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Overexpression of FABP3 Inhibits Human Bone Marrow Derived Mesenchymal Stem Cell Proliferation but Enhances their Survival in Hypoxia

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Abstract

Studying the proliferative ability of human bone marrow derived mesenchymal stem cells in hypoxic conditions can help us achieve the effective regeneration of ischemic injured myocardium. Cardiac-type fatty acid binding protein (FABP3) is a specific biomarker of muscle and heart tissue injury. This protein is purported to be involved in early myocardial development, adult myocardial tissue repair and responsible for the modulation of cell growth and proliferation. We have investigated the role of FABP3 in human bone marrow derived mesenchymal stem cells under ischemic conditions. MSCs from 12 donors were cultured either in standard normoxic or modified hypoxic conditions, and the differential expression of FABP3 was tested by quantitative ¹PCR and western blot. We also established stable FABP3 expression in MSCs and searched for variation in cellular proliferation and differentiation bioprocesses affected by hypoxic conditions. We identified: 1) the FABP3 differential expression pattern in the MSCs under hypoxic conditions; 2) over-expression of FABP3 inhibited the growth and proliferation of the MSCs; however, improved their survival in low oxygen environments; 3) the cell growth factors and positive cell cycle regulation genes, such as PCNA, APC, CCNB1, CCNB2 and CDC6 were all down-regulated; while the key negative cell cycle regulation genes TP53, BRCA1, CASP3 and CDKN1A were significantly up-regulated in the cells with FABP3 overexpression. Our data suggested that FABP3 was up-regulated under hypoxia; also negatively regulated the cell metabolic process and the mitotic cell cycle. Overexpression of FABP3 inhibited cell growth and proliferation via negative regulation of the cell cycle and down-regulation of cell growth factors, but enhances cell survival in hypoxic or ischemic conditions.

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This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Conflict of interest disclosures

None.

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Keywords

FABP3; cell proliferation; human mesenchymal stem cell; hypoxia; cell cycle

Introduction

Over the last decade, autologous adult mesenchymal stem cell (MSC) transplantation therapy has emerged to the forefront of regenerative medicine for patients with ischemic heart disease based both on theory and clinical trial results [1–4]. The advancement of cell-based therapy provides optimism for repairing or replacing damaged myocardium. In theory, the injected exogenous MSCs may have a capacity to restore or stimulate the resident/endogenous stem cells' capacity to regenerate damaged heart tissue [5–7]. However, despite the initial enormous expectation, recent clinical trials have shown only moderate improvement of left ventricular function but no obvious evidence of therapeutic effectiveness [8–12]. Our pre-clinical animal research also demonstrated that direct injection of autologous MSCs promotes angiogenesis and improves left ventricular ejection fraction following chronic myocardial ischemia, but no myocardiogenesis was observed [13, 14].

The cellular biologic activities are determined by a dynamic interplay of signals within a defined microenvironment. Our study sought to identify specific factors that play a role in mesenchymal stem cells in response to an ischemic myocardial environment. Cardiac-type fatty acid-binding protein (FABP3, NM004102.3) is a cytosolic protein specifically expressed in distinct tissues such as myocardium and skeletal muscles [15, 16]. This protein has been reportedly upregulated during terminal differentiation of mouse cardiomyocytes, and relative overexpression in human ventricular septal defect myocardium when compared to normal myocardium as well. In addition, FABP3 arrests growth of mammary epithelial cells and promotes apoptosis of embryonic myocardial cells [17, 18]. Therefore, it has been presumed that FABP3 might play a role in heart development but precise role of FABP3 in human MSCs have not been studied.

Ischemia or oxygen starvation influences cell proliferation and differentiation by influencing hypoxic targeted activities of MSCs and embryonic stem cells [19, 20]. Our hypoxic porcine genome-wide cDNA expression data showed FABP4 and FABP3 were co-overexpressed with HIF1A and VEGF [21]. HIF1A and VEGF are well-studied multi-functional hypoxia induced transcripts that play an essential role in cell growth and angiogenesis [22–24]. And FABP4, one of family members of FABP3 has been recognized as a target of VEGF and may be a regulator of cell proliferation in endothelial cells [25]. Hence, FABP3 may associate with those factors in deprived microenvironments, but the role in human MSCs remains unclear.

This present study described the biological role of FABP3 mRNA and protein expression in human mesenchymal stem cells under hypoxic condition.

Materials and Methods

Cells and cell culture methods

1. Human cells—Human bone marrow-derived mesenchymal Stem Cells (MSCs) from 12 healthy donors were either purchased from Lonza Walkersville Inc. (Lonza, Walkersville, MD) or obtained from National Institutes of Health (Bethesda, MD) at passage 2–4. Human aortic endothelial cells (hAECs) from two different batches were purchased from Lifeline Cell Technology (Lifeline, Walkersville, MD). The cell donors were 18–43 years of age with a median of 24.4 years (see the Table 1 for details).

2. In vitro ischemic model—The MSCs were seeded at a density of 2×10^5 cells/well in a 6-well plate and cultured with 10% FBS/Dulbecco's Modified Eagle Medium (DMEM) in a 37°C and 5% CO₂ humidified incubator overnight. Then the cells (H–) were placed with either 2 ml of fresh 1% FBS/DMEM and incubated in a monitored hypoxic humidified chamber at 37°C, 1% O₂, 8% CO₂ (BioSpherix, New York) for the hypoxic group (H–) or replaced with fresh 10% FBS/DMEM in a normal conditions (37°C, 20% O₂, 5% CO₂) as the normoxic control group (N) as we previously described [21].

3. Stable FABP3 expression in MSCs—Lentiviral-FABP3 (FABP3LV, human pFABP3-Lv-105), lentiviral vector (CLV, pNEG-Lv105) and lentiviral-RFP vector (RFPLV, pMCHR-Lv105) were purchased from GeneCopoeia Inc. To establish a stable FABP3 expression in MSCs, we performed the experiment using the vectors mentioned above: 1×10^5 /well of MSCs were seeded in 6-well plates for a 24 hour (hr) period prior to transduction, and then transduced FABP3LV or the controls (CLV, RFPLV) into MSCs by using 5µg of Hexadimethrine bromide (Sigma-Aldrich) for 24hr. The stable transfected cells, MSC^{FABP3lv}, MSC^{Clv} and MSC^{RFPlv} were selected by incubation in fresh medium containing 5µg/ml of puromycin for another 3 days, followed by culturing the puromycin-resistant colonies in a reduced concentration of puromycin medium (2.5µg/ml) for expansion and analysis.

Identification and characterization of FABP3 in hMSCs

1. RNA preparation and reverse transcription PCR (RT-PCR)—Total RNAs from all samples were isolated using RNeasy Kit. First-strand cDNAs were synthesized from 0.5µg of total RNA using SuperScript[™] III kit. The PCR amplification and gene differentiation expression were performed with SYBR Green I on a multicolor Real-Time PCR detection system (IQ[™] 5, Bio-Rad Lab.). Subsequently, data were normalized and analyzed through the system software of IQ[™] 5. Gene expression was determined relative to two housekeeping genes GAPDH and B2M using the C_t methods [21, 26]. All the oligonucleotides are listed on supplemental table 1.

2. Immunofluorescence and histological analysis—Cells cultured on glass chamber slides were fixed with 3.7% paraformaldehyde in PBS. The specific primary antibodies recognizing HIF-1A (mouse, Invitrogen) and FABP3 (rabbit, Abcam) probed with the cells at a concentration of 1:200 dilution in T-PBS overnight at 4°C. The immunofluorescence conjugated secondary antibodies were all used at a concentration of 4µg/ml for 1hr

incubation at room temperature. The fluorescence protein expression level was viewed microscopically.

3. Western Blotting—Whole-cell lysates were harvested from the experimental cells with a RIPA buffer and centrifuged at 1.36k xg for 30min at 4°C to separate soluble from insoluble fractions. Twenty to 100µg of total protein were fractionated by 4–20% gradient or 12% SDS polyacrylamide gel electrophoresis and transferred to PVDF membranes and probed with specific antibodies: rabbit anti-FABP3 (1:200, Abcam, Cambridge, MI), goat anti-PCNA (1:500, Santa Cruz, Dallas, TX) and mouse anti-β-actin (1:1000, Santa Cruz, TX) overnight at 4°C. Then the membranes were incubated for 1hr in diluted appropriate secondary antibodies. Immune complexes were visualized with the enhanced chemiluminescent western blotting detection system (GE Healthcare, Piscataway, NJ). The protein signal density was analyzed using ImageJ software (<http://rsb.info.nih.gov/ij/>).

4. Cell proliferation assay— 1.0×10^3 /well of MSC^{FABP3lv} and MSC^{Clv} were seeded in 96-well plates with 100µl of EGM2 medium and cultured either normoxia or 2% hypoxia for 7days. Cell growth assays were performed daily. Briefly, after 24hr of incubation, 10µl of cell counting solution (Cell Counting Kit-8, Dojindo Laboratories) was added and incubated the cells for 3hr at 37°C, and then the absorbance was measured at 450nm by a plate reader. The cell proliferation rate indicated by optical density value (OD) was normalized with plate background OD value.

Cell proliferation and differentiation analysis

A set of human Cell Cycle (PAHS-020Z) and Stem Cell (PAHS-405Z) arrays were obtained from SABiosciences (Human RT² Profiler™ PCR Array Rockville, MD). Each array profile contains 84 well-known key genes that are involved in the regulation and enzymatic pathways of cell cycle and stem cell proliferation and/or differentiation, respectively. Four sets of 12 RNA samples were isolated from transfected MSC^{FABP3lv}, MSC^{Clv} and normoxic hMSCs (N-MSCs) and then amplified with the PCR array on Bio-Rad PCR system. The data normalization and analysis were performed via an integrated web-based software package for the PCR Array System, which automatically performs all C_t based fold-change calculations. The significant expressions defined as a cut off at ± 2 fold-changes of a mean value in each hypoxic group.

Statistical analysis

Average data are presented as a mean value $\pm$ SE unless otherwise stated. Statistical analysis was performed with GraphPad Prism6. The differences between mean values of two groups were analyzed with the student t-test. Statistical significance was set as $p < 0.05$.

Results

The FABP3 mRNA expression in MSCs

To identify the differential expression level in adult MSCs, human MSCs (n=12) cultured in hypoxia or normoxia for 6, 24 and 48hours (hr) were analyzed using q^{rt}PCR. As shown in Figure 1A, FABP3 upregulated in 11 of 12 healthy donor cells during 24hr hypoxic

exposure compared to normoxic conditions. The remaining sample had a higher initial FABP3 expression level compared to other samples (Fig. 1A, a), yielding a lower relative change of cell growth number and unhealthy cell shapes compared to the other 11 samples (Fig. 1A, b–c).

The FABP3 protein expression in MSCs

To search for FABP3 protein modulation and localization, MSCs were cultured in either hypoxic or normoxic conditions and then probed with rabbit anti-FABP3 antibodies and analyzed by immunofluorescence and western blot. We also used a hypoxic target protein, HIF1A as a hypoxic sensor control. We found that FABP3 protein expression levels clearly increased in the MSCs exposed to hypoxia, along with HIF1A expression (Fig. 1B). The positive green-FABP3 proteins were overexpressed and localized in the nucleus and cytoplasm, and positive HIF1A proteins were located in nucleus. Notably, the FABP3-positive cells appeared in various morphologies, with no evidence of apoptosis was observed.

FABP3-positive protein was found in eight H-MSC samples after 30hr hypoxic exposure; for the total samples (n=12), three samples were FABP3-negative, 5 weak positive (number of positive cells <10%) and 3 moderate positive (over 10% and less than 50%), but no positive FABP3 cells were found in aortic endothelial cells (AECs).

To further define the differential expression associated with oxygen levels, a group of 24hr H-MSCs were re-oxygenized for another 6, 24, and 48hr time points followed by real-time PCR assays to observe changes in gene expression. Figure 1C demonstrated that this gene's differential expression level was up- or down-regulated due to fluctuating low or high oxygen levels during culture. In contrast, no significant changes of FABP3 were found in hypoxic AECs and HeLa cells (Fig. 1D), indicating that FABP3 regulation is specific to H-MSCs. Therefore FABP3 may serve as a novel biosensor to observe MSCs response to variation in oxygen concentration exposure.

Characterization of FABP3 protein in MSCs

To search for FABP3's biological function, a stable overexpression MSC^{FABP3lv} was established and examined. As shown in Fig. 2A, FABP3 is overexpressed in MSC^{FABP3lv}, and Fig. 2B, a-b, the FABP3 proteins recognized by anti-FABP3 antibody clearly appeared in MSC^{FABP3lv}'s cytoplasm but not in any MSC^{RFPIv} control cells (red).

We also treated the cells in hypoxia for 24hr, Fig. 2C, the overexpressed FABP3 protein in the normoxic cells was localized surrounding the nucleus; however in hypoxic cells, the protein was distributed throughout the cytoplasm as well as the nucleus. These results also further confirmed our previous finding, suggesting that a low oxygen condition stimulates FABP3 overexpression and partial translocation into the nucleus.

Overexpression of FABP3 suppresses MSC proliferation

Figure 2 not only demonstrates the FABP3 protein overexpression in stable transduced cells but also shows that there are fewer cells MSC^{FABP3lv} cells even though identical numbers of

cells were seeded into cell culture plates. This phenomenon indicates that the growth rate of the FABP3-overexpressing cells was lower than the control cells from passage 1 to 3 under normoxic condition. Meanwhile, the cell morphology was also appeared irregular, larger and thinner than usual, in addition to having bubbles present in the cellular plasma and nucleus for 2–3 days but disappearing after (Figure 3A). Western blots showed that Proliferating Cell Nuclear Antigen (PCNA) protein expression level was more than ten times lower than that of controls (fig. 3B, a–b). Therefore, we further performed cell proliferation assays by using CCK-8 and repeating it four times. 1000 cells/well, MSC^{FABP3lv} or, control cells, were tropically seeded in a 96-well and cultured with 1% FBS/DMEM either in a normoxic (N–) or hypoxic (H–) incubator for 7 days. The cell proliferation rates were measured via an optical density value plate reader every day. As shown in the summarized outcomes of cell growth curves in Figure 3C, the normal control cells, N-MSCLV had the highest proliferation rate in the testing groups ($p < 0.01$); both N- and H-MSCLV were growing slowly, but persistently. Compared to H-MSCLV, H-MSCLV^{FABP3lv} cell density was significantly lower on day 2 and 3 ($p < 0.05$) but steadily rose on days 4 to 7. For that reason, the 7-day cell growth curves presented the two diverse growth patterns of H-MSCLV^{FABP3lv} and H-MSCLV ($f < 0.01$). It seems the FABP3 overexpressed cells were more tolerant of the low oxygen environment, suggesting that FABP3 inhibits cellular proliferation, yet enhances the ability of cells to adapt to hypoxic conditions.

Overexpression of FABP3 inhibits cell growth by down-regulation of cell cycle regulators and growth factors

To further investigate FABP3's function, we performed and analyzed specific stem cell (Stem Cell PCR Array, SC array) and cell cycle pathway PCR arrays (CC array). Of the total 168 genes, 53 and 26 were differentially up- and down-regulated over 2-fold in the FABP3-overexpressing cells respectively (Table 2 and supplemental table 2). The genes were further categorized through gene ontology analysis (<http://david.abcc.ncifcrf.gov/>) and some of the genes were validated by PCR and real-time PCR (Figure 4A–C). Figure 4A and B were the summarized the results of SC array (qRT-PCR) and RT-PCR that showed the differential expression of stem cell genes between MSC^{FABP3LV} and MSC^{CLV}, which mostly down-regulated in MSC^{FABP3LV} comparing to control. Fig. 4C illustrated a summarized network correlation of the regulating factors in the cell cycle; the genes with red or blue marker represented up- or down-expressed genes of MSC^{FABP3LV} vs. MSC^{CLV}, respectively. As shown in Fig. 4C and Table 2, TP53, CDK5R, CDKN1A, CASP3 and ATR/ATM were overexpressed, which are positive regulators of programmed cell death, cell cycle arrest and negative cell proliferation factors [27–29]. On the other hand, certain genes that enhance positive cell metabolic regulation, as well as growth and proliferation factors, such as CDK1, CDC6, CCNB1 and MCM were down-regulated [30–34]. These expression patterns highly suggest that incident of cell proliferation in MSC^{FABP3LV} may be resulted by cell cycle arrest and inhibition of growth factors.

Discussion

The goal of this study was to identify the FABP3 expression patterns in a human MSCs data set and to further examine the influence of this protein on the MSCs behaviors in vitro. We

expected the results could lead us to better understand how the cells adapt themselves to low oxygen surroundings. Herein, our data for the first time demonstrated that hypoxia specifically induces FABP3 mRNA regulation in human adult bone marrow derived mesenchymal stem cells, but not in AECs and HeLa cells. The FABP3 modulation mostly occurred in cells after 24hr of exposure to a low oxygen microenvironment and may be associated with cell injury and wound repair. The results suggest that FABP3 is highly sensitive to oxygen level changes and the modulation is specific to MSCs.

Protein localization can provide valuable information about its molecular function, as well as the biological pathway in which it participates. FABP3 is a binding protein in cellular sarcoplasm and cytosol. Activation of FABP3 inhibits cell growth by inducing programmed cell death [18]. In this study, we verified that FABP3 protein is normally localized in the cellular cytoplasm, but spread over in the cellular cytoplasm and nucleus under hypoxic sitting. This occurrence also has been observed by Dr. Cataltepe's group in a study of FABP4 regulation in endothelial cells [25]. They found overexpression of FABP4 in the cytoplasm and nucleus, suggesting the protein may interact with a nuclear target in endothelial cells. Since FABP4 and FABP3 belong to the same family and are highly homologous in protein structure, this interaction may also exist in mesenchymal cells. Because FABP3 is a small intracellular fatty acid binding protein participating in the uptake and intracellular transport of long-chain fatty acid and acyl-CoA in response to energy demands, we presume that FABP3 aggressively transports the fuel into the cellular nucleus from the cytoplasm under hypoxic conditions. This translocation event stimulates a negative growth signal to inhibit cell proliferation and slows cellular metabolism in order to help the cells survive in an ischemic microenvironment.

In order to characterize FABP3's role in the MSCs, we established a stable FABP3 expressing cell line, MSC^{FABP3LV} and analyzed the cell proliferating and differential activity. Unlike the HIF1A and VEGF positive role in cell growth, the FABP3 expressing cells grew very slow and proliferating cells were especially rare but had better survival rates under hypoxic microenvironment.

To further investigate FABP3's role in stem cell, SC array and CC array used in this study contained 168 genes that involved in cell cycle regulation, cell growth and proliferation, communication and differentiation in the stem cells. We also found that overexpression of FABP3 led to TP53 over 4-times upregulation. TP53 encodes the tumor suppressor protein containing transcriptional activation. The encoded protein responds to diverse cellular stresses to down-regulate expression of PCNA and up-regulate expression of BCL2, CASP3 and SERTAD1, thereby inducing cell cycle arrest, and enhances programmed cell death or changes in metabolism [35–38]. In contradistinction, genes promoting mitosis, nuclear division and DNA synthesis were down regulated, such as CDK1, CDC6, CCNB1, CCNB2, MCM and APC. Moreover, most of the SC and CC genes are not only participating in cell cycle regulation, but also in cellular metabolic processes such as MYC, FGF4 and VEGF, as well as differentiation (http://www.sabiosciences.com/rt_pcr_product/HTML/PAHS-082Z.html). Therefore, the complex interplay of genes not only grounds the cell cycle at the G1 to S phase, but also promotes advanced intracellular energy utilization for cell better survival under ischemia. The array data seems corresponds with the FABP3

localization results, but if the tiny advanced phenomenon could promote the MSCs proliferation and differentiation remains unknown and needs further investigation.

In conclusion, our data provides a systematic FABP3 expression profile of human MSCs and demonstrated that FABP3 is highly enhanced in H-MSCs, but negatively regulates cell growth and proliferation by down regulating cell cycle and growth factors. Our data demonstrates that FABP3 is a hypoxic target that inhibits human mesenchymal stem cell growth by preventing cellular proliferation pathways while simultaneously stabilizing cells subjected to hypoxic or ischemic conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- FABP3 expression pattern was studied in 12 human hypoxic-MSCs
- FABP3 mRNA and proteins are upregulated in the MSCs under hypoxic conditions
- Overexpression of FABP3 inhibited cell growth but advanced the MSC survival under hypoxia
- Overexpression of FABP3 down-regulated the cell cycle and stem cell signaling pathways.

Figure 1A

Figure 1B

Figure 1C

Figure 1D

Figure 1.

FABP3 expression pattern in MSCs. (A, a) FABP3 mRNA expression pattern in hypoxic human MSCs: Y axis: relative gene expression level, and X axis: cell group (n=12), * stands for p value, H vs. N: * $p < 0.05 > 0.01$ and ** $p = 0.002$; the circle symbolizes each individual sample, the red solid diamonds and the bar figure; (b) normal human MSC in cell cultures and (c) FABP3 overexpressed hMSCs in cell culture, the blue start represented a corresponding cell and RNA in Fig. A, a–c. (B) FABP3 protein expression and localization: the HIF1A (red, e, f) and FABP3 proteins (green, g, h) appeared in hypoxic MSC nucleus only, nucleus and cytoplasm, respectively; but HIF1A and FABP3 were all absent in the normoxic control cells (a, b, c, d). (C), Figure 1C FABP3 expression associated with oxygen condition. (D) No Relative regulation of FABP3 in no MSCs (Aorta endothelial cells, and human cancer cells).

Figure 2A

Figure 2.

(A) Evidence of FABP3 overexpression in MSC^{FABP3LV}(rtPCR). (B) Immunocytochemical images of FABP3 protein (green, b) present in MSC^{FABP3LV}, but not in vector control MSCs (red, a) and (C) MSC^{FABP3LV} proteins were around the cellular nucleus under normoxic condition (green), but distributed to the cellular nucleus and cytoplasm under hypoxic condition (green).

Figure 3A

Figure 3B

Figure 3C

Figure 3. Overexpressing FABP3 inhibited cell growth and proliferation. (A) Cell growth image. (B) Western Blot result and (C), a summary of 7-days MSC^{FABP3LV} growth curve of normoxia and hypoxia. The cell growth curve indicates the cell proliferation rate. ** = $p < 0.01$ and ^{xx} = $f < 0.01$.

Figure 4 A

Figure 4B

Fig. 4C

Figure 4.

(A & B) A summared ^tPCR and q^tPCR results * indicates the gene regulation was confirmed by both ^tPCR and q^tPCR. APC, JAG1, HSPA9 and FGF4 were down- or up-regulated in MSC^{FABP3LV} comparing to vector control cells, respectively. (C) Illustration of the FABP3 induced genes employed in the cell cycle pathway (KEGG database). The red and blue symbols represent a relative up- or down regulation of MSC^{FABP3LV} and MSC^{CLV}, respectively.

Table 1

Human sample information

Lab ID	Lot ID	Sex	Age	Race	Passage	Cell surface marker	Cause of death	Source
1	hMSC1	M	30	unknown	2			LONZA
2	hMSC2	M	43	C	2			LONZA
3	hMSC3	F	24	H	2			LONZA
4	hMSC4	M	43	unknown	2			LONZA
5	hMSC5	M	21	B	2			LONZA
6	hMSC6	F	22	unknown	2	CD105, CD166, CD29, CD44 90% positive.		LONZA
7	hMSC7	M	22	unknown	2			LONZA
8	hMSC8	M	18	B	2	CD14, CD34, CD45 <10% Negative		LONZA
9	hMSC9	M	22	C	2			LONZA
10	hMSC10	F	22	B	2			LONZA
11	hMSC11	F	42	H	3			NIH
12	hMSC12	M	27	B	3			NIH
1	hAEC1	M	61	C	2	SMAa (-) Factor III (+)	ICH-stroke	Lifeline
2	hAEC2	F	62	C	2		Anoxia/cardia arrest	Lifeline

Table 2

The list of differential regulated genes in FABP3 expressed MSCs.

Unigene ID	Official Gene Symbol	Fold Regulation	Biological processing (GOTERM-BP, level 4)
Hs.376071	CCND2	30.0	G1/S transition of mitotic cell cycle, positive regulation of cell proliferation
Hs.500015	CDK5R1	7.7	positive regulation of programmed cell death
Hs.150749	BCL2	7.5	regulation of cell growth, apoptosis
Hs.370771	CDKN1A	7.5	G1/S t& G2/M transition of mitotic cell cycle, cell cycle arrest
Hs.523852	CCND1	7.2	G1/S transition of mitotic cell cycle, response to endoplasmic reticulum stress, DNA damage stimulus & fat cell differentiation
Hs.292524	CCNH	5.1	DNA metabolic process, positive regulation of cellular metabolic process
Hs.269898	SERTAD1	3.8	negative regulation of cell growth & cell size
Hs.654481	TP53	3.3	cell cycle checkpoint, cell cycle arrest, negative regulation of cell cycle, proliferation and growth; cellular response to starvation, positive regulation of cell death...
Hs.484551	MDM2	3.3	G1 phase, positive regulation of cell proliferation & cell cycle, positive
Hs.141125	CASP3	3.2	negative regulation of cell proliferation; regulation of programmed cell death
Hs.431048	ABL1	3.2	S phase, positive regulation of programmed cell death & cellular process
Hs.534307	CCND3	3.2	positive regulation of cyclin-dependent protein kinase activity, positive regulation of cellular metabolic process, positive regulation of cell cycle
Hs.1973	CCNF	-3.1	M phase of mitotic cell cycle, nuclear division, re-entry into mitotic cell cycle
Hs.591697	MAD2L1	-3.6	M phase, cell cycle checkpoint, negative regulation of cell cycle process & nuclear division,
Hs.146806	CUL1	-3.6	G1/S transition of mitotic cell cycle, cell cycle arrest
Hs.179565	MCM3	-4.1	DNA metabolic process, regulation of gene expression
Hs.386189	GTSE1	-5.4	G2 phase, positive regulation of cellular process
Hs.84113	CDKN3	-5.8	G1/S transition of mitotic cell cycle, cell cycle arrest
Hs.442658	AURKB	-6.5	M phase of mitotic cell cycle, cellular protein metabolic process
Hs.728893	BIRC5	-6.6	G2/M, positive regulation of mitotic cell cycle
Hs.594238	KPNA2	-6.7	M phase and G2 phase, regulation of DNA metabolic process
Hs.656	CDC25C	-7.1	G1 and M phase, regulation of nuclear division
Hs.689823	MKI67	-8.0	M phase of meiotic cell cycle
Hs.194698	CCNB2	-8.0	M phase of mitotic cell cycle, nuclear division
Hs.524947	CDC20	-8.9	M phase of mitotic cell cycle, nuclear division
Hs.477481	MCM2	-13.1	DNA metabolic process, chromatin organization, nucleosome assembly
Hs.58974	CCNA2	-13.1	M phase of mitotic cell cycle, cell cycle checkpoint
Hs.23960	CCNB1	-17.5	G2/M transition of mitotic cell cycle

* partial list of the significant differentially regulated genes (± 3 -fold regulation), the completed gene list in supplement table 1.