Comparative Profiling of mRNA and microRNA Expression in Human Mesenchymal Stem Cells Derived from Adult Adipose and Lipoma Tissues

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Abstract: Human mesenchymal stem cells have been isolated from adult adipose tissue and lipoma that is a benign neoplasm of normal fat cells. The lipoma-derived LM6 cells exhibited a higher cumulative population doubling levels as compared with the adipose-derived AM3 cells. The expression profiles of both mRNAs and microRNAs (miRNAs) from AM3 and LM6 cells were found to exhibit considerable similarities except that miRNAs miR-99a and miR-152 were abundantly expressed in AM3, but absent in LM6 cells. The abundantly differentially expressed genes HAS2, VNN1, SLC16A6 and COL11A1 in LM6 cells were shown to be targets of miRNAs miR-99a and/or miR-152. These highly up-regulated miRNA target genes, as well as several other abundantly differentially expressed genes such as sushi domain containing 2, keratin associated proteins and tumor necrosis factor family, may explain a higher proliferation potential in LM6 cells compared with AM3 cells.

Key Words: Human adipose, mesenchymal stem cells, profiling, mRNAs, miRNAs.

INTRODUCTION

Mesenchymal stem cells (MSCs) have been shown to have ability to differentiate into multiple mesodermal lineages such as adipocytes, osteoblasts and chondrocytes, as well as non-mesodermal lineages such as neural cells [1-3]. Thus, MSCs are potentially very useful for tissue engineering and regenerative medicine [4-6]. Human MSCs have been isolated from several tissue sources, including bone marrow, amniotic fluid, amniotic membrane, umbilical cord blood, placenta, and adipose tissues [7-15].

Human adult adipose tissues are highly abundant and relatively easy to procure with low risk. Two human adipose-derived MSC cultures AD-MSC-3 and AD-MSC-5 had been established [12]. Two additional human MSC cultures LD-MSC-3 and LD-MSC-6L had also been isolated from lipoma that is a benign neoplasm of normal fat cells [15]. The lipoma-derived LD-MSCs showed very similar stem cell characteristics to adipose-derived AD-MSCs. However, LD-MSCs exhibited higher cumulative population doubling levels as compared with AD-MSCs, suggesting that LD-MSCs possess a potent proliferation potential.

Genome-wide mRNA expression profiling has recently been used to identify the core features of several MSCs and the signature genes of each group of MSCs derived from different origins [16-18]. MicroRNAs (miRNAs) are single-stranded non-coding RNAs of approximately 22 nucleotides that have been identified in various organisms, including mammals. Mammalian genomes encode many hundreds of miRNAs, which are predicted to regulate negatively expression of as many as 30% of protein-coding genes [19-25]. The impact of miRNAs on protein output was recently shown that although some targets were repressed without detectable changes in mRNA levels, those translationally repressed by more than a third also displayed detectable mRNA destabilization, and, for the more highly repressed targets, mRNA destabilization usually comprised the major component of repression [26]. Although the biological functions of most miRNAs are not yet known, some miRNAs appear to participate in control of cell proliferation, differentiation and apoptosis in animals [27-29]. Thus, miRNAs may play a key role in self-renewal and differentiation of MSCs.

In this investigation, the expression profiles of both mRNAs and miRNAs from the same RNA samples of previously reported human AD-MSC3 (designated as AM3) and LD-MSC6L (designated as LM6) cells [12,15] were compared in order to understand the genetic bases for their similarities and differences.

MATERIALS AND METHODOLOGY

Cell Culture

Human AM3 (AD-MSC3) and LM6 (LD-MSC6L) cells established from female adult adipose and lipoma tissues, respectively [12,15], were cultured in the K-NAC medium that is a modified MCDB 153 medium (Keratinocyte-SFM,

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GIBCO-Invitrogen) supplemented with N-acetyl-L-cysteine (NAC; Sigma A8199) (2 mM) and L-ascorbic acid 2-phosphate (Asc 2P; sigma A8960) (0.2 mM).

Profiling of mRNAs

Total RNAs from AM3 and LM6 cells were extracted using TRIZOL reagent, and the same total RNAs from each sample were used for both mRNA microarray analysis and miRNA quantitation. The mRNA profiling of duplicate samples was analyzed using Affymetrix Human Genome U133 plus 2.0 GeneChip according to the Manufacturer's protocols (Santa Clara, CA, USA, http://www.affymetrix.com) by the Microarray Core Facility of National Research Program for Genomic Medicine of National Science Council in Taiwan. This Affymetrix GeneChip contains 54,675 probe sets to analyze the expression level of 47,400 transcripts and variants, including 38,500 well-characterized human genes. GeneChips from the hybridization experiments were read by the Affymetrix GeneChip scanner 3000. It may be noted that Affymetrix GeneChip expression analysis can be used as a stand-alone quantitative comparison, since the correlation between Affymetrix GeneChip results and TagMan RT-qPCR results was shown in a good linearity of $R^2 = 0.95$ by the MicroArray Quality Control Study, a collaborative effort of 137 scientists led by the US-FDA [30, 31]. The original data were processed using GC-RMA algorithm and GeneSpring GX software version 7.3.1 (Silicon Genetics, Redwood City, CA, USA, http://www.sigenetics.com). The mRNAs of AM3 and LM6 cells were also analyzed for network and signaling pathways by using MetaCore Analytical Suite (GeneGo Inc., St Joseph, MI, USA). The MetaCore includes a curated database of human protein interaction and metabolism, and thus it is useful for analyzing a cluster of genes in the context of regulatory network and signaling pathways.

Profiling of miRNAs

The expression level of 250 human miRNAs was determined using the TagMan MicroRNA Assays (Applied Biosystems, Foster City, California, USA, http://www.appliedbiosystems.com) as described previously [32, 33]. In brief, TagMan MicroRNA Assays include two steps: stem loop RT followed by real-time PCR. (90 ng/Rx, with 24-multiplex primers) Each 10 ul RT reaction that includes 90 ng total RNA, 50 nM stem-loop RT primers, 1x RT buffer, 1.25 mM each of dNTPs, 0.25 U/ul RNase inhibitor, and 10 U/ul MultiScribe Reverse Transcriptase was incubated in the PTC-225 Peltier Thermal Cycler (MJ Research, Watertown, MA, USA) for 30 min each at 16°C and at 42°C, followed by 5 min at 85°C, and then held at 4°C. RT products were diluted twenty times with H_2O prior to setting up PCR reaction. Real-time PCR for each miRNA was carried out in triplicates, and each 10 ul reaction mixture included 2 ul of diluted RT product, 5 ul of 2x TagMan Universal PCR Master Mix and 0.2 uM TagMan probe, respectively. The reaction was incubated in an Applied Biosystems 7900HT Sequence Detection System at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The threshold cycle (Ct) is defined as the fraction cycle number at which the fluorescence exceeds the fixed threshold of 0.2. Total RNA input was normalized based on the Ct values of the TagMan U6 snRNA assay as an endogenous control. The fold change was calculated as $2^{-\Delta CT} \times K$, where $-\Delta CT = -[CT_{miRNA} - CT_{U6\ snRNA}]$ and K is a constant.

Target Identification of miRNAs

The potential target genes of miRNAs were predicted using the TargetCombo open source software (http://www.diana.pcbi.upenn.edu/cgi-bin/miRGen/v3/Targets.cgi) which predicts targets by the union of miRanda (http://microrna.org), PicTar (4-way, http://pictar.bio.nyu.edu/) and TargetScanS (http://www.targetscan.org/) with a cutoff p-value less than 0.05 [34]. The expression levels of the predicted target mRNAs were then analyzed by the Volcano plot using parametric test and Benjamini-Hochberg false discovery rate for multiple testing correction. The differentially expressed mRNAs were defined by fold-changes of more than 3 and a p-value cutoff of 0.05. Thus, the miRNA targets were identified by inverse relationships between expression levels of miRNAs and their target mRNAs in AM3 and LM6 cells [21-26].

RESULTS

Expression Profiling of mRNAs

The genome-wide mRNA expression profiles of human adipose-derived AM3 and lipoma-derived LM6 cells were determined using Affymetrix human genome U133 plus 2.0 GeneChip. The original data have been deposited to NCBI database, and the GEO series number is GSE12843. The mRNA expression of AM3 and LM6 cells are compared in a scatter plot (Fig. **1**), and very similar patterns with Pearson correlation R^2 of 0.974 were observed. The 974 most abundantly (more than 3-folds of overall mean) expressed genes, including matrix metallopeptidases 1 and 3, gremlin 1, and chemokine ligand 5, in AM3 cells, as well as the corresponding values in LM6 cells, are summarized in Supplementary Table **S1**. Using MetaCore Analytical Suite, the 1,137 gene probes commonly expressed between AM3 and LM6 cells were found to be involved in regulating five cell adhesion processes among the top ten GeneGo canonical pathway maps (Supplementary Fig. **S1**). As indicated in Table **1**, 15 most abundantly (more than 3-folds of overall mean) expressed genes, especially SUSD2 encoding sushi domain containing 2, in AM3 cells were up-regulated more than 3-folds in LM6 cells, whereas 8 extremely abundantly (more than 20-folds of overall mean) expressed genes in AM3 cells were down-regulated more than 3-folds in LM6 cells.

Expression Profiling of miRNAs

The expression profiles of 250 human miRNAs in AM3 and LM6 cells were quantitated using TagMan MicroRNA Assays as described previously [30-31], and the expression level of each miRNA was indicated as folds over U6 snRNA. The mean expression levels of triplicate analyses for 250 miRNAs from AM3 and LM6 cells were compared in a scatter plot (Fig. **2**), and a very close correlation R^2 of 0.999 was found. The mean expression levels of 250 miRNAs from AM3 and LM6 cells are given in Supplementary Table **S2**, and the levels of 38 most abundantly (more than 20-fold U6 snRNA) expressed miRNAs in AM3 cells, as well as the corresponding values in LM6 cells, are summarized in Table **2**. It is of interest that miRNAs miR-99a and miR-152 were abundantly expressed in adipose-derived AM3, but not

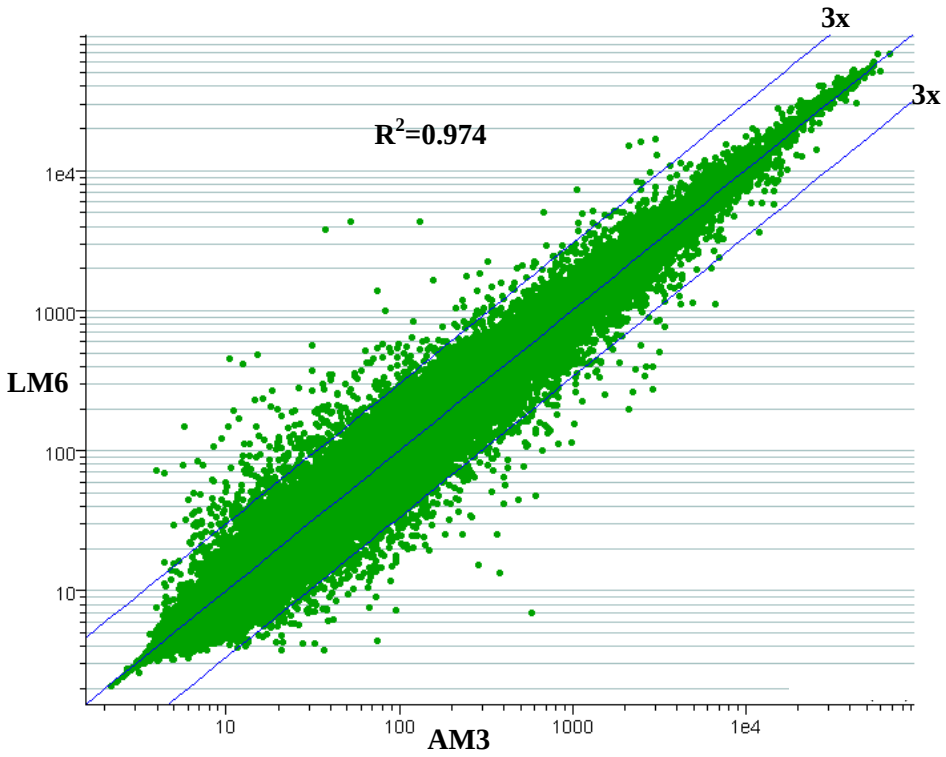

Fig. (1). Scatter plot and correlation analysis of mRNAs between AM3 and LM6 cells.

The average mRNA expression levels of duplicate samples from each cell type were determined using Affymetrix Human Genome U133 plus 2.0 GeneChip. The expression levels of more or less than 3-folds were indicated by lines of 3X. The standard correlation between the mRNA expression levels from AM3 and LM6 cells was found to be $R^2 = 0.974$.

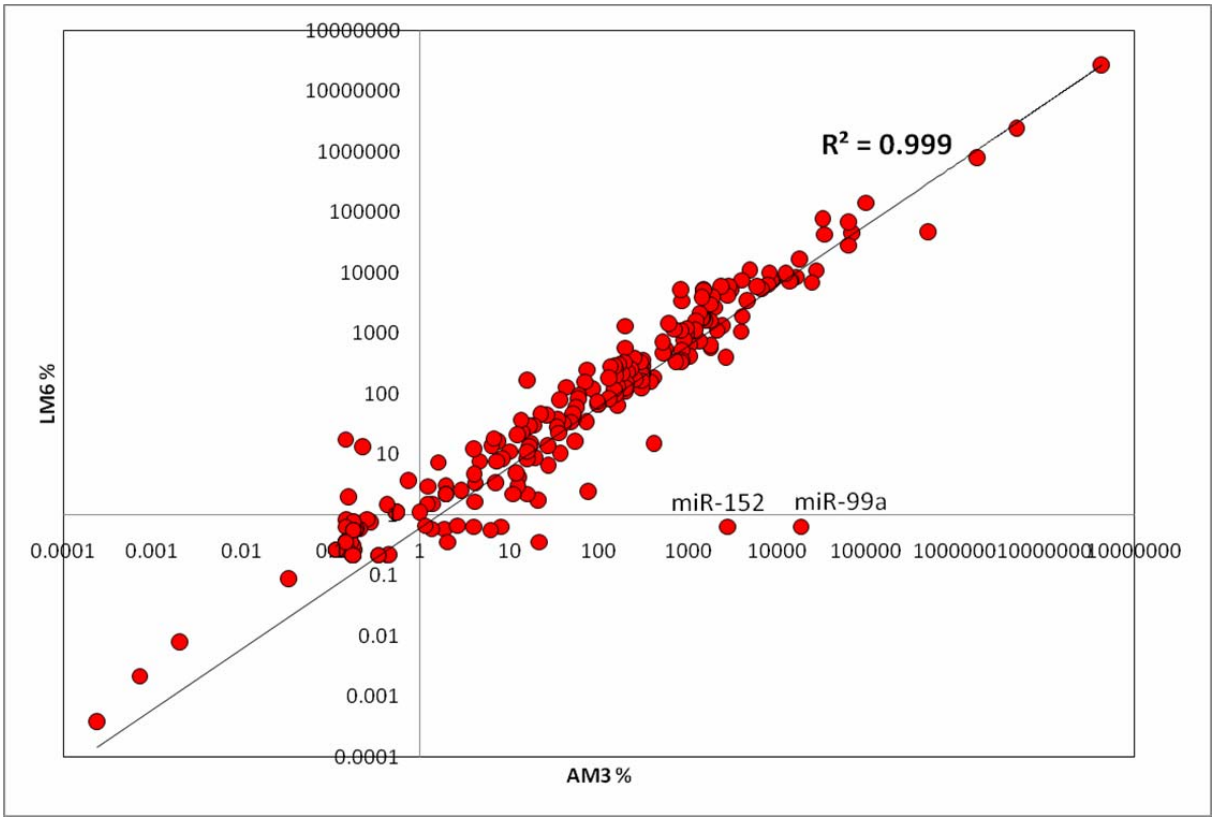

Fig. (2). Scatter plot and correlation analysis of miRNAs between AM3 and LM6 cells.

The miRNA expression levels from each cell type were determined using Applied Biosystems TagMan MicroRNA Assays with stem loop RT followed real-time PCR. The mean miRNA expression levels of triplicate samples are indicated by % folds of U6 snRNA. The standard correlation between the miRNA expression levels from AM3 and LM6 cells was found to be $R^2 = 0.999$. miRNAs miR-99a and miR-152 were abundantly expressed in AM3, but not expressed in LM6 cells.

Table 1. Expression Levels of Abundantly Differentially Expressed Genes in AM3 and LM6 Cells

A. 15 Up-Regulated Genes in LM6 Cells

Gene Symbol	AM3	LM6	LM6/AM3	Description	UniGene	Probe ID
LOC728285 /// LOC728934	92.24	714.90	7.75	keratin associated protein 2-4	---	1555673_at
SLC16A6	67.47	238.20	3.53	solute carrier family 16, member 6	Hs.42645	230748_at
HOXC10	53.00	192.30	3.63	homeobox C10	Hs.44276	218959_at
KRTAP1-5 /// LOC728956	21.01	166.90	7.94	keratin associated protein 1-5	Hs.534499	233533_at
SUSD2	4.26	86.22	20.24	sushi domain containing 2	Hs.131819	227480_at
COL11A1	16.24	76.85	4.73	collagen, type XI, alpha 1	Hs.523446	37892_at
RGS4	15.31	69.49	4.54	regulator of G-protein signalling 4	Hs.386726	204337_at
SNAP25	9.09	40.21	4.42	synaptosomal-associated protein	Hs.167317	202508_s_at
TNFSF4	5.28	36.24	6.86	tumor necrosis factor (ligand) superfamily, member 4	Hs.181097	207426_s_at
CCL7	9.55	35.34	3.70	chemokine (C-C motif) ligand 7	Hs.251526	208075_s_at
TRPA1	5.91	31.24	5.29	transient receptor potential cation channel, subfamily A1	Hs.667156	217590_s_at
COL8A1	4.53	26.33	5.81	collagen, type VIII, alpha 1	Hs.654548	214587_at
ZNF804A	5.37	23.75	4.43	zinc finger protein 804A	Hs.159528	215767_at
TRHDE	3.77	21.33	5.65	thyrotropin-releasing hormone degrading enzyme	Hs.199814	219937_at
CYGB	6.04	20.99	3.48	cytoglobin	Hs.95120	226632_at

B. 8 Down-Regulated Genes in LM6 Cells

Gene Symbol	AM3	LM6	AM3/LM6	Description	UniGene	Probe ID
IL13RA2	368.40	108.00	3.41	interleukin 13 receptor, alpha 2	Hs.336046	206172_at
IL24	255.50	38.21	6.69	interleukin 24	Hs.658964	206569_at
TMEM176B	64.98	10.68	6.08	transmembrane protein 176B	Hs.647090	220532_s_at
ANKRD28	58.23	16.07	3.62	ankyrin repeat domain 28	Hs.335239	229307_at
NKD2	55.62	9.80	5.68	naked cuticle homolog 2 (Drosophila)	Hs.240951	232201_at
TMEM176A	29.33	3.13	9.38	transmembrane protein 176A	Hs.647116	218345_at
ZBTB38	26.35	3.37	7.82	zinc finger and BTB domain containing 38	Hs.518301	1558733_at
TRIB3	22.43	4.96	4.53	tribbles homolog 3 (Drosophila)	Hs.516826	1555788_a_at

expressed in lipoma-derived LM6 cells. Four miRNAs miR-199a, miR-339, let-7i and let-7g were also down-regulated more than 3-folds in LM6 cells, whereas four miRNAs miR-134, miR-155, miR-212 and miR-374 were up-regulated in LM6 cells compared with AM3 cells (Supplementary Table **S2**). It may be further noted that neither AM3 nor LM6 cells expressed the embryonic stem cell- and tissues- (liver, muscle, pancreas, placenta and testis) specific miRNAs.

Target Identification of miRNAs

The targets of six down-regulated miRNAs miR-99a, miR-152, miR-199a, miR-339, let-7g and/or let-7i, as well as four up-regulated miRNAs miR-134, miR-155, miR-212, and/or miR-374, in LM6 cells were identified by inverse relationships between expression levels of miRNAs and their target mRNAs in AM3 and LM6 cells (Table **3**). 36 genes were found to be up-regulated more than 3-folds by the six

Table 2. Levels of 38 Most Abundantly Expressed miRNAs in AM3 Cells as well as the Corresponding Values in LM6 Cells

MicroRNAs	AM3	LM6	AM3/LM6	Chromosome
hsa-miR-19b	433406.50	269515.70	1.61	13q31.3, Xq26.2
hsa-miR-320	48901.56	23829.83	2.05	8p21.3
hsa-miR-186	17453.68	7862.26	2.22	1p31.1
hsa-miR-199a	4943.80	473.29	10.45	19p13.2
hsa-miR-24	999.06	1407.42	0.71	9q22.32, 19p13.12
hsa-miR-20a	695.43	449.79	1.55	13q31.3
hsa-miR-31	634.22	688.63	0.92	9q21.3
hsa-miR-16	632.81	281.59	2.25	3q25.33, 13q14.2
hsa-miR-125b	341.94	419.63	0.81	11q24.1, 21q21.1
hsa-miR-221	329.37	776.09	0.42	Xp11.3
hsa-miR-146b	275.96	108.26	2.55	10q24.32
hsa-miR-339	246.21	67.88	3.63	6p22.3
hsa-miR-99a	188.03	0.01	30017.15	21q21.1
hsa-miR-92	178.21	166.66	1.07	13q31.3, Xq26.2
hsa-let-7b	165.44	84.03	1.97	22q13.31
hsa-miR-93	145.10	73.98	1.96	7q22.1
hsa-miR-125a	139.38	71.12	1.96	19q13.41
hsa-let-7a	125.68	97.92	1.28	9q22.32, 11q24.1, 22q13.31
hsa-miR-26a	89.85	73.97	1.21	3p22.3, 12q14.1
hsa-miR-191	86.28	74.65	1.16	3p21.31
hsa-miR-21	82.87	99.75	0.83	17q23.2
hsa-miR-146a	79.64	62.91	1.27	5q33.3
hsa-miR-27a	67.42	53.46	1.26	19p13.13-13.12
hsa-miR-29a	60.19	58.90	1.02	7q32.3
hsa-miR-214	49.86	109.61	0.45	1q24.3
hsa-miR-30c	46.60	34.02	1.37	1p34.2, q136
hsa-miR-365	41.19	18.77	2.20	16,17
hsa-miR-19a	40.72	74.24	0.55	13q31.3
hsa-let-7i	40.06	10.58	3.79	12q14.1
hsa-miR-342	30.48	52.20	0.58	14q32.2
hsa-miR-140	28.72	58.88	0.49	16q22.1
hsa-miR-152	28.21	0.01	4503.31	17q21.32
hsa-miR-181d	28.15	41.35	0.68	19p13.12
hsa-let-7g	26.81	3.98	6.74	3p21.12
hsa-miR-376a	24.45	13.28	1.84	14q32.31
hsa-miR-127	23.75	59.46	0.40	14q32.31
hsa-miR-26b	21.36	10.93	1.95	2q35
hsa-miR-106b	20.11	26.32	0.76	7q22.1

Table 3. Expression Levels of Abundantly Differentially Expressed Genes Targeted by miRNAs in AM3 and LM6 Cells

A. Targets of Down-Regulated miRNAs

miR- , let-	Gene Symbol	AM3	LM6	LM6/AM3	Description	UniGene	Probe ID
99a, 152, 7g, 7i	HAS2	0.73	6.28	8.61	hyaluronan synthase 2	Hs.571528	206432_at
99a, 152	VNN1	1.52	19.22	12.61	vanin 1	Hs.12114	205844_at
99a	COL11A1	16.24	76.85	4.73	collagen, type XI, alpha 1	Hs.523446	37892_at
99a	POSTN	1.70	12.52	7.35	periostin, osteoblast specific factor	Hs.136348	210809_s_at
99a	NT5E	1.93	7.79	4.04	5'-nucleotidase, ecto (CD73)	Hs.153952	1553994_at
99a	SLC16A3	0.88	7.43	8.49	solute carrier family 16, member 3	Hs.696009	202855_s_at
99a	COPA	1.77	7.34	4.16	coatomer protein complex, subunit alpha	Hs.162121	214337_at
99a	FAM82B	0.96	4.78	5.00	Transcribed locus, weakly similar to XP_519844.1	Hs.145386	229843_at
152	SLC16A6	67.47	238.20	3.53	solute carrier family 16, member 6	Hs.42645	230748_at
152	SPESP1	6.71	21.83	3.25	sperm equatorial segment protein 1	---	229352_at
152	CORIN	0.51	14.23	28.12	corin, serine peptidase	Hs.518618	239260_at
152	HOXA2	3.80	14.12	3.72	Homo sapiens, clone IMAGE:5019307, mRNA	Hs.445239	1557051_s_at
152	EGR3	3.90	13.34	3.42	early growth response 3	Hs.534313	206115_at
152	ULBP2	2.96	12.98	4.39	UL16 binding protein 2	Hs.656778	238542_at
152	C9orf95	2.34	7.15	3.06	chromosome 9 open reading frame 95	Hs.494186	219147_s_at
152	CADPS	2.14	6.59	3.07	Ca2+-dependent secretion activator	Hs.654933	1568603_at
152	SOX9	0.92	6.05	6.55	SRY (sex determining region Y)-box 9	Hs.694731	202936_s_at
152	FAM43A	1.42	5.72	4.02	family with sequence similarity 43, member A	Hs.435080	227410_at
152	NF2	0.78	5.60	7.21	neurofibromin 2 (bilateral acoustic neuroma)	Hs.187898	204991_s_at
152	TSPAN7	0.74	3.92	5.31	tetraspanin 7	Hs.441664	202242_at
152	DKK1	0.18	3.56	20.24	dickkopf homolog 1 (Xenopus laevis)	Hs.40499	204602_at
152	CCL8	0.87	3.03	3.49	chemokine (C-C motif) ligand 8	Hs.271387	214038_at
199a, 7g, 7i	TRHDE	3.77	21.33	5.65	thyrotropin-releasing hormone degrading enzyme	Hs.199814	219937_at
199a	CTGF	1.71	7.95	4.65	connective tissue growth factor	Hs.591346	209101_at
199a	NOX4	0.72	5.16	7.17	NADPH oxidase 4	Hs.371036	219773_at
7g, 7i	SCUBE3	0.41	55.78	134.73	signal peptide, CUB domain, EGF-like 3	Hs.12923	228407_at
7g, 7i	EGR3	3.90	13.34	3.42	early growth response 3	Hs.534313	206115_at
7g, 7i	LOXL4	3.94	12.79	3.24	lysyl oxidase-like 4	Hs.306814	227145_at
7g, 7i	GHR	1.52	5.92	3.90	growth hormone receptor	Hs.125180	205498_at
7g, 7i	CEECAM1	1.61	5.39	3.34	cerebral endothelial cell adhesion molecule 1	Hs.495230	224794_s_at
7g, 7i	ADAMTS5	0.91	5.25	5.78	ADAM metallopeptidase with thrombospondin type 1 motif, 5 (aggrecanase-2)	Hs.58324	229357_at
7g, 7i	CCND1	1.36	4.25	3.13	cyclin D1	Hs.523852	208712_at
7g, 7i	NRK	0.62	3.94	6.38	Nik related kinase	Hs.209527	227971_at
7g	NMNAT2	1.26	4.30	3.42	nicotinamide nucleotide adenylyltransferase 2	Hs.497123	1556029_s_at

(Table 3) contd….

miR- , let-	Gene Symbol	AM3	LM6	LM6/AM3	Description	UniGene	Probe ID
7i	THBS1	4.50	29.45	6.54	thrombospondin 1	Hs.164226	235086_at
7i	CCNE2	0.72	3.53	4.89	cyclin E2	Hs.567387	205034_at

B. Targets of up-regulated miRNAs

miR-	Gene Symbol	AM3	LM6	AM3/LM6	Description	UniGene	Probe ID
134	AMMECR1	5.26	1.12	4.69	Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region, gene 1	Hs.656243	1553219_a_at
134	RUNX1T1	3.08	1.02	3.02	runt-related transcription factor 1; translocated to, 1 (cyclin D-related)	Hs.368431	216831_s_at
374	NFIB	3.27	1.09	3.01	nuclear factor I/B	Hs.644095	211467_s_at

down-regulated miRNAs in LM6 cells compared with AM3 cells. Of these 36 genes, three genes COL11A1, SLC16A6 and TRHDE were among the 15 most highly expressed genes in LM6 cells (Table **1**). The COL11A1 and SLC16A6 genes were the targets of miRNAs miR-99a and miR-152, respectively. The TRHDE gene was the common target of miRNAs miR-199a, let-7g and let-7i. On the contrary, only three genes were down-regulated more than 3-folds by the four up-regulated miRNAs in LM6 cells compared AM3 cells (Table **3**).

DISCUSSION

Human adipose-derived AM3 and lipoma-derived LM6 cells were previously reported to exhibit similar stem cell characteristics and to be readily induced to differentiate into adipocytes, osteoblasts, and chondrocytes [12,15]. In this investigation, the expression profiles of both mRNAs and miRNAs from AM3 and LM6 cells were found to have a considerable similarity, although some differences were observed between them. The abundantly expressed genes such as matrix metallopeptidases and chemokine ligand (Supplementary Table **S1**) in both AM3 and LM6 cells indicate that the up-regulation of extracellular matrix and adhesion is a prominent feature of both MSCs. Indeed, five of the top ten network and signaling pathways are involved in cell adhesion processes (Supplementary Fig. **S1**). These results are in agreement with the previous reports that the core signature transcriptomes of the MSCs isolated from bone marrow, cord blood, amniotic fluid and amniotic membrane include genes involved in the regulation of extracellular matrix and adhesion [18, 35].

Human miRNA changes during MSC differentiation has recently been studied, and 27 miRNAs were identified as regulated during differentiation into adipocytes, osteocytes or chondrocytes [36]. In this investigation, the abundantly differentially expressed genes HAS2, VNN1, COL11A1 and SLC16A6 in LM6 cells were shown to be candidate targets of miR-99a and/or miR-152, which were abundantly expressed in AM3, but not in LM6 cells. In addition, TRHDE gene was also found to be a common target of miR-199a, let-7g and let-7i abundantly expressed in AM3 cells but down-regulated more than 3-folds in LM6 cells. The miR-199a and miR-199a* (processed from the same miRNA precursor) were recently reported to down-regulate the MET proto-oncogene and its downstream effector extracellular signal-regulated kinase 2 (ERK2) gene resulting in inhibiting cell proliferation of tumor cells [37]. Therefore, the highly up-regulated expression of miRNA target genes such as HAS2, VNN1, COL11A1, SLC16A6 and TRHDE, as well as several other abundantly differentially expressed genes such as sushi domain containing 2, keratin associated proteins and tumor necrosis factor family (Table **1**), may explain the higher proliferation potential in LM6 cells compared with AM3 cells. Finally, it will be of interest to express stably miRNAs miR-99a and miR-155 in LM6 cells to see if they can be "converted" into AM3-like cells.

CONCLUSION

The expression profiles of both mRNAs and microRNAs (miRNAs) from human adipose-derived mesenchymal stem cells AM3 (previously designated as AD-MSC3A) and lipoma-derived LM6 (previously designated as LD-MSC6L) cells were found to exhibit considerable similarities except that miRNAs miR-99a and miR-152 were abundantly expressed in AM3, but absent in LM6 cells. The highly up-regulated expression of miRNA target genes such as HAS2, VNN1, COL11A1, SLC16A6 and TRHDE, as well as several other abundantly differentially expressed genes such as sushi domain containing 2, keratin associated proteins and tumor necrosis factor family, may explain the higher proliferation potential in LM6 cells compared with AM3 cells.

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ABBREVIATIONS

MSC	=	Mesenchymal stem cell
AM3	=	Adipose-derived AD-MSC-3
LM6	=	Lipoma-derived LD-MSC-6L
miRNAs	=	microRNAs

SUPPORTIVE/SUPPLEMENTARY MATERIAL

Supplementary material can be viewed at: http://www.bentham.org/open/toscj.

Fig. (S1). Comparison of gene expression and GeneGo canonical pathway maps between AM3 and LM6 cells.

A. The parameters for comparison are set at threshold of 3 with p-value of 0.05. The common genes are indicated by blue/white strips. The unique genes are marked as color band: (1) AM3, orange; (2) LM6, blue. No genes from "similar" set are present.

B. The top 10 common GeneGo canonical pathway maps between AM3 and LM6 cells. The degree of "relevance" to different GeneGo ontology categories is defined by p-value, so that the lower random p-value gets higher priority.

Table S1. Levels of 974 most abundantly expressed mRNAs in AM3 cells.

Table S2. Expression levels of 250 miRNAs in AM3 and LM6 cells.

DATA BASE AND ACCESSION NUMBER

The original data obtained from Affymetrix human genome U133 plus 2.0 GeneChip have been deposited to NCBI database, and the GEO series number is GSE12843.

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