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Research Paper: Lectin Histochemistry Showed a Heterogeneous Population of Cells Among Human Mesenchymal Stem Cells Isolated From Adipose Tissue

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ABSTRACT

Objectives: Adipose tissue as an appropriate source of Mesenchymal Stem Cells (MSCs) has the potential to differentiate into multiple lineages. Glycoconjugates content of the MSCs can be considered as biomarkers in self-renewal, pluripotency and differentiation processes. In this study, the lectin profile of MSCs isolated from adipose tissue was detected and according to that, a subpopulation was determined.

Materials & Methods: MSCs were isolated from adipose tissue by explanting of the tissue pieces. The FITC-conjugated lectins, WGA, UEA, PNA, BSA and PWM were used to detect the terminal sugar residues. The cells were then counterstained with DAPI. The intensity of the reaction was evaluated by ImageJ software. The cells were also stained with PAS method.

Results: MSCs were reacted with all lectins with different intensity of the reactions. The cells reacted with WGA, UEA, and BSA "strongly" and with PWM "moderately" and with PNA with "weak" intensity. The morphological analysis of the isolated MSCs revealed the existence of the two different cell types in the cultures. Two types of cells were detected according to nucleus size and lectin reactivity. The cells with large nuclei constitute 20.62% of the total cells and stained significant more intensity by UEA and less intense with PWM (both P=0.014) and PNA (P=0.044). Flow cytometry with CD34 shows that these large cells were not endothelial cells.

Conclusion: The MSCs derived from adipose tissue seem to be a heterogeneous populations and lectin profile of the cells showed that they are different in the expression of the glycoconjugates.

1. Introduction

Mesenchymal Stem Cells (MSCs) represented a reliable source for clinical cell therapy in recent decades [1]. This quiescent population of the cell has various niches in different specific tissues and they have self-renewal properties [2]. Also, MSCs derived from these sources can differentiate into various cell lineages [3]; and therefore, have roles in tissue regeneration. These cells can

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be isolate from different sources such as bone marrow, umbilical cord and adipose tissue. However, they are not a homogenous cell population. Previously, heterogeneity was shown in cord blood derived-MSCs [4] Wharton's jelly MSCs [5] and adipose-derived MSCs [6] Adipose tissue as a non-invasive source has less ethical concern. Comparing with bone marrow-derived MSC, adipose tissue contains a higher number of MSCs and showed the highest colony forming frequency [1].Adipose derived-MSCs (AD-MSC) have been shown to be a heterogeneous population according to the surface marker expression pattern, granularity and cell size [7] and nuclei size [4] They have different functional features, for instance, the cells with small nuclei has been reported to proliferate with higher rate [4].

As the cell surface covered by cell coat formed from carbohydrate motifs, they are the first part of the cells that encounter to the microenvironment including the other cells, extracellular matrix and growth factors and as a result they contribute in many cell functions and cell signaling [8]. Although there is no specific marker for MSCs [7], it has been suggested that glycans bind to proteins or lipids may introduce a surface marker for some type of stem cells. Any alternation in surface glycoconjugate pattern of the stem cells has been suggested to change cell behavior [9] Glycoconjugates can be considered as biomarkers during self-renewal, pluripotency and differentiation stages [10-12]. Glycomics was used to detect the cell surface carbohydrate content in MSCs, and it was found that expression level of the surface glycoconjugate depended on the type of extracellular matrix constitutes. It also demonstrated that the differentiated and undifferentiated MSCs contain different glycoconjugates [13] . Lectins, as a tool to recognize glycoconjugate, can influence the differentiation potential of mouse mesenchymal cells isolated from limb bud [14] and regulate cell proliferation of placental-derived MSCs [15]. Cell surface glycoconjugateaffinity to various lectinscan be used for isolating a specific cell type in a cell suspension [16].

Heterogeneous nature of the MSCs was reported by detecting of various surface CD markers. The presence of different morphological phenotypes also reported within MSC population and it has been suggested that this diversity may be due to the contamination with endothelial cells [7].The lack of a specific marker for MSCs makes it difficult to distinguish the subpopulations of MSCs. Therefore, the present study was undertaken to identify the presence of different lectin reactivity in the MSCs isolated from adipose tissue.

2. Material and Methods

This research is a descriptive study.

MSC isolation

Adipose tissue was collected from the patient undertaken liposuction surgery with informed consent. After washing the tissue with Phosphate Buffer Saline (PBS), they exposed to the equal volume of erythrocyte lysis solution and agitated for one min by vortex. Then, adipose tissue samples were incubated at 37℃ at a humid atmosphere containing 5% CO_2 for 15 min. Thereafter, centrifugation was done at 900g for 15 min. The tissue pieces were seeded into culture dishes and be allowed to attach to the base of it for 10 min. α-minimal essential medium (α-MEM, Gibco, USA) containing 10% of fetal bovine serum (FBS, Gibco, USA), 100U/mL penicillin/streptomycin(Gibco, UK) and 1% L-glutamine (Bioidea, Iran) was added to the plates. After 24 h, non-adherent tissues were removed by washing the cultures with PBS. The medium was changed every 5-7 days for 14-17 days till the cells reach 90% confluence.

MSC characterization

The isolated cells were harvested at passage three and suspended in the PBS containing 2% FBS. The cells were then resuspended in PBS containing FITC-conjugated-anti CD44, PerCp-conjugated anti CD105 and CD34 antibodies (all from Abcam, UK, Cambridge) for 25min at room temperature. The cells were then washed in PBS and subjected to the flow cytometry. To exclude background staining, the matched isotype control antibodies were used. The data were analyzed by WinMDI 2.9.

Lectin histochemistry

The cells were harvested and the smear was prepared. The cells allowed being air dried, washed in PBS and then were fixed in buffer formalin. After washing, the cultures were incubated with FITC-conjugated lectins (Sigma) at a dilution of 10µg/mL for 2 h at room temperature and dark condition. The lectins were Wheat Germ Agglutinin (WGA), Peanut Agglutinin (PNA), Banderiea Simplicifolia Agglutinin (BSA), Ulex Europeus Agglutinin (UEA) and Phytolacca Americana (PWM) that bind to sialic acid, galactose/N-acetylgalactoseamine, N-acetylglucoseamine (and also galactose), fucose and N-acetyl-β--glucosamine, Respectively. WGA also reacts to N-acetyl glucoseamine [15, 16]. The cells were then counterstained with Diamino-2-Phenylindole (DAPI) for 1 min and were mounted. The slides were

observed under the florescent microscope. Along with the lectin-treated slides, one smear was incubated with the solvent of the lectin for the same period of time and this slide was considered as negative control. The cell count and pixel intensity was evaluated by the free software ImageJ[1]. Then, they scored as arbitrary scoring system as follows: the pixel intensity up to 10 considered as "negative", the pixel intensity 11-25 "weak", the pixel intensity 26-40 "moderate" and the pixel intensity more than 40 was considered as "strong".

Periodic Acid Schiff (PAS)

The smears were fixed with buffer formalin and incubated in 1% periodic acid for 5 min. Then, the samples were incubated in Schiff reagent for 15 min. the samples were washed with tap water for 5 min.

Statistical analysis

The data analyzed by independed-sample t-test. SPSS 16.0 for windows was used to analyze the data and prism was used to depict the graphs. A P-value less than 0.05 was considered as significant difference. All experiments performed in triplicated.

3. Results

Flow cytometry analyses showed that the frequency of the adipose-derived MSCs expressed CD44 was 92.8% and CD105 was 46.12%. They fail to react with hematopoietic, CD34, marker (1.99%) (Figure 1). In the primary culture, the cells showed a fibroblast-like appearance. DAPI staining revealed two subpopulations among the adipose-derived MSCs according to the size of the nuclei. A subpopulation with large nuclei constituted 20.62% of the cells. The rest of the cells had smaller nuclei. Lectin histochemistry showed MSCs could react with all lectins; however, the intensity of the reactions was different among two subpopulatios. Both type of the cells reacted with WGA and BSA "strongly". The subpopulation with larger nuclei stained with PNA and PWM "weakly" and "moderately", respectively, and they showed a significantly lower staining intensity than small cells (P=0.044 and P=0.005, respectively) (Figure 2). The subpopulation with larger nuclei also reacted with UEA with "strong" intensity and they revealed a significant higher intensity than small cells (P=0.014). The subpopulation with small nuclei reacted with PNA "moderately" and with PWM "strongly". The Figure 3 compares the pixel intensity value for each subpopulation. PAS staining revealed the small cells stored neutral glycoconjugates in the cytoplasm; while, the cytoplasm of the cells with large nuclei remained unstained (Figure 3). The pixel intensity value of each lectin was summarized in Table 1.

1. http://imagej.nih.gov/ij/index.html

4. Discussion

The current study revealed the presence of a subset of cells according to the nuclear size and lectin reactivity. The heterogeneity in the MSC population from different resources was previously reported and it has been detected that cell contamination such as macrophages or endothelial cells dose not remain anymore. The heterogeneity was reported according to various criteria. According to the differentiation state, this heterogeneous cell population divided into two subgroups, stem cells and partially differentiated progenitor cells [7].

The subpopulation of progenitors also showed distinct self-renewal properties and various differentiation potentials [16]. Adipose-derived MSCs also are heterogeneous according to CD marker expression pattern [17]. They also contain subpopulations with different differentiation potential toward various lineages including osteoblasts and chondroblasts [18]. Besides, subpopulations of adipose-derived MSCs have been demonstrated

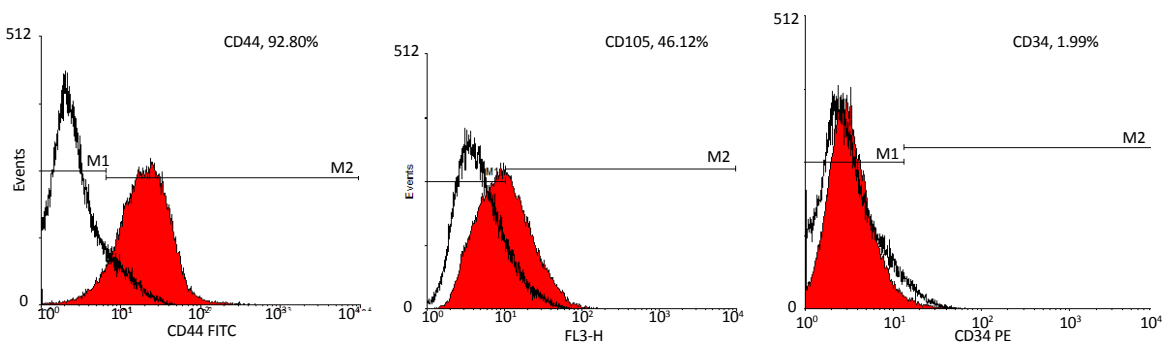

Figure 1. The flow cytometry shows adipose-derived MSCs express CD105 and CD44 but not CD34.

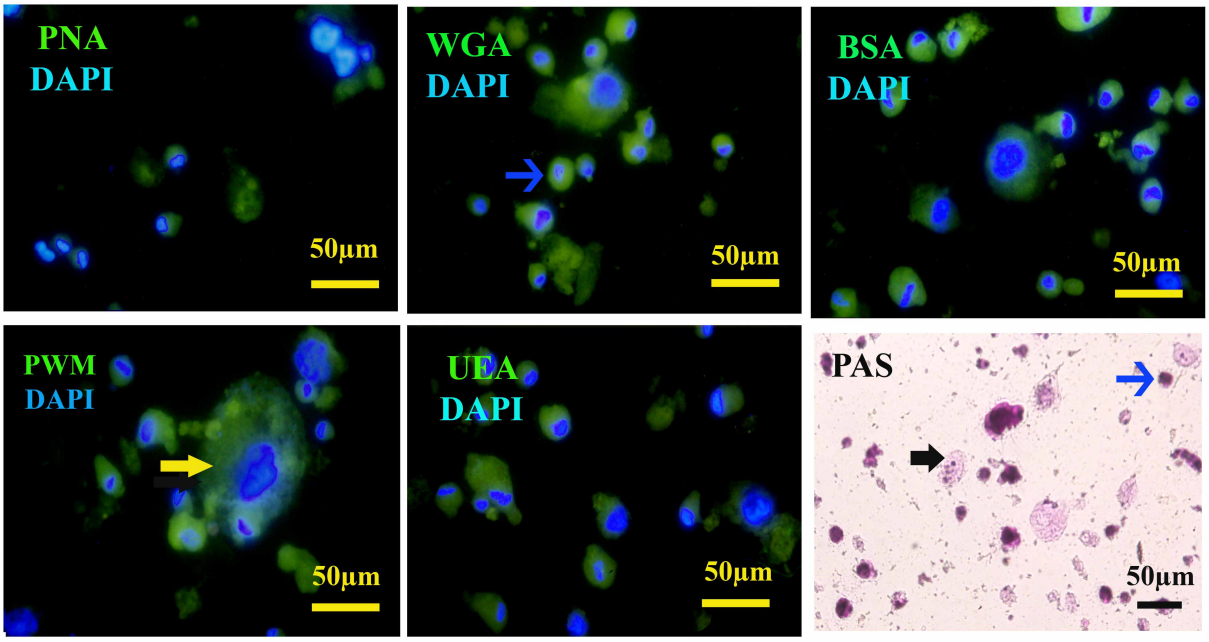

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Figure 2. Stained slides with FITC-conjugated lectins. Two types of the cells can be observed; the cells with large nuclei showed expanded and flat morphology (yellow arrow) and expressed a lower amount of PNA and PWM and higher amount of UEA-reacted glycoconjgugate. PAS staining also shows that the cells with small nuclei are PAS-Positive (blue arrow) and those with large nuclei are PAS-negative (black arrow).

to inhibit T-cell proliferation [19]. The current study confirms the heterogeneity according to lectin reactivity.

We found heterogeneity according to the nucleus size among adipose–derived MSCs. A previous report also detected the presence of these two subpopulations in colonies of adipose derived MSCs according the nuclear size. It has been shown that the cells with small nuclei had higher proliferation rate [6]. Besides, the data from present study showed that the cells with small nuclei were PAS-positive.

The PAS-positive cell subpopulation in cord blood derived MSCs has been introduced as stem cell [4]. With regard to this consideration, it is possible that the PAS-positive cells with small nuclei are stem cells. The glycoconjugates have different structural and functional roles in tissues [11]. They participate in cell proliferation, differentiation and cell-cell or cell-matrix interactions [12]. Many CD markers are glycoconjugates and contain sugar residues in their structure. Also, the cell with small nuclei has been reported to have higher proliferation rate [4]. PNA was reported to have mitogenic activity [20]. As our data indicated the cell subpopulation with small nuclei expressed a significant higher PNA reacted glycoconjugates. At the same time, we found higher number of such a cell in cultures and this may be due to the possible function of PNA-reacted glycoconjugates in cell proliferation.

UEA-positive cells have been reported in to be present in Wharton's jelly [21] and cord blood-derived MSC [22] populations. This subset of cells also detected in adipose derived MSCs and it was showed that they could differentiate into endothelial cells. However, the endothelial progenitor cells reported to have higher proliferation rate than MSCs and expressed CD34 [23]. Our data showed that the cell with larger nuclei expressed a significant higher level of UEA and it may indicate the presence of an endothelial progeni-

Table 1. The comparison of the pixel intensity and the arbitrary staining intensity of two adipose-derived subpopulations

	PNA	BSA	WGA	UEA	PWM
Large cells	24.19±4.7 Weak	49.9±22.2 Strong	40.56±15.67 Strong	50.89±22.08 Strong	30.67±12.64 Moderate
Small cells	29.87±7.93 Moderate	50.4±11.29 Strong	46.88±18.07 Strong	39.95±12.9 Moderate	45.91±18.6 Strong

JAMSAT

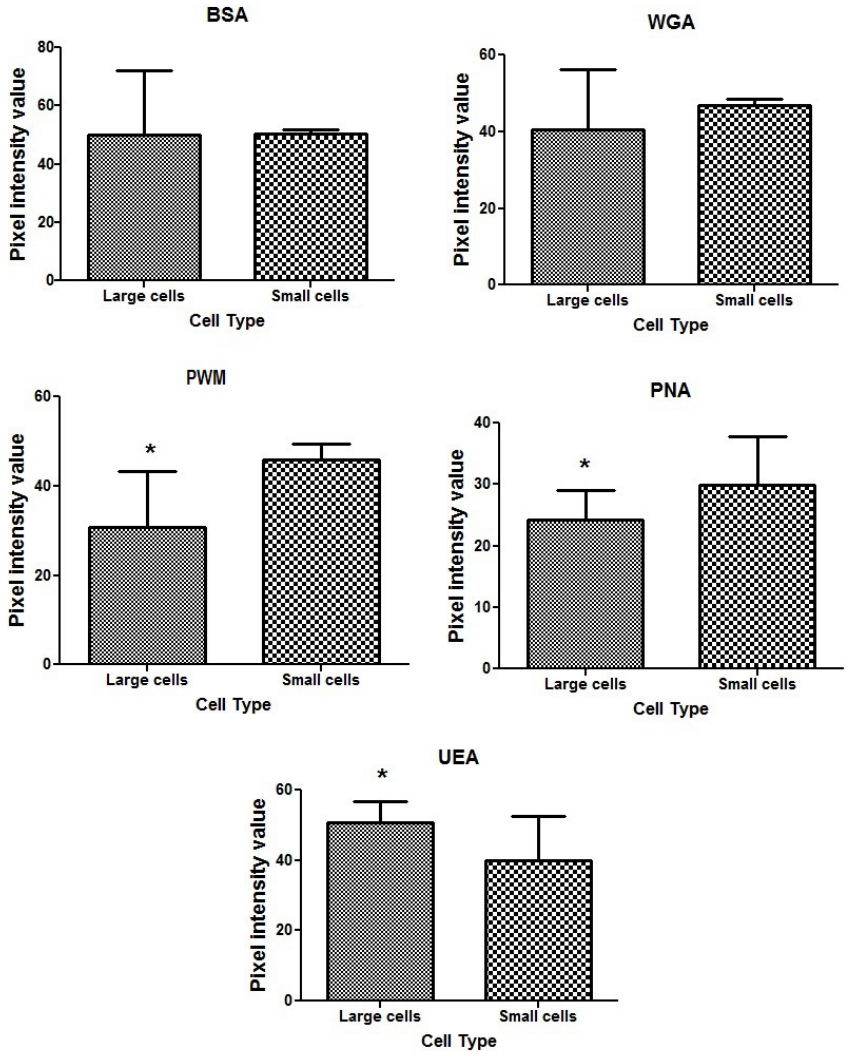

Figure 3. The comparison of the pixel intensity of the reaction of the cells with large and small nuclei (Significant difference with small cells (P=0.014))

tor cell subset. Nevertheless, this cells form a minor fraction of the population and also all populations were negative for CD34. However, to judge about the nature of such a cell, it needs to detect the other features including expression of endothelial marker expression and their attachment behavior.

Cell surface glycoconjugates can be used for isolating a specific cell type in a cell suspension [16]. The cell affinity to different lectins has been suggested as a tool to separate the cell subsets [24]; however, the expression of some markers depends on the isolation or flow cytometry techniques [25].

Therefore, if the culture condition standardized, lectin can be suggested as a tool for cell separation. Adipose derived MSCs have been reported to be heterogeneous according to the level of the expression of CD markers such as CD105 or CD73. CD105 negative cells has been demonstrated to be more susceptible to differentiate toward adipogenic and osteogenic cell lineages and also could inhibit the proliferation of $CD4^+$ T cells more effectively compared with the MSCs express CD105 [19]. The flow cytometry data also showed the frequency of CD105 positive cells was just 46.12%.

5. Conclusion

MSCs derived from adipose tissue showed heterogeneity according to their reaction with lectins and nuclear size. Lectins including PNA, PWA and UEA may be used to distinguish and separate the subpopulation.

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Conflict of Interest

The authors declared no conflicts of interest.

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