ORIGINAL ARTICLE

Received: May 2013
Accepted: December 2013

The study of LPL gene expression during differentiation of Human BM-MSC into adipocytes

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BCCR
2014; 6(1): 16-21
www.bccrjournal.com

ABSTRACT

Background: Many types of cancer cells require a supply of fatty acids (FA) for growth and survival. LPL, in the presence of triglyceride-rich lipoproteins, accelerates the growth of these cells. Recent evidence shows that LPL plays important roles in inflammation and obesity, implies that it is an appropriate general target for chemo preventive and chemotherapeutic agents. Thus, we are going to report LPL expression before and after BM-MSCs differentiation into adipocytes.

Methods: In this study, we isolated mesenchymal stem cells from human bone marrow by Ficoll - gradient and then their surface markers by flow cytometry was confirmed and osteoblastic and adipocytes differentiation by Dexamethasone were carried out and it was confirmed by staining. Then qualitative expression of LPL gene was carried out by RT-PCR before and after of its differentiation into adipocytes. Statistical analysis was performed by paired t test and by using Pfaffl and graph pad software.

Results: After 14 days, analysis of morphology with invert microscopy indicated that BM-MSCs have ability of differentiation capacities into adipocytes. Investigations of expression of LPL showed that BM-MSC served as negative control with no expression of LPL while in BM-MSCs derived- adipocyte have found significant expression of LP.

Conclusion: BM-MSCs can differentiate into either adipocytes or osteoblastic cells that in decision between the two lineages, LPL has the important role through activation inhibiting osteoblastic in favor of adipogenic differentiation. LPL could be an effective agent on tumor suppressing with catalyzes the hydrolysis of plasma TG. LPL gene deficiency, such as due to chromosome 8p22 loss, LPL gene polymorphism, and epigenetic changes increases cancer risk, especially in the prostate.

Keywords: Mesenchymal stem cell, Differentiation, Adipocyte, Lipoprotein lipase.

Introduction

The existence of lipoprotein lipase was first realized in 1943 when Paul Hahn observed that an intravenous injection of heparin could abolish the lipaemia associated with the absorption of a fatty meal.[1] These findings led to the proposed existence of a so-called heparin-releasable 'clearing factor'. Subsequent studies revealed this factor to be a lipolytic enzyme, and it was therefore named 'clearing factor lipase'.[2] The activity of this clearing factor lipase' was found in several extrahepatic tissues, including heart and adipose tissue, but was shown to have only a limited action on artificial lipid emulsion substrates in the absence of serum lipoproteins.[3] It was not until 1966 that apolipoprotein C2 (apoC2), a component of high-density lipoprotein and very low-density lipoprotein (VLDL) was demonstrated to be an essential cofactor/activator for the action of the enzyme. As a result of this study, the clearing factor lipase' was renamed as lipoprotein lipase.[4] The enzyme has subsequently been shown to play a central role in the overall lipid metabolism and transport LPL has the ability to bind simultaneously to both lipoproteins and cell surface receptors/proteoglycans, and this allows it to carry out a non-catalytic bridging function, which leads to the accumulation and cellular uptake of lipoproteins.[5] LPL has also been found to play key roles in a number of physiological and pathophysiological conditions, with abnormal LPL expression and/or function being associated, either directly or indirectly, with atherosclerosis, obesity, diabetes, chylomicronaemia, Alzheimer's disease (AD) and cachexia. Recent evidence shows that LPL plays important roles in inflammation and obesity implies that it is an appropriate general target for chemopreventive and chemotherapeutic agents. Bone marrow MSCs (BM-MSCs) exhibit patterns of gene expression similar to preadipocytes that can be developed to mature adipocytes. Potential therapeutic use of mesenchymal stem cells (MSCs) is likely to require large-scale in vitro expansion of the cells before transplantation. MSCs from adipose tissue can be cultured extensively until senescence.[6] However, little is known on the differentiation potential of adipose stem cells (ASCs) upon extended culture. However, during the differentiation process, expression of LPL has not been investigated and is incompletely characterized.[7] Thus, for the first time, we are going to report LPL expression before and after BM-MSCs differentiation into adipocyte under controlled conditions.

Matrials and Methods

Isolation and culture of MSC from BM

MScs were obtained from BM aspirates of healthy human at the Tehran Taleghani Hospital. The aspirates were diluted 1:5 with 2 mM ethylene deamine tetra acetic acid (EDTA)-phosphate-buffered saline (PBS). The MNC fraction was isolated by density gradient centrifugation at 435g for 30 minutes at room temperature using Ficoll-Hypaque-Plus solution and seeded at a density of 1 × 106 cells per cm2 into T75 or T175 cell culture flasks. The first change of medium was accomplished within 3 days after isolation. The resulting fibroblastoid adherent cells were termed BM-derived fibroblastoid adherent cells (BM-FACs) and were cultivated at 37°C at a humidified atmosphere containing 5% CO2. The expansion medium consisted of either mesenchymal stem cell growth medium Dulbecco's modified Eagle's medium-low glucose (DMEM-lg) containing 10% mesenchymal stem cell growth supplements (MSCGS).

Adipogenic Differentiation

In order to induce adipogenic differentiation, the cells were seeded at a density of 3X103 cells per cm2 into T25 flasks until reaching 70% confluence. Then, the cells were induced by adipogenic induction medium for 14 days. For the negative control, the cells were kept in DMEM-hg +10% FBS. Adipogenic differentiation was demonstrated by the accumulation of neutral lipid vacuoles indicated by the Oil Red O staining.

RNA isolation:

1) After differentiation, total RNA was extracted and cDNA was performed and analyzed by reverse-transcribed and finally RT-PCR d 200ul chloroform / 1mL TRIZOL (originally used), vortex for 15 seconds, and leave at room temperature for 2-3 minutes.

2) Centrifuge samples at 12,000g for 15 minutes at 2-8 C.

3) Following centrifugation, there will be three phases visible within the tube. Transfer the aqueous phase (top) to a fresh tube, being careful not to contaminate the solu-

tion with the other phases. Contamination will be obvious by the presence of any flakes or unclear liquid.

4) Add 500ul isopropanol / 1mL TRIZOL (originally used) to the new tube and incubate at room temp for10 minutes.

5) Centrifuge samples at 12,000g for 10 minutes at 2-8 C.

6) Following centrifugation, remove the supernatant.

7) Wash RNA pellet with 80% EtOH / 1ml TRIZOL (originally used) and vortex.

8) Centrifuge samples at 7,500g for 5 minutes at 2-8 C.

9) Remove supernatant. Allow remaining EtOH to air dry for 2-3 minutes.

10) Transfer tubes to 70 C heat block and let sit for 2-3 minutes.

11) Re-dissolve the pellet in 81ul of DEPC water.

cDNA Synthesis Protocol

1) 500ng RNA of each sample prepared in volume of 10ul and placed on ice.

2) 9ul of mix added to each sample and 2 negative controls.

3) All samples heated to 65C for 5 minutes and returned to ice

4) 1ul RT enzyme added to all samples.

5) All samples heated at 37C for 60 minutes.

RT-PCR (Qualitative PCR)

Qualitative the using performed was analysis PCR protocol of cinagene kit.

After 14 days, analysis of morphology with microscope indicated that BM-MSCs have ability of differentiation capacities into adipocytes. Investigations of expression LPL showed BM-MSC that served as negative control have no expression of LPL while in BM-MSCs derived- adipocyte have found significant expression of LPL.

Results

Results from mesenchymal stem cells towards osteoblast

As it can be observed from **Figure 1**, separated mesenchymal stem cells have differentiation capability V cells.

Flow cytometry results

In order to approve that obtained cells are mesenchymal, flow cytometry was carried out for markers such as CD34, CD31, CD105, CD90, which their results in **Figure 2**.

Staining BM-MSCs during differentiation towards adipocytes

As it can be observed from **Figure 3**, after differentiation MSCsgot morphologically adipocytes cells specification.

Results from qualitative expression of LPL gene

In **Figure 4** from qualitative expression of LPL gene before and after BM-MSCs differentiation can be observed. As it has been shown, this gene has no expression before differentiation, but after differentiation LPL gene expression is clearly obvious.

1: beta actin before differentiation
2: LPL before differentiation
3: LADER 50bp
4: beta actin after differentiation
5: LPL after differentiation
6: NTC

Conclusion

In this study, we isolated mesenchymal stem cells

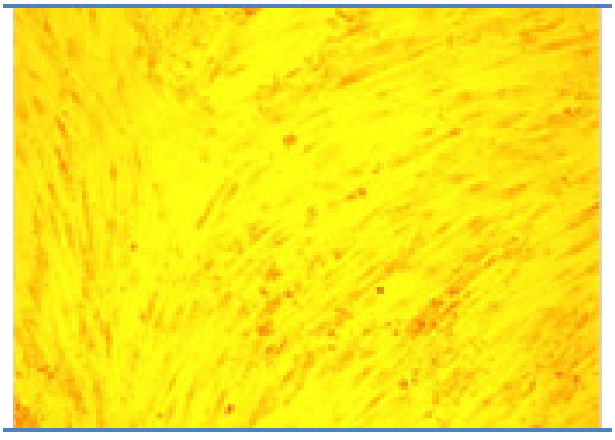

Figure 1: Alizarin red staining of differentiated mesenchymal stem cells into osteoblast after 21 days.

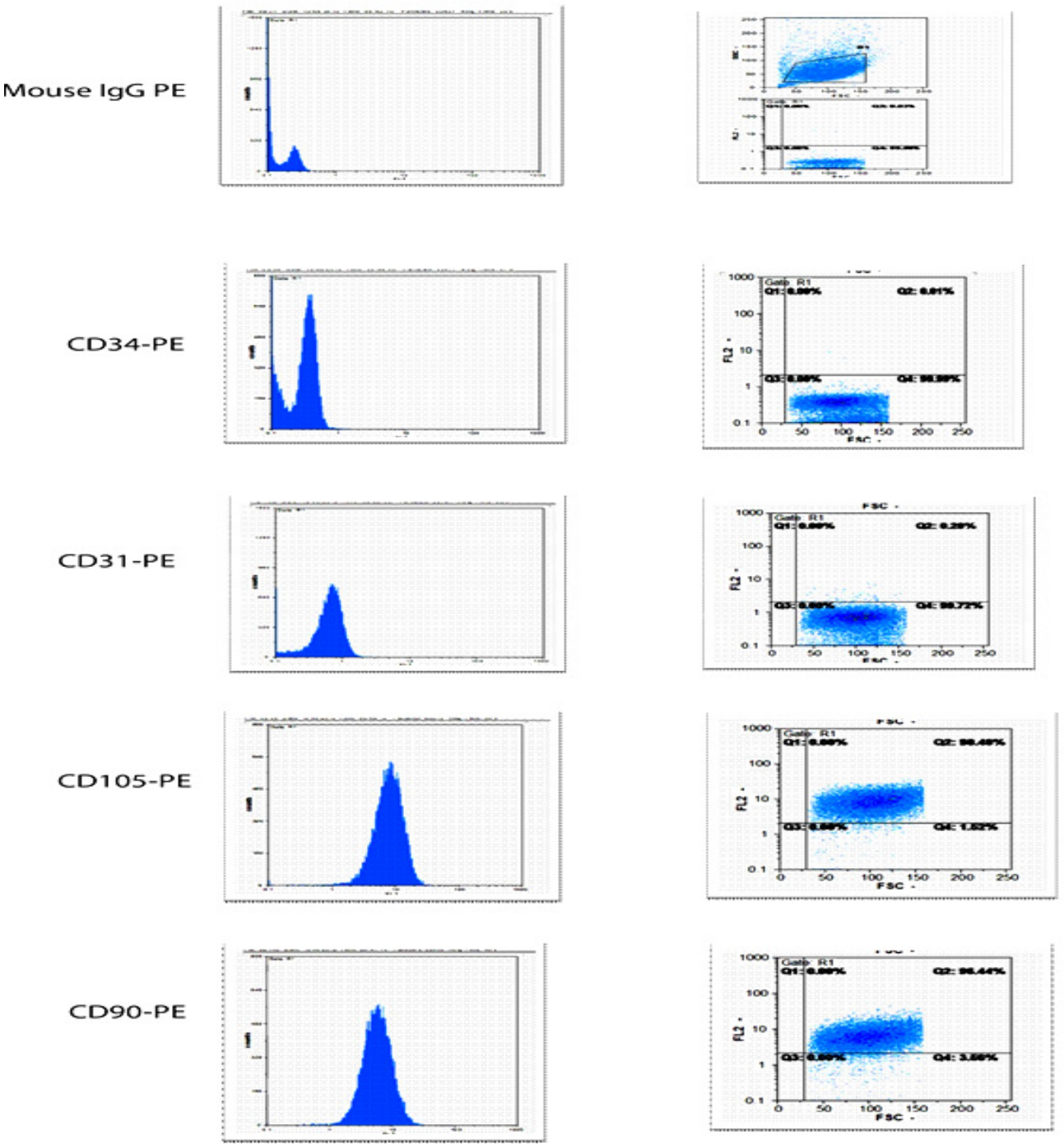

Figure 2: result of mesenchymal stem cells flow cytometry. (CD34- CD31- CD105+ - CD90+).

from human bone marrow by Ficoll - gradient and then their surface markers by flow cytometry was confirmed and osteoblastic and adipocytes differentiation by Dexamethasone protocol were carried out and confirmed by staining. Then qualitative expression of LPL gene was carried out by RT-PCR before and after differentiation into adiposities. Expression of LPL gene showed that BM-MSC served as negative control with no expression

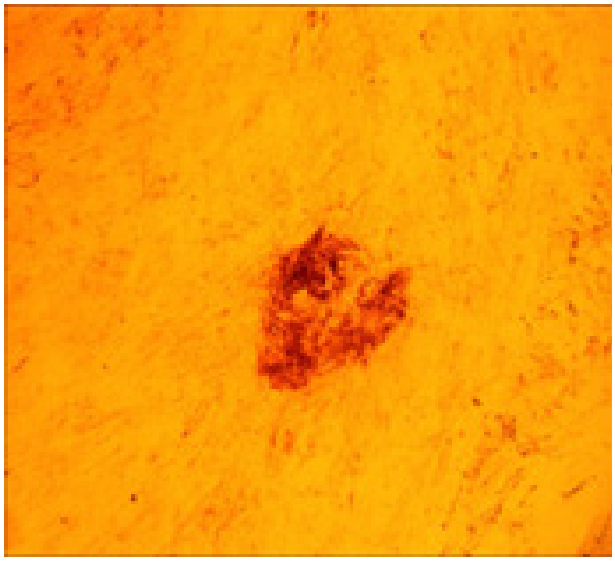

Figure 3: Oil-Red-O staining of differentiated mesenchymal stem cells into adipocytes after 14 days.

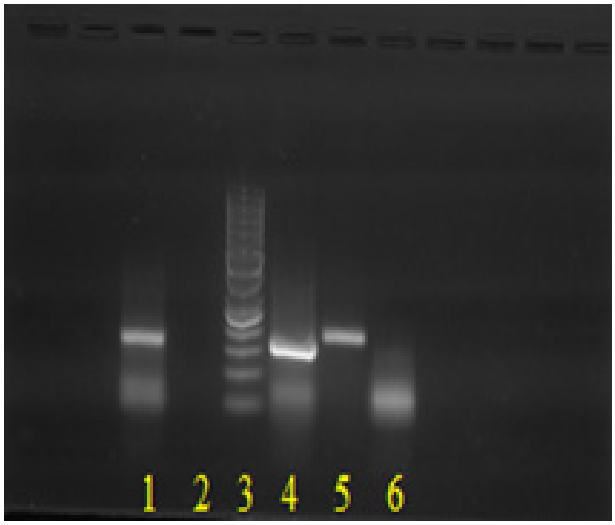

Figure 4: result of RT-PCR.

of LPL while in BM-MSCs derived- adipocyte have found significant expression of LPL. Many types of cancer cells require a supply of fatty acids (FA) for growth and survival, LPL, in the presence of triglyceride-rich lipoproteins, accelerates the growth of these cells transplantation. Lipoprotein lipase (LPL) catalyses the hydrolysis of the triacylglycerol component of circulating chylomicrons and very low density lipoproteins, thereby providing non-esterified fatty acids. Research carried out over the past two decades have not only established a central role for LPL in the overall lipid metabolism and transport, but have also identified additional non-catalytic functions of the enzyme. Furthermore, abnormalities in LPL function have been found to be associated with a number of pathophysiological conditions, including atherosclerosis, chylomicronaemia, and obesity, Alzheimer's disease, and insulin resistance diabetes. When preadipocytes differentiate into adipocytes, several differentiation-linked genes are activated. Lipoprotein lipase (LPL) is one of the first genes induced during this process. According to the results, the LPL gene acts as one of the important factors in the differentiation of MSCs into adipocytes. In brief it is suggested that the inhibition of this gene expression can prevent many common cancers such as prostate cancer as a future idea to cure.

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