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Mini Review

Cell culture purity issues and DFAT cells

Shengjuan Wei [a,b], Werner G. Bergen [c], Gary J. Hausman [d], Linsen Zan [a,*], Michael V. Dodson [b,*]

[a] College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi Province 712100, China
[b] Department of Animal Sciences, Washington State University, Pullman, WA 99164, USA
[c] Program in Cellular and Molecular Biosciences/Department of Animal Sciences, Auburn University, Auburn, AL 36849, USA
[d] Animal Science Department, University of Georgia, Athens, GA 30602-2771, USA

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ABSTRACT

Dedifferentiation of mature adipocytes, *in vitro*, has been pursued/documented for over forty years. The subsequent progeny cells are named dedifferentiated adipocyte-derived progeny cells (DFAT cells). DFAT cells are proliferative and likely to possess mutilineage potential. As a consequence, DFAT cells and their progeny/daughter cells may be useful as a potential tool for various aspects of tissue engineering and as potential vectors for the alleviation of several disease states. Publications in this area have been increasing annually, but the purity of the initial culture of mature adipocytes has seldom been documented. Consequently, it is not always clear whether DFAT cells are derived from dedifferentiated mature (lipid filled) adipocytes or from contaminating cells that reside in an impure culture.

1. Introduction

Experimental observations obtained through the use of cell cultures for cellular and molecular studies may be plagued with inconsistencies. For example, primary cell cultures may contain co-cultured (contaminating) cells which may alter desired outcomes from experiment to experiment, cell lines may alter their growth/developmental potential with prolonged culture, and cell clones may exhibit high rates of cell death influenced by improper substratum and/or media used for propagation. While numerous learned societies [such as the Society for *In Vitro* Biology] have attempted to establish standards for cell culture research, few workers appear to follow such recommendations or report specific details of their cell culture method/protocol to enable others to repeat their findings. Consequently, while numerous reports are available in developmental cell culture work using DFAT cells, experimental protocols and findings are difficult to be confirmed. One such area of *in vitro* work is the reported ability of mature adipocytes to dedifferentiate to form proliferative-competent progeny DFAT cells. DFAT cells reportedly express several embryonic stem cell markers [1,2], and possibly possess mutilineage potential, including the capacity to redifferentiate into adipocytes, or transdifferentiate into endothelial cells, chondrocytes, osteoblasts, skeletal myocytes, smooth muscle cells or cardiomyocytes [3]. Thus, DFAT cells possess potential value for various applications for tissue engineering and as potential vectors for the alleviation of several disease states.

Among recent studies to explore DFAT cells, specific purification steps to insure the purity of the initial culture of mature adipocytes has seldom been reported [4–10]. Indeed, 96%–100% purity of isolated mature adipocyte samples utilizing fluorescence activated cell sorting (FACS), immunostaining or confocal microscopy was reported in several studies [1,11–16]. It is not always clear from such work whether proliferative-competent DFAT cells are derived from dedifferentiated mature (lipid filled) adipocytes. This is a key point as studies with "unsuspected-heterogeneous" cell preparations which were unwittingly described as DFAT cells, could provide misleading information. The impact of small numbers of contaminating cells may be more serious than hitherto thought in downstream work.

Methods for acquiring DFAT cells described by various publications are similar (Table 1) [4–10,1,11–31,2] and include cell dispersion, filtration, centrifugation, washing and ceiling culture (a common method to acquire lipid-containing adipocytes based on their lipid buoyancy) steps. Dispersed cell preparations of adipose tissue when centrifuged, separate into a pellet containing sedimentary cells and an upper, floating fraction containing lipid-laden adipocytes, free lipids, and a small number of contaminating cells. After ceiling culturing the upper (fat) layer of the centrifugate, mature adipocytes (the mother cell of future DFAT cells) float and attach to the ceiling of inverted cultureware. Subsequently, attached mature (lipid-filled) adipocytes change their morphology and undergo dedifferentiation without any inducement reagent, resulting in fibroblastic and proliferative DFAT cells [29–31,33]. However, the upper, floating fraction of centrifugate is not a pure pupulation

* Corresponding authors. Fax: +86 29 87091148 (L. Zan), +1 509 335 1082 (M.V. Dodson).
E-mail addresses: zanls@yahoo.com.cn (L. Zan), dodson@wsu.edu (M.V. Dodson).

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Table 1
Research progress on DFAT cells with diverse cell isolation methods and purification tests.

Purification tests	Cell isolation methods	Main contributions in DFAT cell research	References
None	Digest[a]; remove undispersed pieces; centrifuge at 400 g for 1 min; wash twice; pipette; plate in monolayers for 4 h; add small amount of media and keep undisturbed for 5–7 d	Lipid-laden human adipocytes which became delipidated in culture were found to be capable of division; the rate and number of cell divisions were age dependent	[17,18]
	Digest[a]; filter; centrifuge at 135 g for 3 min, wash 2–3 times, pipette; 7 d ceiling culture	DFAT cells transdifferentiated into osteoblasts, skeletal myocytes cardiomyocytes *in vitro* and/or *in vivo*; Transplantation of DFAT cells repaired infarcted cardiac tissue and improved urethral sphincter contractility in rats; Differentially expressed genes during dedifferentiation were revealed; Clonal expansion of rat DFAT cells formed adipogenic differentiation capable (PPARγ abundant) and incapable (Collagen Type-1 abundant) cell strains	[4–10]
Unknown[b] (More than 98% or 99% of the isolated cells were mature adipocytes)	Digest[a]; filter; centrifuge at 600 g for 10 min, wash, pipette; culture mature adipocytes between two coverslips for 4 to 5 d	DFAT cells rapidly acquired the endothelial phenotype *in vitro* and promoted neovascularization in ischemic tissue and vessel-like structure formation in Matrigelplug, suggesting cells of endothelial and adipocyte phenotypes may have a common precursor	[19]
	Digest[a]; filter; centrifuge at 135 g for 3 min, wash 2–3 times, pipette; 7 d ceiling culture	Mature adipocytes dedifferentiated and redifferentiated *in vitro* and *in vivo*; Implantation of DFAT cells ameliorates habu snake venom-induced chronic renal dysfunction in tenascin-C- deficient mice	[20,21]
Examination under a microscope by the cell shape	Digest[a]; filter; centrifuge; wash thrice; pipette; ceiling culture until fat cells attached firmly	Unilocular fat cells proliferated *in vitro* with different division modes	[22]
Examination by a microscope; S-100[c] immuno-staining (contamination rate with preadipocytes was around 1/350)	Digest[a]; filter; centrifuge; wash thrice at 700 rpm for 5 min, ceiling culture until fat cells attached firmly or perform three-dimensional collagen gel matrix culture	Unilocular fat cells from the bone marrow dedifferentiated, proliferated and redifferentiated in culture	[23]
Based on examination under a microscope and purified methods	Digest[a]; filter; centrifuge at 180 g for 10 min; wash, pipette; ceiling culture with purified methods including differential platings, cell surgery and clone techniques	Human/pig/bovine derived mature adipocytes dedifferentiated and redifferentiate *in vitro*; Bovine derived DFAT cells displayed protracted adipogenesis; Gene expression patterns were revealed during the redifferentiation of bovine-derived DFAT cells; PPARγ and SREBP-1 play a key role to regulate the redifferentiation; Different adipose depot derived DFAT cells displayed diverse gene expression patterns during the adipogenesis process	[24–28]
Based on examination under a microscope, purified methods and single cell system	Digest[a]; filter; centrifuge at 180 g for 10 min; wash, pipette; ceiling culture with serial differential platings and other purified methods	Mature adipocytes asymmetrically divided *in vitro*; Angus-derived adipocytes may extrude lipid prior to proliferation *in vitro*; Mature pig adipocytes extruded lipid before proliferation *in vitro*	[29–31]
Confocal microscopy analysis (100% of the floating cells were mature adipocytes)	Digest[a]; filtered once, wash four times at 250 g for 5 min, pipette; 8–10 d ceiling culture	Human DFAT cells showed similar properties to bone marrow-derived mesenchymal stem cells	[1]
Fluorescence-activated cell sorting (FACS) assessed the purity of isolated adipocytes was over 99%	Digest[a]; centrifuge at 670–700 g for 10 min, wash twice, pipette; 7–14 d ceiling culture	DFAT cells differentiated into bone-forming cells *in vitro* and *in vivo*	[11]
	Digest[a]; filter; centrifuge at 135 g for 3 min, wash thrice, pipette; 7 d ceiling culture	DFAT cells differentiated into mature adipocytes *in vivo* and *in vitro*; DFAT cells transdifferentiated into cardiomyocyte-like cells	[12,13]
Stain[d] and microscopic analysis (96% – 995% of the cells were lipid-filled mature adipocytes with a single nucleus)	Digest[a]; filter; centrifuge at 135 g for 3 min; wash thrice; pipette; 7 d ceiling culture	DFAT cells exhibited multilineage potential, including adipocytes, chondrocytes, osteoblasts and smooth muscle-like cells; DFAT cells contributed to bladder tissue regeneration; DFAT cell lines could be stable subcultured and might be a substitute for preadipocytes in research	[14–16]
	Digest[a]; filter; centrifuge at 220 g for 5 min; wash thrice; pipette; 10 d ceiling culture	DFAT cells expressed some embryonic stem cells markers like SV cells	[2]

[a] Fat issue was cut and then digested with collagenase.
[b] Purification tests were not shown in text.
[c] S-100: preadipocyte marker.
[d] Stain the neutral lipid by AdipoRed® and the nuclei by Hoechst 33342.

[23,24,32,33]. Common problems associated with obtaining a mixture of cells during cell isolation and initial ceiling culture have been documented and may include improper cell dispersement during isolation resulting in clumping together of spindle-shaped SV cells with mature adipocytes [33]. As the mature adipocytes float and attach to the ceiling of flasks, they may "drag" contaminating cells with them [24]. Moreover, small totipotent or pluripotent stem cells or preadipocytes with uncharacterized boundaries, latent appearance, but similar buoyancy might be co-isolated and attach together with mature adipocytes [24]. Such problems may result in mixed (cell) cultures which will produce inconclusive results in experimental work [34].

During the mature adipocyte isolaion process, if 99 lipid-filled adipocytes and 1 potential unknown contaminating cell (might be one fibroblast cell/preadipocyte/stem cell/other cell) were isolated together (99% purity) the one contaminating cell could supply all proliferative cells. In our recent, yet unpublished culture experiments, about 40% of lipid-filled adipocytes (40 cells out of each 100) attach to the ceiling of the cultureware. For these cells studies we found that only 4–10 mature adipocytes actually

dedifferentiate and start to proliferate on d 4 after cell isolation (mature adipocytes took the first 3 days to attach firmly, stretch the cell membrane and dedifferentiate). This varies according to donor animals or adipose depots used to obtain cell isolations [25], and overall numbers of viable, homogeneous mature adipocytes may be increased through the use of substratum coatings or different cell culture ware [35]. Even so, should only one attached contaminating cell be present in the culture, it is possible for proliferation of the contaminating cell to begin immediately thus dominating the mature lipid filled cells contribution to the (supposed) DFAT cell population. In other words, during the mature fat cell attachment period, alone, even if only a few contaminating cells with strong proliferative potential are present, such cells could generate a huge population of non-DFAT cells possessing the ability to proliferate, replicate or to produce different daughter cells. As such, the initial purity of isolated mature adipocytes plays an important role for acquiring purified DFAT cells, and cultures that are 99% pure are not reliable for the subsequent experimental work to study DFAT cell properties [32].

In summary, DFAT cells are defined as progeny cells from dedifferentiation of mature (lipid-filled) adipocytes. When ceiling culturing mature adipocytes in order to obtain DFAT cells, due to attachment and dedifferentiation efficiency as well as the required time for these processes to occur, a small number of co-cultured contaminating cells could soon replace the DFAT cell status and become the major cell type, resulting in a seriously contaminated population. Studies by using this so-called 'DFAT' cells in downstream work are not reliable. Primary cell culture purity is vital if using DFAT cells for any application.

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