

Comparison of the properties of bone marrow-derived dedifferentiated fat cells and mesenchymal stem cells in humans

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Research

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Abstract

Background

Dedifferentiated fat (DFAT) cells have been studied as a potential tool for various aspects of tissue engineering. However, the understanding of DFAT cells is mainly derived from studies of inguinal subcutaneous or visceral white adipose tissues rather than human bone marrow (BM). Here, we focused on DFAT cells dedifferentiated from bone marrow adipocytes.

Methods

A plate ceiling culture method and light microscope were used to observe the morphological changes in bone marrow adipocytes. RT-qPCR analysis, immunofluorescence and immunocytochemistry were used to compare gene and protein expression before and after the adipogenic and osteogenic differentiation of BM-DFAT cells and mesenchymal stem cells from bone marrow (BM-MSCs). CCK8 assays and annexin V-PI staining were used to assess the effect of BM-DFAT cells on the proliferation and chemoresistance of leukaemic cells.

Results

We found that bone marrow adipocytes can dedifferentiate into fibroblast-like multipotent cells, and BM-DFAT cells exhibited similar a morphology, immunophenotype, multipotent gene expression, proliferation ability and osteogenic differentiation in comparison with BM-MSCs. Interestingly, BM-DFAT cells possessed a higher potential for adipogenic differentiation than BM-MSCs, since they expressed a lower level of Pref-1, an inhibitor of adipogenesis. Meanwhile, our results revealed the significantly enhanced proliferation of leukaemic cell lines in the BM-DFAT cell group compared with that in the BM-MSC group. However, the effects of BM-DFAT cells were not as obvious as those of BM-MSCs on the chemoresistance of leukaemic cells.

Conclusions

This is the first study to illustrate the properties of BM-DFAT cells. We determined that BM-DFAT cells may be a useful cell source to study the role of bone marrow adipocytes and might be utilized for clinical applications in regenerative medicine.

Introduction

Bone marrow (BM) aspiration is the most routine and standard method used for diagnosing several haematological diseases, including leukaemia, multiple myeloma, aplastic anaemia and myelodysplastic syndrome. BM fluid is an important resource for mesenchymal stem cells (MSCs); however, bone marrow

adipocytes in BM fluid are usually ignored. Recently, by using ceiling culture methods, mature adipocytes were shown to convert into lipid-free fibroblast-like cells in vitro that are known as dedifferentiated fat (DFAT) cells [1, 2]. DFAT cells are highly proliferative and have been successfully redifferentiated or transdifferentiated into several cell lineages, including lipid-filled adipocytes, osteoblasts, chondrocytes, myocytes and other cell types [3–7]. As a consequence, these cells may be useful as a potential tool for various aspects of tissue engineering and attractive for use in new stem cell research. For in vitro research, DFAT cells are mainly derived from inguinal subcutaneous or visceral white adipose tissue from various species, including humans, rats, mice and cattle [8–10]. Shigematsu M et al reported that the unilocular fat cells from the bovine metacarpal could dedifferentiate into fibroblast-like cells [11], but to date, the understanding of DFAT cells derived from human bone marrow adipocytes has been lacking.

Subcutaneous or abdominal adipose tissues are abundant, and the harvest of mature adipocytes utilizes a minimally invasive method [12], which makes them a major source for DFAT cell research. However, bone marrow adipocytes are a specific type of active cell in the BM niche, where their phenotype and function remain incompletely known. It has been reported that bone marrow adipocytes possess some characteristics of brown fat or represent an intermediate tissue between brown and white fat [13, 14], which suggests that the function of bone marrow adipocytes may be different from that of subcutaneous or abdominal adipose tissue. Additionally, bone marrow adipocytes are not grouped in lobules but scattered within the haematopoietic tissue and are not correlated with weight, body mass index or body fat [15–17]. This leads us to suppose that it is more reasonable to study bone marrow adipocytes from the perspective of BM-DFAT cells or BM-MSCs, since both of these cell types can differentiate into bone marrow adipocytes.

As multipotent cells, BM-MSCs are present in adult bone marrow and can be induced to differentiate into adipocytes, chondrocytes, or osteocytes [18]. At present, the bone marrow adipocytes studied in vitro are mainly derived from the adipogenic differentiation of BM-MSCs. Considering that the cycle of adipogenic differentiation is long, the rate of adipogenesis is not high, and the isolation of adipocytes from BM fluid is inconvenient, BM-DFAT cells may represent a new cellular model for adipocyte regulation and physiological studies. Additionally, it has been reported that BM-MSCs are involved in the proliferation and chemotherapeutic resistance of leukaemic cells [19, 20]. Compared to MSCs, DFAT cells dedifferentiated from human omental and subcutaneous fat tissues showed many similar properties, including similar DNA methylation conditions and similar immunomodulatory and haematopoietic supportive properties [12]. However, the differences in the properties of BM-DFAT cells and BM-MSCs have not been reported.

In this study, we investigated the physiological process of mature bone marrow adipocyte dedifferentiation and compared the morphology, immunophenotypes, proliferative ability and expression of multipotent genes between BM-DFAT cells and BM-MSCs, which were isolated from the same samples. Furthermore, we detected their redifferentiation and transdifferentiation capacity and their effect on the proliferation and chemoresistance of leukaemic cells. The results demonstrated that bone marrow

adipocytes can dedifferentiate into multipotent cells and that BM-DFAT cells may be a useful cell source for the study of bone marrow adipocytes.

Materials And Methods

Cell culture

The leukaemic cell lines THP-1 and NALM6 (Chinese Academy of Sciences Cell Bank, Shanghai, China) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) and penicillin-streptomycin at 37 °C in 5% CO₂. BM was harvested from the iliac crest of five patients diagnosed with lymphoma without BM infiltration after informed consent was obtained. These experiments were approved by the Ethics Committee of the Shanghai Jiaotong University School of Medicine (2018-KY-023(K)). BM-MSCs were obtained as described previously [21]. Briefly, the bone marrow was diluted twice with phosphate-buffered saline (PBS) and then isolated by Ficoll-Hypaque (Axis-Shield Diagnostics, Dundee, Scotland, UK) density gradient centrifugation. Monocytes were collected by adherence to a plastic flask and incubated with low-glucose Dulbecco's Modified Eagle Media (LG-DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% FBS for 48 h.

Isolation And Culture Of Bone Marrow Adipocytes

To efficiently acquire homogeneous mature adipocytes from bone marrow, we used the plate ceiling culture method that Wei Shengjuan et al previously reported [22]. Briefly, after two centrifugations, the top fat layer of the bone marrow was transferred into a 150 mm × 25 mm cell culture dish containing a small amount of media (high-glucose DMEM + 10% FBS) by Pasteur pipettes. Then, an inverted 100 mm × 15 mm cell culture dish was placed into the large dish by forceps. A proper amount of media was added to make the solution surface attach to the inner surface of the small dish. The large dish was covered with a lid and incubated at 37 °C in 5% CO₂. The medium was removed and replaced with fresh medium, and the dishes were inverted after approximately 4–6 days. When the cells reached 80%-90% confluence, they were trypsinized and subcultured at an initial seeding density of 2×10^4 cells per square centimetre.

Immunocytochemistry

Cells grown on coverslips were fixed with 4% formaldehyde for 30 minutes and rinsed with PBS. After 1 h of incubation in blocking solution (5% bovine serum albumin in PBS), the cells were rinsed with PBS and then incubated overnight at 4 °C with primary antibodies against Pref-1 (1:800, ab21682, Abcam), FAP (1:600, HPA059739, Sigma-Aldrich), and FSP-1 (1:600, HPA007973, Sigma-Aldrich). After DAB staining, the nuclei were revealed by haematoxylin staining. Images of the sections stained for various markers were obtained at 40 × magnification using an optical microscope (Olympus Co., Tokyo, Japan).

Immunofluorescence

Cells grown on coverslips were fixed with 4% paraformaldehyde and blocked with goat serum. The process was carried out as previously described [23]. Cells were stained with β -tubulin (1:200, ab6046, Abcam) and anti-rabbit osteopontin (OPN) (1:500, ab8448, Abcam). Cells were incubated for 1 h with Alexa Fluor 647 (AF647)-conjugated and Alexa Fluor 488 (AF488)-conjugated anti-rabbit IgG (Beyotime, 1:500). Adipocytes were stained with a neutral lipid-specific BODIPY® 493/503 (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a, 4a-diaza-s-indacene) dye. After rinsing, the nuclei were stained for 15 minutes with 4,6-diamidino-2-phenylindol (DAPI, Sigma, 1:100). Images of fluorescent cells were captured with a confocal laser microscopy system (Leica SP2).

Immunophenotype Analysis

BM-MSCs and BM-DFAT cells from the same five patients were characterized by flow cytometry after the third passage. Cells were trypsinized and stained with anti-CD34-PE, anti-CD31-APC, anti-CD45-FITC, anti-CD73-PE-Cy5, anti-CD90-PE, anti-CD105-PerCP and anti-HLA-DR-PE (eBioscience). Negative isotype antibodies were used as control antibodies. Cells were incubated with the primary antibodies at 4 °C for 30 minutes. Thereafter, the cell fluorescence was evaluated by flow cytometry using a FACSCalibur instrument (Becton Dickinson). The data were analysed using FlowJo Software.

Adipogenic And Osteogenic Differentiation

BM-MSCs (n = 5) and BM-DFAT cells (n = 5) were cultured in differentiation medium after the third passage. For adipogenic and osteogenic differentiation, cells were plated in 6-well plates at a density of 2×10^4 cells/cm² and grown to confluency. Samples were processed in duplicate, and cells maintained in regular medium were used as a negative control.

For adipogenic differentiation, cells were incubated for 3 weeks in DMEM-HG containing 10% FBS, 0.01 mg/ml insulin, 1 mM dexamethasone, 0.5 mM 3-isobutyl-methylxanthine and 0.2 mM indomethacin (Cyagen Biosciences, USA). The presence of intracellular lipid droplets indicated adipogenic lineage differentiation. The redifferentiated adipocytes were stained with Oil Red O (ORO). To quantify lipid accumulation, 200 μ l/cm² isopropanol (Wako) was added to each washed and dried cell sample, which was agitated for 15 minutes on a shaker. The extracted ORO absorbance was read at 492 nm with a spectrophotometer (Thermo Fisher, Multiscan FC).

For osteogenic differentiation, cells were incubated for 3 weeks in DMEM-HG containing 10% FBS, 100 nM dexamethasone, 10 mM β -glycerophosphate, and 50 mM L-ascorbic acid-2-phosphate (Cyagen Biosciences, USA). The induction medium was replaced every 3 days. The differentiation potential of the osteogenic cell lineage was evaluated by calcium accumulation, which was assessed by alizarin red S staining (Sigma, St. Louis, MO, USA).

Real-time Quantitative Pcr (RT-qPCR)

Total RNA was extracted from BM-MSCs and BM-DFAT cells using TRIzol (Invitrogen, Paisley, UK), and the RNA was converted into cDNA using the Primer Script™ RT Reagent Kit (Takara Bio Inc, Otsu, Shiga, Japan). The sequences used for RT-qPCR (forward/reverse) are shown in Table 1. All RT-qPCR reactions were performed using an ABI 7500 real-time PCR system (Biosystems, Foster City, CA, USA) and the SYBR Premix Ex Taq reagent kit (Takara Bio Inc, Otsu, Shiga, Japan). Housekeeping gene (GAPDH) expression was measured in each sample.

Table 1
Oligonucleotide primers used for real time PCR analysis of gene expression

Gene name	Forward primers	Reverse primers
c-MYC	TCAAGAGGTGCCACGTCTCC	TCTTGGCAGCAGGATAGTCCTT
NANOG	CCTCCTCCATGGATCTGCTTATTCA	CAGGTCTTCACCTGTTTGTAG
OCT-4	CGAAAGAGAAAGCGAACCAGTAT	AGCAGCCTCAAAATCCTCTCGTT
SOX2	GGAGAGAGAAAGAAAGGGAGAGA	GAGGCAAACCTGGAATCAGGA
FABP4	AACCTTAGATGGGGGTGTCCTG	TCGTGGAA GTGACGCCTTTC
C/EBPα	GAACAGCAACGAGTACCGGGTA	GCCATGGCCTTGACCAAGGAG
PPARγ	TCTCTCCGTAATGGAAGACC	GCATTATGAGACATCCCCAC
BMP-6	CAATCTGTGGGTTGTGACTCC	GAAGGGCTGCTTGTGCTAAG
OPN	GTGATTTGCTTTTGCCTCCT	GAGATGGGTCAGGGTTTAGC
RUNX2	ATGCTTCATTCGCCTCAC	ACTGCTTGCAGCCTTAAAT
HSL	TTTGAGATGCCACTGACTGC	CATAGGAGATGAGCCTGACGA
Adiponectin	CCCATTGCTTTACCAAGAT	GGCTGACCTTCACATCCTTC
Adipsin	CTGAGGTGGGAGGATGACTT	AGACAAGGTTTCACTCTGTTGC
GAPDH	CGGAGTCAACGGATTTGGTCGTAT	AGCCTTCTCCATGGTGGTGAAGAC
Abbreviation: SOX-2, SRY-box transcription factor 2; FABP4, fatty acid binding protein 4; C/EBPα, CCAAT enhancer binding protein alpha; PPARγ, peroxisome proliferator activated receptor gamma; BMP-6, bone morphogenetic protein 6; RUNX2, RUNX family transcription factor 2; HSL, hormone sensitive type; GAPDH, glyceraldehyde-3-phosphate dehydrogenase		

Cell Proliferation Assay

Leukaemic cell lines (THP-1 and NALM 6 cells) were cultured in direct contact with BM-MSCs or BM-DFAT cells in 6-well plates for 3 days. BM-MSCs or BM-DFAT cells (2×10^4 /ml) were precultured for 3 days

before 1×10^6 leukaemic cells were added to each well. Then, a CCK8 kit (Dojindo, Japan) was used to evaluate cell proliferation. For the CCK8 assay, cells (3×10^3) were seeded into each well of 96-well plates, and CCK8 reagent was then added to each well; then, each plate was incubated for 2 h at 37 °C. The measurement of absorption was performed using a microplate reader at 450 nm (Multiscan FC, Thermo Fisher, USA).

Apoptosis Assay Using Annexin V-PI Staining

After THP-1 and NALM 6 cells were cocultured with BM-MSCs or BM-DFAT cells, the leukaemic cells were collected and treated with Ara-C (10 μ M, Tokyo Chemical Industry, Tokyo, Japan) for 48 h. Apoptosis assays were performed according to the manufacturer's instructions using a flow cytometer after staining with propidium iodide (PI) and fluorescent phycoerythrin-conjugated annexin V antibodies (Beyotime, China). Briefly, after staining with PI/anti-annexin V, flow cytometric analysis was used to identify the viable annexin V-PI-, early apoptotic annexin V + PI-, and late apoptotic/necrotic annexin V + PI + cell subsets.

Statistical Analyses

The data are represented as the mean \pm standard deviation, and $P \leq 0.05$ was considered significant. The statistical analyses were performed using a two-sample, two-tailed Student's t-test. All statistical analyses were conducted by using the SPSS 20.0 software program (SPSS, Chicago, IL, USA).

Results

BM adipocytes can dedifferentiate into fibroblast-like multipotent cells

Using plate ceiling culture, mature adipocytes from BM were successfully dedifferentiated for the purposes of this study. As illustrated in Fig. 1a, the cellular morphology of mature adipocytes undergoing dedifferentiation was observed at various time points. After 2 days of ceiling culture, the mature adipocytes were round and adhered loosely to the support. Then, the cytoplasm began to spread on the ceiling surface. On day 4, the cell population was heterogeneous, containing both round cells and elongated cells. On day 7, the adipocytes exhibited attachment, and the lipid droplets shrank. Adipocytes grew in clusters and became flattened, and the tentacle-like cells showed increased growth on the tenth day. On day 13, most lipid droplets in the adipocytes had disappeared, and the round cells had become fibroblast-like cells, which are BM-DFAT cells.

The BM-DFAT cells were cultured under conditions that were favourable for adipogenic differentiation to detect their differentiation potential. After adipogenic induction for 3 weeks, the accumulation of lipid-rich vacuoles within the cytoplasm was observed, and cells were stained positively for lipid vacuoles with

ORO, indicating adipogenic differentiation (Fig. 1b). When BM-DFAT cells were cultured in the osteogenic induction medium, mineralized matrix aggregates were observed after 1 week and gradually increased until 3 weeks, as indicated by alizarin red S staining (Fig. 1c). BM-DFAT cells were successfully redifferentiated into osteoblasts. These results showed that BM adipocytes can dedifferentiate into fibroblast-like multipotent cells.

Many properties of BM-DFAT cells are similar to those of BM-MSCs

To further compare the morphology of BM-DFAT cells and BM-MSCs, β -tubulin, a cytoskeletal protein, was detected through immunofluorescence in these two cells. As shown in Fig. 2a, adherent BM-DFAT cells and BM-MSCs exhibited a similar appearance; both were spindle-shaped or protuberant regardless of whether they were observed with light microscopy (left) or immunofluorescence (right). To identify protein expression at the surfaces of cells, BM-DFAT cells were compared to BM-MSCs from the same passage using flow cytometry. Our results showed that BM-DFAT cells were uniformly positive for CD73 (ecto-5-nucleotidase), CD90 (Thy-1) and CD105 (endoglin) but negative for CD31 (platelet endothelial cell adhesion molecule), CD34, CD45 and HLA-DR. This expression profile was consistent with that of BM-MSCs (Fig. 2b). Quantitative analysis showed that the difference in surface antigens between BM-MSCs and BM-DFAT cells was not significant (Table 2).

Table 2
Comparison of the surface markers of dedifferentiated fat cells and mesenchymal stem cells from bone marrow

markers	Percentage of positive cells (mean \pm SD %)			
	other names	BM-MSCs	BM-DFAT cells	P
CD31	PECAM1	2.33 \pm 0.76	1.07 \pm 0.31	0.09
CD34	L-selectin ligand	0.27 \pm 0.25	1.27 \pm 0.64	0.1
CD45	PTPRC	1.11 \pm 0.66	0.13 \pm 0.15	0.119
CD73	NT5E	89.33 \pm 6.03	96.33 \pm 2.08	0.173
CD90	THY1	90.33 \pm 1.53	88.53 \pm 1.55	0.184
CD105	ENG	89.01 \pm 3.04	84.37 \pm 5.13	0.261
Abbreviation: BM, bone marrow; MSC, mesenchymal stem cells; DFAT, dedifferentiated fat cells;PECAM1, platelet and endothelial cell adhesion molecule 1; PTPRC, protein tyrosine phosphatase, receptor type C; NT5E,5'-nucleotidase ecto; ENG, endoglin.				

Pluripotency-associated genes are important for the maintenance of stem cells. RT-qPCR was performed for three stem cell markers (c-MYC, NANOG and OCT-4) to compare the pluripotent states of BM-DFAT cells. We found that BM-DFAT cells expressed levels of these markers similar to those of BM-MSCs (Fig. 2c). CCK8 detection showed that the proliferation capacity of BM-DFAT cells and BM-MSCs was not significantly different (Fig. 2d). Since BM-MSCs and BM-DFAT cells present a fibroblast-like morphology,

fibroblast-associated genes were detected in these two cells. Immunocytochemistry assays showed that both cell lines positively expressed FAP and FSP-1 and there was no significant difference between these two groups after quantification analysis (Fig. 2e, 2f). These results suggested that the morphology, immunophenotype, multipotent gene expression and proliferative ability were similar between BM-DFAT cells and BM-MSCs.

BM-DFAT Cells Have A High Adipogenic Differentiation Ability

As multipotent cells, BM-DFAT cells and BM-MSCs from the five patients were successfully redifferentiated into mature adipocytes under similar culture conditions. As illustrated in Fig. 3a, the morphology of the mature adipocytes derived from these two cell types was multilocular, as indicated by immunofluorescence staining with BODIPY and bright field microscopy. After ORO staining, the content of adipocyte lipid droplets in cells redifferentiated from BM-DFAT cells was higher than that in cells redifferentiated from BM-MSCs; the former accumulated approximately 40% more lipid droplets than the latter (Fig. 3b, 3c). Subsequently, RT-qPCR analysis revealed that DFAT-derived mature adipocytes expressed increased levels of transcription factors involved in the regulation of adipocyte differentiation, including PPAR γ , C/EBP α and FABP4 (Fig. 3d). In addition, the expression of mature adipocyte markers, such as HSL, adiponectin and adipsin, was also compared in mature adipocytes derived from BM-DFAT cells and BM-MSCs. The results showed that the expression of adiponectin and adipsin was more abundant in the former group, whereas there was no significant difference in the expression of HSL between the two groups (Fig. 3e). Since BM-DFAT cells were redifferentiated into mature adipocytes, a special marker of preadipocytes, Pref-1, was detected in BM-DFAT cells and BM-MSCs. The results of immunocytochemistry staining and quantification analysis showed that the expression of Pref-1 was significantly higher in BM-MSCs than in BM-DFAT cells (Fig. 3f, 3g). Since Pref-1 is an inhibitor of adipogenesis [24], this might explain why BM-DFAT cells possess an increased adipogenic differentiation potential.

BM-DFAT cells are similar to BM-MSCs in their capability for osteogenesis

It has been reported that DFAT cells can not only redifferentiate into mature adipocytes but also can transdifferentiate into osteoblasts. Therefore, we compared the osteogenesis capability of BM-DFAT cells and BM-MSCs. After osteogenic induction for 3 weeks, mineralized matrix aggregates were observed through alizarin red S staining and immunofluorescence staining of OPN (a protein widely expressed in bone). Representative photos are presented in Fig. 4a. To further compare the differences in osteogenesis between BM-DFAT cells and BM-MSCs, the expression of osteoblast-associated genes was evaluated by RT-qPCR. BM-DFAT cells and BM-MSCs expressed similar levels of RUNX2, BMP6 and OPN (Fig. 4b). These results revealed that there was no significant difference in osteogenesis between BM-DFAT cells and BM-MSCs.

The effect of BM-DFAT cells and BM-MSCs on leukaemic cells

It is well known that BM-MSCs can regulate the proliferation and chemotherapeutic resistance of leukaemic cells [19, 20]. Subsequently, we compared the effect of BM-DFAT cells and BM-MSCs on leukaemic cells. As shown in Fig. 5a, the proliferation of leukaemic cell lines (THP-1 and NALM 6 cells) showed a greater increase after coculturing with BM-DFAT cells compared to that with BM-MSCs starting on the third day. Both BM-DFAT cells and BM-MSCs inhibited the apoptosis of leukaemic cell lines after treatment with Ara-c for 48 h (Fig. 5b, c). However, the apoptosis rate of leukaemic cell lines was higher in the BM-DFAT cell group than in the BM-MSC group, suggesting that the potential effect of BM-DFAT cells on the chemotherapeutic resistance of leukaemic cells was not as obvious as that of BM-MSCs.

Discussion

In the present study, we investigated the properties of mature adipocytes isolated from BM. First, we discovered that bone marrow adipocytes can dedifferentiate into multipotent fibroblast-like cells. Moreover, the morphology, proliferative ability and immunophenotype of BM-DFAT cells and BM-MSCs were similar. Interestingly, we found that the adipogenic ability of BM-DFAT cells was higher than that of BM-MSCs, indicating that BM-DFAT cells may be an effective tool for studying the function of bone marrow adipocytes. Intriguingly, BM-DFAT cells can significantly enhance the proliferation of leukaemic cells.

Under physiological stimuli, mature adipocytes of mice or humans are able to reversibly change their phenotypes and directly transform into cells with a different morphology and physiology [2]. In line with these data, we found that mature adipocytes from bone marrow could revert to a more primitive phenotype and gain cell proliferative capability when they were subjected to an *in vitro* dedifferentiation method referred to as a plate ceiling culture. DFAT cells obtained from subcutaneous and visceral adipose tissues could redifferentiate into adipocytes and transdifferentiate into chondrocytes and osteoblasts under appropriate culture conditions [5]. Our results showed that BM-DFAT cells could also differentiate into adipocytes and osteoblasts, indicating that these cells are multipotent.

As reported, DFAT cells can re-establish their expression of MSC markers during the dedifferentiation process [12]. Moreover, DFAT cells from human subcutaneous fat and adipose-derived stem cells (ADSCs) share common phenotypes [25]. In addition, it was noted that the profile of DFAT cells from human omental and subcutaneous adipose tissues was consistent with that of BM-MSCs [26]. Our results showed that the protein expression at the surface of BM-DFAT cells was not significantly different from that of BM-MSCs, suggesting that both BM-DFAT cells and BM-MSCs contain the same types of cells. DFAT cells not only express stem cell antigens but also express stem cell genes and reprogramming genes [27]. Our analysis showed that BM-DFAT cells and BM-MSCs expressed the same levels of stem cell genes, such as NANOG, OCT4 and c-MYC, indicating that BM-DFAT cells retain the properties of stem cells. This may be caused by adipocytes, since previous studies have demonstrated that isolated mature murine or human adipocytes express stem cell genes and reprogramming genes [28, 29].

It is important to find proper sources for the study of adipocytes. Certain murine cell lines, such as 3T3-L1 and its subclonal cell line 3T3-F442A, have been used as cell culture models, since they can be induced to differentiate into adherent, lipid-storing adipocytes under adipogenic stimulation, making them extremely useful in in vitro studies of adipocyte differentiation [29]. However, these cells originate from mouse embryo tissue and may not fully reflect the physiology of human adipocytes [29, 30]. DFAT cells were identified as a more homogenous cell group in most reports [31, 32, 5–9] and as more similar to BM-derived MSCs based on their epigenetic signature [26]. They have been considered a novel preadipocyte cell line derived from mature adipocytes. James E et al. compared the markers and functional attributes of human DFAT cells and ADSCs from the subcutaneous fat of an obese diabetic donor and found that DFAT cells showed higher efficiency in terms of their adipogenic capacity [25]. However, other studies showed different results. Daisuke Akita et al. found that the adipogenic potential of DFAT cells was similar to that of ADSCs from periodontal tissues in rats [33]. In comparison to human BM-MSCs, human DFAT cells derived from omental and subcutaneous fat tissues showed similar properties [26]. However, unlike MSCs, we found that under the same conditions, BM-DFAT cells induced the formation of a greater number of mature adipocytes than BM-MSCs since they expressed low levels of Pref-1. This indicated to us that we should use BM-DFATs as a substitute for human bone marrow preadipocytes.

As important bone marrow stromal cells, osteoblasts and adipocytes share a common progenitor cell, and both play critical roles in the bone marrow microenvironment [34, 35]. The commitment of MSCs to the adipocyte and osteoblast lineages has been specifically implicated in pathological conditions involving abnormal bone remodelling [36]. Therefore, we mainly compared the ability of adipogenesis and osteogenesis of DFAT cells and BM-MSCs in this study. In terms of osteogenesis, when compared to that of ADSCs from subcutaneous fat, DFAT cells from humans showed higher efficiency in terms of osteogenic capacity [37, 25]. Similar results were also found for DFAT cells from rats, which may be related to the fact that rats have a very high capability for regenerating bone [33]. However, the reason why the osteoblastic differentiation ability of DFAT cells and ADSCs was different was unknown [38]. Our results showed that the osteogenesis potential of BM-DFAT cells was similar to that of BM-MSCs. This may reflect the fact that the bone marrow adipocytes were different from the peripheral adipocytes.

It has been reported that MSCs are essential components of the BM microenvironment and have antiapoptotic and growth-enhancing effects on acute myeloid leukaemic cells [19, 20]. They can protect leukaemic cells from apoptosis through c-MYC-mediated drug resistance and support the long-term proliferation of AML cells by altering the cytokine network [19, 20]. However, until now, studies on the effect of DFAT cells on tumour cells have been lacking. In our study, we also found that BM-DFAT cells could promote the proliferation and inhibit the apoptosis of AML cells. Surprisingly, the ability of BM-MSCs and BM-DFAT cells to promote the proliferation and inhibit the apoptosis of AML cells was different. The underlying mechanisms need to be further studied.

Conclusion

In conclusion, bone marrow adipocytes can dedifferentiate into fibroblast-like multipotent cells. Furthermore, the BM-DFAT cells showed several advantages as a model of adipocyte differentiation. First, we can take advantage of the bone marrow fluid, owing to the fact that adherent cells can be used to acquire BM-MSCs and floating fat cells can be used to obtain BM-DFAT cells. Our study revealed that the BM-DFAT cells showed a higher adipogenic ability than BM-MSCs and could therefore be a useful source for the study of bone marrow adipocytes. Finally, considering that bone marrow adipocytes are different from peripheral adipocytes, mature adipocytes redifferentiated from BM-DFAT cells may better represent the characteristics of bone marrow adipocytes.

Abbreviations

ADSCs: adipose-derived stem cells; BM: Bone marrow; DAPI: 4, 6-diamidino-2-phenylindol; DFAT: dedifferentiated fat; FBS: fetal bovine serum; LG-DMEM: low-glucose Dulbecco's Modified Eagle Media; MSCs: mesenchymal stem cells; OPN: osteopontin; ORO: Oil Red O; PBS: phosphate-buffered saline; PI: propidium iodide; RT-qPCR: Real-time quantitative PCR

Declarations

Acknowledgments

Not applicable.

Authors' contributions

WL performed the immunocytochemistry and wrote the manuscript. YY collected patients' samples and isolated the BM-MSC and BM adipocytes. CZ performed the RT-qPCR. SY analyzed the immunophenotype of cells. YW induced the differentiation of cells. JS designed the experiments. All authors read and approved the final version of the submitted manuscript.

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Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval and consent to participate

Human MSCs and adipocytes were isolated from bone marrow, after written informed consent. All protocols were approved by the Ethics Committee of the Shanghai Jiaotong University School of Medicine and in compliance with the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Ethical statement

There are no animal experiments carried out for this article.

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Figures

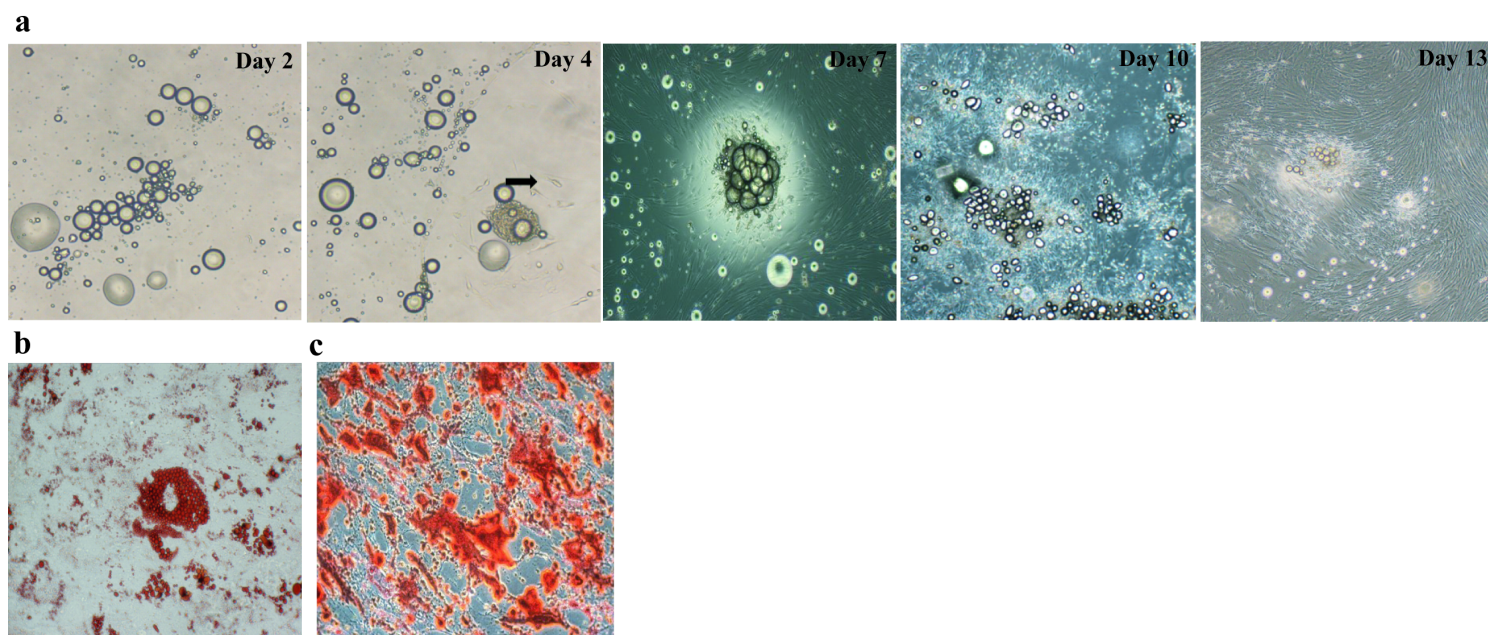


Figure 1

Mature adipocytes of bone marrow dedifferentiated into DFAT cells by ceiling culture. a. Morphology of adipocytes from day 2 to day 13 after ceiling culture. The black arrow shows the protrusions of the adherent adipocytes. c. The adipogenic differentiation of BM-DFAT cells. c. The osteoblastic differentiation of BM-DFAT cells. All images were obtained at a magnification of 400 \times .

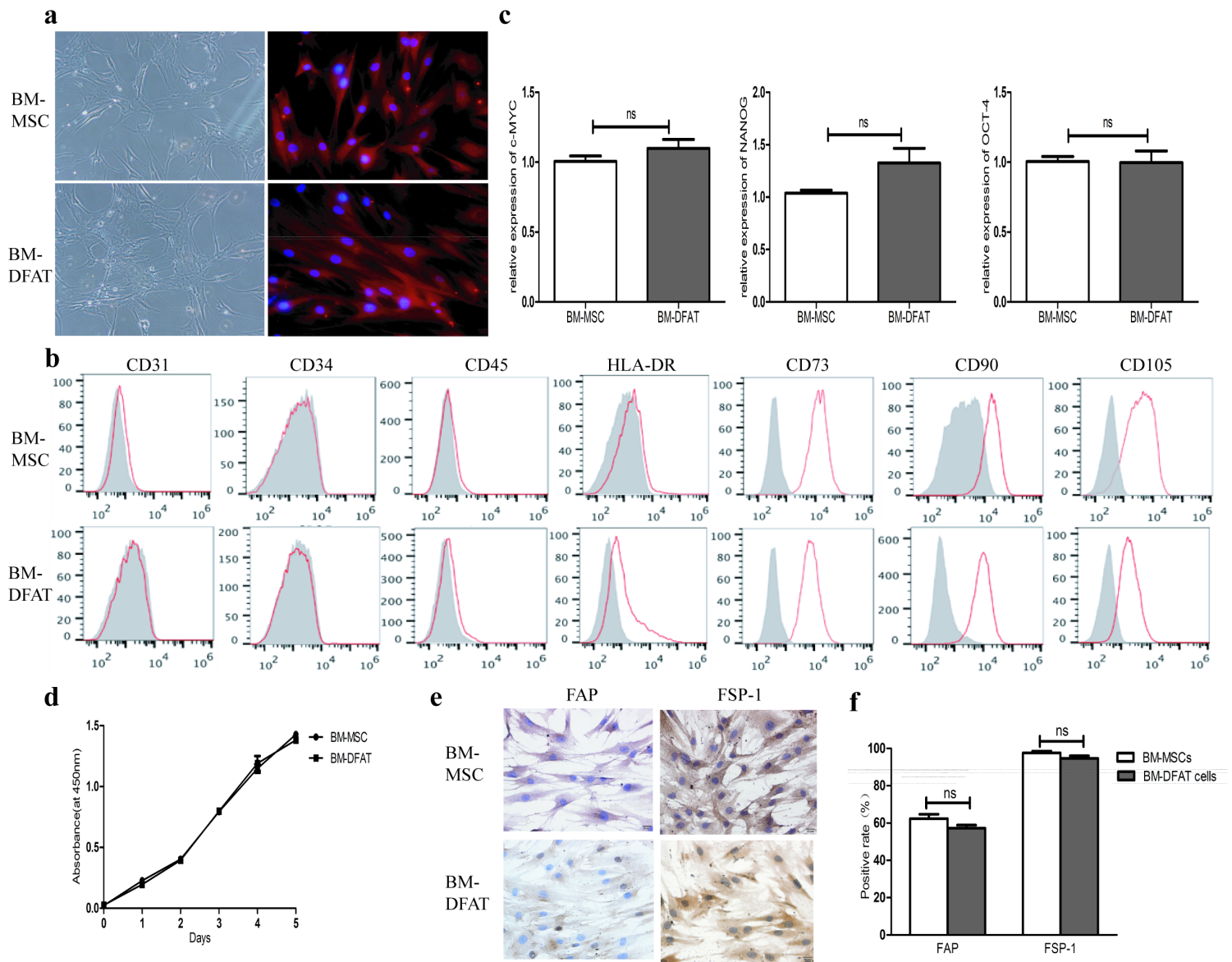


Figure 2

Comparison of the properties of BM-DFAT cells and BM-MSCs. a. Morphology of BM-MSCs and BM-DFAT cells was shown by light microscopy (left) and immunofluorescence staining of β -tubulin (right). b. Phenotypic analysis of BM-MSCs and BM-DFAT cells by flow cytometry. c. Expression of pluripotency-associated genes in BM-MSCs and BM-DFAT cells was analysed by RT-qPCR. Values are the mean \pm SD of triplicate samples. d. The proliferation of BM-MSCs and BM-DFAT cells detected by CCK8 assay. e. The expression of fibroblast-associated genes (FSP-1 and FAP) in BM-MSCs and BM-DFAT cells detected by immunocytochemistry. f. The percentages of positive cells that expressed FAP and FSP-1 in the two groups. The scale bar represents 20 μ m. NS: not significant

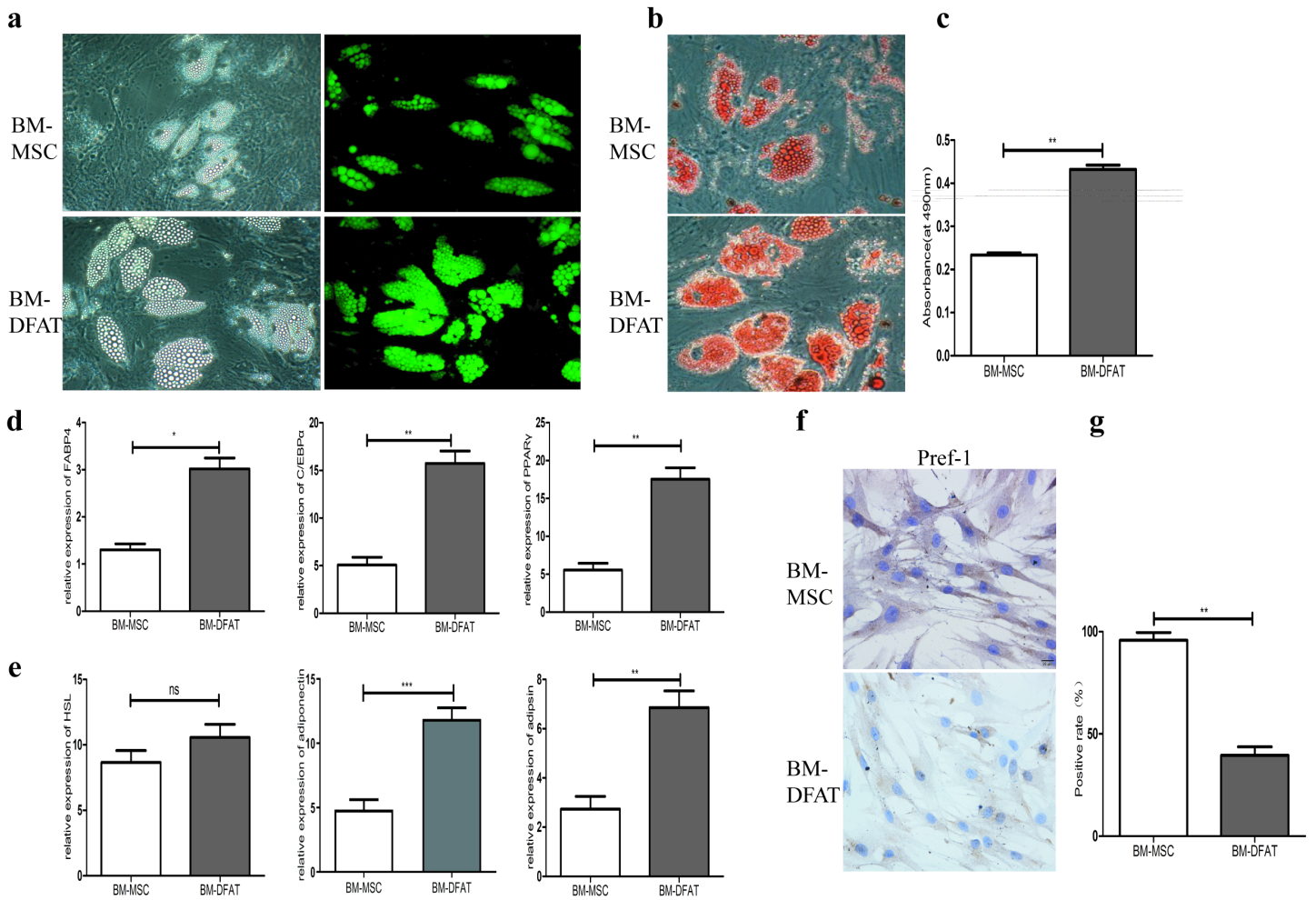
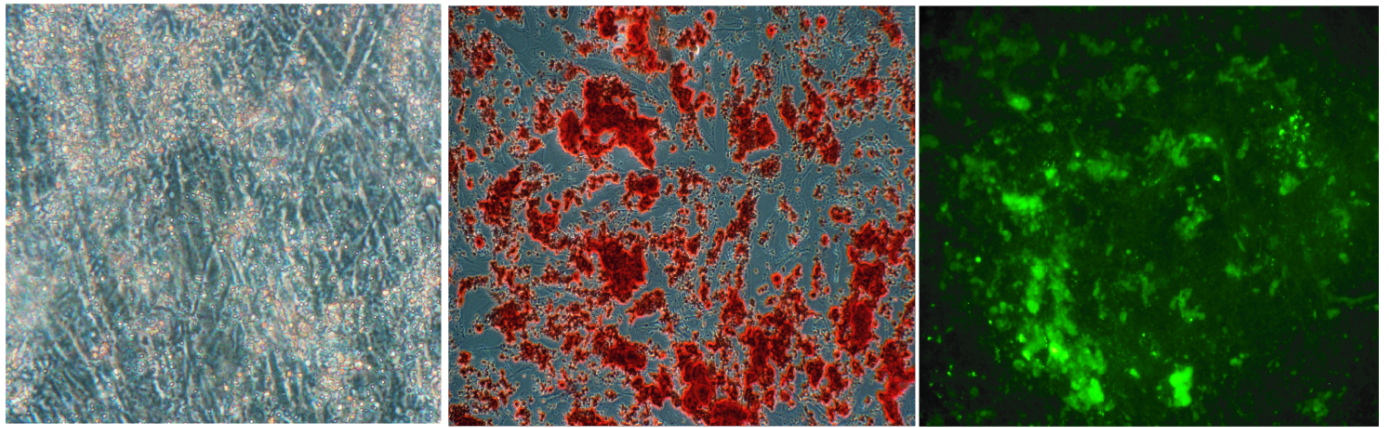
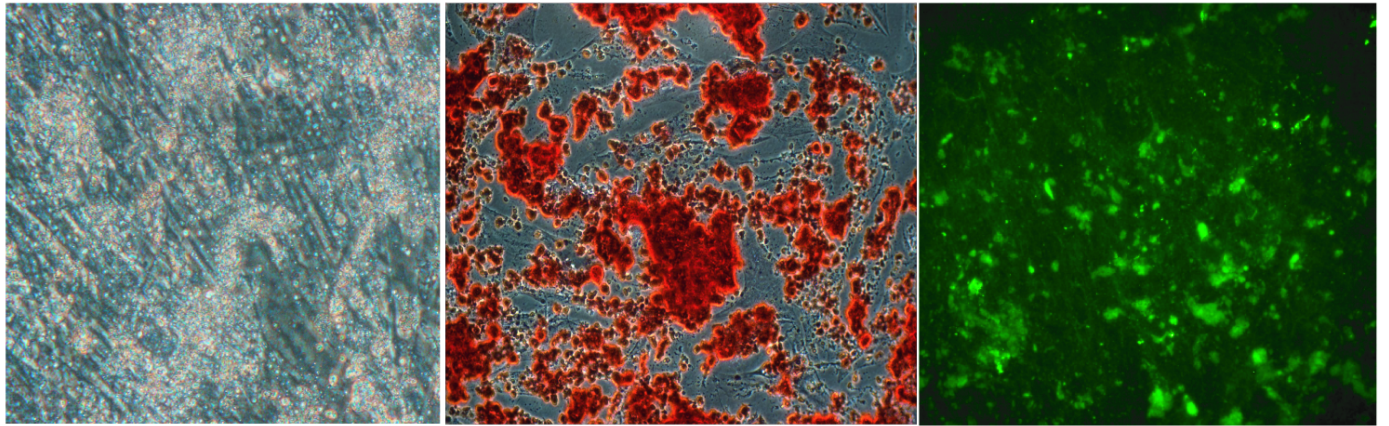
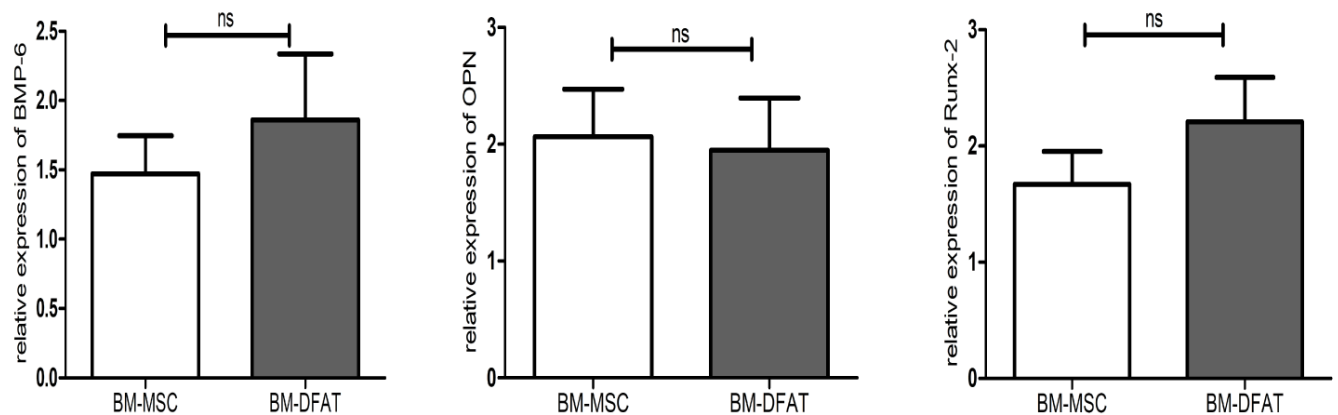


Figure 3

The capability for adipogenesis of BM-DFAT cells and BM-MSCs. a. Morphology of mature adipocytes redifferentiated from BM-MSCs and BM-DFAT cells shown by bright field microscopy (left) and immunofluorescence staining with BODIPY (right). b-c. The morphology (b) and OD values (c) of mature adipocytes redifferentiated from BM-MSCs and BM-DFAT cells after Oil Red O staining. d-e. Relative expression of transcription factors involved in adipocyte differentiation (d) and adipogenic genes (e) in mature adipocytes redifferentiated from BM-MSCs and BM-DFAT cells as analysed by RT-qPCR. Values are the mean \pm SD of triplicate samples. f. The expression of Pref-1 in BM-MSCs and BM-DFAT cells was detected by immunocytochemistry. g. The percentages of positive cells that expressed Pref-1 in these two groups. The scale bar represents 20 μ m. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

aBM-
MSCBM-
DFAT**b****Figure 4**

The osteogenic capability of BM-DFAT cells and BM-MSCs. a. The mineralized matrix of osteoblasts transdifferentiated from BM-MSCs and BM-DFAT cells is shown by bright field microscopy (left), alizarin red staining (middle) and immunofluorescence staining of OPN (right). b. Relative expression of osteogenic genes in BM-MSCs and BM-DFAT cells was analysed by RT-qPCR. Values represent the mean \pm SD of triplicate samples. NS: not significant

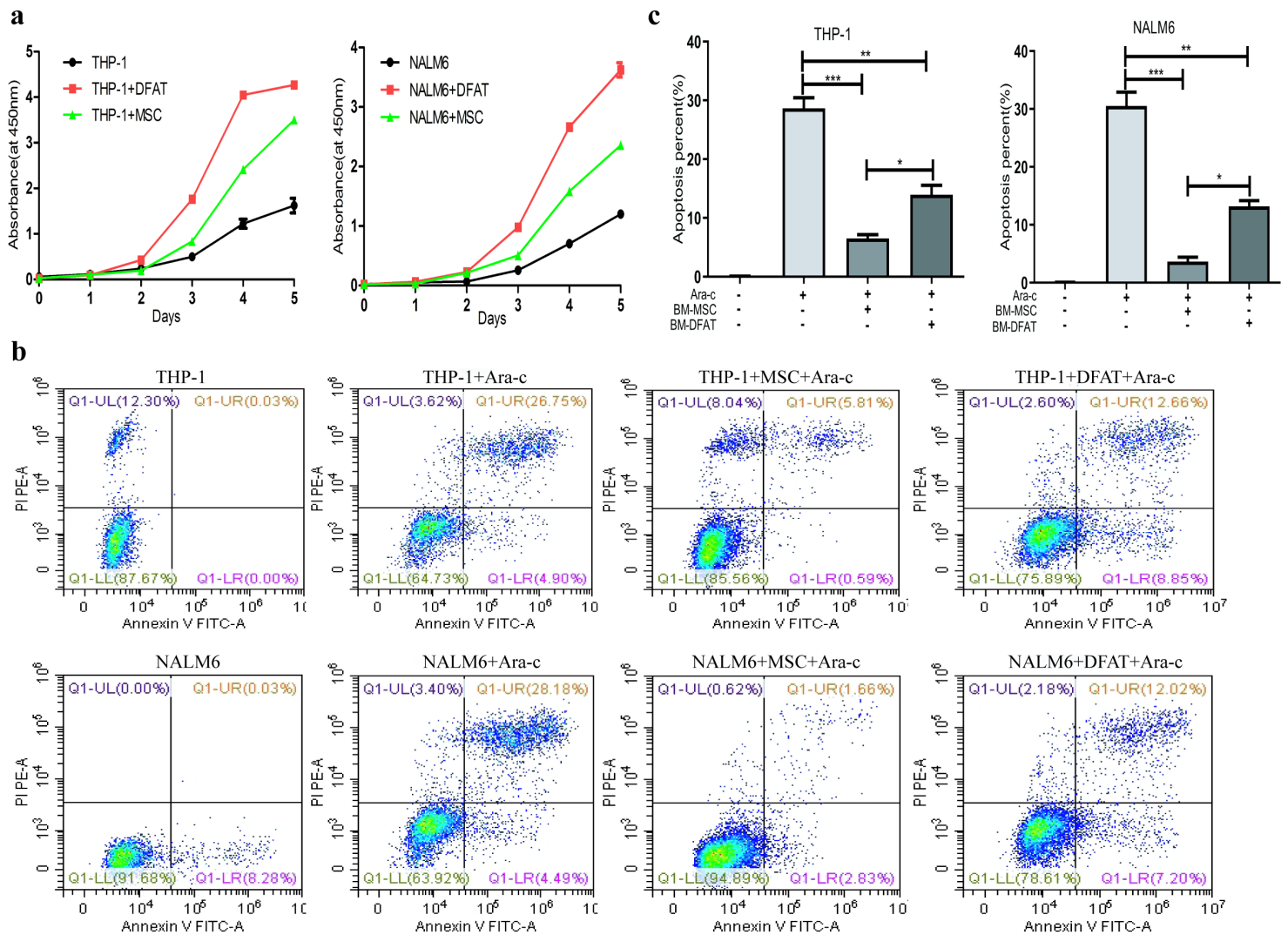


Figure 5

The effect of BM-DFAT cells and BM-MSCs on leukaemic cells. a. The proliferation of THP-1 and NALM6 cells that were cocultured with BM-DFAT cells and BM-MSCs was detected through CCK8 assays. b. Bar plots illustrating the viability of THP-1 and NALM6 cells that were cultured in medium alone or directly cocultured with BM-DFAT cells or BM-MSCs with treatment with Ara-C (10 μ M) for 48 h. c. The percentage of apoptotic THP-1 and NALM6 cells in the groups. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.