Minireview

Cell line models for differentiation: preadipocytes and adipocytes

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Abstract

In vitro **models have been invaluable in determining the mechanisms involved in adipocyte proliferation, differentiation, adipokine secretion and gene/protein expression. The cells presently available for research purposes all have unique advantages and disadvantages that one should be aware of when selecting cells. Established cell lines, such as 3T3-L1 cells, are easier and less costly to use than freshly isolated cells, even though freshly isolated cells allow for various comparisons such as the *in vitro* evaluation of different *in vivo* conditions that may not be possible using cell lines. Moreover, stem cells, transdifferentiated cells or dedifferentiated cells are relatively new cell models being evaluated for the study of adipocyte regulation and physiology. The focus of this brief review is to highlight similarities and differences in adipocyte models to aid in appropriate model selection and data interpretation for successful advancement of our understanding of adipocyte biology.**

Keywords: adipose, adipocyte, preadipocyte, stromal–vascular, differentiation, *in vitro*

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Introduction

Adipocyte biology is intensely studied, due to a global rise in obesity-associated health issues. Technologies such as microarrays, protein arrays and genetic manipulation allow for rapid identification of genes, proteins and pathways essential for understanding adipocyte regulation.[1] However, these techniques are of limited value if the most appropriate model is not used in research efforts. This review will highlight similarities and differences in adipocyte models to aid in appropriate model selection and data interpretation for successful advancement of our understanding of adipocyte biology. As advances in cell isolation and identification, stem cell technology, and transdifferentiation allow for the adipogenic evaluation of unique systems, this review provides a recent comparison of commonly used cell lines and builds on previous reports by including recent advances in technologies for adipocyte study.[2–4]

Adipogenic cells – freshly isolated cells, primary cell lines, established cell lines

White (mature) adipocytes contain single, large lipid droplets that appear to comprise the majority of cell volume, while the cytoplasm and nucleus are found at the cell periphery. The lipid droplet increases adipocyte buoyancy, making cell culture methods used for other cell types fairly ineffective as they rely on cells attaching to a culture plate whereas lipid-containing adipocytes float. Recent reports have documented methods to utilize floating adipocytes for the study of adipogenesis,[5] and short-term maintenance of these cells for several hours has been achieved for the assessment of metabolic parameters. For example, the acute effects of insulin exposure on intracellular glucose transporter 4 cycling and regulation[6] and hexose uptake[7] have been evaluated in freshly isolated, mature adipocytes. More frequently, preadipocytes that do not yet contain a significant amount of lipid and resemble fibroblasts are cultured and after differentiation is induced, the cell cultures may be used for metabolic studies. Brown adipocytes, which are characterized by multilocular lipid droplets and high mitochondrial content, are derived from distinct adipose tissue depots that are highly vascular and innervated, and several cell lines and methods to isolate and study these cells have been developed but will not be reviewed here.[8]

Adipogenesis, lipid metabolism and apoptosis

Of mesenchymal origin, adipocytes are generated through the process of adipogenesis.[2,4] Adipogenesis is both a prenatal and a postnatal physiological event. However, after adipocytes begin to fill with lipid, they may/may not be

considered to be fully differentiated.[2] Main research areas of adipogenesis have been in altering rates of adipogenic cell proliferation and/or reducing differentiation of cells committed to the adipose lineage. Adipocytes possess the capacity to store a large volume of lipid for subsequent release for use by higher priority tissues, and much of the research on adipocytes has stemmed from interest in regulating lipid metabolism (especially deposition). Although less information about apoptosis is known, adipose depots appear, at times, to become afflicted with autoimmune dysfunctions that, in part, may result in apoptosis (Dodson *et al.*, in preparation).

Accessory cells of adipose tissue

It is well-established that adipose tissue is also comprised of various other cell types including mesenchymal stem cells, T regulatory cells, endothelial precursor cells, preadipocytes and macrophages. Although the full complement of cell types found in adipose tissue has not been confirmed, these various cells present in adipose tissue can be isolated and cultured from freshly isolated adipose tissue. Methods to isolate the various fractions of cells from adipose tissue have been reported.[9,5] For example, maturation of preadipocytes to adipocytes can be evaluated using stromal–vascular (SV) cell fractions freshly isolated from adipose tissue and cultured as compared with studies using mature adipocytes. In addition, these cultures offer the ability to culture various cell types present in adipose tissue as compared with culturing a single cell type. While single cell types may offer advantages in identifying novel pathways and players, cultures with multiple cell types may be more predictive of *in vivo* conditions more closely for those whose long-term goal is to evaluate adipose tissue and adipogenesis *in vivo*.

Stromal–vascular cells

The pellet of cells that can be isolated following the enzymatic digestion and centrifugation of adipose tissue, termed SV cells, can be used for a multitude of *in vitro* experiments. Interestingly, the proportion of SV cells that can be induced to differentiate into adipocytes appears to vary by species, age of donor and depot (Table 1). This may be due, in part, to the exposure of various hormones and growth factors *in vivo*. Alternatively, because SV cell cultures contain various cell types, the *in vitro* conditions may mimic *in vivo* conditions of adipose tissue complexity more closely than cultures of a single cell type. Another potential advantage of SV cells is that they can be obtained following *in vivo* treatments and from various species, ages and depots allowing for various layers of evaluation. As such, one consideration is the increased time and financial cost often incurred on behalf of the tissue donor.

Commercially available adipogenic cells

An alternative is now available due to recent advances in cell harvesting and storage. Primary human preadipocytes can now be purchased from several companies. Similar to established rodent cell lines, human preadipocyte cells may be subcultured several times, although their ability to differentiate declines with each passage.

Cell lines

Classic cell types to study adipogenesis are established cells lines, such as the 3T3-L1 cell line, which were developed through clonal expansion of rodent-derived cells and only contain a single cell type.[2] Although often thought to be immortalized, it is known that the capacity of 3T3-L1 cells to differentiate into adipocytes declines with increasing number of passages. These cell lines have been quite useful in identifying key molecular markers, transcription factors and various interactions that are required for preadipocyte differentiation, and are thus frequently used to rapidly screen and assess the adipogenic potential of various agents or cellular perturbations.

Unique cell models

Cell lines established from knockout mice can be invaluable in determining the role of specific genes in adipogenesis.[10,11]

Mature adipocytes as a source of cells for adipogenesis

Mature adipocytes isolated after the enzymatic digestion of adipose tissue are less frequently used but have the potential for providing valuable information. Mature adipocytes lose their lipid[12] and return to a proliferative competent cell, *in vitro*.[2,5,13] Proliferative-competent progeny cells are presently being evaluated for the ability to re-differentiate into lipid-assimilating adipocytes,[14] ability to produce adipogenic regulatory factors,[15,16] and ability to transdifferentiate into other types of cells.[17,18]

Differentiation potential

The patterns and models of adipogenesis derived from established cell lines can dramatically differ from those seen with primary cells and many adipogenic changes observed in these cells are not observed with further *in vivo* evaluation (Table 2). For example, primary cells that have been isolated from an adipose tissue depot have different developmental patterns than those observed in differentiation of established cell lines.[14] Both *in vivo* and *in vitro* studies have clearly shown the complexity of adipose biology that may account for some of these differences. Numerous studies have shown that endocrine and metabolic perturbations have long-lasting influences on preadipocyte development in *in vitro* systems and have been hypothesized to 'imprint' disease risk in later life.[4] Interestingly, the proportion of SV cells that differentiate into adipocytes can be altered by modifying media cocktails and extra-cellular matrix components,[19] which may mimic the various factors that adipocytes are exposed to *in vivo*. Common adipogenic cocktails have emerged with some components known to induce adipogenesis *in vivo*

Table 1 Adipogenic cell models vary greatly in their origin and capacity to proliferate and differentiate

	Clonally expanded unipotent cell lines derived from mice				Primary S–V cells					
Cell type	**3T3-L1**	**3T3-F422A**	**TA1**	**OB1771**	**Mouse**	**Rat**	**Pig**	**Bovine**	**Chicken**	**Human**
Methods used to derive cells	Clonally isolated from Swiss 3T3 cells from disaggregated 17–19 d embryos	Clonally isolated from Swiss 3T3 cells from disaggregated 17–19 d embryos	5-azacytidine-treated 10T1/2 embryo fibroblasts	Subcloning of Ob17 cells from obese C57BL/6J ob/ob epididymal adipose tissue	Isolation and enzymatic digestion of adipose tissue, centrifugation to harvest stromal–vascular cells					
Plating density, cells (cm^2)	$2–3 \times 10^3$	2×10^2		3×10^3	1.5×10^4	1×10^4			$1–5 \times 10^4$	$3–4 \times 10^4$
Proliferation media	10% FCS		10% FCS	10% FCS	5% FBS	10% FBS	10% FBS	20% FBS	10% FCS	10% FCS for 4 h then ITT medium
Time until confluency (d)	3	5		3			3		3–4	4–5
Maximal differentiation media cocktail required	FBS, INS or IGF-1, glucocorticoid and IBMX	FBS and INS	DEX and INS. Spontaneous differentiation after 10–14 d	INS, T3, IBMX and DEX	DME/WAJC404A media with INS, transferrin and FGF	ITS and T3	FBS and DEX (0-3 d). ITS (3-6 d)	FBS, IBMX, DEX, biotin, acetic acid, sodium octanate, BSA and ITS	Serum-free medium with transferrin, INS and BSA	Serum-free medium with MIX, INS, TZD and DEX

BSA, bovine serum albumin; DEX, dexamethasone; FBS, fetal bovine serum; FCS, fetal calf serum; FGF, fibroblast growth factor; IBMX, 3-isobutyl-1-methylxanthine; IGF-1, insulin-like growth factor 1; INS, insulin; ITS, insulin, transferrin, selenium; ITT, insulin and triiodothyronine; MIX, 1- methyl-3-isobutylxanthine; S–V, stromal–vascular; T3, triiodothyronine; TZD, thiazolidinedione
References[24,46–57]

Table 2 Adipogenic cell models vary in their expression of transcription factors associated with adipogenesis

Transcription factor	Clonally expanded unipotent murine cell lines	Primary S–V cells				
	3T3-L1	Rat	Pig	Bovine	Chicken	Human
C/EBPα						
Initial expression	2–3 d after confluency	By 1 d after seeding with 75% of cells being immunoreactive 2 d after seeding	11% immunoreactive cells 1 d after seeding		Fatty acids increased gene expression compared with basal media by 4 d	Up-regulated within 4 h of INS and MIX exposure
Maximal expression	5–6 d after confluence	4 d after seeding	By 2 d after seeding if treated with DEX	6 d of culture then decreased through 12 d	12 d after culture	Protein levels are maximal at 4 d after seeding while mRNA levels increase
Maintenance of maximal expression	DEX, indirect, relayed	INS-dependent	DEX-dependent			
C/EBPβ						
Initial expression	mRNA present after plating. Protein present after plating and increases by 2 d					Within 4 h. Transient increase with DEX treatment
Maximal expression						
Maintenance of maximal expression	Gradual decline with ~0% of maximal level expression by 8 d				Slight and gradual increase in gene expression over 48 h	
C/EBPδ						
Initial expression	mRNA present after plating. Protein present after plating and increases by 2 d.					
Maximal expression	Expression decreases over 48 h after removal of MIX and DEX					
Maintenance of maximal expression					Slight and gradual increase in gene expression over 48 h	
PPARγ						
Initial expression					4 d	First detected on 2 d
Maximal expression					Fatty acids increased expression compared with basal medium. Gene expression increased from 4 to 8 d of culture and then declined by 12 d of culture	4 d after plating
Maintenance of maximal expression						DEX included in differentiation medium

Continued

Table 2 Continued

Transcription factor	Clonally expanded unipotent murine cell lines	Primary S–V cells				
	3T3-L1	Rat	Pig	Bovine	Chicken	Human
aP2						
Initial expression						By 2 d with MIX + INS + DEX treatment
Maximal expression			Cells treated with fatty acids shown aP2 transcript concentration increased concomitantly with PPARγ		4 d after culture and reduced to basal levels by 8 d	mRNA increased linearly through day 8
Maintenance of maximal expression					Achieved with fatty acids	

aP2, adipocyte fatty acid binding protein; C/EBPα, CCAAT/enhancer binding protein α; C/EBPβ, CCAAT/enhancer binding protein β; C/EBPδ, CCAAT/enhancer binding protein δ; DEX, dexamethasone; INS, insulin; MIX, 1-methyl-3-isobutylxanthine; PPARγ, peroxisome proliferator-activated receptor γ, S–V, stromal–vascular
References[50,51,53,54,57–59]

(Table 3). It should be noted that both the basal and maximal proportion of cells whose differentiation can be induced varies by species.[14] Not surprisingly, studies comparing the profile of genes expressed during adipogenesis also show a number of differentially expressed genes during adipogenesis which are quite different between *in vitro* and *in vivo* samples.[20] Differences in the time required for cell monolayers to become confluent hint that mitotic regulation may differ (Table 1). In addition, there appear to be differences in the capacity and regulation of differentiation in various cell models.

Since this has been the focus of more extensive evaluation, it is now known that several transcription factors are important regulators of adipocyte differentiation. In particular, the CCAT/enhancer binding protein family (C/EBP) and the peroxisome proliferator-activated receptors (PPARs) family are known to play important roles in adipose tissue development *in vivo* and in adipocyte differentiation *in vitro*.[21] Studies in established cell lines, including 3T3-L1 cells, suggest adipocyte differentiation is a result of sequential expression of transcription factors. The cascade is initiated by extracellular signals resulting in a transient increase in C/EBPβ and C/EBPδ expression within 24–48 h following cell exposure to medium containing a differentiation cocktail. Recent research in 3T3-L1 cells suggests C/EBPδ has a role in mitotic clonal expansion.[22] This is thought to be necessary for the stimulation of PPARγ expression. Adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding protein increases PPARγ activity through primary or secondary stimulation of PPARγ ligands which activate the PPARγ–RXRα complex. Studies suggest that C/EBPα expression is subsequently induced and PPARγ expression is maintained while C/EBPβ and C/EBPδ expression decline.[23] In addition, it appears that C/EBPα and PPARγ reciprocally regulate one another to ensure that adipocytes are maintained in a differentiated state. However, numerous studies using primary SV cells from rats, pigs and humans have shown dramatically different levels of these transcription factors than the transcriptional cascade observed with immortalized cells. For example, mRNA and protein levels of PPARγ, C/EBPβ, C/EBPα and protein levels of C/EBPδ are all present at the initiation of differentiation of SV cell cultures from rats, pigs and humans.[4] PPARγ and C/EBPα mRNA expression in bovine SV cells have been observed without the induction of adipogenesis.[4] SV cell cultures and fetal adipose tissue do not show a temporal expression of C/EBPα, β and δ as reported in numerous studies using the 3T3-L1 cell lines.[4]

Previously frozen cells isolated from human adipose tissue are readily available and are now being used for extensive evaluation of adipogenesis. Although differentiation potential depends on the age of the donor, up to 70% of human SV cells can be induced to differentiate into adipocytes using serum-free medium supplemented with insulin, triiodothyronine and glucocorticoids.[24] Human SV cells seem to differentiate in a manner more similar to animal SV cells than established cell lines. For example, the expression profile of the transcription factors PPARγ, C/EBPβ, C/EBPα and C/EBPδ is more closely matched to other primary cells (Table 2). Since cells from established cell lines lack recent exposure to the hormone and growth factor milieu and various cell types found in adipose tissue known to impact adipogenesis, it should come as no surprise that adipogenesis is regulated differently in primary SV cells as compared with established cell lines.

Adipogenic response to hormones and growth factors

It is well established that changes in circulating hormone concentrations, beginning in fetal life and continuing throughout adulthood, can result in dramatic differences in adipose tissue growth.[4] For example, *in vivo* thyroxine

Table 3 Adipocyte response to endocrine stimulation

	Clonally expanded unipotent cell lines				Primary S–V cells				
	3T3-L1	**3T3-F422A**	**TA1**	**OB1771**	**Rat**	**Pig**	**Bovine**	**Chicken**	**Human**
Glucocorticoid	Differentiation is induced through increased expression of C/EBP-δ	Inhibit differentiation by down-regulating GH receptors	Differentiation induced with or without INS	Adipogenic-mitogenic stimulation triggers differentiation	Decreases cell proliferation and induces differentiation. DEX more potent than cortisol and corticosterone	Differentiation induced. Combination of INS and DEX is most effective		Differentiation induced. Combination of INS and DEX is most effective	Glucocorticoid and PPARγ agonist sequentially promote differentiation. Glucocorticoid effects are insulin dependent.
Insulin		Differentiation is INS and GH dependent				Induces differentiation		Stimulates differentiation	Supraphysiological insulin concentrations induce differentiation
Thyroid hormone		Induces differentiation		Induces differentiation		Differentiation induced if insulin present		No effect on differentiation	
Leptin					Biphasic effect on proliferation. Low concentrations increase proliferation and high concentrations decrease proliferation. No effect on differentiation	Proliferation stimulated but no inhibition of differentiation induced by INS, DEX, or IGF-I			
IGF-1	Exogenous IGF-I is obligatory and sufficient for differentiation through MAPK signaling					Induces differentiation. Anti-IGF-1 reduces adipocyte number		Stimulates proliferation but not differentiation	
Growth hormone		Adipogenesis is induced with 2 nmol/L met-hGH			Inhibits differentiantion	Inhibits differentiation			

C/EBPδ, CCAAT/enhancer binding protein δ; DEX, dexamethasone; GH, growth hormone, IGF-1, insulin-like growth factor 1; INS, insulin; S–V, stromal–vascular; T3, Triiodothyronine
References[24,55,57,60–72]

exposure of hypophysectomized fetal pigs and *in vitro* thyroxine exposure of adipose tissue SV cells enhance adipogenesis.[25,26] Changes in endocrine status throughout life, such as hypothyroidism, are also known to greatly impact adipogenesis.[27] Similarly, glucocorticoids such as dexamethasone are commonly used to stimulate adipocyte differentiation in multiple culture systems (Table 3) and are known to impact adipose development in people.[28] While glucocorticoids appear to induce adipogenesis in various culture systems, the adipogenic effects of insulin, growth hormone and thyroid hormones appear to vary greatly depending on the cell model used. It is plausible that these differences to responses are characteristic of various cell types but may also represent *in vivo* differences in response due to age, species, etc. Regardless, metabolic response may be relevant to a particular study and needs to be incorporated into the selection criteria while designing a research study.

Tissue engineering and transdifferentiation

Emerging research in the study of tissue regeneration, stem cells and transdifferentiation suggest alternative cell sources are available. Interest in adipose tissue as a source of various cell types for tissue regenaration therapies has developed; partly because it is a tissue of high abundance, a tissue that is easily accessible, and also because it has a relatively high abundance of mesenchymal stem cells.[18,29,30] It is accepted that adipose tissue contains various cell populations *in vivo* and more recent studies have shown the *in vitro* potential of angiogenic, osteogenic, hematopoietic, cardiomyocyte and neurogenic progenitor cells of adipose tissue cells.[31] For example, chondrogenesis can be stimulated by altering oxygen exposure,[32] while osteogenesis[33] and myogenesis[34] have been stimulated by modifying supplements in the media. Mesenchymal cell differentiation into various cell types including adipocytes, myocytes, chondrocytes or osteoblasts was demonstrated a decade ago.[35,36] The differentiation of adipocytes from mesenchymal stem cells derived from bone marrow requires a number of signaling pathways, growth factors and transcription factors,[37] while high levels of adipocytes from adipose tissue derived stem cells formed in serum-free media within 14 days.[38] Again, this supports the notion that the *in vivo* environment from which cells were isolated can significantly impact *in vitro* studies.

Studies evaluating adipocytes found within other tissue have also begun evaluating differences in cell development. For example, adipogenic cells isolated from skeletal muscle[9,12,39] and from intramuscular adipose tissue[12,40] have been used to study adipocytes naturally present within skeletal muscle. Initial indications suggest differences in adipogenic potential may exist as has previously been reported when adipocytes from various adipose tissue depots have been compared.[41] These cells are naturally found within skeletal muscle and do not represent the ectopic fat stored in non-adipocytes that is observed with several disease states. However, the physiologic and metabolic role of these adipocytes is poorly understood.

Lastly, there are reports of adipocyte transdifferentiation, defined as the function and phenotypic change of a particular differentiated cell into a different cell type without undergoing dedifferentiation.[42–44]

Conclusion

Recent studies assessing the role of epigenetic regulation in adipogenesis expand on the research on transcriptional regulation of adipocyte differentiation.[45] While studies, such as those conducted in 3T3-L1 cells, are invaluable in identifying new regulatory mechanisms, one should always be cognizant of both the metabolic and physiologic differences between various cell types used in experiments. This review provides an initial comparison of various adipocyte cell lines in the hope that selection of appropriate cell models for a given study aim will be considered. Lastly, while *in vitro* experiments are invaluable, one must appreciate the limitations when attempting to transfer the information from such studies to whole-body physiology, metabolism and development. The selection and use of an *in vitro* system must consider all known levels of regulation of proliferation, differentiation and function to ensure relevant results.

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