Pluripotency and Differentiation Capacity of Human Adipose-Derived Stem Cells from Subcutaneous and Visceral Fat

Abdul Malik Setiawan
Department of Anatomy, Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University
Fairus Ahmad
Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia
Adila A. Hamid
Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia
Abdul Kadir Abdul Karim
Department of Obstetrics and Gynecology, Faculty of Medicine, Universiti Kebangsaan Malaysia
Taty Anna Kamarudin (tatykamarudin@ukm.edu.my)
Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia

Short Report

Keywords: ADSC, pluripotent, BMP4, subcutaneous, visceral, OCT4

Posted Date: November 30th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3653093/v1

Additional Declarations: No competing interests reported.

Abstract

Adipose derived stem cells (ADSC), a type of mesenchymal stem cell (MSC) found in the vascular stroma of human fat tissues. It showed promising potentials as an alternative source for tissue engineering. Octamer-binding transcription factor 4 (OCT4) regulates pluripotency in ADSC, while bone morphogenetic protein 4 (BMP4) signalling is essential for the differentiation of ADSC towards ectodermal lineages.

Objective: The expression of BMP4 and OCT4 in primary ADSCs from different adipose tissue depots were assessed.

Method: Subcutaneous and visceral adipose tissues were harvested from the abdomen of 6 females, processed and ADSCs were isolated. The expression of *BMP4*and *OCT4* were assessed at P4 and compared to ADSC cell line as a control group.

Results: The expression of *OCT4* and *BMP4* were higher in primary ADSC from both subcutaneous and visceral locations compared to the control. The *OCT4* and *BMP4* expression in visceral and subcutaneous ADSCs showed a different pattern. Majority (67% and 100%) of the visceral ADSCs expressed higher OCT4 and BMP4 respectively compared to subcutaneous ADSCs. These findings suggest a potential correlation between the ADSCs' differential capacity and its location, explained by the interplays between underlying regulatory pathways of pluripotency and ectodermal differentiation.

Introduction

Corneal blindness is a major public health concern worldwide, affecting millions of people [1, 2]. The most common treatment for corneal blindness is corneal transplantation using donated human corneal tissue [3]. However, the shortage of corneal donors and the risk of graft rejection have limited the success of this approach [4]. Corneal tissue differentiation using adipose-derived stem cells (ADSC) has been proposed as a potential alternative to human donor as it is abundant and easily accessible [5]. Previous studies have shown that ADSC can be differentiated into corneal epithelial cells under appropriate conditions [6]. Several methods have been developed to differentiate ADSC into corneal-like cells, including the use of BMP4 signalling pathway modulators [7–9]. However, the efficacy of differentiation method still needs to be improved to achieve a viable corneal epithelial layer for transplantation [10].

Therefore, elucidating the expression of *BMP4* and *OCT4* in ADSC and its potential role in therapeutic applications for corneal epithelial tissue engineering may have important implications for the development of new cell-based therapies for corneal diseases. This study aimed to investigate the expression of *OCT4* and *BMP4* in primary ADSC from different adipose tissue depots. The findings may offer useful insights for the development of tissue engineering strategies for corneal epithelial regeneration.

Background

ADSC is found in adipose tissues and it is widely distributed throughout the body. Adipose tissue can be classified into two main types: subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). SAT is located directly under the skin, while VAT is located around the organs in the abdominal cavity [11]. Studies have shown that VAT has a higher risk of metabolic dysfunction, inflammation, and insulin resistance compared to SAT [12]. Additionally, it has been suggested that the location of adipose tissue may affect the characteristics of ASCs, including their differentiation potential [13]. A study by Tang in 2017 showed that ADSC isolated from VAT had greater proliferation capacity compared to ADSC isolated from SAT. However, ADSC isolated from SAT had greater differentiation capacity compared to ADSC isolated from VAT [14].

Octamer-binding transcription factor 4 (OCT4) is a member of the POU (Pit, Oct, Unc) transcription factor family and plays a central role in pluripotency regulation. It is highly expressed in pluripotent cells, but its expression is silenced during differentiation. OCT4 is naturally expressed at low levels in early passages of human ADSC, and their levels progressively decrease as the cells' passage number increases. It has been demonstrated that the overexpression of OCT4 in ADSC increases their proliferative activity and their capacity to differentiate into adipocytes or osteoblasts.

Several studies have examined the role of BMP4 in ADSC and its potential therapeutic applications specifically for corneal epithelial tissue engineering [7, 15–18]. Bone morphogenetic protein 4 (BMP4) is a member of the transforming growth factor beta (TGF-β) superfamily that regulates a variety of cellular processes, including differentiation, proliferation, and apoptosis [19]. BMP4 signalling is essential for the differentiation of ADSC into corneal epithelial cells, and manipulating BMP4 signalling can enhance the efficiency of corneal epithelial tissue engineering [20, 21]. However, the role of BMP4 signalling in ADSC differentiation into corneal epithelial cells has not been fully investigated. Therefore, understanding the role of BMP4 in ADSC differentiation may provide insights into the mechanisms underlying corneal epithelial tissue engineering and the development of new therapeutic strategies.

Method

Materials and reagents

Unless otherwise specified, all cell culture reagents were obtained from Invitrogen-Gibco (Inchinnan, UK). TRIzol® reagent (Invitrogen, USA) was utilized for RNA extraction, whereas LunaScript RT SuperMix and qPCR Master mix (New England Biolabs, UK) were utilized for cDNA synthesis and quantitative polymerase chain reaction (qPCR). All the primers used are listed in Table S1 in a supplemental document.

Adipose tissue collection

Subcutaneous and visceral adipose tissue were collected from consented 6 females (mean age 34.3 ± 7.4 years, range 27–47 years) undergo caesarean section or laparotomy at Obstetrics and Gynecology department of Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia (UKM). The procedure

was reviewed and approved by the Universiti Kebangsaan Malaysia Medical Research and Ethics committee (JEP-2019-705) and completed with signed patient's informed consent. Inclusion criteria were female gender, age below 50 years. Exclusion criteria were having cancer, on chemotherapy treatment and having peritoneal dialysis. Approximately 2–5 mg of subcutaneous and visceral adipose tissue samples were collected from each patient. The samples were kept in cold 50 ml of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM:F12) supplemented with 1% of antibiotic-antimitotic and processed immediately.

Isolation, culture and expansion of human adipose-derived stem cells

The subcutaneous and visceral adipose tissue were processed and isolated using stromal vascular fraction (SVF) isolation method as previously described with some modification [22, 23]. In brief, the adipose tissue samples were minced and washed with sterile PBS to remove blood. Sample were then digested using 0.3% collagenase type I solution and incubated for 4 hours at 36°C. Tissue samples were then centrifuged at 700 g for 10 minutes to isolate the SVF. The SVF cells were cultured in DMEM:F12 supplemented with 10% foetal bovine serum (FBS), 1% vitamin C, 1% ascorbic acid, 1% of antibiotic-antimitotic and 1% glutamax in 6-well plates. The cells were seeded at 10,000 cells/cm^2 until confluence and expanded up to passage 4 (P4).

ADSC cell line purchased from the Centre for Tissue Engineering and Regenerative Medicine (CTERM), UKM was used as a positive control. The cells were cultured in DMEM:F12 supplemented with 10% foetal bovine serum (FBS), 1% vitamin C, 1% ascorbic acid, 1% of antibiotic-antimitotic and 1% glutamax until confluence and multiplied up to P6 and used as a positive control of pluripotent cells.

RNA Isolation and RT-PCR

RNA was extracted from the cultured cells using the Trizol method according to the company guideline. The concentration and quality of RNA were determined using a Nanodrop spectrophotometer. The expression levels of *OCT4* and *BMP4* genes were evaluated by quantitative real-time PCR (qPCR) using the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene as a reference gene. Results were analysed using the $2^{-\Delta\Delta Ct}$ method and expressed as the fold change in gene expression relative to the ADSC cell line [24, 25].

Statistical Analysis

Standard error of the mean (SEM) was used to present the gene expression results. GraphPad Prism version 9.0.0 for Windows, (GraphPad Software, San Diego, California, USA, www.graphpad.com) was used to analyse the data. One-way analysis of variance (ANOVA) was used to determine the statistical significance for comparisons made within the groups while student t-test was used to evaluate the statistical significance between subcutaneous and visceral ADSC groups. P values of 0.05 or less were considered as statistically significant.

Results

Adipose tissue samples and ADSC morphology

A total of six donors have consented to participate in this study. Adipose tissues samples from the subcutaneous and visceral layers were collected from the six patients as detailed in Table S2 as in a supplemental document.

Histological morphology observations at passage 4 showed that subcutaneous ADSC had spindle-shaped form similar to the ADSC cell line, while the visceral ADSC exhibit multipolar or stellate shapes. The subcutaneous ADSC had similar oval or elongated nucleus shape with the ADSC cell line while visceral ADSC had rounded or irregular nucleus shape as shown in Fig. 1.

OCT4 expression

The pooled expression of *OCT4* in subcutaneous and visceral ADSC groups were significantly higher compared to the control (ADSC cell line) ($p < 0.0001$) (results not included). However, individual comparisons showed 4 out of 6 subcutaneous samples (66%) significantly expressed higher *OCT4* compared to the control. Only 2 visceral samples (33%) significantly expressed higher *OCT4* compared to the control (Figure S1 in the supplemental documents). Interestingly, four patients showed higher expression of *OCT4* in visceral ADSC compared to their respective subcutaneous samples (Fig. 2).

BMP4 expression

The pooled expression of the *BMP4* in subcutaneous ADSC and visceral groups were significantly different when compared to the control ($p < 0.0001$) (results not included). However, individual comparisons of *BMP4* expression of each sample to the control showed only 4 out of 6 (66%) subcutaneous ADSC samples had higher expression of *BMP4*, while all the visceral ADSC expressed higher *BMP4* (Figure S2 in the supplemental documents). Furthermore, the *BMP4* expression in visceral ADSC from all patients were significantly higher compared to their respective subcutaneous samples (Fig. 3).

Discussion

OCT4 gene has long been recognized as a crucial regulator of pluripotency in embryonic stem cells, and its expression in adult stem cells has gained interest in recent years [26]. However, conflicting findings regarding *OCT4* expression in stable and differentiated adult cell populations have been reported [27]. Our study focused on ADSCs, which are considered as an attractive source of stem cells for regenerative therapies [5, 13].

Our results indicated that the relative expression of the *OCT4* gene in primary ADSC from both subcutaneous and visceral groups were significantly higher compared to that of ADSC cell line. Four out of 6 patients showed higher *OCT4* expression in visceral ADSC than the subcutaneous ADSC. The higher

relative expressions of *OCT4* in visceral ADSCs suggests that ADSC from this depot may possess a higher pluripotency as previously discussed by Takahashi and colleagues [28]. Interestingly, the higher expressions of *BMP4* were observed in all visceral ADSCs. These suggest that the visceral ADSC may have higher differentiation potential as discussed previously by Setiawan and colleagues [9].

The BMP family of proteins plays a crucial role in regulating cell differentiation and tissue development [19]. In our study, we found significant differences in the relative expression of BMP4 in subcutaneous and visceral ADSCs compared to the ADSC cell line. However, the proportion of visceral ADSC samples that showed a significantly higher expression on *BMP4* were larger compared to the subcutaneous ADSC (100% vs 50%). This suggests that the BMP pathway may be differentially regulated between subcutaneous ADSC and visceral ADSC. In addition, 4 out of 6 samples showed a significantly higher expression of *BMP4* in visceral ADSC when compared to the respective subcutaneous ADSC of the same patient. These findings highlight a promising differentiation capacity of visceral ADSC. The enhanced expression of *BMP4* in visceral ADSCs may be indicative of depot-specific molecular characteristics and could contribute to the observed functional differences between subcutaneous and visceral adipose tissues [29].

The different expression of *BMP4* between subcutaneous and visceral ADSCs suggests that adipose tissue depots may harbour distinct regulatory mechanisms governing their respective stem cell populations [30]. Depot-specific differences in the microenvironment, including variations in cell-cell interactions, paracrine signalling, and extracellular matrix composition, may contribute to the observed disparities in *BMP4* expression [31]. Understanding these depot-specific regulatory mechanisms could provide valuable insights into the functional variations of both subcutaneous and visceral adipose tissues and aid in the development of finely tailored regenerative therapies.

Conclusion

This study offers an insight into the expression patterns of *BMP4 and OCT4* genes in primary ADSCs from subcutaneous and visceral depots. The different *BMP4* expression levels exhibited by the ADSC from the two depots suggested that they were regulated differently in the same individual. Further investigations are warranted to elucidate the underlying molecular mechanisms and to explore the functional implications of these findings in the context of ADSC-based regenerative medicine.

Limitations

This study was limited to microscopy observation and *OCT4 and BMP4* gene expressions analyses. Sample collection process were complicated due to the COVID-19 restrictions in the hospital. Therefore, only 6 samples were utilized in this study.

Declarations

Ethics approval and consent to participate

All methods were caried out conducted in accordance with relevant guidelines and regulation reviewed and approved by the Universiti Kebangsaan Malaysia Medical Research and Ethics committee (UKMMREC) with approval number JEP-2019-705. All participants agreed to participate in the study and signed an informed consent form. The signed consent forms were kept confidential and safe and available upon request.

Availability of data and material

The data used to support the findings of this study are available from the corresponding author upon request.

Funding

This research was funded by the Fundamental Research Grant, awarded by the Faculty of Medicine Universiti Kebangsaan Malaysia, FF-2019-540.

Acknowledgements

We would like to thank the Department of Anatomy and the surgeons from the Department of Obstetrics and Gynaecology, Hospital Chancellor Tuanku Muhriz for the help in sample collection and the laboratory works.

Consent for publication

Not applicable.

Competing interests

Authors declare that they have no competing interests.

Author’s contributions

AMS, FA, AAH, AKAK and TK conceived and designed experiments. AMS conducted the experiment. AMS and TK analysed the data. AMS and TK wrote the manuscript. All authors read and approved the final manuscript.

References

1. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli M V., et al. Global causes of blindness and distance vision impairment 1990–2020: a systematic review and meta-analysis. Lancet Glob Heal. 2017;5:e1221–34.
2. Wang EY, Kong X, Wolle M, Gasquet N, Ssekasanvu J, Mariotti SP, et al. Global Trends in Blindness and Vision Impairment Resulting from Corneal Opacity 1984–2020: A Meta-analysis. Ophthalmology. 2023;:1–9.

3. Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F, et al. Global survey of corneal transplantation and eye banking. JAMA Ophthalmol. 2016;134:167–73.
4. Williams AM, Muir KW. Awareness and attitudes toward corneal donation: Challenges and opportunities. Clin Ophthalmol. 2018;12:1049–59.
5. Alió del Barrio JL, De la Mata A, De Miguel MP, Arnalich-Montiel F, Nieto-Miguel T, El Zarif M, et al. Corneal Regeneration Using Adipose-Derived Mesenchymal Stem Cells. Cells. 2022;11:1–21.
6. Bandeira F, Goh TW, Setiawan M, Yam GHF, Mehta JS. Cellular therapy of corneal epithelial defect by adipose mesenchymal stem cell-derived epithelial progenitors. Stem Cell Res Ther. 2020;11:1–13.
7. Petry L, Kippenberger S, Meissner M, Kleemann J, Kaufmann R, Rieger UM, et al. Directing adipose-derived stem cells into keratinocyte-like cells: impact of medium composition and culture condition. J Eur Acad Dermatology Venereol. 2018;32:2010–9.
8. Venugopal B, Shenoy SJ, Mohan S, Anil Kumar PR, Kumary T V. Bioengineered corneal epithelial cell sheet from mesenchymal stem cells—A functional alternative to limbal stem cells for ocular surface reconstruction. J Biomed Mater Res - Part B Appl Biomater. 2020;108:1033–45.
9. Setiawan AM, Kamarudin TA, Abd Ghafar N. The role of BMP4 in adipose-derived stem cell differentiation: A minireview. Front Cell Dev Biol. 2022;10 October:1–8.
10. Zhang A, Zhang W, Backman LJ, Chen J. Advances in Regulatory Strategies of Differentiating Stem Cells towards Keratocytes. Stem Cells Int. 2022;2022 Figure 1.
11. Oikonomou EK, Antoniades C. The role of adipose tissue in cardiovascular health and disease. Nat Rev Cardiol. 2019;16:83–99.
12. Chait A, den Hartigh LJ. Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. Front Cardiovasc Med. 2020;7 February:1–41.
13. Arnhold S, Elashry MI, Klymiuk MC, Geburek F. Investigation of stemness and multipotency of equine adipose-derived mesenchymal stem cells (ASCs) from different fat sources in comparison with lipoma. Stem Cell Res Ther. 2019;10:1–20.
14. Tang Y, Pan ZY, Zou Y, He Y, Yang PY, Tang QQ, et al. A comparative assessment of adipose-derived stem cells from subcutaneous and visceral fat as a potential cell source for knee osteoarthritis treatment. J Cell Mol Med. 2017;21:2153–62.
15. Lin J, Zhu Q, Huang J, Cai R, Kuang Y. Hypoxia Promotes Vascular Smooth Muscle Cell (VSMC) Differentiation of Adipose-Derived Stem Cell (ADSC) by Regulating Mettl3 and Paracrine Factors. Stem Cells Int. 2020;2020.
16. Hasani S, Javeri A, Asadi A, Taha MF. Cardiac differentiation of adipose tissue-derived stem cells is driven by BMP4 and bFGF but counteracted by 5-azacytidine and valproic acid. Cell J. 2020;22:273–82.
17. Maeda Y, Hasegawa T, Wada A, Fukai T, Iida H, Sakamoto A, et al. Adipose-derived stem cells express higher levels of type VII collagen under specific culture conditions. Arch Dermatol Res. 2017;309:843–9.

18. Yogi A, Rukhlova M, Charlebois C, Tian G, Stanimirovic DB, Moreno MJ. Differentiation of adipose-derived stem cells into vascular smooth muscle cells for tissue engineering applications. Biomedicines. 2021;9:1–16.
19. Grafe I, Alexander S, Peterson JR, Snider TN, Levi B, Lee B, et al. TGF-β family signaling in mesenchymal differentiation. Cold Spring Harb Perspect Biol. 2018;10:1–49.
20. Shen S, Wang S, He Y, Hu H, Yao B, Zhang Y. Regulation of bone morphogenetic protein 4 on epithelial tissue. J Cell Commun Signal. 2020;14:283–92.
21. Hu H, Wang S, He Y, Shen S, Yao B, Xu D, et al. The role of bone morphogenetic protein 4 in corneal injury repair. Exp Eye Res. 2021;212:108769.
22. Hamid AA, Idrus RBH, Saim A Bin, Sathappan S, Chua KH. Characterization of human adipose-derived stem cells and expression of chondrogenic genes during induction of cartilage differentiation. Clinics. 2012;67:99–106.
23. Ritter A, Friemel A, Fornoff F, Adjan M, Solbach C, Yuan J, et al. Characterization of adipose-derived stem cells from subcutaneous and visceral adipose tissues and their function in breast cancer cells. Oncotarget. 2015;6:34475–93.
24. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 2001;25:402–8.
25. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55:611–22.
26. Sohn EJ, Moon HJ, Lim JK, Kim DS, Kim JH. Regulation of the protein stability and transcriptional activity of OCT4 in stem cells. Adv Biol Regul. 2021;79 December 2020:100777.
27. Zangrossi S, Marabese M, Broggini M, Giordano R, D'Erasmo M, Montelatici E, et al. Oct-4 Expression in Adult Human Differentiated Cells Challenges Its Role as a Pure Stem Cell Marker. Stem Cells. 2007;25:1675–80.
28. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell. 2007;131:861–72.
29. Russo V, Yu C, Belliveau P, Hamilton A, Flynn LE. Comparison of Human Adipose-Derived Stem Cells Isolated from Subcutaneous, Omental, and Intrathoracic Adipose Tissue Depots for Regenerative Applications. Stem Cells Transl Med. 2014;3:206–17.
30. Pan Z, Zhou Z, Zhang H, Zhao H, Song P, Wang D, et al. CD90 serves as differential modulator of subcutaneous and visceral adipose-derived stem cells by regulating AKT activation that influences adipose tissue and metabolic homeostasis. Stem Cell Res Ther. 2019;10:1–18.
31. Ritter A, Friemel A, Roth S, Kreis NN, Hoock SC, Safdar BK, et al. Subcutaneous and visceral adipose-derived mesenchymal stem cells: Commonality and diversity. Cells. 2019;8:1–23.

Figures

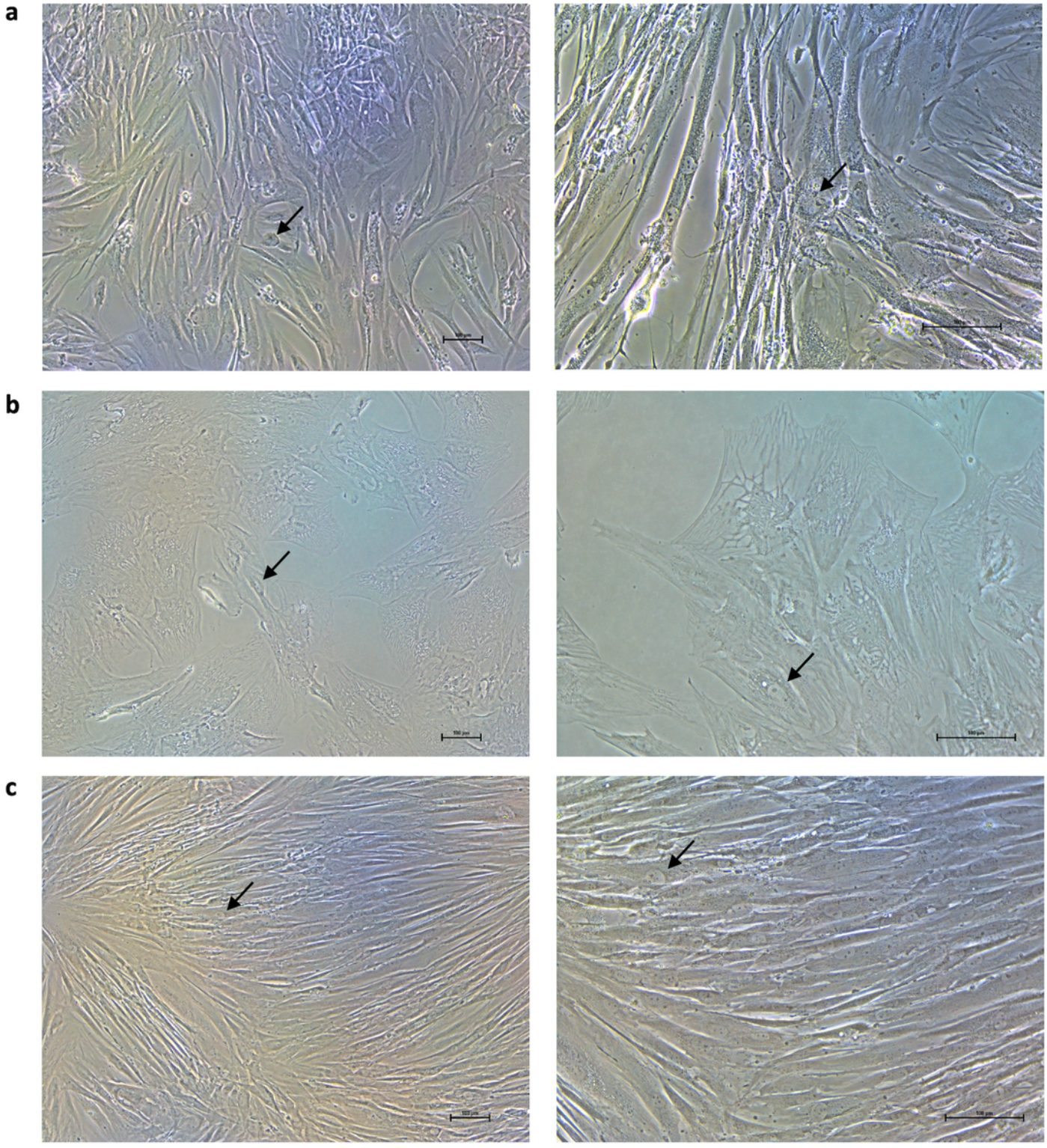

Figure 1

Subcutaneous ADSC show a typical spindle like cells similar with the ADSC cell line compared to the visceral ADSC that more rounded. Arrows are showing cell nuclei. a = subcutaneous ADSC (10x and 20x objectives respectively). b = visceral ADSC (10x and 20x objectives respectively). c = ADSC cell line (10x and 20x objectives respectively). Scale bar = 100 mm for all image.

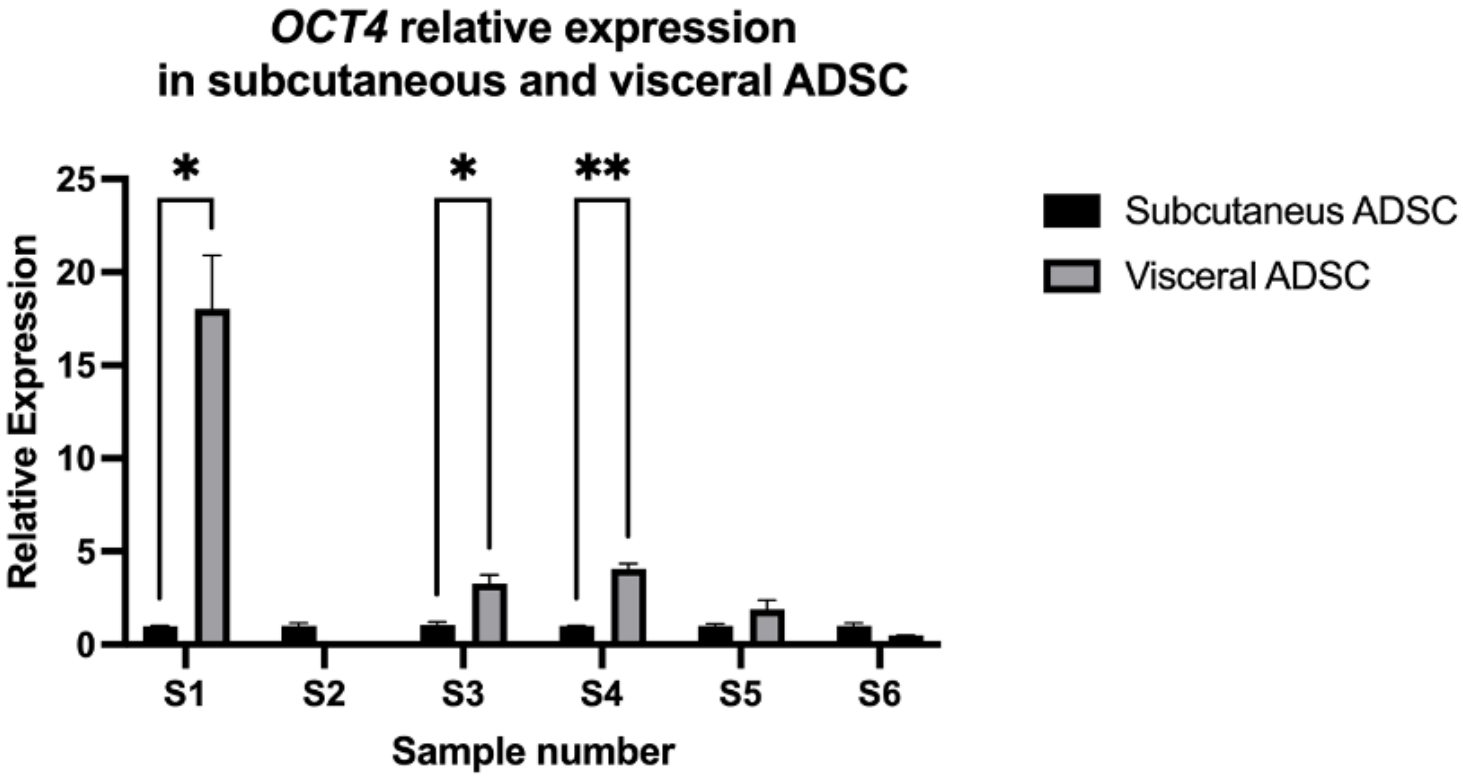

Figure 2

Relative expressions of OCT4 in subcutaneous and visceral ADSC. *p<0.05, **p<0.

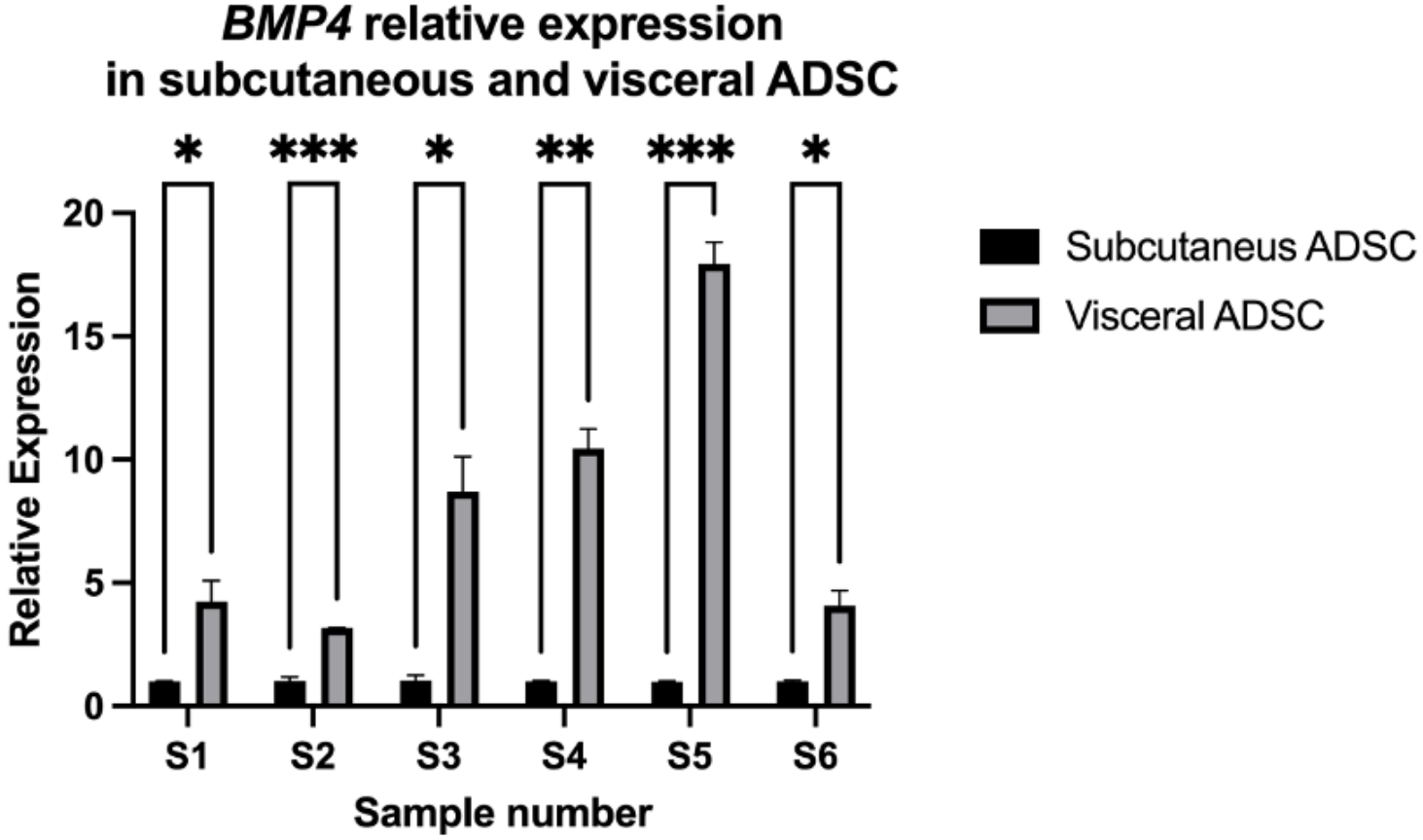

Figure 3

Relative expressions of *BMP4* in subcutaneous and visceral ADSC. *p<0.05, **p<0.01, ***p<0.005.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.png
- FigureS2.png
- TableS1.Primerdesigndocx.docx
- TableS2.Donorsanthropometriccharacteristics.docx
- VisualAbstract.png