Journal of Cellular Biochemistry 103:1198–1210 (2008)

Age-Related Gene Expression Profiles of Rhesus Monkey Bone Marrow-Derived Mesenchymal Stem Cells

Joseph G. Hacia,[1] C. Chang I. Lee,[2] Daniel F. Jimenez,[2] Mazen W. Karaman,[1] Vincent V. Ho,[1] Kimberley D. Siegmund,[3] and Alice F. Tarantal[2,4]*

[1]Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, California 90089
[2]California National Primate Research Center, University of California, Davis, California 95616
[3]Department of Preventive Medicine, University of Southern California, Los Angeles, California 90089
[4]Departments of Pediatrics and Cell Biology and Human Anatomy, University of California, Davis, California 95616

Abstract The objective of this study was to elucidate age-related differences in gene expression profiles of rhesus monkey bone marrow-derived mesenchymal stem cells (rhMSC) obtained from fetal, infant, and adult donors relevant to their growth and other properties. Although a high degree of similarity was observed in the rhMSC gene expression profiles when comparing the three age groups, significant differences were found that strongly parallel gene expression profiles of human MSC. In general, there was a trend towards increased abundance of transcripts associated with differentiation and growth arrest with increasing donor age. Conversely, transcripts involved in RNA processing and the negative regulation of gene expression showed a downward trend with increasing donor age. Overall, the observed gene expression profiles were found to be similar to observations that MSC from older individuals show diminished proliferative capacity. These data highlight the importance of use of non-human primates to study the properties of stem and progenitor cells, and for future therapies. J. Cell. Biochem. 103: 1198–1210, 2008.

Key words: mesenchymal stem cells (MSC); microarray; rhesus monkey; transcriptional profiling

Mesenchymal stem cells (MSC) have been under consideration for tissue regenerative purposes based on studies in humans and various animal models [Keating, 2006; Giordano et al., 2007]. The capability to greatly expand these cells in vitro and differentiate into mesenchymal lineages (adipogenic, chondrogenic, osteogenic) and the proposed role in immune regulation provides the possibility that MSC can be used in cellular transplantation for specific diseases and to enhance hematopoietic recovery [Stagg, 2006]. While the potential of these cells has been reported, questions remain on the use of MSC for tissue repair, as well as the optimal source for transplantation purposes [English, 2007].

Our prior studies have described the isolation, expansion, characterization, differentiation, and transduction of bone marrow-derived rhesus monkey MSC (rhMSC) [Lee et al., 2004; Lee et al., 2006]. Studies have also shown that fetal cells have significantly greater population doubling times when compared to cells from other age groups, particularly adults [Lee and Tarantal, 2006]. In this study, the gene expression profiles of bone marrow-derived rhMSC from fetal, infant, and adult rhesus monkeys were evaluated. Although all three groups displayed similar expression profiles, age-related differences in specific groups of genes pertinent to MSC biology and cell differentiation were observed. These trends are consistent with human MSC gene expression

This article contains supplementary material, which may be viewed at the Journal of Cellular Biochemistry website at http://www.interscience.wiley.com/jpages/0730-2312/suppmat/index.html.

Grant sponsor: NIH; Grant numbers: #HL73220, #RR00169.

*Correspondence to: Alice F. Tarantal, California National Primate Research Center, University of California, Pedrick and Hutchison Roads, Davis, CA 95616-8542.
E-mail: aftarantal@primate.ucdavis.edu

Received 3 February 2007; Accepted 20 June 2007
DOI 10.1002/jcb.21498

profiles and support rhMSC as a model system for studying stem and progenitor cell biology and the future use of these cells for tissue regeneration purposes.

MATERIALS AND METHODS

Animals

All animal procedures conformed to the requirements of the Animal Welfare Act and protocols were approved prior to implementation by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Davis. Cells were obtained from third trimester fetuses (N = 4; 120–160 days of gestation; term 165 ± 10 days), infants (N = 4; 3–6 months of age), and adults (N = 4; 7–8 years) (Table I). Normally cycling, adult female rhesus monkeys (*Macaca mulatta*) with a history of prior pregnancy were bred and identified as pregnant, using established methods to obtain fetuses and infants (N = 8), as previously reported [Tarantal, 2005]. Activities related to animal care (diet, housing), and screening animals for endogenous retroviruses (SRV, STLV) prior to assignment to the study were performed as per standard California National Primate Research Center (CNPRC) operating procedures.

rhMSC Culture

rhMSC were collected by flushing the long bones at tissue harvest (fetuses) or by bone marrow aspiration under ketamine (10 mg/kg) and local lidocaine (~1–3 ml marrow; infants, adults), and grown as previously described [Lee et al., 2004; Lee et al., 2006]. Briefly, the mononuclear cell fraction was enriched by gradient centrifugation and plated at 5×10^5 cells/cm^2 in DMEM culture media (Invitrogen Corp., Carlsbad, CA), supplemented with 20% fetal bovine serum (FBS) (Invitrogen Corp.). After 3 days non-adherent cells were discarded. The medium was changed every 3–4 days thereafter, and when the cells achieved approximately 80% confluence. The cells were then plated at a density of 4×10^5 cells/cm^2 on tissue culture dishes, cultured to the second passage, collected before confluence was achieved, and stored at $\leq -80^\circ$C until processing.

TABLE I. rhMSC Samples Analyzed—Donor Age

Category	Code	Donor age
Fetal	Fetal 1	Early third trimester
	Fetal 2	Early third trimester
	Fetal 3	Term
	Fetal 4	Term
Infant	Infant 1	6 months
	Infant 2	6 months
	Infant 3	3 months
	Infant 4	5 months
Adult	Adult 1	7 years
	Adult 2	7 years
	Adult 3	8 years

Gene Expression Profiling

The cells were washed three times with phosphate-buffered saline (PBS), and then TRIzol Reagent (Invitrogen Corp.) was added for total RNA extraction. Isolated RNA was cleaned using the QiaAmp isolation RNA kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Two micrograms of total RNA was amplified and labeled according to a standard protocol (Affymetrix, Inc., Santa Clara, CA). Total RNA was subjected to analysis on Affymetrix U133A microarrays, designed to study the relative abundance of approximately 12,500 human transcripts. Microarray Suite version 5.0 software (Affymetrix, Inc.) was used to generate .dat and .cel files for each experiment. The latter were imported into ArrayAssist software (Stratagene Corp., La Jolla, CA) and the robust multi-array average (RMA) algorithm [Irizarry et al., 2003] was used to generate background-adjusted normalized log-transformed gene expression scores for each experiment.

Due to differences in rhesus 3′-UTRs and the oligonucleotide probes (approximately 11–12 perfect match probes per gene) designed for human sequences, certain rhesus transcripts will be more readily detected than others [Karaman et al., 2003; Nagpal et al., 2004]. Thus, it is not possible to compare expression levels of distinct transcripts within a given sample. Based on gene expression data from cultured human and non-human primate fibroblasts [Karaman et al., 2003] and unpublished observations, it was anticipated that accurate measurements would be obtained of approximately 60% of the genes that were expressed in the rhMSC and present in the microarray. However, we have empirically determined that, on average, we are interrogating approximately 20% (2,500) of the transcripts represented in the microarray. Lastly, it should be noted that the RMA algorithm is well-suited for cross-species microarray analyses since it only considers

hybridization data from perfect match and not mismatch probes [Irizarry et al., 2003]. The latter are especially confounding for cross-species analyses since they likely contain more than one mismatch with the intended transcript and thus do not provide good measures of cross-hybridization [Nagpal et al., 2004].

To identify genes that showed trends towards increased or decreased expression associated with increasing age, we conducted a linear trend test and list all trend *P*-values in Supplemental Table 1. In Tables II and III, we report data for transcripts showing (i) a trend *P*-value ≤ 0.005 and (ii) at least 1.5-fold differential expression in the fetal versus adult comparisons. The latter requirement imposes a minimal threshold for the magnitude of the trends observed in our analyses.

With the exception of the *CHI3L1*, the application of the Benjamini–Hochberg multiple hypothesis correction factor for the trend or paired *t*-tests resulted in no transcripts showing differential expression (corrected *P*-value ≤ 0.1). However, commonly used correction factors are not especially well suited to address the current study conditions wherein (i) a significant subset of probe tilings cannot detect rhesus transcripts and (ii) the human and rhesus genes can show different levels of divergence depending upon their function [Yu et al., 2006]. As such, there could be a bias towards measuring highly related and/or co-regulated genes. If true, the application of commonly used multiple hypothesis correction factors could result in a high Type II (false negative) rate and thus explain the inability to identify differentially expressed transcripts despite the different biological properties of these cultures.

To minimize the effects of Type I (false positive) errors in our analyses, we emphasize the functional implications of groups of transcripts showing an age-related trend in abundance. We used the WebGestalt platform (http://bioinfo.vanderbilt.edu/webgestalt/) to determine if specific sets of differentially expressed genes were enriched for Gene Ontology (GO) categories, taking into account the composition of the microarray [Zhang et al., 2005] (Supplemental Table 2). We have defined enriched categories as being comprised of at least four probe tilings with $P < 0.001$ based on a hypergeometric test. As noted above, these analyses could be skewed if highly related and/or co-regulated genes are over-represented in our data set.

In addition to the trend *t*-tests, we conducted a one-way analysis of variance (ANOVA) of the microarray data and report ANOVA *P*-values in Supplemental Table 1. We report data for transcripts showing (i) an ANOVA *P*-value ≤ 0.005 and (ii) at least 1.5-fold differential expression with a Student's *t*-test *P*-value ≤ 0.005 in the fetal versus adult (Supplemental Table 3), fetal versus infant (Supplemental Table 4), and infant versus adult (Supplemental Table 5) comparisons. For similar reasons discussed for the trend *t*-test, a multiple hypothesis correction factor was not applied to any of these ANOVA or Student's *t*-test *P*-values. The data in Supplemental Tables 3–5 are not discussed in the text since we focus our discussion on transcripts that show trends towards increased or decreased expression associated with increasing age.

All scaled fluorescent intensity values and .cel files are available at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/projects/geo/) under Series Accession Number GSE6814. In addition, all processed fluorescent intensity values are available in Supplemental Table 1.

Real-Time PCR

The difference in the levels of gene transcripts identified in the oligonucleotide microarray-based analysis was independently tested by quantitative RT-PCR. The human cDNA sequences of interest were retrieved from the NCBI website, then entered into the BLAST-Like Alignment Tool (BLAT) on the UCSC Genome Bioinformatics website (http://genome.ucsc.edu/) to obtain available *M. mulatta* genomic sequence. Primers were designed using Primer Express software (Applied Biosystems, Foster City, CA) then synthesized (Invitrogen Corp.). Primers were designed to span an intron and thus eliminate the possibility of amplifying off of genomic DNA templates remaining in the RNA preparations (Table IV). Amplicon sizes ranged between 90 and 150 base pairs in length. Real-time PCR was carried out in 96-well plates using the 7900 ABI Sequence Detection System (Applied Biosystems) and the QuantiTect™ SYBR® Green PCR Kit (Qiagen) according to the manufacturer protocols. PCR reactions contained 1× SYBR Green master

TABLE II. Transcripts Showing Decreased Expression With Advancing Age in Cultured rhMSC

			Median value			Trend-line	Fetal vs. adult		Fetal vs. infant		Infant vs. adult	
Probe ID[a]	Gene	Gene description	Fetal	Infant	Adult	*P*-value[b]	FC[c]	*P*-value[d]	FC[c]	*P*-value[d]	FC[c]	*P*-value[d]
203186_s_at	*S100A4*	S100 calcium binding protein A4	1813	746	469	0.0006	3.9	0.0015	2.4	0.0080	1.6	0.2194
206561_s_at	*AKR1B10*	Aldose reductase	2420	2063	759	0.0011	3.2	0.0013	1.2	0.3031	2.7	0.0037
205990_s_at	*WNT5A*	Wingless-type MMTV integration site family	610	452	229	0.0017	2.7	0.0045	1.4	0.2384	2.0	0.0160
204114_at	*NID2*	Nidogen 2 (osteonidogen)	1337	1101	519	0.0011	2.6	0.0046	1.2	0.2079	2.1	0.0063
214803_at	—	CDNA clone IMAGE: 4152983	527	279	212	0.0028	2.5	0.0149	1.9	0.0417	1.3	0.1250
209156_s_at	*COL6A2*	Collagen, type VI, alpha 2	1427	803	598	0.0015	2.4	0.0012	1.8	0.0298	1.3	0.2565
206074_s_at	*HMGA1*	High mobility group AT-hook 1	1275	999	531	0.0015	2.4	0.0094	1.3	0.2602	1.9	0.0014
202410_x_at	*IGF2*	Insulin-like growth factor 2	775	508	321	0.0016	2.4	0.0008	1.5	0.0933	1.6	0.1308
210881_s_at	*IGF2*	Insulin-like growth factor 2	1149	831	480	0.0026	2.4	0.0067	1.4	0.2043	1.7	0.0417
207826_s_at	*ID3*	Inhibitor of DNA binding 3	299	212	131	0.0003	2.3	0.0001	1.4	0.0774	1.6	0.0436
212473_s_at	*MICAL2*	Microtubule associated monoxygenase	733	347	320	0.0034	2.3	0.0127	2.1	0.0004	1.1	0.7233
203440_at	*CDH2*	Cadherin 2, type 1, N-cadherin (neuronal)	407	279	188	0.0001	2.2	0.0050	1.5	0.0264	1.5	0.0027
202855_s_at	*SLC16A3*	Solute carrier family 16, member 3	615	338	282	0.0011	2.2	0.0075	1.8	0.0072	1.2	0.2146
201545_s_at	*PABPN1*	Poly(A) binding protein, nuclear 1	525	309	235	0.0049	2.2	0.0098	1.7	0.0293	1.3	0.3747
212091_s_at	*COL6A1*	Collagen, type VI, alpha 1	1471	1001	689	0.0004	2.1	0.0005	1.5	0.0381	1.5	0.0936
201430_s_at	*DPYSL3*	Dihydro-pyrimidinase-like 3	428	309	202	0.0020	2.1	0.0119	1.4	0.1349	1.5	0.0326
209386_at	*TM4SF1*	Transmembrane 4 L six family member 1	449	338	227	0.0004	2.0	0.0018	1.3	0.0746	1.5	0.0223
214085_x_at	*GLIPR1*	GLI pathogenesis-related 1 (glioma)	938	665	466	0.0010	2.0	0.0102	1.4	0.0547	1.4	0.0421
201770_at	*SNRPA*	Small nuclear ribonucleoprotein polypeptide A	1257	752	628	0.0025	2.0	0.0135	1.7	0.0106	1.2	0.3341
202396_at	*TCERG1*	Transcription elongation regulator 1	407	238	215	0.0013	1.9	0.0079	1.7	0.0033	1.1	0.2581
203131_at	*PDGFRA*	Platelet-derived growth factor receptor	892	675	460	0.0015	1.9	0.0069	1.3	0.1303	1.5	0.0353
204256_at	*ELOVL6*	ELOVL family member 6	276	183	144	0.0024	1.9	0.0250	1.5	0.0550	1.3	0.0073
221059_s_at	*COTL1*	Coactosin-like 1 (dictyostelium)	358	282	191	0.0029	1.9	0.0085	1.3	0.1783	1.5	0.0581
201853_s_at	*CDC25B*	Cell division cycle 25B	486	357	269	0.0003	1.8	0.0041	1.4	0.0252	1.3	0.0354
203820_s_at	*IMP-3*	IGF-II mRNA-binding protein 3	185	122	105	0.0005	1.8	0.0048	1.5	0.0022	1.2	0.1915
216236_s_at	*SLC2A3*	Solute carrier family 2, member 3	792	594	430	0.0008	1.8	0.0018	1.3	0.0540	1.4	0.0897
200073_s_at	*HNRPD*	Heterogeneous nuclear ribonucleoprotein D	1482	1109	807	0.0010	1.8	0.0086	1.3	0.0284	1.4	0.0935
203476_at	*TPBG*	Trophoblast glycoprotein	496	426	280	0.0017	1.8	0.0046	1.2	0.2093	1.5	0.0232
210513_s_at	*VEGF*	Vascular endothelial growth factor	574	486	327	0.0022	1.8	0.0092	1.2	0.1743	1.5	0.0337
201431_s_at	*DPYSL3*	Dihydro-pyrimidinase-like 3	473	384	261	0.0023	1.8	0.0193	1.2	0.1108	1.5	0.0318
200610_s_at	*NCL*	Nucleolin	2848	2209	1692	0.0003	1.7	0.0007	1.3	0.0371	1.3	0.0708
218543_s_at	*ZC3HDC1*	Zinc finger CCCH type domain containing 1	242	179	141	0.0005	1.7	0.0094	1.4	0.0362	1.3	0.0039
221481_x_at	*HNRPD*	Heterogeneous nuclear ribonucleoprotein D	2920	2159	1732	0.0006	1.7	0.0079	1.4	0.0278	1.2	0.0484
200020_at	*TARDBP*	TAR DNA binding protein	1164	880	702	0.0008	1.7	0.0091	1.3	0.0241	1.3	0.0875
211737_x_at	*PTN*	Pleiotrophin	242	189	143	0.0009	1.7	0.0055	1.3	0.0601	1.3	0.0605
204729_s_at	*STX1A*	Syntaxin 1A (brain)	425	268	248	0.0013	1.7	0.0012	1.6	0.0016	1.1	0.5564
201585_s_at	*SFPQ*	Splicing factor proline/glutamine rich	553	387	326	0.0020	1.7	0.0061	1.4	0.0388	1.2	0.1855

(*Continued*)

TABLE II. (*Continued*)

Probe ID[a]	Gene	Gene description	Median value			Trend-line	Fetal vs. adult		Fetal vs. infant		Infant vs. adult	
			Fetal	Infant	Adult	*P*-value[b]	FC[c]	*P*-value[d]	FC[c]	*P*-value[d]	FC[c]	*P*-value[d]
202814_s_at	*HIS1*	HMBA-inducible	457	389	263	0.0021	1.7	0.0078	1.2	0.1049	1.5	0.0525
205534_at	*PCDH7*	BH-protocadherin (brain-heart)	482	429	276	0.0022	1.7	0.0034	1.1	0.3814	1.6	0.0087
203441_s_at	*CDH2*	Cadherin 2, type 1, N-cadherin (neuronal)	513	418	307	0.0024	1.7	0.0193	1.2	0.1578	1.4	0.0117
217983_s_at	*RNASET2*	Ribonuclease T2	263	215	159	0.0029	1.7	0.0219	1.2	0.1100	1.4	0.0487
211375_s_at	*ILF3*	Interleukin enhancer binding factor 3	579	363	346	0.0034	1.7	0.0101	1.6	0.0025	1.0	0.6932
200916_at	*TAGLN2*	Transgelin 2	3234	2404	1941	0.0035	1.7	0.0126	1.3	0.0801	1.2	0.1501
210002_at	*GATA6*	GATA binding protein 6	225	130	133	0.0050	1.7	0.0027	1.7	0.0026	−1.0	0.8559
208649_s_at	*VCP*	Valosin-containing protein	1566	1270	1008	0.0005	1.6	0.0050	1.2	0.0481	1.3	0.0318
212414_s_at	*SEPT6*	Septin 6	250	196	159	0.0007	1.6	0.0015	1.3	0.0336	1.2	0.1289
218401_s_at	*ZNF281*	Zinc finger protein 281	338	261	218	0.0009	1.6	0.0004	1.3	0.0434	1.2	0.1703
219025_at	*CD248*	CD248 antigen, endosialin	614	511	386	0.0014	1.6	0.0119	1.2	0.1092	1.3	0.0251
220607_x_at	*TH1L*	TH1-like (drosophila)	453	386	291	0.0018	1.6	0.0095	1.2	0.1991	1.3	0.0117
208815_x_at	*HSPA4*	Heat shock 70 kDa protein 4	410	276	263	0.0019	1.6	0.0070	1.5	0.0042	1.0	0.3175
202189_x_at	*PTBP1*	Polypyrimidine tract binding protein 1	2277	1587	1461	0.0020	1.6	0.0020	1.4	0.0101	1.1	0.4663
206385_s_at	*ANK3*	Ankyrin 3, node of Ranvier (ankyrin G)	228	165	143	0.0028	1.6	0.0113	1.4	0.0210	1.2	0.3000
214728_x_at	*SMARCA4*	SWI/SNF related	471	319	302	0.0028	1.6	0.0103	1.5	0.0092	1.1	0.3881
202933_s_at	*YES1*	Sarcoma viral oncogene homolog	403	312	258	0.0031	1.6	0.0254	1.3	0.0709	1.2	0.0524
200896_x_at	*HDGF*	Hepatoma-derived growth factor	1810	1180	1127	0.0045	1.6	0.0029	1.5	0.0063	1.0	0.7466
208433_s_at	*LRP8*	Apolipoprotein e receptor	405	306	271	0.0008	1.5	0.0063	1.3	0.0117	1.1	0.1324
211730_s_at	*POLR2L*	Polymerase (RNA) II polypeptide L	1048	910	713	0.0010	1.5	0.0134	1.2	0.0526	1.3	0.0252
209835_x_at	*CD44*	CD44 antigen	827	717	566	0.0019	1.5	0.0166	1.2	0.1606	1.3	0.0088
201243_s_at	*ATP1B1*	ATPase, Na+/K+ transporting	799	727	529	0.0020	1.5	0.0019	1.1	0.3250	1.4	0.0188
207157_s_at	*GNG5*	G protein, gamma 5	1884	1469	1246	0.0024	1.5	0.0107	1.3	0.0150	1.2	0.2606
215438_x_at	*GSPT1*	G1 to S phase transition 1	438	359	289	0.0029	1.5	0.0229	1.2	0.0462	1.2	0.1120
209025_s_at	*SYNCRIP*	Cytoplasmic RNA interacting protein	450	359	297	0.0031	1.5	0.0238	1.3	0.0290	1.2	0.1627
222047_s_at	*ARS2*	Arsenate resistance protein ARS2	550	374	356	0.0032	1.5	0.0132	1.5	0.0011	1.0	0.6565
200872_at	*S100A10*	S100 calcium binding protein A10	1221	962	823	0.0034	1.5	0.0019	1.3	0.0647	1.2	0.2749
202856_s_at	*SLC16A3*	Solute carrier family 16, member 3	257	207	167	0.0035	1.5	0.0072	1.2	0.1278	1.2	0.1217
201242_s_at	*ATP1B1*	ATPase, Na+/K+ transporting, beta 1	335	280	229	0.0037	1.5	0.0111	1.2	0.1023	1.2	0.1388
210794_s_at	*MEG3*	Maternally expressed 3	310	208	203	0.0038	1.5	0.0130	1.5	0.0010	1.0	0.8082
217025_s_at	*DBN1*	Drebrin 1	816	564	539	0.0041	1.5	0.0122	1.4	0.0047	1.0	0.6513
209022_at	*STAG2*	Stromal antigen 2	374	352	258	0.0047	1.5	0.0068	1.1	0.3691	1.4	0.0351
213470_s_at	*HNRPH1*	Heterogeneous nuclear ribonucleoprotein H1	374	290	249	0.0048	1.5	0.0230	1.3	0.0847	1.2	0.1032
202484_s_at	*MBD2*	Methyl-CpG binding domain protein 2	695	640	449	0.0049	1.5	0.0018	1.1	0.4574	1.4	0.0378

[a]Affymetrix U133A probe designation.
[b]Based on trend *t*-test.
[c]Fold change.
[d]Based on two-tailed Student's *t*-test.

TABLE III. Transcripts Showing Increasing Expression With Advancing Age in Cultured rhMSC

Probe ID[a]	Gene	Gene description	Median expression value			Trend-line, *P*-value[b]	Fetal vs. adult		Fetal vs. infant		Infant vs. adult	
			Fetal	Infant	Adult		FC[c]	*P*-value[d]	FC[c]	*P*-value[d]	FC[c]	*P*-value[d]
209395_at	*CHI3L1*	Chitinase 3-like 1	259	885	3000	1.45×10^{-5}	−11.6	0.0009	−3.4	0.0025	−3.4	0.0122
209396_s_at	*CHI3L1*	Chitinase 3-like 1	828	2087	6175	3.15×10^{-7}	−7.5	0.0001	−2.5	0.0011	−3.0	0.0004
211663_x_at	*PTGDS*	Prostaglandin D2 synthase	668	2630	3829	0.0014	−5.7	0.0026	−3.9	0.0092	−1.5	0.3691
218638_s_at	*SPON2*	Spondin 2, extracellular matrix protein	457	666	1795	0.0007	−3.9	0.0020	−1.5	0.1430	−2.7	0.0140
212354_at	*SULF1*	Sulfatase 1	229	452	785	0.0001	−3.4	0.0014	−2.0	0.0094	−1.7	0.0343
204298_s_at	*LOX*	Lysyl oxidase	667	969	2184	0.0030	−3.3	0.0021	−1.5	0.2296	−2.3	0.0695
211748_x_at	*PTGDS*	Prostaglandin D2 synthase	494	1039	1517	0.0023	−3.1	0.0083	−2.1	0.0296	−1.5	0.2421
204457_s_at	*GAS1*	Growth arrest-specific 1	291	641	891	0.0040	−3.1	0.0274	−2.2	0.0042	−1.4	0.3373
209758_s_at	*MFAP5*	Microfibrillar associated protein 5	144	159	382	0.0011	−2.7	0.0002	−1.1	0.4071	−2.4	0.0029
209291_at	*ID4*	Inhibitor of DNA binding 4	141	222	347	0.0029	−2.5	0.0214	−1.6	0.0544	−1.6	0.1148
216526_x_at	*HLA-C*	Major histocompatibility complex	1901	2520	4801	0.0032	−2.5	0.0067	−1.3	0.2854	−1.9	0.0294
212187_x_at	*PTGDS*	Prostaglandin D2 synthase	447	836	1139	0.0048	−2.5	0.0098	−1.9	0.0570	−1.4	0.2948
211385_x_at	*SULT1A2*	Sulfotransferase family, cytosolic	509	1036	1143	0.0030	−2.2	0.0107	−2.0	0.0030	−1.1	0.5989
204480_s_at	*C9orf16*	Chromosome 9 open reading frame 16	392	557	826	0.0036	−2.1	0.0278	−1.4	0.1104	−1.5	0.0464
212509_s_at	*TMAP1*	Transmembrane anchor protein 1	1214	1796	2464	0.0004	−2.0	0.0013	−1.5	0.0368	−1.4	0.0707
203881_s_at	*DMD*	Dystrophin (muscular dystrophy)	128	149	260	0.0013	−2.0	0.0077	−1.2	0.2269	−1.7	0.0043
209147_s_at	*PPAP2A*	Phosphatidic acid phosphatase type 2A	272	308	534	0.0020	−2.0	0.0010	−1.1	0.3517	−1.7	0.0186
213519_s_at	*LAMA2*	Laminin, alpha 2	262	398	515	0.0026	−2.0	0.0128	−1.5	0.0311	−1.3	0.2156
203951_at	*CNN1*	Calponin 1, basic, smooth muscle	269	317	482	0.0001	−1.8	0.0003	−1.2	0.0250	−1.5	0.0028
215299_x_at	*SULT1A1*	Sulfotransferase family, cytosolic	301	391	536	0.0048	−1.8	0.0381	−1.3	0.1078	−1.4	0.0575
221796_at	*NTRK2*	Neurotrophic tyrosine kinase, receptor	99	117	172	0.0001	−1.7	0.0010	−1.2	0.0003	−1.5	−0.0042
201058_s_at	*MYL9*	Myosin, light polypeptide 9, regulatory	390	438	657	0.0034	−1.7	0.0182	−1.1	0.4009	−1.5	0.0001
205547_s_at	*TAGLN*	Transgelin	11810	16073	19507	0.0044	−1.7	0.0362	−1.4	0.0860	−1.2	0.0012
219525_at	*FLJ10847*	Hypothetical protein FLJ10847	143	182	225	3.28×10^{-5}	−1.6	0.0004	−1.3	0.0053	−1.2	0.0355
41047_at	*C9orf16*	Chromosome 9 open reading frame 16	675	818	1069	0.0001	−1.6	0.0023	−1.2	0.0340	−1.3	0.0013
213640_s_at	*LOX*	Lysyl oxidase	233	289	383	0.0041	−1.6	0.0016	−1.2	0.1855	−1.3	0.1311
215849_x_at	*TTC18*	Tetratricopeptide repeat domain 18	188	228	283	0.0003	−1.5	0.0011	−1.2	0.0512	−1.2	0.0295
215230_x_at	*EIF3S8*	Eukaryotic translation initiation factor 3	655	735	1001	0.0006	−1.5	0.0039	−1.1	0.1983	−1.4	0.0014
210106_at	*RDH5*	Retinol dehydrogenase 5	239	272	365	0.0025	−1.5	0.0134	−1.1	0.0620	−1.3	0.0621
212503_s_at	*KIAA0934*	KIAA0934	195	223	298	0.0031	−1.5	0.0211	−1.1	0.1354	−1.3	0.0376
218656_s_at	*LHFP*	Lipoma HMGIC fusion partner	287	311	418	0.0033	−1.5	0.0057	−1.1	0.3591	−1.3	0.2130
217977_at	*SEPX1*	Selenoprotein X, 1	464	520	700	0.0033	−1.5	0.0197	−1.1	0.0614	−1.3	0.0553

[a]Affymetrix U133A probe designation.
[b]Based on trend *t*-test.
[c]Fold change.
[d]Based on two-tailed Student's *t*-test.

TABLE IV. Primer Sequences and Lengths of Expected PCR Products

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Amplicon length
CD47	TGGTAGCGGCGCTGTTG	TTGTGCCTCCATATTAGTAACAAAG	136
S100A10	GTTTCCTGGATTTTTGGAAAATCAA	AGCTCTGGAAGCCCACTTTG	101
S100A4	TCAAGCTCAACAAATCAGAGCTAAA	TCATCTGTCCTTTTCCCCAAGA	76
HexiM1	GAGATTATTCCCTCCTGTCACTTTG	AGTGATTTGAGCAACGCAGTTG	92
CHI3	GCTCCAGTGCTGCTCTGCATA	AAGCGGTCAATGGCATCTG	102
HPNRD	GGGTTTTGGCTTTGTGCTATTT	CACCTTCCCATTCAATTTATGTTCT	85
EGR1	CCGCAGGTCTTTTCCTGACA	GGGCTCGGGCCATAAGG	157
STXA1	GCAGGACCACGACCAGTGA	AGATGCTGGAGTCCATGATGATC	93
FOS	GAATCCGAAGGGAAAGGAATAAGA	GTCTGTCTCGCTTGGAGTGTATCA	88
RPL13	GAAGCCTACAAGAAAGTTTGCCTATC	CTTGGCTTTCTCTTTCCTCTTCTC	108
HPRT1	TTTTATCAGACTGAAGAGCTATTGTAATGA	CATCGTTTTGCCAGTGTCAATTAT	135
TBP	TGCACAGGAGCCAAGAGTGAA	CACATCACAGCTCCCCACCA	132
SDHA	TGGGAACAAGAGGGCATCTG	AGCTTTGTAACACATGCTGTATGAAA	88

mix and 500 nM of forward and reverse primers in a 25 μl reaction volume. The PCR protocol consisted of one cycle of 2 min at 50°C, 15 min at 94°C, followed by 40 cycles at 15 s at 95°C, 30 s at 56°C, and 30 s at 72°C. All PCR reactions were performed in duplicate.

RESULTS

Gene expression analyses were performed on rhMSC obtained from three age groups (Table I) with fetal, infant, and adult bone marrow donors included. Unsupervised hierarchical clustering analyses of gene expression data clearly separated the adult #1 and adult #2 rhMSC groups from the adult #3, fetal, and infant rhMSC groups (Fig. 1). The fetal and infant groups were separated, except for fetal #4 which clustered with several of the infant donors. Overall there was a limited tendency for the three age groups to separate based on gene expression profiles.

Next, we conducted linear trend analyses to identify candidate transcripts showing age-related gene expression patterns. These included 71 transcripts with significant trends towards decreasing expression with advancing donor age (Table II). Conversely, we identified 32 transcripts with significant trends towards increasing expression with advancing donor age. In addition, pair-wise comparisons of the fetal, infant, and adult rhMSC gene expression profiles are provided in order to highlight the relative significance of differential gene expression between groups (Table III).

In light of the cross-species analyses of rhesus transcripts on oligonucleotide microarrays designed to interrogate human sequences, we sought to verify a group of transcripts where differential expression, even if only by paired *t*-tests, was observed (Supplemental Table 1). Quantitative RT-PCR was utilized to validate the expression levels of *S100A4* and *S100A10* identified in the fetal versus adult comparison, and *EGR1*, *FOS*, and *STX1A* identified in the fetal versus infant comparison. For normalization, three genes were used (*SDHA*, *HPRT1*, and *RPL13a*) that were predicted not to show any changes in the gene expression analyses. Overall, the qRT-PCR and oligonucleotide microarray-based gene expression data for all these genes were in excellent agreement (Table V).

Transcripts Showing Decreasing Expression With Age

Based on GO analyses of the 71 transcripts that showed decreasing rhMSC expression with increasing donor age, there was enrichment for transcripts associated with the general themes of RNA biology and gene silencing (Fig. 2). The former RNA-related grouping included the following categories: RNA metabolism (10 probe tilings $P = 5 \times 10^{-8}$), mRNA metabolism (7 probe tilings, $P = 1.1 \times 10^{-4}$), RNA processing, (8 probe tilings, $P = 5.1 \times 10^{-4}$), and RNA binding genes (12 probe tilings, $P = 7.1 \times 10^{-6}$). The latter gene silencing grouping included the following categories: negative regulation of cellular metabolism (6 probe tilings, $P = 6.8 \times 10^{-4}$), negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism (6 probe tilings, $P = 3.4 \times 10^{-4}$), and negative regulation of transcription (6 probe tilings, $P = 2.4 \times 10^{-4}$). The identity of the transcripts associated with the above-mentioned probe tilings are provided in Supplemental Table 2.

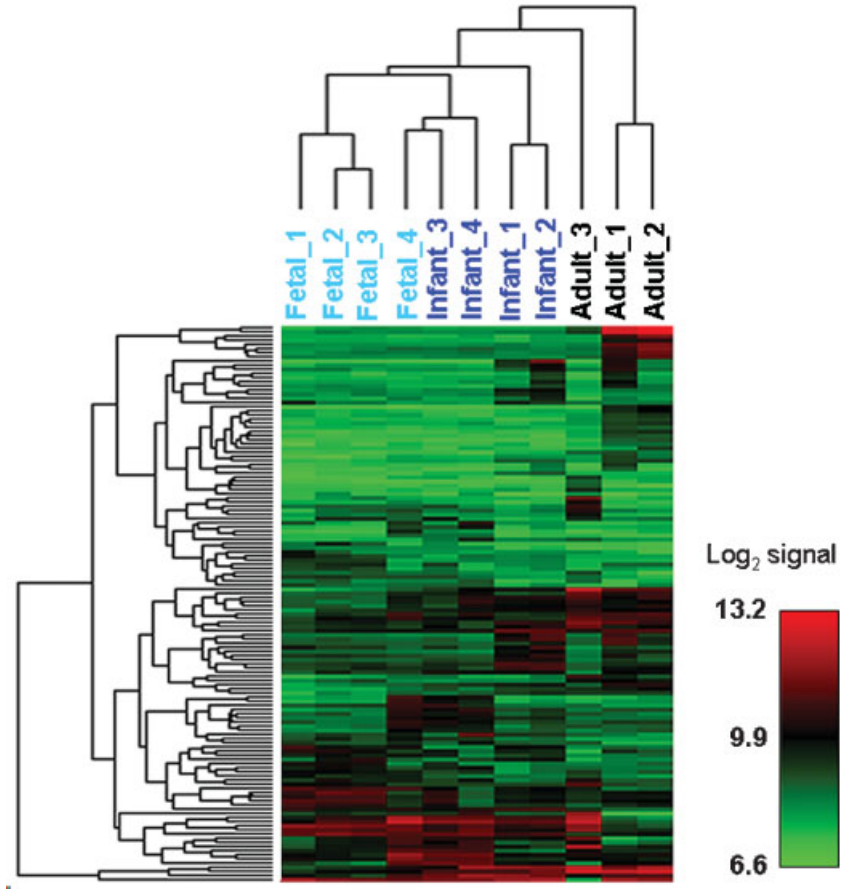

Fig. 1. Hierarchical clustering analysis of gene expression data from rhMSC cultures. The dendrograms were generated based on average linkage hierarchical clustering of expression data from 134 transcripts whose coefficient of variation was greater than 0.07 across all groups. Sample names are color-coded to indicate if they are derived from fetal (light blue), infant (blue), or adult (black) donors.

In the general area of ribonucleic acid metabolism, there were eight (*HNRPH1*, *PABPN1*, *IGF2BP3*, *SNRPA*, *SFPQ* [aka *PSF*], *PTBP1*, *SYNCRIP*, *HNRPD*) key genes involved in global RNA processing and/or trafficking. Interestingly, at least three of these proteins (SFPQ, PTPB1, and SNRPA) are known to encode factors that interact with one another. Both PSF and PTB can bind the polypyrimidine tract of mammalian introns either separately or as part of a complex [Patton et al., 2002]. In addition, SNRPA was reported to bind PTBP1 in yeast two-hybrid analyses [Rual et al., 2005].

In the realm of gene silencing, there were six (*ILF3*, *TH1L*, *HEXIM1* [aka *HIS1*], *ZNF281*, *ID3*, and *MBD2*) key genes identified as showing higher expression in rhMSC from younger relative to older donors. *MBD2* is an interesting

TABLE V. Comparison of the Fold Changes in Transcript Levels in Individual Samples Assessed by Oligonucleotide Microarray and Real-Time PCR Analyses

Comparison	Gene	Probe tiling	Microarray	Real-time PCR
Fetal vs. adult	*CD47*	211075_s_at	−1.63	−3.05
	S100A4	203186_s_at	3.65	8.18
	S100A10	200872_at	1.48	2.50
Fetal vs. infant	*FOS*	209189_at	−3.26	−5.63
	EGR1	201694_s_at	−2.21	−2.20
	STX1A	204729_s_at	1.57	1.17

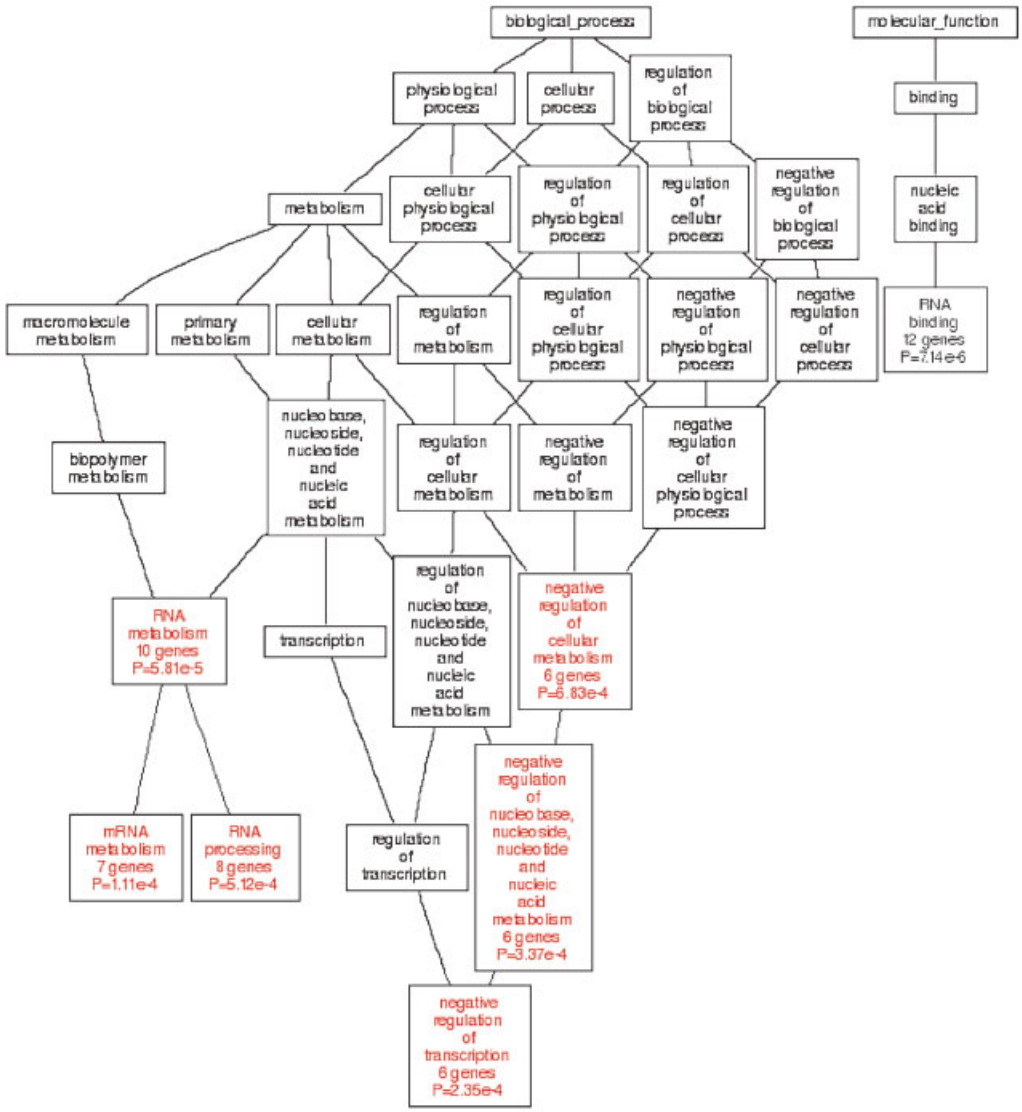

Fig. 2. Gene Ontology (GO) analysis of transcripts showing down-regulation with increasing age. The 71 transcripts with significant trends towards decreasing expression with advancing donor age were subjected to GO analysis using WebGestalt software, as described in the Methods. On the provided Directed Acyclic Graph (DAG), the functional categories showing significant enrichment ($P < 0.001$ based on a hypergeometric test) in this dataset are colored in red. The identities of these probe tilings are provided in Supplemental Table 2. GO analyses performed on the 32 transcripts with significant trends towards decreasing expression with advancing donor age yielded only four highly related enriched functional categories that are provided in Supplemental Table 2.

candidate given its role in epigenetic regulation of gene expression via its interactions in the methyl cytosine-binding complex MeCP1 [Kransdorf et al., 2006] (Table III). On a similar note, *ID3* is a transcriptional repressor that is a down-stream target of MeCP2, methyl-CpG-binding protein 2 [Peddada et al., 2006]. The fact that *ID3* is an inhibitor of differentiation [Kowanetz et al., 2004] could reflect the increased pluripotency of rhMSC in younger relative to older donors. Lastly, HEX1M1, a growth inhibitor and promoter of neuronal differentiation, acts in concert with 7 SK snRNA to inhibit the activity of the positive transcriptional elongation factor [Turano et al., 2006]. This could serve as a link between gene silencing and the ribonucleic acid metabolism grouping discussed above.

In addition, there were also multiple differentially expressed transcripts relevant to MSC biology not directly involved in nucleic acid metabolism or gene silencing. For example,

the decreased expression of CD44 with age, a known MSC surface marker [Kulterer et al. 2007], could reflect loss of pluripotency. The fact that *S100A4* and *S100A10* genes were more highly expressed with advancing age is similar to a human study that showed dramatic changes in gene expression of two other family members (*S100A8* and *S100A9*) in bone marrow relative to umbilical cord-derived human MSC [Panepucci et al., 2004]. Interestingly, *SEPT6*, whose gene product is reported to bind *S100A4* [Koshelev et al., 2003], also is more abundantly expressed in fetal relative to adult rhMSC. The differential expression of collagen family members *COL6A1* and *COL6A2* were also observed in fetal versus adult rhMSC comparisons. The higher expression of *COL6A1* in fetal relative to adult rhMSC could reflect higher differentiation potential in the former group.

Transcripts Showing Decreasing Expression With Age

GO analyses of the 32 transcripts showing increasing rhMSC expression with increasing age of the donor showed enrichment for transcripts associated with extracellular matrix biology (Supplemental Table 2). This was subdivided in the following categories: extracellular region (7 probe tilings $P = 5.2 \times 10^{-4}$), extracellular matrix (5 probe tilings, $P = 4.9 \times 10^{-5}$), extracellular region part (6 probe tilings, $P = 3.6 \times 10^{-4}$), and extracellular matrix (sensu Metazoa) (5 probe tilings, $P = 4.6 \times 10^{-5}$). All the above categories included a subset of the following transcripts: *CHI3L1*, *SPON2*, *PTGDS*, *DMD*, *LOX*, *PTGDS*, *SULF1*, and *MFAP5*. The increased expression of the lysyl oxidase (*LOX*) gene with age, required for cross-linking extracellular collagen, may be related to higher *LOX* expression in differentiated relative to uncommitted human MSC [Pochampally et al., 2004]. Similarly, the increased expression of *CH13L1* with advancing age may relate to its higher expression in cultured chondrocytes during differentiation [Imabayashi et al., 2003].

Further, there were also several differentially expressed transcripts relevant to MSC biology that showed increasing expression with rhMSC donor age. The increased levels of HLA-G transcript could relate to a previous report that undifferentiated human MSC express HLA class I but not class II genes [Le Blanc et al., 2003]. On a related note, *CDH2* is known to be up-regulated during the differentiation of mouse embryonic stem cells [Bouhon et al., 2005]. *CNN1* and *MYL9* are both associated with smooth muscle cell gene expression and thus may indicate lowered pluripotency of adult cells. In keeping with their properties in cell culture, the decreased expression of the growth arrest-specific 1 (*GAS1*) gene in fetal rhMSC is consistent with their higher growth potential relative to infant rhMSC.

DISCUSSION

The promise of MSC resides in their capability for self-renewal and to differentiate into multiple mesenchymal lineages. Encouraging results in the experimental and clinical setting include the use of MSC for regeneration of bone and cartilage [Noel et al., 2002] including the partial reversion of osteogenesis imperfecta, the amelioration of experimental autoimmune encephalitis, and the co-transplantation with human hematopoietic stem cells to enhance engraftment and provide a lower incidence of graft-versus-host-disease [Nilsson et al., 1999; Koc et al., 2000; Horwitz et al., 2001; Lazarus et al., 2005]. However, recent studies have shown that the biology of these cells may be influenced by factors such as the anatomical site of origin and the age of the donor [Campagnoli et al., 2001; Lee and Tarantal, 2006]. It has been suggested that human and non-human primate MSC derived from an older donor cohort group have a shorter life-span, diminished proliferative and differentiation potential, and more frequent expression of age-related markers [Lee and Tarantal, 2006; Sethe et al., 2006]. The potential greater self-renewal capabilities of stem cells obtained from younger sources is especially important for biomedical applications.

Here, we found that gene profiles of bone marrow-derived rhMSC cultures obtained from fetal, newborn, and adult donors reflect age-related differences in pluripotency and proliferative capacity. Furthermore, our observations of differential expression of genes involved in gene silencing and epigenetic phenomena is in keeping with their pivotal role in stem cell biology [see review, Zhang et al., 2006]. However, we emphasize our results are only suggestive in nature. The current study also demonstrates increased fetal rhMSC expression of genes directly involved in stem

cell function and immunogenicity, including *S100A8, S100A4, COL6A1,* and *COL6A2* (Supplemental Table 2) relative to adult rhMSC. SAGE-based analyses demonstrated that *S100A8* is among the highest expressed genes in bone marrow-derived human MSC and not expressed in CD34+ hematopoietic progenitors [Silva et al., 2003]. Importantly, *S100A4* is involved in mesenchymal cell shape and enabling cellular motility [Xue et al., 2003]. In addition to *S100A8* and *S100A4*, fetal rhMSC showed significantly higher levels of *COL6A1* and *COL6A2* transcripts compared to adult cells. Interestingly, *COL6A1* is down-regulated in human bone marrow-derived MSC after chondrogenic induction [Winter et al., 2003]. Thus, the higher expression of *COL6A1* in fetal relative to adult rhMSC could reflect higher differentiation potential in the former group. Furthermore, *COL6A2* is more highly expressed in pre-adipocytes cells relative to mature adipocytes [Ibrahimi et al., 1992]. Likewise, the lower-expression of HLA-G in rhMSC from younger donors may be related to the fact that HLA class I expression appears to be inversely related to the state of differentiation [Le Blanc et al., 2003]. Taken together, our results suggest that fetal rhMSC possess greater self-renewal, differentiation, and possibly engraftment potential than their older counterparts.

Our data provide evidence to suggest that fetal cells express a significantly higher level of genes involved in global RNA metabolism and gene regulator pathways compared to cells obtained from older animals. The global RNA metabolism post-transcription includes splicing, mRNA export, and mRNA stability, and has been suggested to be a key post-transcriptional mechanism in controlling mature transcript levels [Lundgren et al., 1996; Ben-Yehuda et al., 2000]. These findings may also be important for transcriptome homeostasis and hematopoietic stem cell activation in vivo [Bowman et al., 2006]. Several studies have also suggested a correlation between transcript stability, processing, and the proliferative state of cells [Ash et al., 1993; Darville and Rousseau, 1997; Pryor et al., 2004; Bowman et al., 2006]. Although genes involved in global RNA metabolism and processing are not well studied in stem cells, these data suggest that they may play an important role in proliferation and regulation of stem cells in their microenvironment, where these cells are required to maintain homeostasis of their respective organ systems.

In summary, this study corroborates global gene expression of rhMSC markers in accordance with the human MSC literature [Silva et al., 2003]. These differences, however, should be viewed with caution because the expression analysis was based on cultured cells, although all bone marrow-derived rhMSC were grown to the second passage under identical conditions. Results must also be viewed from the perspective that large-scale gene expression profiles aid in developing hypothesis-driven studies rather than to provide a direct explanation for cell function and behavior [Peng et al., 2002]. Future gene expression profiling experiments involving microarrays specific for rhesus macaque transcript analyses [Magness et al., 2005] and using cells grown in single cell (clonal) assays will provide a means to expand upon the results obtained in these studies.

ACKNOWLEDGMENTS

The authors thank Samantha Yeligar at the University of Southern California and the animal care staff at the Primate Center. This study was supported by NIH Grants #HL73220 and the Primate Center base operating grant #RR00169.

REFERENCES

Ash J, Ke Y, Korb M, Johnson LF. 1993. Introns are essential for growth-regulated expression of the mouse thymidylate synthase gene. Mol Cell Biol 13:1565–1571.

Ben-Yehuda S, Dix I, Russell CS, McGarvey M, Beggs JD, Kupiec M. 2000. Genetic and physical interactions between factors involved in both cell cycle progression and pre-mRNA splicing in *Saccharomyces cerevisiae*. Genetics 156:1503–1517.

Bouhon IA, Kato H, Chandran S, Allen ND. 2005. Neural differentiation of mouse embryonic stem cells in chemically defined medium. Brain Res Bull 68: 62–75.

Bowman TV, McCooey AJ, Merchant AA, Ramos CA, Fonseca P, Poindexter A, Bradfute SB, Oliveira DM, Green R, Zheng Y, Jackson KA, Chambers SM, McKinney-Freeman SL, Norwood KG, Darlington G, Gunaratne PH, Steffen D, Goodell MA. 2006. Differential mRNA processing in hematopoietic stem cells. Stem Cells 24:662–670.

Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. 2001. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 98:2396–2402.

Darville MI, Rousseau GG. 1997. E2F-dependent mitogenic stimulation of the splicing of transcripts from an S phase-regulated gene. Nucleic Acids Res 25:2759–2765.

English D. 2007. Preserve mesenchymal stem cell research. Stem Cells Dev 16:1–7.

Giordano A, Galderisi U, Marino IR. 2007. From the laboratory bench to the patient's bedside: An update on clinical trials with mesenchymal stem cells. J Cell Physiol 211:27–35.

Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, McCarville ME, Orchard PJ, Pyeritz RE, Brenner MK. 2001. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. Blood 97:1227–1231.

Ibrahimi A, Bonino F, Bardon S, Ailhaud G, Dani C. 1992. Essential role of collagens for terminal differentiation of preadipocytes. Biochem Biophys Res Commun 187: 1314–1322.

Imabayashi H, Mori T, Gojo S, Kiyono T, Sugiyama T, Irie R, Isogai T, Hata J, Toyama Y, Umezawa A. 2003. Redifferentiation of dedifferentiated chondrocytes and chondrogenesis of human bone marrow stromal cells via chondrosphere formation with expression profiling by large-scale cDNA analysis. Exp Cell Res 288:35–50.

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4:249–264.

Karaman MW, Houck ML, Chemnick LG, Nagpal S, Chawannakul D, Sudano D, Pike BL, Ho VV, Ryder OA, Hacia JG. 2003. Comparative analysis of gene-expression patterns in human and African great ape cultured fibroblasts. Genome Res 13:1619–1630.

Keating A. 2006. Mesenchymal stromal cells. Curr Opin Hematol 13:419–425.

Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, Lazarus HM. 2000. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol 18:307–316.

Koshelev YA, Kiselev SL, Georgiev GP. 2003. Interaction of the S100A4 (Mts1) protein with septins Sept2, Sept6, and Sept7 *in vitro*. Dokl Biochem Biophys 391:195–197.

Kowanetz M, Valcourt U, Bergstrom R, Heldin CH, Moustakas A. 2004. Id2 and Id3 define the potency of cell proliferation and differentiation responses to transforming growth factor ß and bone morphogenetic protein. Mol Cell Biol 24:4241–4254.

Kransdorf EP, Wang SZ, Zhu SZ, Langston TB, Rupon JW, Ginder GD. 2006. MBD2 is a critical component of a methyl cytosine-binding protein complex isolated from primary erythroid cells. Blood 108:2836–2845.

Kulterer B, Friedl G, Jandrositz A, Sanchez-Cabo F, Prokesch A, Paar C, Scheideler M, Windhager R, Preisegger KH, Trajanoski Z. 2007. Gene expression profiling of human mesenchymal stem cells derived from bone marrow during expansion and osteoblast differentiation. BMC Genomics 8:70.

Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, Shpall EJ, McCarthy P, Atkinson K, Cooper BW, Gerson SL, Laughlin MJ, Loberiza FR, Jr., Moseley AB, Bacigalupo A. 2005. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant 11: 389–398.

Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O. 2003. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 31:890–896.

Lee CC, Ye F, Tarantal AF. 2006. Comparison of growth and differentiation of fetal and adult rhesus monkey mesenchymal stem cells. Stem Cells Dev 15:209–220.

Lee CI, Kohn DB, Ekert JE, Tarantal AF. 2004. Morphological analysis and lentiviral transduction of fetal monkey bone marrow-derived mesenchymal stem cells. Mol Ther 9:112–123.

Lundgren K, Allan S, Urushiyama S, Tani T, Ohshima Y, Frendewey D, Beach D. 1996. A connection between pre-mRNA splicing and the cell cycle in fission yeast: cdc28+ is allelic with prp8+ and encodes an RNA-dependent ATPase/helicase. Mol Biol Cell 7:1083–1094.

Magness CL, Fellin PC, Thomas MJ, Korth MJ, Agy MB, Proll SC, Fitzgibbon M, Scherer C, Miner DG, Katze MG, Iadonato SP. 2005. Analysis of the *Macaca mulatta* transcriptome and the sequence divergence between *Macaca* and human. Genome Biol 6:R60. Epub June 30.

Nagpal S, Karaman MW, Timmerman MM, Ho VV, Pike BL, Hacia JG. 2004. Improving the sensitivity and specificity of gene expression analysis in highly related organisms through the use of electronic masks. Nucleic Acids Res 32:e51.

Nilsson SK, Dooner MS, Weier HU, Frenkel B, Lian JB, Stein GS, Quesenberry PJ. 1999. Cells capable of bone production engraft from whole bone marrow transplants in nonablated mice. J Exp Med 189:729–734.

Noel D, Djouad F, Jorgense C. 2002. Regenerative medicine through mesenchymal stem cells for bone and cartilage repair. Curr Opin Investig Drugs 3:1000–1004.

Panepucci RA, Siufi JL, Silva WA, Jr., Proto-Siquiera R, Neder L, Orellana M, Rocha V, Covas DT, Zago MA. 2004. Comparison of gene expression of umbilical cord vein and bone marrow-derived mesenchymal stem cells. Stem Cells 22:1263–1278.

Patton JG, Porro EB, Galceran J, Tempst P, Nadal-Ginard B. 2002. PSF and p54(nrb)/NonO–multi-functional nuclear proteins. FEBS Lett 531:109–114.

Peddada S, Yasui DH, LaSalle JM. 2006. Inhibitors of differentiation (ID1, ID2, ID3 and ID4) genes are neuronal targets of MeCP2 that are elevated in Rett syndrome. Hum Mol Gen 15:2003–2014.

Peng CF, Wei Y, Levsky JM, McDonald TV, Childs G, Kitsis RN. 2002. Microarray analysis of global changes in gene expression during cardiac myocyte differentiation. Physiol Genomics 9:145–155.

Pochampally RR, Smith JR, Tlostalo J, Prockop DJ. 2004. Serum deprivation of human marrow stromal cells (hMSCs) selects for a subpopulation of early progenitor cells with enhanced expression of *OCT-4* and other embryonic genes. Blood 103:1647–1652.

Pryor A, Tung L, Yang Z, Kapadia F, Chang TH, Johnson LF. 2004. Growth-regulated expression and G0-specific turnover of the mRNA that encodes URH49, a mammalian DExH/D box protein that is highly related to the mRNA export protein UAP56. Nucleic Acids Res 32: 1857–1865.

Rual JF, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, Li N, Berriz GF, Gibbons FD, Dreze M, Ayivi-Guedehoussou N, Klitgord N, Simon C, Boxem M, Milstein S, Rosenberg J, Goldberg DS, Zhang LV, Wong SL, Franklin G, Li S, Albala JS, Lim J, Fraughton C, Llamosas E, Cevik S, Bex C, Lamesch P, Sikorski RS, Vandenhaute J, Zoghbi HY, Smolyar A, Bosak S, Sequerra R, Doucette-Stamm L, Cusick ME, Hill DE, Roth FP, Vidal M. 2005. Towards a proteome-scale map of the human protein-protein interaction network. Nature 437:1173–1178.

Sethe S, Scutt A, Stolzing A. 2006. Aging of mesenchymal stem cells. Ageing Res Rev 5:91–116.

Silva WA, Jr., Covas DT, Panepucci RA, Proto-Siqueira R, Siufi JL, Zanette DL, Santos AR, Zago MA. 2003. The profile of gene expression of human marrow mesenchymal stem cells. Stem Cells 21:661–669.

Stagg J. 2006. Immune regulation by mesenchymal stem cells: Two sides of the coin. Tissue Antigens 69:1–9.

Tarantal AF. 2005. Ultrasound imaging in rhesus (*Macaca mulatta*) and long-tailed (*Macaca fascicularis*) macaques: Reproductive and research applications. In: Wolfe-Coote S, editor. The Laboratory Primate. San Diego, CA: Elsevier Academic Press. pp 317–351.

Turano M, Napolitano G, Dulac C, Majello B, Bensaude O, Lania L. 2006. Increased HEXIM1 expression during erythroleukemia and neuroblastoma cell differentiation. J Cell Physiol 206:603–610.

Winter A, Breit S, Parsch D, Benz K, Steck E, Hauner H, Weber RM, Ewerbeck V, Richter W. 2003. Cartilage-like gene expression in differentiated human stem cell spheroids: A comparison of bone marrow-derived and adipose tissue-derived stromal cells. Arthritis Rheum 48:418–429.

Xue C, Plieth D, Venkov C, Xu C, Neilson EG. 2003. The gatekeeper effect of epithelial-mesenchymal transition regulates the frequency of breast cancer metastasis. Cancer Res 63:3386–3394.

Yu XJ, Zheng HK, Wang J, Wang W, Su B. 2006. Detecting lineage-specific adaptive evolution of brain-expressed genes in human using rhesus macaque as outgroup. Genomics 88:745–751.

Zhang B, Kirov S, Snoddy J. 2005. WebGestalt: An integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res 33:W174–W178.

Zhang B, Pan X, Anderson TA. 2006. MicroRNA: A new player in stem cells. J Cell Physiol 209:266–269.