibroblasts. Since a *MEST* gene, hosting *mir-335* within the intron sequence (Liang et al. 2007), was one of up-regulated genes in MSCs (Supplementary Table 1), this data adds credence to the previous observation of the co-expression pattern of microRNAs and their host genes (Baskerville and Barttel 2005).

Confirmation of comparative gene and microRNA profiles by RT-PCR

To verify the comparative mRNA and microRNA expression profiles, RT-PCR analysis was performed for the top 10 differentially expressed genes and *pre-mir-335*. The results showed that the band intensities for all PCR probes were higher in MSCs than in fibroblasts (Fig. 4), demonstrating that the gene expression pattern was in a good agreement with the microarray-generated expression data. Therefore, all experimental and analytical procedures had probably beene properly employed to generate comparative gene and microRNA profiles.

Discussion

Human MSCs are multifunctional in that they not only self-renew to a large extent and differentiate into a variety of

Table 2 MicroRNA signature of MSCs in comparison with fibroblasts (*BM-MSC* bone-marrow-derived mesenchymal stromal cells)

| microRNA number	miRBase identifier	Accession number	Intensity in BM-MSCs	Intensity in fibroblasts	Ratio of intensity
1	hsa-miR-335	MI0000816	44.5	1.0	44.5
2	hsa-miR-520f	MI0003146	4.2	1.0	4.2
3	hsa-miR-181a-2	MI0000269	24.7	6.4	3.9
4	hsa-miR-340	MI0000802	4.4	1.3	3.3
5	hsa-miR-431	MI0001721	4.9	1.5	3.2
6	hsa-miR-140	MI0000456	76.3	27.4	2.8
7	hsa-miR-520e	MI0003143	2.7	1.0	2.7
8	hsa-miR-181c	MI0000271	3.2	1.2	2.7
9	hsa-miR-519b	MI0003151	2.6	1.0	2.6
10	hsa-miR-491	MI0003126	2.6	1.0	2.6
11	hsa-miR-520g	MI0003158	2.3	1.0	2.3
12	hsa-miR-143	MI0000459	1061.5	456.9	2.3
13	hsa-miR-337	MI0000806	4.1	1.9	2.2
14	hsa-miR-122a	MI0000442	2.2	1.0	2.2
15	hsa-miR-409–5p	MI0001735	2.1	1.0	2.1
16	hsa-miR-132	MI0000449	21.6	10.2	2.1
17	hsa-miR-652	MI0003667	3.9	1.8	2.1
18	hsa-miR-181a*	MI0000289	5.5	2.7	2.1
19	hsa-miR-145	MI0000461	1987.9	961.1	2.1
20	hsa-miR-18b	MI0001518	2.0	1.0	2.0
21	hsa-miR-21	MI0000077	8641.5	4311.0	2.0
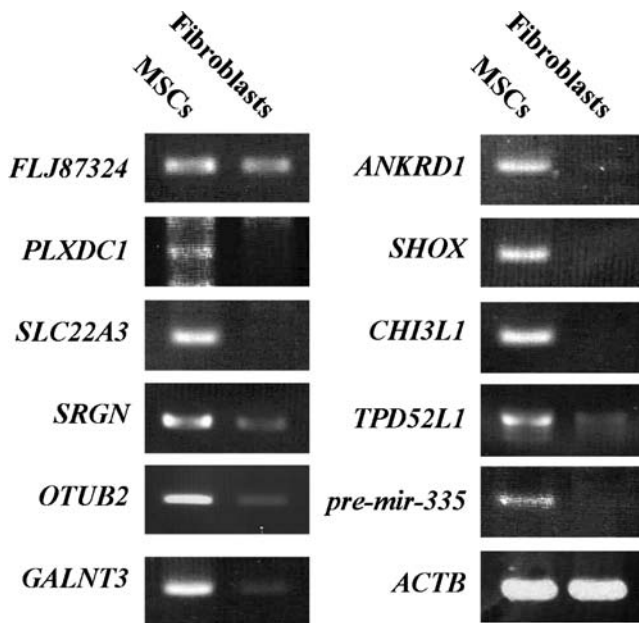
Fig. 4 RT-PCR analysis of the differentially expressed genes and microRNA. The expression levels of the top ten differentially expressed genes and *pre-mir-335* in MSCs and fibroblasts were verified by RT-PCR with the same RNA samples as those employed in gene and microRNA expression profiling studies and with *β-actin* as an internal control (*ACTB*)

cell types, but also support hematopoiesis and suppress immune responses, offering great hope to meet diverse therapeutic needs. Further advantages are that they are the most ample and accessible stem cell types (da Silva Meirelles et al. 2006) and can be routinely obtained, without ethical and safety concerns for their clinical uses, from BM, neonatal blood, and adipose tissues. Their homogeneous culture, however, remains as a challenging task, because of the lack of MSC-specific markers, without which the standardization of the cell isolation protocol with fluorescence-activated cell sorting or magnetic cell sorting is not permitted. Alternative approaches, mostly based on plastic adherence and/or negative selection, are currently in widespread use but entail multi-step processing and the risk of contamination with other tissue cells.

In particular, skin fibroblasts are the contaminant of the greatest concern, because they share striking phenotypic and functional resemblances with MSCs, and hence, once introduced into tissue harvests, might be difficult to eliminate from MSC culture. Molecular markers that can reliably distinguish MSCs from fibroblasts would therefore be of practical value. This study has hence been performed to compare simultaneously the gene and microRNA expression profiles of the two cells at the whole-genome scale and to extract any genetic messages that can reliably discriminate MSCs from fibroblasts. For analytical simplicity, we have chosen two cell populations, one each for the two cell types, from donors of similar age. Our studies have previously

shown little donor-to-donor variation in the global transcriptional profile of MSC samples (Jeong et al. 2005, 2007). Likewise, the gene expression pattern of fibroblasts has been reported to be independent with respect to age, sex, or culture conditions (Rinn et al. 2006). Thus, any significant findings drawn from this study cab be generalized to cells from other donors.

In this study, we have observed, for the first time, that the global gene expression profile of MSCs is markedly similar to that of fibroblasts. This similarity might well account for their extensive homology in phenotype, function, and developmental status. Accordingly, the salient differences in mesengenic differentiation observed between the two cell types is likely to be the outcome of the differential expression of a small, rather than large, set of molecular factors. To identify such molecular factors at a high confidence level, we have sorted out genes whose expression is at least ten-fold higher in MSCs compared with fibroblasts.

Intriguingly, many genes in this MSC-specific signature have turned out to be associated with tumors and metastasis, suggesting that MSCs might be more plastic and migratory in nature than fibroblasts. This finding agrees with the observation that MSCs, but not fibroblasts, possess a multilineage differentiation and migration potential. Nevertheless, the way that such MSC-specific functionalities are mediated by these genes is currently unknown.

Among MSC-enriched genes, we have paid particular attention to those genes encoding transmembrane proteins, because most, if not all, of them could serve as surface marker candidates to discriminate MSCs from fibroblasts. In order to confirm their suitability as MSC-specific biomarkers, however, the preferential expression of these proteins on the surface of MSCs compared with fibroblasts, and possibly with other blood cell types, should be verified. The transmembrane protein genes are also important, because they may provide clues as to how the MSC-specific communication interface is organized between the extracellular milieu and intracellular signaling machineries. In particular, the receptor proteins for canonical signaling pathways, such as two receptor tyrosine kinases, EPHA3 and FGFR2, and a G-protein-coupled receptor GPR177, are possible molecular candidates that may endow MSCs with "stemness" and thus deserve further in-depth functional study.

In addition, we have observed that MSCs and fibroblasts share similar microRNA expression profiles, indicating that microRNAs might not play such significant roles in either MSC- or fibroblast-specific functions, as observed in other biological processes, including embryo development (Darell et al. 2007), carcinogenesis (Calin and Croce 2006), apoptosis (Cheng et al. 2005), viral infection (Sullivan and Ganem 2005), and hematopoietic stem cell

differentiation (Chen et al. 2004). A notable exception to the homologous microRNA pattern shared by MSCs and fibroblasts has been found in *mir-335* whose expression has been measured to be about 44-fold higher in MSCs than in fibroblasts. Why this microRNA is solely up-regulated at such higher level in MSCs in comparison with fibroblasts is unknown, but one plausible explanation can be deduced from a recent study demonstrating its function as a suppressor of breast cancer metastasis (Tavazoie et al. 2008). Therefore, high *mir-335* expression might be required for the post-transcriptional regulation of metastasis-associated genes expressed by MSCs.

In summary, we have compared the global gene and microRNA expression profiles between MSCs and fibroblasts and have identified a catalog of genetic messages preferentially expressed by MSCs versus fibroblasts. These data should provide the molecular basis for the discovery of novel MSC-specific biomarkers and "stemness" factors.

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