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The perivascular phenotype and behaviors of dedifferentiated cells derived from human mature adipocytes

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ABSTRACT

Derived from mature adipocytes, dedifferentiated fat (DFAT) cells represent a special group of multipotent cells. However, their phenotype and cellular nature remain unclear. Our study found that human DFAT cells adopted perivascular characteristics and behaviors. Flow cytometry and immunofluorescent staining revealed that human DFAT cells positively expressed markers highly related to perivascular cell lineages, such as CD140b, NG2 and desmin, but were negative for common endothelial markers, including CD31, CD34, and CD309. Furthermore, DFAT cells displayed vascular network formation ability in Matrigel, and they noticeably promoted and stabilized the vessel structures formed by human umbilical vascular endothelial cells (HUVECs) in vitro. These results provide novel evidence on the pericyte nature of human DFAT cells, further supporting the recent model for the perivascular origin of adult stem cells, in which tissue-specific progenitor cells in mesenchymal tissues associate with blood vessels, exhibiting perivascular characteristics and functions.

1. Introduction

Mature adipocytes exhibit dynamic plasticity and can be cultured into a special type of multipotent, proliferating cells, called dedifferentiated fat (DFAT) cells [1]. At first, the source of DFAT cells was adipose tissue from the bone marrow of metacarpal bones in neonatal calves [2,3]. Subsequent research has demonstrated that subcutaneous white adipose tissue (WAT) from several species, including human [4–6], rat [7], mouse [8], cattle [9,10], porcine [11], ovine [12], and rabbit [13], can give rise to DFAT cells. In addition, mature adipocytes derived from intermuscular or perimuscular fat can also give rise to DFAT cells [9,14]. The ceiling culture technique, first reported by Sugihara at 1986, has frequently been used to obtain DFAT cells [2].

Because of their dynamic multipotency, DFAT cells are considered a new source of adult stem cells. Our team and several other groups have reported that DFAT cells are able to differentiate into adipocytes [6,8,15,16], osteoblasts [8,15], chondrocytes [15], skeletal myocytes [17], cardiomyocytes [7] and smooth muscle-like cells [4,18]. However, their cellular nature and differentiation stage remained unclear. Yagi et al. and Nobusue H et al. established a novel pre-adipocyte cell line from subcutaneous fat tissue of mice via ceiling culture, suggesting that DFAT cells shared a similar shape and characteristics with 3T3-L1 pre-adipocytes [19,20]. A subsequent study from the same group demonstrated that DFAT–P cells derived from porcine subcutaneous fat tissue were pre-adipocytes akin to the 3T3-L1, 3T3-F442A, and RMB-AD pre-adipocyte cell lines [21]. Our previous study also showed that DFAT cells are more able to differentiate adipogenically than osteogenically [22].

Interestingly, other reports have asserted that human DFAT cells can trans-differentiate into endothelial cells in vivo [23] or in vitro [24]. By contrast, in this study, we did not find that DFAT cells demonstrated endothelial phenotypes even in the presence of several key angiogenic factors and cytokines. However, for the first time, we proved that human DFAT cells exhibited a series of pericytic characteristics. Furthermore, these cells could significantly promote and stabilize endothelial networks formed by human umbilical vascular endothelial cells (HUVECs). These findings provide direct evidence supporting the hypothesis that pericytes might serve as a common reservoir of multiple tissue-specific progenitors, in which the stemness of different tissue-specific

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progenitors is recruited to nascent microvascular walls during development and postnatal growth [25,26].

2. Materials and methods

2.1. Establishment of human DFAT cells

Our study was approved by the Ethics Committee of State Key Laboratory of Oral Diseases, Sichuan University. Samples of human white adipose tissue were obtained from the buccal fat pad area of patients undergoing maxillofacial surgery in the West China Hospital of Stomatology, Sichuan University. Written informed consent was obtained from patients before surgery.

The isolation protocol resembles the standard method described previously [7]. To avoid contamination with other cells, the sample was carefully washed three times in phosphate-buffered saline, and then, white adipose tissues were minced and treated with 0.1% Type II collagenase at 37 °C for 40 min. After filtration and repeated centrifugation, the top layer, containing unilocular adipocytes, was collected. The in vitro dedifferentiation method was the ceiling culture technique reported by Sugihara in 1986 [2]. After the cells were transferred into culture flasks completely filled with high-glucose DMEM containing 20% fetal bovine serum (FBS, J R Scientific, Inc., Canada), the flasks were inverted and incubated at 37 °C in a humidified atmosphere with 5% CO_2. The floating adipocytes attached to the top surface of the flasks.

Purchased HUVECs (Lonza Walkersville Inc., Walkersville, MS, USA) were maintained in EGM-2 medium (EBM-2 endothelial culture medium supplemented with the EGM-2 MV kit, Lonza Walkersville Inc., Walkersville, MS, USA).

2.2. Flow cytometry

When DFAT cells and HUVECs reached 85–90% confluence, flow cytometry analysis was conducted. DFAT cells, both cultured in DMEM with 20% FBS and EGM-2 MV, and HUVECS were detached with 0.25% trypsin-EDTA (Invitrogen, Canada), before being suspended in PBS at a density of 1×10^6 cells/ml. For each sample, 500 μl of the cell suspension was blocked by PBS with 1% bovine serum albumin (BSA, Vector Laboratories, Inc., USA) and then incubated for 30 min with the following mouse anti-human phycoerythrin (PE)-conjugated antibodies: PE-CD31 (platelet endothelial cell adhesion molecule, PECAM); PE-CD34; PE-CD105 (endoglin); PE-CD106 (vascular cell adhesion molecule-1, VCAM-1); PE-CD140b (platelet-derived growth factor receptor beta, PDGFRβ); PE-CD146 (melanoma cell adhesion molecule, MCAM); PE-α-SMA (alpha-smooth muscle actin); and PE-NG2 (neuron-glial 2, a chondroitin sulfate proteoglycan). The corresponding PE-conjugated mouse IgG antibodies were used as isotype controls. Anti-α-SMA and anti-NG2 antibodies were obtained from R&D Systems (USA), whereas the others were purchased from BD Biosciences (USA). Flow cytometry recording and analysis were performed with the Guava EasyCyte Mini System and CytoSoft Software (Guava Technologies, USA).

2.3. Angiogenic induction and analysis

Angiogenic induction was induced by the EGM-2 MV culture medium, which contained vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and epidemic growth factor (EGF). At the same time, DFAT cells and HUVECs were also cultured in DMEM with 20% FBS and EGM-2 MV, respectively. At indicated time, cells were fixed with 2% formaldehyde at 4 °C for 20 min and blocked with 1% BSA in PBS for 30 min. Then, the cells were incubated at 37 °C for 3 h with mouse anti-human NG2 (R&D System Inc., USA) or mouse anti-human desmin (BD Biosciences, USA). After 3 washes with PBS, Alexa-488 goat anti-mouse immunoglobulin (Molecular Probes, Invitrogen, USA) was employed as a secondary antibody. Finally, the nuclei were counterstained with DAPI for 3 min. All of the above incubations were performed at 4 °C with proper light protection. Images were obtained by laser-scanning microscopy (LSM710, Zeiss, Germany). Flow cytometry analysis was also conducted with antibodies against CD31, CD309, CD140b, CD146, α-SMA and NG2.

2.4. Matrigel assay

For Matrigel assays, 24-well plates were coated with 200 μl/well of Matrigel (BD Bioscience, USA), and the cells were added at a density of 1×10^4/well. DFAT cells and HUVECs were planted separately or combined at a 1:1 ratio in DMEM with 10% FBS or in EGM-2 MV medium. At the indicated times, the cells were blocked with PBS with 1% BSA and incubated with primary vWF and NG2 antibodies for 3 h at 37 °C and 5% CO_2, followed by incubation with Alexa-488- and Alexa-568-conjugated secondary antibodies. Sprouting and vascular formation were monitored and recorded by inverted microscopy and LSM710 microscopy.

2.5. Statistical analyses

The data are expressed as the mean ± SD. Statistical analysis was performed with two-tailed Student's t-tests for pairwise comparisons or one-way ANOVA for multiple comparisons, using SPSS13.0; $P < 0.05$ was considered significant.

3. Results

3.1. Establishment of human DFAT cells

At first, the lipid portion within the adipocytes broke into small droplets, decreasing gradually. After 2–3 weeks of ceiling culture, all lipid droplets eventually disappeared. At the same time, the cells lost their round contour, shifting into a fibroblast-like morphology with flat bodies and multiple processes. These phenomena resembled the reports of previous studies [6,7,19,20]. The adipocytes were considered dedifferentiated when they displayed fibroblast-like, lipid-free morphology and regained the ability to proliferate (Fig. 1).

3.2. Characteristics of human DFAT cells

Flow cytometry analysis displayed that human DFAT cells in this study did not express endothelial cells or endothelial progenitor cells makers, such as CD31, CD34, CD106, and CD146. However, they were demonstrated markers typically associated with perivascular cell lineages, such as CD140b and NG2. Those results implied that DFAT cells might resemble the characteristics of pericytes (Fig. 2). In our experiments, this expression pattern remained stable and persistent from the 1st passage as far as the 25th passage.

3.3. Angiogenic induction and analysis

Although the plasticity of human ASCs toward endothelial cells has previously been verified by several studies [27,28], the angiogenic differentiation ability of DFAT cells has not been thoroughly studied. In Planat-Bernard's report, Matrigel plugs with human DFAT cells were injected into mouse ischemic muscle models, and the neovascularization elicited was found to be similar to the implantation of human adipose stromal vascular fraction (SVF) cells [23]. In our in vitro study, DFAT cells were induced in an endothelial

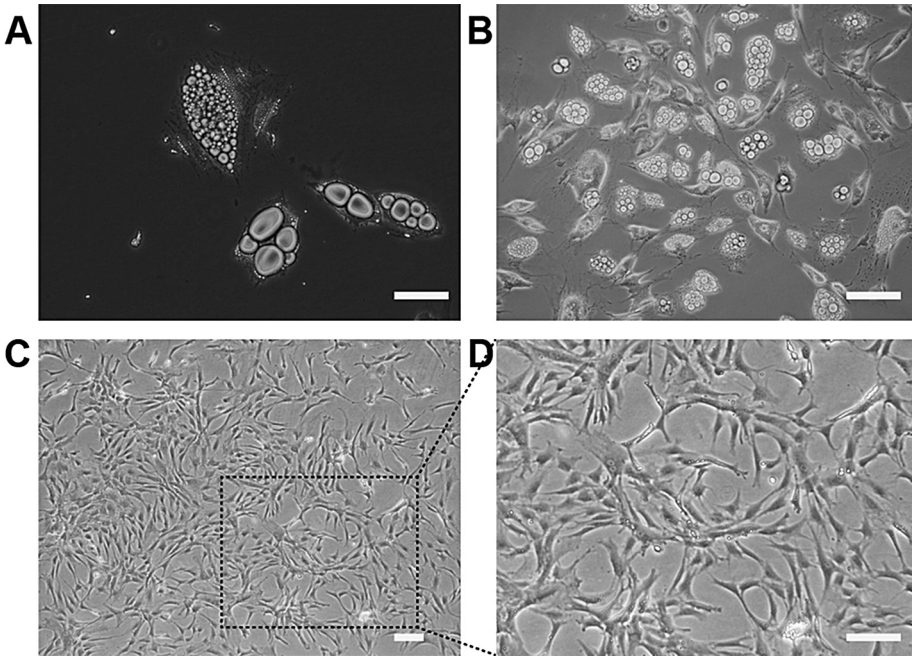

Fig. 1. Establishment of human DFAT cells. (A) At the 5th–7th day, the lipid portion within the adipocytes began to break into small droplets, and the cells lost their round contour. (B) The lipid amount decreased significantly, and the cells became flat with multiple processes extended. (C) (D) After approximately two weeks of ceiling culture, the liquid droplets disappeared, and the adipocytes shifted into a fibroblast-like morphology. (Bar length, 100 μm).

cell medium containing angiogenic growth factors, including bFGF and VEGF, which are commonly used in the angiogenesis of ASCs and other MSCs. Consistent with our previous findings, briefly, all cells extended elongated processes and aligned along a certain direction after induction. Flow cytometry analysis also demonstrated that DFAT cells did not express the endothelial cell markers like CD31, and CD309 before or after induction. However, both immunofluorescent staining and flow cytometry analysis showed strong expression of pericyte related markers, including NG2, CD146, CD140b, and desmin, in all groups of DFAT cells (Fig. 3), although NG2-positive cells decreased from the original 97.69 ± 3.32% to 57.14 ± 6.41% after culture in EGM-2 MV medium for 7 days (Fig. 3). The findings in this section suggest that the human DFAT cells in this study exhibited pericyte characteristics without the potential to differentiate toward endothelial cells.

3.4. Network formation ability of human DFAT cells on Matrigel

Under the influence of angiogenic cytokines, human DFAT cells demonstrated strong networking formation capacity on Matrigel (Fig. 4, A). In particular, DFAT cells sprouted out immediately after being seeded and established interconnections within the first 6 h, compared with the 12 h noted for HUVECs. In the following 6 h, more DFAT cells converged at the intersections, and the sprouts of several cells joined together to form branches. Approximately 12 h after seeding, the vascular clusters stopped increasing and connecting. The entire network structure produced by the DFAT cells remained stable for another 24 h, after which, it began to break. The interconnection process of HUVECs appeared to be slower, and the network reached stability after the first 24 h and remained stable for at least 48 h. In addition, the average size of the grids formed by

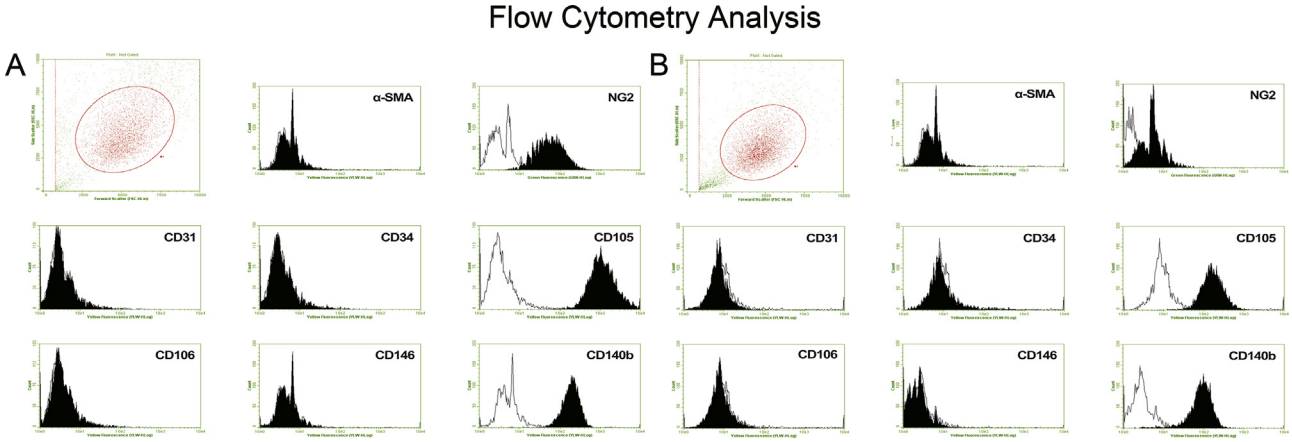

Fig. 2. Phenotype of human DFAT cells by flow cytometry. (A) DFAT cells cultured in DMEM. (B) DFAT cells cultured in EGM-2 MV. After being cultured in DMEM or EGM-2 MV, the human DFAT cells in this study positively expressed NG2 (97.69 ± 1.32% and 57.14 ± 6.41%, respectively), CD140b (95.53 ± 0.33% and 89.38 ± 0.49%, respectively), CD105 (96.53 ± 0.44% and 90.01 ± 1.65%, respectively), but negative for CD31 (3.85 ± 0.45% and 2.84 ± 0.17%, respectively), CD34 (2.87 ± 0.38% and 1.46 ± 0.57%, respectively), CD106 (3.68 ± 1.03% and 6.91 ± 2.26%, respectively), CD146 (2.11 ± 0.84% and 1.02 ± 0.21%, respectively) and α-SMA (1.24 ± 0.78% and 2.54 ± 0.12%, respectively).

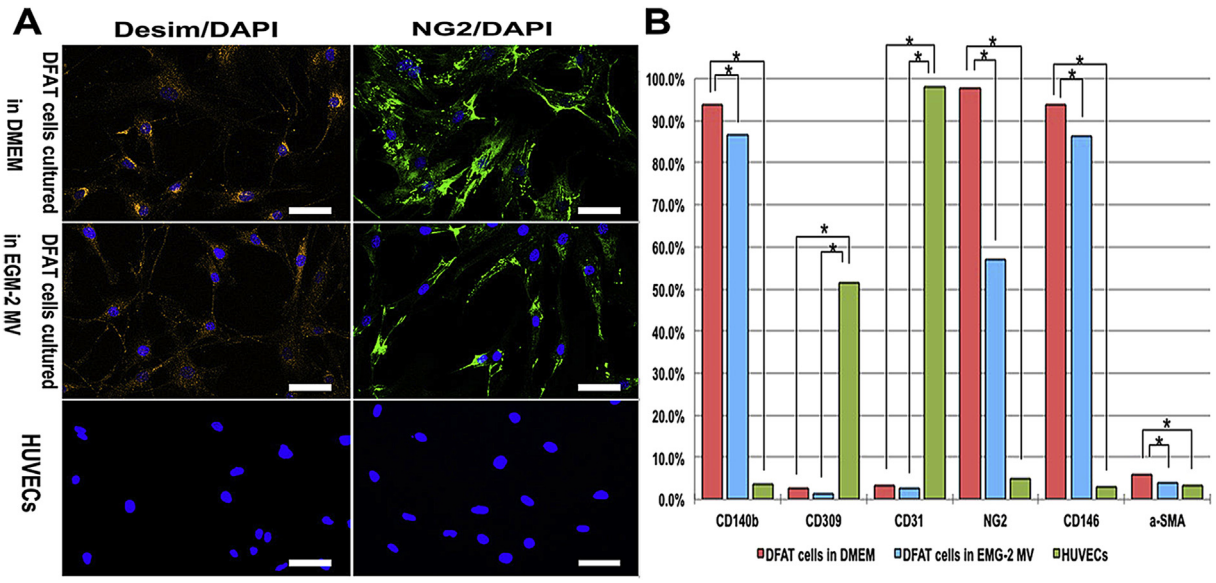

Fig. 3. Angiogenic induction of human DFAT cells. (A) Immunofluorescent staining and confocal microscopy evaluation after 7 days of angiogenic induction (bar length, 50 μm). DFAT cells cultured in endothelial medium were positive for the perivascular lineage markers NG2 and desmin, which were not expressed by HUVECs. (B) Flow cytometry analysis was performed before and after angiogenic induction for 7 days. DFAT cells in this study were consistently negative for the endothelial cell markers CD31 (3.18 ± 0.62% and 2.74 ± 0.20%, respectively) and CD309 (2.73 ± 0.41% and 1.38 ± 0.26%, respectively) but positive for pericyte markers CD140b (93.86 ± 1.61% and 86.36 ± 6.62%, respectively) and NG2 (97.69 ± 1.32% and 57.14 ± 6.41%, respectively). The HUVECs showed the opposite trends. *$P < 0.05$.

HUVECs was significantly smaller than that of DFAT cells. When the two cell groups were cultured together in a 1:1 ratio, both the formation speed and the duration of the vascular network were considerably improved. The subsequent immunofluorescent microscopy indicated that the vascular structures were positive for NG2 and vWF, which confirmed that both DFAT cells and HUVECs participated in the vascularization process (Fig. 4, B). When DFAT cells were grown on Matrigel in DMEM, many fewer interconnections between the cells were observed than in the results above (data not shown), suggesting that pro-vascular cytokines such as VEGF [29], bFGF or EGF might be indispensible to DFAT cells for the activation of the perivascular ability.

4. Discussion

DFAT cells have not been thoroughly characterized, but the multipotency of DFAT cells has been relatively accepted. Their high similarity to pre-adipocytes, except for the multilineage differentiation, also drew researchers' attention [19,20]. However, our study focused on the perivascular nature and behaviors of DFAT cells. Perivascular cells, also called pericytes, are contractile cells that wrap around the endothelial cells of capillaries and venules throughout the body. Pericytes become embedded in the basement membrane, where they communicate with endothelial cells via both direct physical contact and paracrine signaling [30]. Our further findings support the pericytic phenotype and behaviors of DFAT cells.

In our study, flow cytometry (Fig. 2) and immunofluorescence (Fig. 4, B) indicated that DFAT cells were nearly a homogenous $NG2^{+}CD140b^{+}Desmin^{+}\alpha\text{-}SMA^{-}CD146^{-}$ cell group. Although there is no specific molecular marker for pericytes, several combinations of markers have helped identify them [30,31]. Commonly used markers include surface proteins, such as NG2, PDGFRβ (CD140b) and MCAM (CD146), as well as certain intracellular proteins, such as desmin, α-SMA, regulator of G protein signaling 5 (RGS-5), and aminopeptidases A and N [30,32,33]. The expression patterns of those markers above have been found to be dynamic and origin dependent. Most previous research on pericytes used NG2 for cell identification, and PDGFRβ (CD140b) has been the most widely studied molecule in pericytes [30]. However, α-SMA has appeared to be less definitive in the identification of pericytes because it also expressed by vascular smooth muscle cells (VSMCs). Furthermore, α-SMA was not found in the pericytes in skin and brain [32].

The key roles of pericytes in vessel formation include sensing the presence of angiogenic stimuli, regulating the endothelial cell proliferation and differentiation, and supporting vessel growth as a scaffold [30,34]. Several studies have found pericytes to be capable of guiding sprouting formation by migrating ahead of endothelial cells, after which, endothelial cells secrete vascular cytokines to facilitate vessel maturation [30,35,36]. Furthermore, pericytes serve the function of stabilizing, monitoring and contributing to endothelial cell maturation and survival [37]. Interestingly, in our study, DFAT cells exhibited the pericyte function of promoting and stabilizing the neovascularization process. DFAT cells not only independently established a network on Matrigel but also accomplished this task more rapidly than HUVECs. Without endothelial cells, the DFAT cell network was not sustained for very long. However, when the two cell groups worked together, the formation and stabilization of the vascular structures were significantly improved (Fig. 4). These findings suggested that human DFAT cells invested actively in sprouting and modeling vessels in vitro, and subsequent involvement of endothelial cells might lead to vessel maturation and stabilization.

The behaviors of human DFAT cells in the neovascularization process above should not be interpreted as a result of differentiation toward endothelial cells. Before and after induction with several key angiogenic cytokines, our human DFAT cells never expressed any endothelial cell markers such as CD31, CD34, or CD309 (Figs. 2 and 3), which was consistent with previous phenotypic analysis of DFAT cells [15]. However, this result was distinct from Planat-Bernard's report that when DFAT cells were injected into a mouse ischemic muscle model, numerous neovessels were identified by CD31 labeling [23]. Based on the immunofluorescent staining in the Matrigel assay (Fig. 4), we

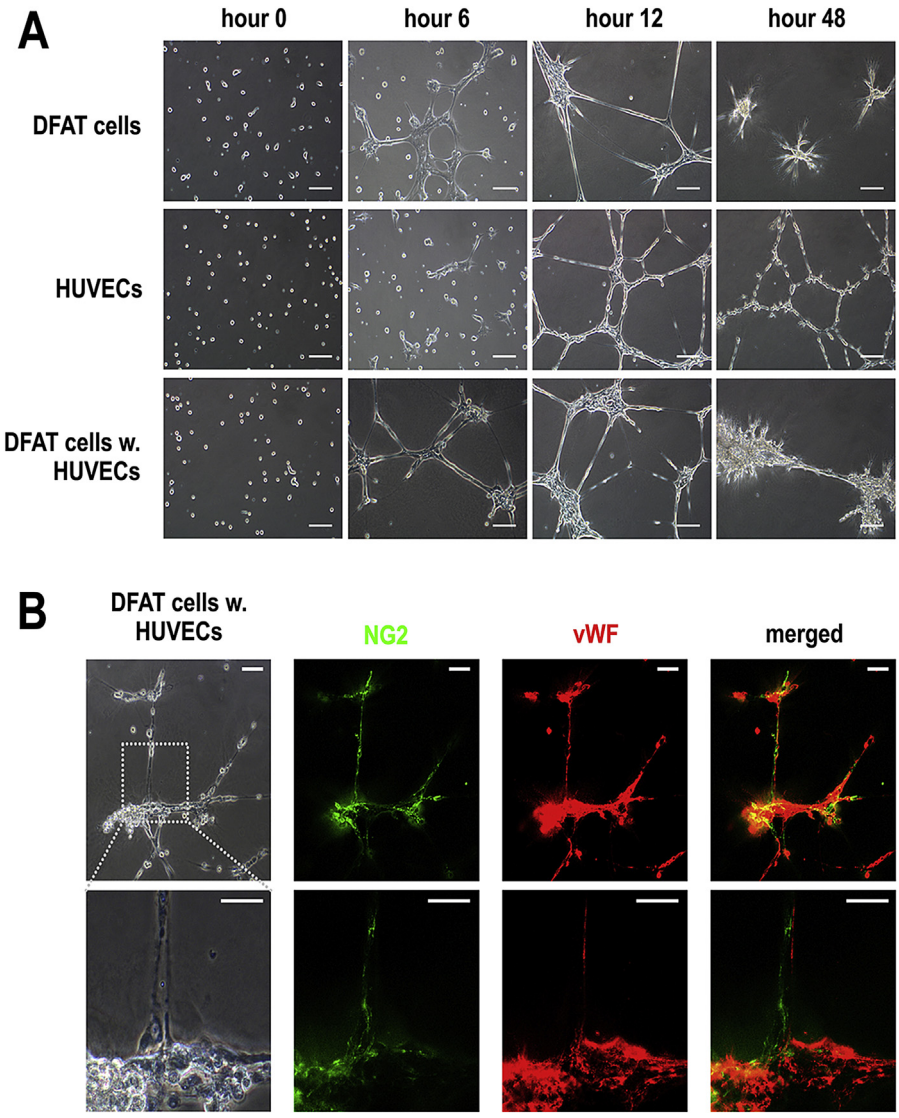

Fig. 4. Vascular network formation ability on the Matrigel. (A) DFAT cells established their interconnections faster than HUVECs, but the connections lasted for a shorter time (bar length, 100 μm). When the two cell types were seeded together, the network grew at the speed of DFAT cells and had a lifespan similar to that of HUVECs. (B) Immunofluorescent analysis of the network formed by DFAT cells and HUVECs, indicated by NG2 (green) and vWF (red), respectively (bar length, 50 μm). Almost all network branches and connections exhibited double staining, which suggested that both cells participated in the building of those structures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

believe that those $CD31^+$ vessels actually represented the endothelial cells migrating into the framework previously established by DFAT cells.

Another notable similarity between DFAT cells and pericytes is their multipotency. As mentioned above, several studies have demonstrated that DFAT cells can differentiate into adipocytes, skeletal myocytes, chondrocytes, osteoblasts, cardiomyocytes, and other cell types. Pericytes in various human tissues and organs also have been reported to express MSC markers, as well as to possess osteogenic, chondrogenic, myogenic and adipogenic potentials [38]. A model that is becoming increasingly popular has linked MSCs to pericytes, maintaining that MSCs from various tissues reside in perivascular locations and that pericytes might even act as MSC progenitors [31]. Because of such multipotency and the wide distribution of blood vessels in the body, pericytes may be an abundant source of stem cells for vascular restoration and other tissue bioengineering [31,38]. However, like ASCs, pericytes also comprise several subgroups and lack specific markers [31], which might hinder their definite purification and further clinical application. Therefore, the homogeneity and perivascular characteristics and functions of DFAT cells revealed in this study provide new insight into tissue repair and regeneration. Autologous DFAT cells would be able to facilitate blood vessel reconstruction in damaged human tissues and organs with higher efficiency and milder immune reactions. However, further in vivo experiments are necessary to confirm the pericytic characteristics of DFAT cells.

Conflict of interest

None.

Acknowledgments

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References

[1] J.F. Shen, A. Sugawara, J. Yamashita, H. Ogura, S. Sato, Dedifferentiated fat cells: an alternative source of adult multipotent cells from the adipose tissues, Int. J. Oral Sci. 3 (2011) 117–124.
[2] H. Sugihara, N. Yonemitsu, S. Miyabara, K. Yun, Primary cultures of unilocular fat cells: characteristics of growth in vitro and changes in differentiation properties, Differentiation 31 (1986) 42–49.
[3] M. Shigematsu, H. Watanabe, H. Sugihara, Proliferation and differentiation of unilocular pat cells in the bone marrow, Cell Struct. Funct. 24 (1999) 89–100.
[4] T. Sakuma, T. Matsumoto, K. Kano, N. Fukuda, D. Obinata, K. Yamaguchi, T. Yoshida, S. Takahashi, H. Mugishima, Mature, adipocyte derived, dedifferentiated fat cells can differentiate into smooth muscle-like cells and contribute to bladder tissue regeneration, J. Urol. 182 (2009) 355–365.
[5] F. Hildner, S. Concaro, A. Peterbauer, S. Wolbank, M. Danzer, A. Lindahl, P. Gatenholm, H. Redl, M. van Griensven, Human adipose-derived stem cells contribute to chondrogenesis in coculture with human articular chondrocytes, Tissue Eng. Part A 15 (2009) 3961–3969.
[6] S.S. Tholpady, C. Aojanepong, R. Llull, J.H. Jeong, A.C. Mason, J.W. Futrell, R.C. Ogle, A.J. Katz, The cellular plasticity of human adipocytes, Ann. Plast. Surg. 54 (2005) 651–656.
[7] M. Jumabay, T. Matsumoto, S. Yokoyama, K. Kano, Y. Kusumi, T. Masuko, M. Mitsumata, S. Saito, A. Hirayama, H. Mugishima, N. Fukuda, Dedifferentiated fat cells convert to cardiomyocyte phenotype and repair infarcted cardiac tissue in rats, J. Mol. Cell. Cardiol. 47 (2009) 565–575.
[8] Y. Oki, S. Watanabe, T. Endo, K. Kano, Mature adipocyte-derived dedifferentiated fat cells can trans-differentiate into osteoblasts in vitro and in vivo only by all-trans retinoic acid, Cell Struct. Funct. 33 (2008) 12.
[9] M. Taniguchi, L.L. Guan, B. Zhang, M.V. Dodson, E. Okine, S.S. Moore, Adipogenesis of bovine perimuscular preadipocytes, Biochem. Biophys. Res. Commun. 366 (2008) 54–59.
[10] M.E. Fernyhough, J.L. Vierck, G.J. Hausman, P.S. Mir, E.K. Okine, M.V. Dodson, Primary adipocyte culture: adipocyte purification methods may lead to a new understanding of adipose tissue growth and development, Cytotechnology 46 (2004) 163–172.
[11] Y. Guo, Y. Chen, Y. Zhang, Y. Zhang, L. Chen, D. Mo, Up-regulated miR-145 expression inhibits porcine preadipocytes differentiation by targeting IRS1, Int. J. Biol. Sci. 8 (2012) 1408–1417.
[12] J.L. Vierck, J.P. McNamara, M.V. Dodson, Proliferation and differentiation of progeny of ovine unilocular fat cells (adipofibroblasts), In Vitro Cell Dev. Biol. Anim. 32 (1996) 564–572.
[13] N. Kishimoto, Y. Momota, Y. Hashimoto, K. Ando, T. Omasa, J. Kotani, Dedifferentiated fat cells differentiate into osteoblasts in titanium fiber mesh, Cytotechnology 65 (2013) 15–22.
[14] S. Wei, W.G. Bergen, G.J. Hausman, L. Zan, M.V. Dodson, Cell culture purity issues and DFAT cells, Biochem. Biophys. Res. Commun. 433 (2013) 273–275.
[15] T. Matsumoto, K. Kano, D. Kondo, N. Fukuda, Y. Iribe, N. Tanaka, Y. Matsubara, T. Sakuma, A. Satomi, M. Otaki, J. Ryu, H. Mugishima, Mature adipocyte-derived dedifferentiated fat cells exhibit multilineage potential, J. Cell. Physiol. 215 (2008) 210–222.
[16] N. Ijichi, K. Ikeda, K. Horieinoue, K. Yagi, Y. Okazaki, S. Inoue, Estrogen-related receptor α modulates the expression of adipogenesis-related genes during adipocyte differentiation, Biochem. Biophys. Res. Commun. 358 (2007) 813–818.
[17] T. Kazama, M. Fujie, T. Endo, K. Kano, Mature adipocyte-derived dedifferentiated fat cells can transdifferentiate into skeletal myocytes in vitro, Biochem. Biophys. Res. Commun. 377 (2008) 780–785.
[18] D. Obinata, T. Matsumoto, Y. Ikado, T. Sakuma, K. Kano, N. Fukuda, K. Yamaguchi, H. Mugishima, S. Takahashi, Transplantation of mature adipocyte-derived dedifferentiated fat (DFAT) cells improves urethral sphincter contractility in a rat model, Int. J. Urol. 18 (2011) 827–834.
[19] K. Yagi, D. Kondo, Y. Okazaki, K. Kano, A novel preadipocyte cell line established from mouse adult mature adipocytes, Biochem. Biophys. Res. Commun. 321 (2004) 967–974.
[20] H. Nobusue, T. Endo, K. Kano, Establishment of a preadipocyte cell line derived from mature adipocytes of GFP transgenic mice and formation of adipose tissue, Cell Tissue Res. 332 (2008) 435–446.
[21] H. Nobusue, D. Kondo, M. Yamamoto, K. Kano, Effects of lysophosphatidic acid on the in vitro proliferation and differentiation of a novel porcine preadipocyte cell line, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 157 (2010) 401–407.
[22] L. Kou, X.W. Lu, M.K. Wu, H. Wang, Y.J. Zhang, S. Sato, J.F. Shen, The phenotype and tissue-specific nature of multipotent cells derived from human mature adipocytes, Biochem. Biophys. Res. Commun. 444 (2014) 543–548.
[23] L. Casteilla, V. Planat-Benard, B. Cousin, J.S. Silvestre, P. Laharrague, G. Charriere, A. Carriere, L. Penicaud, Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives, Circulation 109 (2004) 656–663.
[24] M. Jumabay, R. Abdmaulen, S. Urs, S. Heydarkhan-Hagvall, G.D. Chazenbalk, M.C. Jordan, K.P. Roos, Y. Yao, K.I. Bostrom, Endothelial differentiation in multipotent cells derived from mouse and human white mature adipocytes, J. Mol. Cell. Cardiol. 53 (2012) 790–800.
[25] P. Bianco, P. Robey, P. Simmons, Mesenchymal stem cells: revisiting history, concepts, and assays, Cell. Stem Cell. 2 (2008) 313–319.
[26] G. Lin, M. Garcia, H. Ning, L. Banie, Y.-L. Guo, T.F. Lue, C.-S. Lin, Defining stem and progenitor cells within adipose tissue, Stem Cells Dev. 17 (2008) 1053–1064.
[27] Y. Cao, Z. Sun, L. Liao, Y. Meng, Q. Han, R.C. Zhao, Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo, Biochem. Biophys. Res. Commun. 332 (2005) 370–379.
[28] L.J. Fischer, S. McIlhenny, T. Tulenko, N. Golesorkhi, P. Zhang, R. Larson, J. Lombardi, I. Shapiro, P.J. DiMuzio, Endothelial differentiation of adipose-derived stem cells: effects of endothelial cell growth supplement and shear force, J. Surg. Res. 152 (2009) 157–166.
[29] Y.Q. Yang, Y.Y. Tan, R. Wong, A. Wenden, L.K. Zhang, A.B. Rabie, The role of vascular endothelial growth factor in ossification, Int. J. Oral Sci. 4 (2012) 64–68.
[30] G. Bergers, S. Song, The role of pericytes in blood-vessel formation and maintenance, Neuro Oncol. 7 (2005) 452–464.
[31] M. Corselli, C.W. Chen, M. Crisan, L. Lazzari, B. Peault, Perivascular ancestors of adult multipotent stem cells, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 1104–1109.
[32] A. Armulik, A. Abramsson, C. Betsholtz, Endothelial/pericyte interactions, Circ. Res. 97 (2005) 512–523.
[33] C.L. Maier, B.R. Shepherd, T. Yi, J.S. Pober, Explant outgrowth, propagation and characterization of human pericytes, Microcirculation 17 (2010) 367–380.
[34] H. Gerhardt, C. Betsholtz, Endothelial-pericyte interactions in angiogenesis, Cell Tissue Res. 314 (2003) 15–23.
[35] L.P. Reynolds, A.T. Grazul-Bilska, D.A. Redmer, Angiogenesis in the corpus luteum, Endocrine 12 (2000) 1–9.
[36] U. Ozerdem, W.B. Stallcup, Early contribution of pericytes to angiogenic sprouting and tube formation, Angiogenesis 6 (2003) 241–249.
[37] M. Franco, P. Roswall, E. Cortez, D. Hanahan, K. Pietras, Pericytes promote endothelial cell survival through induction of autocrine VEGF-A signaling and Bcl-w expression, Blood 118 (2011) 2906–2917.
[38] M. Crisan, S. Yap, L. Casteilla, C. Chen, M. Corselli, T. Park, G. Andriolo, B. Sun, B. Zheng, L. Zhang, A perivascular origin for mesenchymal stem cells in multiple human organs, Cell. Stem Cell. 3 (2008) 301–313.