

## Original Article

Methylation Dynamics of the PPAR Gamma (PPAR $\gamma$ ) Gene during Adipogenic Differentiation of Human Bone Marrow Mesenchymal Stem CellsSaeid Kaviani <sup>1</sup>, Aryan Salahi-Niri <sup>2</sup>, Mohammad Hossein Mohammadi <sup>3</sup>, Mohsen\_Hamidpour <sup>3</sup>, Shadi Esmaeili <sup>3\*</sup> <sup>1</sup> Hematology Department, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran<sup>2</sup> Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran<sup>3</sup> Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, IranScan and read the  
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## Abstract

**Background:** Epigenetics is crucial in differentiating mesenchymal stem cells (MSCs) into adipocytes. Specifically, DNA methylation, an epigenetic modification, regulates the expression of genes involved in this process. The peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) gene is a critical player in adipocyte differentiation, with epigenetic changes affecting its expression.**Methods:** We isolated mesenchymal stem cells (MSCs) from the human bone marrow. The isolated MSCs were expanded and cultured in a differentiation medium for two weeks. DNA extraction was performed on undifferentiated and differentiated adipocytes after the culturing process. The methylation status of the promoter region of the PPAR $\gamma$  gene was assessed using methylation-specific primers (M for methylated and U for unmethylated) in a methylation-specific PCR (MSP) assay. This analysis involved the treatment of DNA samples with sodium bisulfite to convert unmethylated cytosine to uracil, thereby enabling the differentiation between methylated and unmethylated regions of the gene.**Results:** The successful differentiation of MSCs into adipocytes was confirmed by the accumulation of lipid droplets within the differentiated cells, as visualized by the oil Red O dye staining. This observation provides strong evidence of the commitment of MSCs towards the adipogenic lineage and their ability to undergo adipocyte differentiation. Surprisingly, the MSP analysis revealed no significant changes in the methylation pattern of this gene following differentiation. The PPAR $\gamma$  gene promoter region exhibited an unmethylated status in both undifferentiated and differentiated states.**Conclusion:** Our study revealed that additional genetic or epigenetic mechanisms control the expression of PPAR $\gamma$  during the adipogenic differentiation of mesenchymal stem cells. These findings highlight the regulatory role of PPAR $\gamma$  in the differentiation pathway from mesenchymal stem cells to adipocytes.

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## 1. INTRODUCTION

The remarkable capacity of stem cells to differentiate and repair tissues has attracted significant attention over the past decade. These cells have been demonstrated to possess a remarkable potential for multipotent differentiation, exhibiting the ability to differentiate into osteogenic, adipogenic, chondrogenic, and myogenic cell lineages under precisely defined conditions and specific environmental cues (1). Although mesenchymal stem cells (MSCs) have been identified in several tissues throughout the body, including adipose tissue, muscle, and dental pulp, the bone marrow remains the primary reservoir of these cells (2). Therefore, MSCs derived from bone marrow are widely considered the benchmark for studying and characterizing MSCs. Laboratory studies on MSCs have significantly contributed to understanding these cells, providing researchers with valuable insights and knowledge (3, 4). Today, researchers employ well-defined culture conditions and growth factors to guide the differentiation of MSCs into specific cell lineages. This enables the utilization of MSCs for various applications in regenerative medicine and tissue engineering (5, 6). Adipogenesis is a tightly controlled process in which mesenchymal stem cells undergo differentiation into mature and functional adipocytes (7). Under optimal conditions, these mesenchymal cells exhibit diverse morphological characteristics and express specific genes associated with adipocyte maturation. It is essential to consider that the differentiation process is a multi-stage and coordinated phenomenon involving mesenchymal stem cells, pre-adipocytes, and mature adipocytes as crucial players (8).

Effective cellular communication, both intracellularly and with the extracellular microenvironment, is imperative for adipogenesis (9). Growth factors, molecular signals, and transcriptional factors mediate this intricate communication. One of the transcription factors involved in this process is the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ). PPAR $\gamma$  belongs to the family of ligand-activated transcription factors and plays a significant role in the regulation of gene expression (10).

Previous studies have proved that the PPAR $\gamma$  gene is crucial in differentiating mesenchymal stem cells towards fully mature adipocytes (11). It is regarded as a fundamental factor in this process. PPAR $\gamma$  comprises two isoforms, both of which are expressed within adipocytes. Analysis of regulatory regions associated with this transcription factor has demonstrated its involvement in the transcriptional regulation of numerous genes involved in adipogenesis (12, 13). Mesenchymal stem cells derived from bone marrow

possess a gene expression profile comparable to that of pre-adipocytes, thereby endowing them with the capacity to differentiate into mature adipocytes (4, 14).

One of the fundamental mechanisms regulating gene expression involves epigenetic processes, including DNA methylation, primarily in gene promoter regions, and various histone modifications, such as acetylation, deacetylation, methylation, and phosphorylation (15). These modifications play a pivotal role in determining the accessibility of genetic information and modulating the expression levels of genes. DNA methylation is a biochemical process that involves adding a methyl group (-CH<sub>3</sub>) to the carbon atom of the cytosine residues within the CpG dinucleotide. CpG islands, which are stretches of DNA with a high frequency of CpG dinucleotide, are particularly susceptible to DNA methylation (16). Adding a methyl group to the cytosine residue leads to epigenetic modifications that can influence gene expression and play a crucial role in various biological processes, including development, genomic imprinting, and X-chromosome inactivation (17).

Research investigations have provided evidence that hypo-methylation status correlates with enhanced gene expression, whereas hyper-methylation is associated with decreased or silenced gene expression (18, 19). Epigenetic alterations, including DNA methylation, present more favorable targets for developing new therapeutic strategies as they exhibit a more significant potential for reversibility when compared to genetic changes (20, 21). These findings underscore the significance of exploring epigenetic modifications as promising avenues for advancing innovative therapies. Given the crucial role of gene-specific methylation in gene expression, assessing methylation status in specific adipogenic genes, such as PPAR $\gamma$ , is essential. The aim of this study was to investigate if DNA methylation of the PPAR $\gamma$  gene is a crucial regulatory mechanism for PPAR $\gamma$  expression during the differentiation of mesenchymal stem cells into adipocytes. To achieve this, we analyzed the promoter methylation status of PPAR $\gamma$  in DNA samples extracted from undifferentiated and differentiated adipocytes using the Methylation-Specific PCR (MSP) method.

## 2. MATERIALS AND METHODS

### 2.1. Ethics Statement

Ethical approval was obtained before conducting the study, and informed consent was acquired from healthy individuals at Taleghani Hospital in Tehran. Bone marrow

aspirations were performed to collect samples." Replaced with these sentences "The Regional Ethics Committee of Shahid Beheshti University of Medical Sciences approved the study, and informed consent was acquired from healthy individuals at Taleghani Hospital in Tehran. Bone marrow aspirations (BMAs) were performed to collect samples.

## 2.2. Cell culture

Ultimately, the resulting mononuclear cells were re-suspended in a concentration of  $1 \times 10^6$  cells/ml and subsequently transferred to a T75 flask for further culturing and analysis. The mononuclear cells were maintained at 37°C and 5% CO<sub>2</sub> under controlled conditions in a T75 flask. The culture medium used for cell replication consisted of DMEM supplemented with 10% fetal bovine serum (FBS). The medium was refreshed thrice weekly to provide optimal nutrient support for cell proliferation.

To achieve this purpose, the flask was decanted to remove the culture medium, and the adhesive cells on the flask surface were subjected to three washes with 20 milliliters of PBSA buffer. The cells were subsequently transferred to a fresh culture medium and incubated for approximately one week until reaching a confluency level of 60-70%. The cells were dissociated using trypsin and passaged into a new flask for subsequent cultivation.

## 2.3. Differentiation of mesenchymal stem cells into osteoblast

Isolation and Cultivation of Mesenchymal Stem Cells from Bone Marrow Mononuclear cells (MNCs) were isolated from the BMA by Ficoll-Hypaque density gradient centrifugation. MNCs were quantified, and their viability percentage was determined by trypan blue staining before the cell culture.

Ultimately, the resulting MNCs were re-suspended in a concentration of  $1 \times 10^6$  cells/ml and a specialized osteogenic differentiation medium was prepared to induce differentiation of the cells into osteoblast. The medium consisted of high glucose DMEM culture medium supplemented with 10% fetal bovine serum (FBS), 8-10 mol/L dexamethasone (Sigma, USA), 3.7 g/L sodium bicarbonate, and 0.05 g/L ascorbic acid (Sigma, USA).

The cells were centrifuged at 1200 rpm for 5 minutes at 25 °C. After centrifugation, the supernatant was carefully decanted to obtain a 1 mL aliquot of the cellular solution. The cells were then seeded onto a 6-well culture plate containing the specific osteogenic differentiation medium and placed inside an incubator.

Subsequently, every 3-4 days, approximately 1 mL of the differentiation medium was aspirated from each well and replaced with fresh differentiation medium to maintain optimal cell differentiation conditions. As a control group, cells from a flask were cultured on plates with a differentiation-free medium. The morphological feature of the cells was periodically evaluated using an inverted microscope. After a 21-day incubation period, when the differentiation process was complete, the cells were stained with Alizarin Red (Sigma, USA), and images were captured for analysis.

## 2.4. Osteoblast staining with Alizarin Red

Osteoblasts were washed with  $1 \times$  PBS (Kantonsapotheke Zürich, Switzerland, Cat. No. A171012) and fixed with 4% (v/v) formaldehyde (Sigma, USA, Cat. No. F8775) in  $1 \times$  PBS for 30 min. After washing twice with ddH<sub>2</sub>O, Alizarin Red staining solution (0.7 g Alizarin Red S (Sigma, USA, Cat. No. A5533) diluted in 50 ml ddH<sub>2</sub>O at pH = 4.2) was added for 20 min. Afterward, cells were washed four times with ddH<sub>2</sub>O, dried, and stored in the dark until image acquisition.

## 2.5. Flow cytometric validation of mesenchymal stem cells

In this experiment, a flask from Passage 3 was transferred to the Research Center of Taleghani Hospital to confirm the stemness of the cells using a flow cytometer.

In this procedure, four antibodies were selected for experimental analysis, namely CD34, CD31, CD90, and CD105. A negative control or unstained control tube was also incorporated as a reference group to establish baseline measurements. After the cells were detached using trypsin following the third passage, they were suspended in 100 µl of PBS at a concentration of  $10^5$  cells. Subsequently, 3 µl of the above antibodies were added to the sample. Following the addition of the antibodies, the samples were subjected to incubation at a temperature of 4 °C for 30 minutes. Then, the cells were incubated with 3 µl of the above antibodies at 4 °C for 30 min. The cells were washed using 500 µl of PBS, re-suspended in 300 µl PBS, and analyzed by BD FCES Calibur flow cytometer from the USA. The acquired data was then analyzed using flow software (version 7.6.1).

## 2.6. Differentiation of mesenchymal stem cells into adipocytes

To achieve this objective, we used the Passage 3 cells. Initially, the cells were cultivated in 6-well plates, and upon reaching confluence, the differentiation process commenced. Subsequently, the standard cell milieu was replaced with a differentiation medium comprising DMEM supplemented with 10% fetal bovine serum (FBS), 100 nM Dexamethasone (Sigma; USA), and 50 µg/mL Indomethacin (Sigma; USA). Certain wells of the 6-well plate were assigned as control groups and received the standard medium consisting of DMEM supplemented with 10% fetal bovine serum. Both the differentiation and control groups were maintained at 37 °C with 5% CO<sub>2</sub> for 14 days. At the end of this incubation period, the samples were evaluated using the Oil Red O staining technique.

## 2.7. Adipocyte staining with Oil red O.

Cells were washed twice with PBS and fixed with 10% formaldehyde for 45 minutes at room temperature. After washing with distilled water twice and 50% isopropanol once, the cells were stained for 1 hour at room temperature with filtered Oil red O/60% isopropanol solution. The cells were washed twice with distilled water and twice with PBS. Adipocytes stained red were recorded by light microscopy.

## 2.8. DNA extraction

Genomic DNA was extracted from cell samples during the first week (Undifferentiated MSCs (UD)) and at the end of the second week of differentiation (Differentiated adipocytes (D)) by the Roche DNA extraction kit. The quality assessment of the extracted DNA involved agarose gel electrophoresis. DNA was quantified using a NanoDrop 1000TMS spectrophotometer (OD 260 nm/OD 280 nm). (Table 1).

**Table 1.** The concentration of extracted DNA and its purity, assessed by OD 260/280 and OD 260/230 ratios

RNA Sample	Concentration (ng)
UD (MSC)	335
D (Adipocyte)	475

## 2.9. DNA Treatment with Sodium bisulfite (SBS) and CpG Methyltransferase (M.SssI).

Before conducting MSP (Methylation-Specific PCR), the extracted DNA was subjected to SBS treatment. The 40 µL of deionized distilled water and 5.5 µL of 2M NaOH were added to 10 µL of DNA and incubated at 37 °C for 20 min in an incubator. The 30 µL of freshly prepared 10 mM hydroquinone and 520 µL of freshly prepared 3 M sodium bisulfite were sequentially added to the tube. The tube was then coated with mineral oil to ensure the stability of the solution and incubated at 50°C for 16 hours. The DNA was extracted from the resulting solution using the Roche kit. The DNA sample underwent desulfonation at this stage of the protocol. To facilitate the process, we added 22 µL of a 3 M NaOH solution to the DNA solution and incubated it at ambient temperature for 5 minutes. The desulfonated DNA was then precipitated using the ammonium acetate-ethanol precipitation method. Subsequently, the DNA pellet was resuspended in 30 µL of double-distilled water (ddH<sub>2</sub>O).

MSP was conducted using custom-designed primers specific to methylated (M) and unmethylated (U) regions for the targeted DNA sequences. The primers were designed using the Methprimer online software, facilitating the differentiation between methylated and unmethylated DNA during the PCR analysis (Table 2). The PCR reaction was prepared by combining a 10x buffer, dNTP mixture (each at 1.25 mM), primers (0.5 µM each), 25 mM MgCl<sub>2</sub>, 1-4% DMSO, 1.25 units of Taq DNA Polymerase (Fermentas), and SBS-treated DNA (50 ng) in a final volume of 25 µL. MSP analysis was repeated for each sample a minimum of three times to ensure reliable results. Thermal cycling was done with the amplification of methylated (PPAR $\gamma$ -M) and unmethylated (PPAR $\gamma$ -U) regions were conducted using specific primer sets, as detailed in Table 2. The high-temperature differences between the forward and reverse primers were carefully considered during the amplification process. The methylated primer set (PPAR $\gamma$ M3-F and PPAR $\gamma$ M3-R) produced a product of 124 base pairs (bp) with a melting temperature (T<sub>m</sub>) of 52.2°C for the forward primer and 46.3°C for the reverse primer. Similarly, the unmethylated primer set (PPAR $\gamma$ U3-F and PPAR $\gamma$ U3-R) generated a product of 128 bp, with a T<sub>m</sub> of 53.2°C for the forward primer and 47.5°C for the reverse primer. All MSP amplifications were visualized on a 2% agarose gel.

MSP reactions included a non-template control sample to exclude possible contaminations as a negative control and a methylated DNA generated by using the M.SssI enzyme according to the protocol of the New England Biolabs as a

**Table 2.** Methylated and unmethylated primers were used for Methylation-Specific PCR (MSP)

Product length(bp)	Tm	sequence	Gene name
124	52.2	AAGACGGTTTGGTCGATC	PPAR $\gamma$ M <sub>3</sub> -F
124	46.3	CGAAAAAAAATCCGAAATTTAA	PPAR $\gamma$ M <sub>3</sub> -R
128	53.2	GGGAAGATGGTTTGGTTGATT	PPAR $\gamma$ U <sub>3</sub> -F
128	47.5	TCCAAAAAAAATCCAAAATTTAA	PPAR $\gamma$ U <sub>3</sub> -R

Abbreviations; F, Forward primer; R, Reverse primer; M, Methylated-specific primer; U, Unmethylated-specific primer; bp, base pair; TM, Annealing temperature

positive control. For this purpose, 10  $\mu$ L of a 10X buffer, 0.5  $\mu$ L of 32 mM S-adenosyl methionine, and 2  $\mu$ L of M.SssI enzyme added 6  $\mu$ L of peripheral blood genomic DNA. The volume was adjusted to 100  $\mu$ L using distilled water and incubated at 37°C for 1.5 hours. Subsequently, the resultant methylated DNA was utilized as a positive control in MSP with the specific-methylated (M) primer.

### 3. RESULTS

#### 3.1. PPAR $\gamma$ expression pattern during the adipogenic differentiation of MSCs

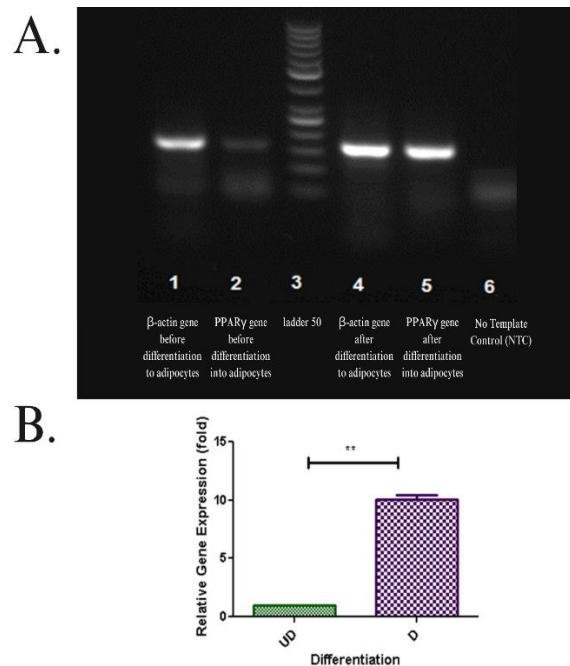
In our previous studies, we conducted gene expression analysis of PPAR $\gamma$  before and after the adipogenic differentiation of MSCs using the RT (reverse transcriptase) and real-time PCR methods, respectively (22). As illustrated in **Figures 1 and 2**, a substantial upregulation of the PPAR $\gamma$  gene expression was observed following differentiation, indicating its pivotal role in the differentiation process.

#### 3.2. Morphology of cultured cells

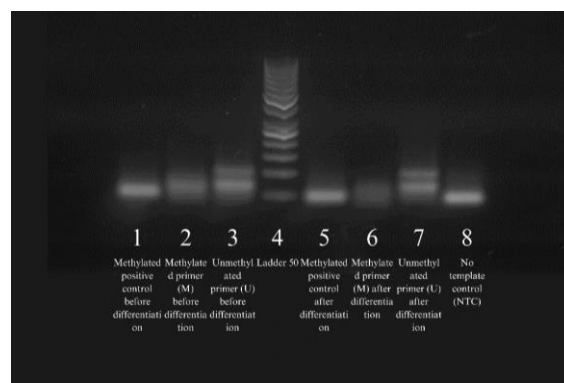
In the primary culture, heterogenous cellular clusters were observed in the human bone marrow-derived cells. By day 7, cells exhibited diverse morphologies, such as flat, elongated, and multipolar. In subsequent passages, there was an increased proportion of elongated cells to the extent that the majority of cells in the third passage displayed an elongated morphology. Furthermore, in the subsequent passages, mesenchymal stem cells exhibited a morphological resemblance to fibroblast-like cells (elongated shape) in their morphology.

#### 3.3. Evaluation of Surface Marker Expression by Flow Cytometry

To substantiate the mesenchymal nature of these cells, in addition to their adhesive characteristics to the culture



**Figure 1.** A. RT-PCR Results of Beta-Actin (Internal Control because it is regarded as a highly stable housekeeping gene) and PPAR $\gamma$  Genes in Undifferentiated MSCs on Day 14 of Differentiation. B. Quantitative Real-Time PCR (qRT-PCR) Results Related to PPAR $\gamma$  Gene ( $P < 0.05$ ).



**Figure 2.** Methylation Analysis of PPAR $\gamma$  Gene Before and After Differentiation

substrate, another method of identification is the utilization of surface markers. Following multiple passages and isolation of the mesenchymal stem cells from other cell populations, their surface markers were analyzed using flow cytometry. The cells demonstrated negative expression for the surface markers CD34 and CD31, indicating their non-hematopoietic and non-endothelial nature, respectively. On the contrary, they exhibited positive expression for the surface markers CD90 and CD105, known characteristic markers of mesenchymal stem cells. Flow cytometry analysis confirms mesenchymal lineage of human bone marrow-derived cells in **Figure 3**.

### 3.4. Staining Results for Differentiation of Cells into the Osteogenic Lineage

Alizarin Red is a calcium-binding dye commonly used for staining mineralized tissues, such as bone. When mesenchymal stem cells differentiate into osteoblasts, they start producing and depositing calcium phosphate, eventually forming a mineralized matrix, i.e., bone.

The staining results using Alizarin Red demonstrated that the cells differentiated into the osteogenic lineage exhibited a distinct red coloration, indicating the presence of a mineralized bone matrix. In contrast, the control culture without any differentiation-inducing agents showed no observable red-colored deposits, as shown in **Figure 4**.

### 3.5. Result of Oil Red O staining of differentiated mesenchymal stem cells into adipocytes

The mechanism of action of Oil Red O staining involves the formation of hydrophobic interactions between the dye and the hydroxyl groups present in lipid molecules. When applied to cells, the dye penetrates the plasma membrane and binds to the intracellular lipid droplets. This binding results in the intense red staining of lipid-rich areas, allowing for easy visualization and evaluation under a microscope.

The red staining of differentiated mesenchymal stem cells into adipocytes with Oil Red O dye revealed the presence of prominent lipid droplet accumulation within the cells. On the 14th and 21st days, alizarin red staining was conducted to confirm the differentiation of mesenchymal stem cells.

### 3.6. Isolation of DNA from Adipose Tissue-Derived Fat Cells and MSP

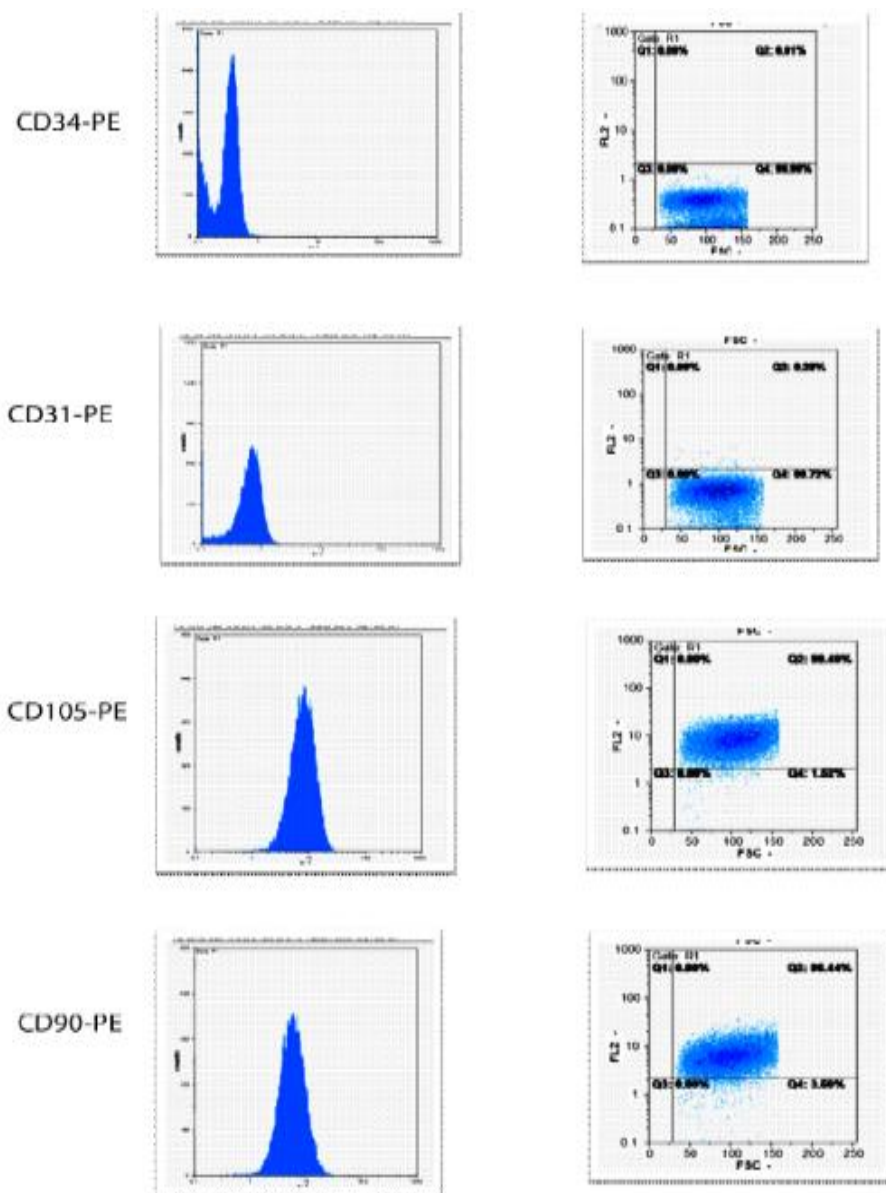
DNA extraction from fat cells, sourced from adipose tissue, involved performing Methylation-Specific PCR (MSP) to control for unmethylated primers. This step followed the DNA extraction procedure outlined in the preceding section, with the distinction that the DNA sample originated from fat cells isolated from adipose tissue. The adipose tissue was acquired with informed consent from Bahman Hospital during a liposuction procedure. The tissue, housed in a 50cc syringe filled with normal saline to maintain freshness, underwent cell separation under a hood. Subsequently, the tissue was subjected to triple washes with PBS, followed by meticulous homogenization through scalpel-assisted cutting. The homogenized tissue was then incubated in a Falcon 50 tube with 1 mg/ml collagenase type 1 for 2 hours in the incubator. Following centrifugation at 1500 RPM for 7 minutes, the supernatant was discarded, and the concentrated contents were utilized for DNA extraction as per the specified protocol. Subsequent to bisulfite treatment, Methylation-Specific PCR was executed, and the resulting product underwent electrophoresis (**Figure 5**).

### 3.7. Comparison of the methylation status of PPAR $\gamma$ between undifferentiated MSCs and differentiated adipocytes

As shown in **Figure 2**, the PPAR $\gamma$  gene retains its unmethylated state in undifferentiated MSCs and differentiated adipocytes. These findings highlight the robustness and conservation of the DNA methylation patterns within the specific loci of the PPAR $\gamma$  gene, irrespective of the cellular differentiation state.

## 4. DISCUSSION

Scientific investigations have demonstrated that DNA methylation patterns, which encompass the addition of methyl groups to cytosine residues in DNA, can play a pivotal role in the etiology and progression of cancer (23, 24). This epigenetic modification exerts regulatory control over gene expression and contributes to carcinogenesis's complex mechanisms. Specifically, research on the PPAR $\gamma$  gene has illuminated its involvement in regulating adipocyte differentiation and its relevance to adipocyte-associated malignancies (25, 26). PPAR $\gamma$  is predominantly expressed in adipocytes, where it exerts essential functions in regulating adipocyte activity, differentiation, and lipid metabolism. Alterations in DNA methylation patterns



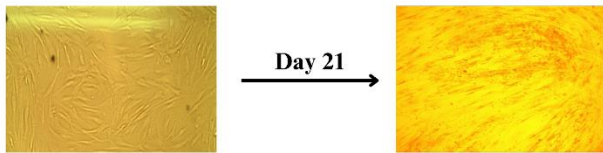
**Figure 3.** Verification of the mesenchymal lineage identity of human bone marrow-derived cells using flow cytometric analysis. The markers CD31 and CD34 showed negative expression, and the CD90 and CD105, known characteristic markers of the MSCs, showed highly positive expression.

within the PPAR $\gamma$  gene have been identified in several types of cancers, including prostate and breast cancers, intimately linked to adipose tissue (27, 28). These perturbations can disturb the delicate balance of PPAR $\gamma$  gene expression, leading to compromised adipocyte differentiation and dysregulated lipid synthesis by cancer cells. The DNA methylation patterns within the PPAR $\gamma$  gene have been identified as critical regulators of various cellular processes, including cell differentiation and disease

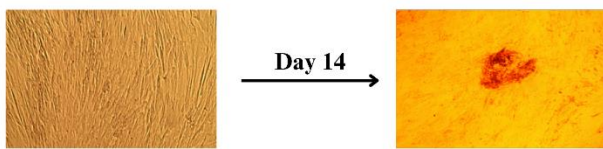
progression (29). Research conducted by L Coutos-Thévenot et al. in the context of bladder cancer has demonstrated that alterations in the DNA methylation patterns within the promoter region of the PPAR $\gamma$  gene can significantly impact its genetic activity and expression. Such dysregulation can impede proper cellular differentiation, thereby contributing to bladder cancer initiation and progression (30).

Similarly, investigations by Kotta-Loizou et al. on breast cancer have revealed the association between DNA methylation changes within the promoter regions of the PPAR $\gamma$  gene and the cell's capacity to mobilize fatty substances to cancer-associated loci. These epigenetic alterations have been linked to the facilitation of angiogenesis, new blood vessel formation, and tumors' observed metastatic behavior (31).

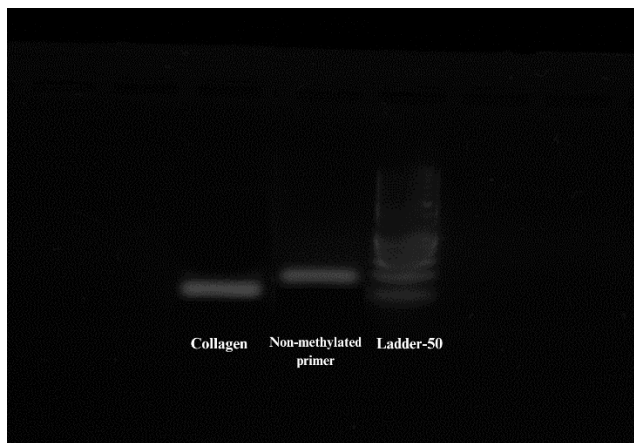
**A.**



**B.**



**Figure 4.** On day 21, Alizarin Red staining confirmed mesenchymal stem cell differentiation into osteoblasts, while on day 14, Oil-Red-O staining confirmed differentiation into adipose tissue.



**Figure 5.** MSP results of tissue-derived somatic cells for unmethylated primers

Further elucidating the regulatory mechanisms, Smith et al. (2018) have emphasized the involvement of DNA methylation within the PPAR $\gamma$  gene in the modulation of adipocyte cellular activity and differentiation (32). Notably, the framework of DNA methylation patterns within the

gene's enhancer regions has been observed to dictate lipid maladjustments and concentration irregularities within breast cancer cells (33).

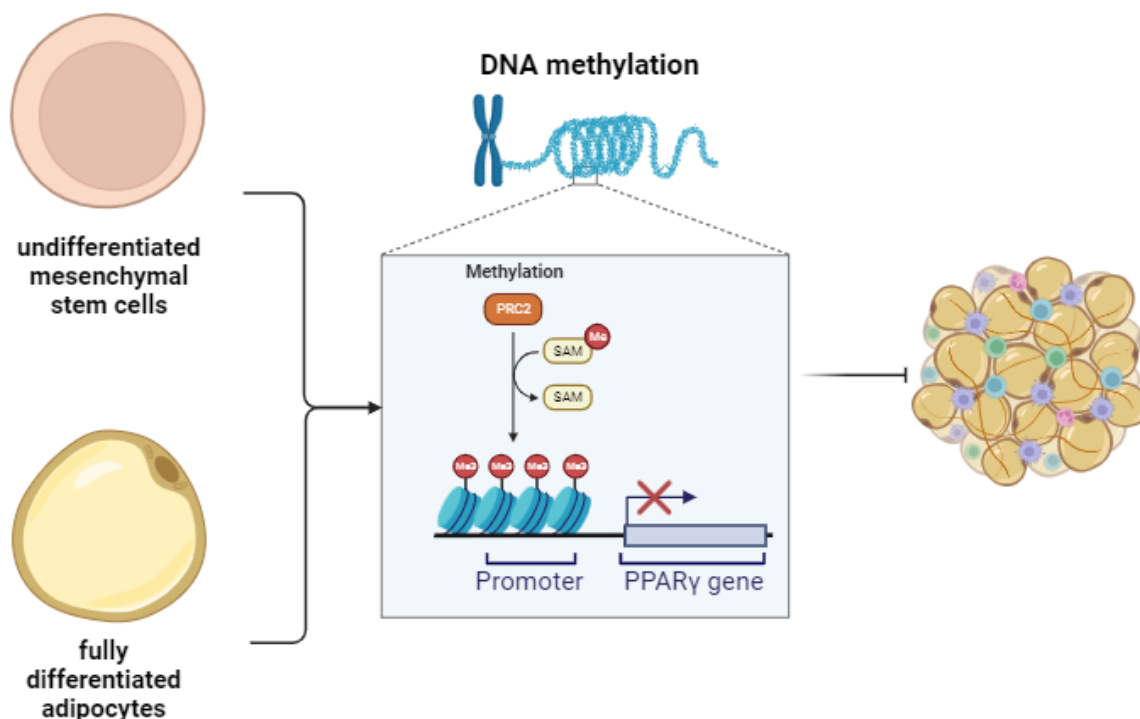
The studies mentioned above highlight the significant impact of DNA methylation patterns within the PPAR $\gamma$  gene on cellular differentiation processes, which is relevant in cancer and other pathological conditions. In the context of aplastic anemia, adipogenesis is crucial because changes in the bone marrow microenvironment can significantly affect disease progression (22, 34). Adipocytes within the bone marrow play a vital role in influencing the hematopoietic niche and the differentiation of blood cells (35). They also regulate hematopoiesis, which affects the maintenance and functionality of HSCs. Furthermore, adipocytes are involved in the secretion of inflammatory cytokines, which may contribute to an inflammatory environment in the bone marrow (36, 37). This can affect the immune response and worsen the progression of aplastic anemia (38).

The pertinence of our recent paper, delving into epigenetic modifications during adipogenic differentiation, is underscored in comprehending the intricate molecular mechanisms governing adipogenesis in bone marrow diseases. Our exploration of DNA methylation changes, notably in genes like PPAR $\gamma$ , provides crucial insights into the regulatory framework during adipogenic differentiation. Concurrent studies on epigenetic modifications during adipogenesis further enrich our understanding of how DNA methylation, histone modifications, and non-coding RNAs collectively influence this process. In the context of aplastic anemia, where disruptions in the bone marrow microenvironment and hematopoiesis are pivotal, these investigations collectively illuminate potential therapeutic targets and diagnostic markers, emphasizing the transformative implications of unraveling the epigenetic intricacies for future interventions.

In this study, we examined the DNA methylation status of the PPAR $\gamma$  gene during the adipogenic differentiation of mesenchymal stem cells. Our results revealed that the promoter region of the PPAR $\gamma$  gene exhibited unmethylated in both undifferentiated mesenchymal stem cells and fully differentiated adipocytes. **Figure 6** illustrates the schematic form. Interestingly, adipocyte differentiation did not exert any discernible effect on the DNA methylation status of this gene, suggesting that the expression of the PPAR $\gamma$  gene remains unaffected by alterations in its methylation patterns.

Investigating the DNA methylation profile of the PPAR $\gamma$  gene holds promise for elucidating the underlying





**Figure 6.** A summary of our study that shows PPAR $\gamma$  gene exhibited complete methylation in both undifferentiated mesenchymal stem cells and fully differentiated adipocytes.

mechanisms driving adipocyte-related carcinogenesis. Enhanced comprehension of how DNA methylation modulates gene expression and cellular differentiation in the context of cancer is crucial for developing targeted therapeutic strategies and improving diagnostic approaches and preventive interventions for adipocyte-associated cancers.

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#### Conflict of interest

The authors declare that they have no conflict of interests.

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