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# DIFFERENCES IN THE EXPRESSION OF OCT4A, SOX2, AND NANOG IN HUMAN MESENCHYMAL STEM/STROMAL CELLS DERIVED FROM ADIPOSE TISSUE AND UMBILICAL CORD

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**Abstract**

Mesenchymal stem/stromal cells (MSCs) derived from adult and perinatal tissues exhibit distinct biological and stemness characteristics due to their different developmental origins. These differences are reflected in their proliferative capacity, differentiation potential, and the expression of key pluripotency-related transcription factors: OCT4A, SOX2, and NANOG. We examined protein-level expression and localization of these factors in early-passage adipose tissue-derived MSCs (AT-MSCs) and umbilical cord-derived MSCs (UC-MSCs). OCT4A was more prevalent in UC-MSCs, while SOX2 was higher in AT-MSCs, and NANOG was predominantly expressed in a small subpopulation ($\sim$ 3%) of cells obtained from both sources. OCT4A was mainly localized in the nucleus, whereas SOX2 and NANOG showed mixed nuclear and perinuclear localization. Notably, UC-MSCs exhibited higher proliferative potential, which correlated with higher OCT4 levels. These findings reveal differences in the expression of the pluripotency-related transcription factors OCT4A and SOX2 depending on the source, whereas NANOG exhibits comparable expression levels across the studied MSCs.

**Key words:** OCT4, NANOG, SOX2, adipose tissue-derived stem/stromal cells, umbilical cord-derived stem/stromal cells

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**Introduction.** Mesenchymal stem/stromal cells (MSCs) are considered a promising therapeutic tool due to their regenerative and immunomodulatory properties. They are used in the treatment of autoimmune diseases, degenerative joint disorders, cardiovascular diseases, and nervous system injuries, with therapeutic effects mediated through tissue integration and paracrine immunomodulatory mechanisms [1]. The developmental origin and source of MSCs can significantly influence their biological characteristics. Perinatal tissues, including the umbilical cord and placenta, are enriched with MSCs that exhibit higher proliferative capacity, lower senescence rates, and distinct molecular profiles compared to MSCs derived from adult tissues, such as bone marrow and adipose tissue [2].

The pluripotency-related transcription factors OCT4, SOX2, and NANOG play critical roles in self-renewal and differentiation in embryonic stem cells and induced pluripotent stem cells [3, 4]. In humans, OCT4 undergoes alternative splicing to produce several isoforms with distinct functions and regulatory mechanisms [5]. Of these, OCT4A has been the most extensively studied; its exogenous expression alone is sufficient to induce and maintain a pluripotent state in differentiated cells [6]. While the expression of OCT4A, SOX2, and NANOG is well-characterized in pluripotent stem cells, emerging evidence suggests that these factors may also be present at lower levels in MSCs. Such expression may contribute to MSC stemness, self-renewal capacity, and differentiation potential. However, the expression patterns and functional relevance of these pluripotency-related transcription factors across MSCs derived from different sources remain incompletely characterized.

Umbilical cord-derived MSCs (UC-MSCs) are considered more primitive and developmentally immature compared to adult tissue-derived MSCs, such as adipose-derived MSCs (AT-MSCs). This has led to the hypothesis that UC-MSCs may retain higher expression levels of pluripotency-related genes than AT-MSCs and exhibit enhanced stemness characteristics. In MSCs, the expression of these genes is generally lower than in pluripotent stem cells, and their biological significance remains controversial. It has been reported that the expression of pluripotency markers may support MSC self-renewal, enhance proliferative potential, and increase differentiation plasticity [7,8]. Direct comparisons of OCT4A, SOX2, and NANOG expression in MSCs derived from umbilical cord and adipose tissue remain limited. Conducting such comparisons could provide valuable insights into the stemness characteristics of UC-MSCs and AT-MSCs.

The present study aims to investigate and compare the expression of OCT4A, SOX2, and NANOG in UC-MSCs and AT-MSCs.

**Material and methods.** ***Ethics statement.*** The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Hospital of Obstetrics and Gynecology "Maichin Dom" (DN 25-05-69, 6 November 2018).

***Isolation and culture of human mesenchymal stem cells.*** UC-MSCs

were isolated from the Wharton's jelly of umbilical cords ($n = 4$) using the explant method, as previously described [9]. AT-MSCs were obtained from human adipose tissues collected as surgical waste ($n = 4$, donor ages 26 to 52 years). The adipose tissue was minced and digested with 0.1% collagenase IA (Sigma-Aldrich, USA) in serum-free low-glucose Dulbecco's Modified Eagle's Medium (DMEM, PAN-Biotech, Germany) at 37 °C for 2 h. Following digestion, the cell suspension was centrifuged at 300×g for 5 min, and the cell pellet was resuspended in DMEM, supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA) and 1% Penicillin/Steptomycin/Amphotericin B Mix (PAN-Biotech, Germany). Cells were cultured at 37 °C in 5% $CO_2$, with the medium changed every 2 days. MSCs at the third passage were used for all experiments.

***Evaluation of cell growth.*** UC-MSCs and AT-MSCs were seeded into 96-well plates (Corning, USA) at a density of $2 \times 10^3$ cells per well. The MTT assay was performed every 24 hours over a 7-day culture period. After a 4-hour incubation with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, USA) the resulting formazan salts were dissolved in 120 µL of dimethyl sulphoxide (DMSO, Sigma Aldrich, USA). Optical density readings were then taken at 544 nm using a FLUOstar Optima microplate reader (BMG Labtech, Germany), with DMSO serving as a blank control. A calibration curve of OD versus known cell number was generated to convert OD values to estimated cell numbers. Population doubling time (PDT) was calculated using:

$$\text{PDT} = \text{duration(hours)} \times \log(2)/\log(\text{FinalConcentration}) - \log(\text{InitialConcentration}).$$

***Flow cytometry.*** To assess the intracellular expression of OCT4A, SOX2, and NANOG, MSCs were harvested, washed with PBS, and fixed with 4% paraformaldehyde (Serva, Germany) at room temperature for 20 min. After permeabilization with 0.1% Triton X-100 (Merck, Germany) at 4 °C for 1 h, the cells were incubated with primary anti-human OCT4A, SOX2, and NANOG antibodies (10 µg/mL; R&D Systems, USA) for 1 h, followed by incubation with FITC- and Cy3-conjugated secondary antibodies (2 µg/ml, Invitrogen, USA) for one h. The stained cells were analyzed using a FACS Calibur flow cytometer (BD Biosciences, USA).

***Immunofluorescence staining.*** MSCs were cultured on glass coverslips for 48 h, washed with PBS, and fixed with 4% formaldehyde (Sigma-Aldrich, USA) at room temperature for 20 min. After a subsequent wash with PBS, the cells were blocked with 1% bovine serum albumin (BSA, Sigma-Aldrich, USA) and permeabilized with 0.1% Triton X-100 (Merck, Germany) in PBS at room temperature for 1 h. The samples were incubated overnight at 4 °C with primary antibodies: monoclonal mouse anti-OCT4A, mouse anti-SOX2, and polyclonal goat anti-NANOG (10 µg/mL, R&D Systems, USA). Following washing with PBS,

the cells were incubated with species-specific secondary antibodies conjugated to Alexa Fluor 488 (2 µg/ml, Invitrogen, USA) at room temperature for 1 h. Nuclei were stained with 10 µg/mL Hoechst 33258 (Sigma-Aldrich, USA) for 5 min. The coverslips were mounted onto glass slides and imaged using a Leica TCS SPE confocal microscope (Leica Microsystems, Germany).

**Results.** UC-MSCs and AT-MSCs exhibited an elongated, fibroblast-like morphology (Fig. 1A). UC-MSCs appeared noticeably larger than AT-MSCs, and statistical analysis confirmed significant differences in both cell length and width (Fig. 1B). Population doubling times (PDTs), calculated from the growth curves of UC-MSCs ($n = 4$) and AT-MSCs ($n = 4$) at passage 3, indicated that UC-MSCs had a shorter PDT ($25.2 \pm 7.5$ h) than AT-MSCs ($56.3 \pm 20.2$ h), with less variability observed in UC-MSCs (Fig. 1C).

Immunofluorescence staining revealed expression of pluripotency-related transcription factors OCT4A and SOX2 in both UC-MSCs and AT-MSCs (Fig. 2). OCT4A expression was predominantly localized in nuclei. In contrast, SOX2 exhibited both nuclear and perinuclear cytoplasmic localization, with its nuclear expression being less distinctly defined than that of OCT4A. Notably, NANOG was detected at very low levels in both UC-MSCs and AT-MSCs, with localization to the nucleus and cytoplasm. These observations were further confirmed by flow cytometry analysis (Fig. 3). UC-MSCs showed a higher percentage of OCT4A-positive cells (58 ± 5%) compared to AT-MSCs (34 ± 7%), whereas SOX2 expression was more prominent in AT-MSCs (43 ± 4%) than in UC-MSCs (32.6 ± 6%). NANOG-positive cells constituted a small fraction of both populations, approximately 3%.

**Discussion.** The OCT4A, SOX2, and NANOG have been suggested to influence the stemness-related properties of MSCs [10]. The expression patterns of these transcription factors show considerable variability among MSCs from different tissue sources as well as between studies [10, 11].

Here, we focused on detecting OCT4A, SOX2, and NANOG at the protein level in AT-MSCs and UC-MSCs at early passage. Protein detection provides stronger evidence for functional relevance, as protein expression is more directly linked to biological activity. It is important to consider that pluripotency-related gene expression in MSCs can change during in vitro culture – for example, OCT4 and SOX2 levels increase with prolonged culture in BM-MSCs and AT-MSCs, although they remain much lower than in pluripotent cells [12]. In our comparative study, all MSCs were cultured under similar conditions, and experiments were performed using third-passage cells. This design minimizes culture and passage-related effects, ensuring that observed differences reflect the intrinsic characteristics of AT-MSCs and UC-MSCs.

The observed nuclear localization of OCT4A in both UC-MSCs and AT-MSCs is consistent with its established role as a transcription factor regulating stemness properties, as previously reported in MSCs from various tissue sources

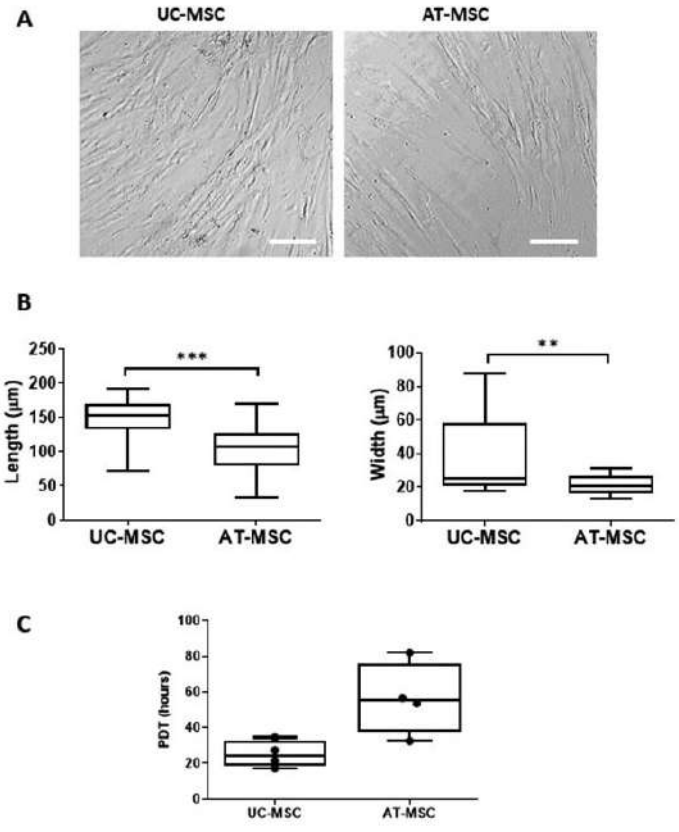



Fig. 1. Morphology and proliferation of UC-MSCs and AT-MSCs. (A) Phase-contrast images of UC-MSCs and AT-MSCs showing elongated, fibroblast-like morphology. Scale bars represent 200 µm. (B) Quantitative analysis of cell length and width. UC-MSCs were significantly longer and wider than AT-MSCs. Box plots represent data expressed as mean ± standard deviation ($n = 150$). Statistical significance is indicated as $p < 0.001$ (***) for length and $p < 0.005$ (**) for width. (C) Population doubling time (PDT) of UC-MSCs and AT-MSCs. AT-MSCs exhibited a longer PDT compared to UC-MSCs, indicating slower proliferation. Box plots represent data expressed as mean ± standard deviation ($n = 4$)

[11]. OCT4A interacts with SOX2 to form a complex that activates pluripotency-related genes while repressing differentiation-associated genes, and the loss of SOX2 can compromise MSC self-renewal and stemness [13]. The mixed nuclear and perinuclear cytoplasmic localization of SOX2 aligns with prior observations in MSCs, where partial cytoplasmic distribution has been associated with regulated transcriptional activity and potential post-translational modifications affecting its nuclear import [14]. Regarding NANOG Trivanović et al. [11] reported nuclear

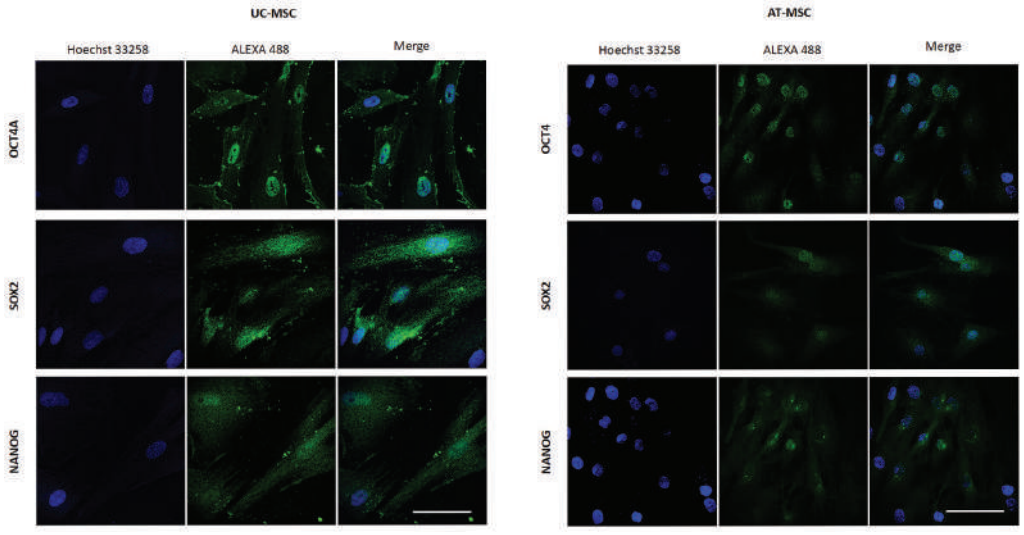



Fig. 2. Immunofluorescence analysis of pluripotency-related transcription factors in UC-MSCs and AT-MSCs. Representative images show the expression of OCT4A, SOX2, and NANOG in UC-MSCs and AT-MSCs. Nuclei were stained with Hoechst 33258 (blue), and markers were visualized using Alexa Fluor 488 (green). Scale bars represent 50 µm

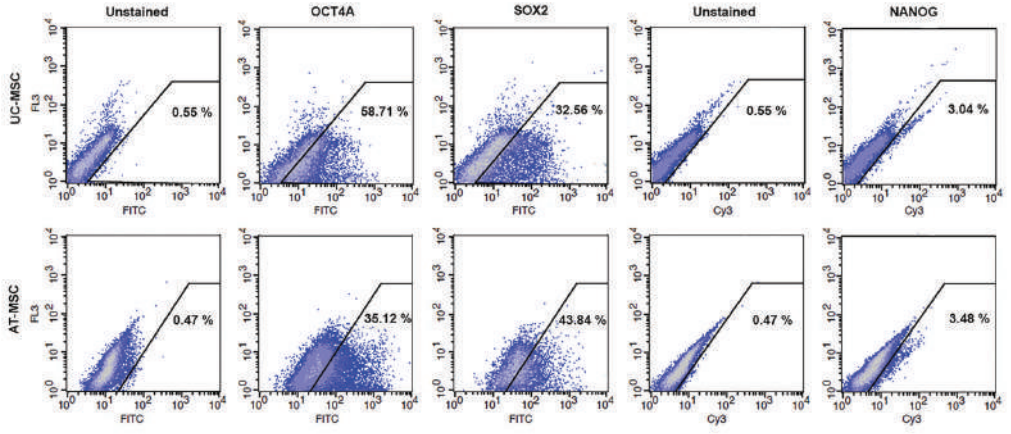



Fig. 3. Flow cytometry analysis of OCT4A, SOX2, and NANOG expression in UC-MSCs and AT-MSCs. Unstained controls define gates, and percentages of positive cells are shown

and cytoplasmic localization of NANOG in both UC-MSCs and AT-MSCs, which is in agreement with our observation.

Furthermore, our analysis revealed that OCT4A was expressed in more than half of UC-MSCs, while fewer than 40% of AT-MSCs exhibited OCT4A positivity. Conversely, we observed higher SOX2 expression in AT-MSCs, detected in approximately 40% of cells, compared to about 30% in UC-MSCs. NANOG expression was consistently low in both UC-MSCs and AT-MSCs, remaining below

4%. Collectively, these data support the concept that perinatal MSCs preserve a more primitive molecular signature than MSCs derived from adult tissues [14].

In human MSCs, SOX2 and NANOG have been extensively investigated at the transcript level; however, comprehensive analyses at the protein level remain limited, and their protein expression patterns are still incompletely characterized. SOX2 expression in MSCs has been implicated in the regulation of cell proliferation [15]. However, evidence regarding SOX2 expression in MSCs is still inconclusive. While Park et al. [15] reported higher expression levels of SOX2 in UC-MSCs compared with AT-MSCs, Pierantozzi et al. [16] did not detect SOX2 expression, as well as OCT4 expression, in AT-MSCs.

The available data suggest that NANOG expression in AT-MSCs follows a passage-dependent pattern, with no detectable expression in freshly isolated cells (P0), a marked increase during early passages (P1–P9), with an average of $36 \pm 16\%$, and a pronounced decline to approximately $3 \pm 3\%$ in late passages (P10–P22) [16]. UC-MSCs have been shown to exhibit very low NANOG protein expression under normoxic conditions ($3 \pm 1\%$), which increases under hypoxia ($26 \pm 6\%$), although the majority of cells remain NANOG-negative [17].

Our observations demonstrate that adult AT-MSCs exhibit a lower proliferative potential than UC-MSCs, consistent with previous reports [18]. We also observed that UC-MSCs displayed a higher proliferative capacity and a greater proportion of OCT4A-positive cells. Interestingly, infant-derived AT-MSCs have been reported to exhibit higher proliferative capacity and lower levels of cellular senescence and replicative stress than UC-MSCs [19]. Overall, adult AT-MSCs demonstrate reduced proliferative capacity compared with UC-MSCs, likely due to age-associated cellular senescence and limited self-renewal potential

**Conclusions.** OCT4A and SOX2 are differentially expressed in UC-MSCs and AT-MSCs: OCT4A is more abundant in UC-MSCs, and SOX2 is higher in AT-MSCs. By contrast, NANOG is present at extremely low levels in both cell types. These findings reveal source-dependent differences in the transcriptional network related to stemness in MSCs, which may influence their regenerative potential and differentiation capacity.

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