PROSPECTS

Journal of Cellular Biochemistry 110:564–572 (2010)

Journal of Cellular Biochemistry

# Cellular Models for Understanding Adipogenesis, Adipose Dysfunction, and Obesity

Andrea Armani,[1,2] Caterina Mammi,[1,3] Vincenzo Marzolla,[1,2] Matilde Calanchini,[1,2] Antonella Antelmi,[1,3] Giuseppe M.C. Rosano,[1,3] Andrea Fabbri,[2] and Massimiliano Caprio[1,2*]

[1] *Centre for Clinical and Basic Research, IRCCS San Raffaele Pisana, Rome, Italy*

[2] *Department of Internal Medicine, Endocrinology Unit, S. Eugenio & CTO A. Alesini Hospitals, University Tor Vergata, Rome, Italy*

[3] *San Raffaele Sulmona, l'Aquila, Italy*

## ABSTRACT

White adipose tissue (WAT) is no longer considered a depot for energy storage in the form of triglycerides, but is a secretory organ that releases factors, known as adipokines, capable of regulating several physiological processes. Alteration of WAT function with subsequent dysfunctional expression and secretion of adipokines plays a key role in the pathogenesis of obesity, diabetes, and other metabolic diseases. For this reason, a deeper understanding of the molecular mechanisms regulating adipocyte function is deemed necessary for planning strategies to treat and prevent obesity and its metabolic complications. This review examines cell culture models currently available for studying adipocyte biology. We focus on advantages, disadvantages and main differences between established preadipocyte cell lines and primary preadipocyte cultures. We revise protocols used to promote adipocyte differentiation and mature adipocytes dedifferentiation into preadipocytes. Finally, we briefly describe co-cultures of adipocytes with other cell types and three-dimensional adipocyte culture systems. These models allow investigation of cell–cell interactions with the cross-talk physiologically occurring between adipocytes and other cell types residing within or outside adipose tissue. J. Cell. Biochem. 110: 564–572, 2010. © 2010 Wiley-Liss, Inc.

**KEY WORDS:** ADIPOCYTE; ADIPOGENESIS; CELL CULTURE; OBESITY

The study of adipocyte development has become an area of intense research in recent years. Adipocyte differentiation has many implications for human diseases. The primary health concerns related to adipocyte development derive from extreme aberration in fat cell number. The largest class of health problems is caused by obesity, due in part to an overabundance of fat cells. The growing obesity epidemic is becoming a serious public health problem all over the world and the coexistence of obesity, type II diabetes, dislipidemia and hypertension, known as metabolic syndrome, determines an increased risk for the development of cardiovascular disease [Wilson et al., 2005].

In mammals, two distinct types of fat exist, white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is the predominant type and serves as a storage depot of lipids, whereas BAT generates heat through mitochondrial uncoupling of lipid oxidation. WAT is a remarkable endocrine organ secreting a number of hormones, known as adipokines, involved in the regulation of metabolic functions. Dysfunctional secretion of adipokines, together with a dysregulated disposal of glucose and lipids, is a pivotal pathogenetic factor in the development of several metabolic disorders [Rosen and Spiegelman, 2006; Lefterova and Lazar, 2009]. Disorders in adipocyte development and function has also been linked to some types of cancers and immune dysfunction [Spiegelman et al., 1993]. The molecular link to cancer has not been strongly established but is believed to be related to an increase in free radical damage of DNA caused by an increase in fatty acid metabolism.

A thorough understanding of the differentiation process could importantly support the potential manipulation of adipocyte cell number in order to control certain diseases. Indeed, a detailed study of differentiation, expansion and endocrine function of adipocytes is necessary for planning therapies against obesity and its metabolic complications.

Grant sponsor: Progetti di Ricerca di Interesse Nazionale Ministero dell'Università e della Ricerca 2007 (PRIN 2007 to A.F.).

*Correspondence to: Massimiliano Caprio, MD, PhD, IRCCS San Raffaele, Centro Ricerche, Via dei Bonacolsi, 81-00163 Rome, Italy. E-mail: massimiliano.caprio@sanraffaele.it

Received 25 February 2010; Accepted 26 February 2010 • DOI 10.1002/jcb.22598 • © 2010 Wiley-Liss, Inc.
Published online 21 April 2010 in Wiley InterScience (www.interscience.wiley.com).

## ADIPOSE TISSUE: A NOVEL RECOGNIZED ENDOCRINE ORGAN

Adipose tissue represents a peculiar endocrine organ, characterized by a discontinuous distribution throughout the body, with several subcutaneous and visceral depots under a coordinated, but still largely unknown, neuro-hormonal control. Moreover, it is characterized by a marked cellular heterogeneity: among its cellular components we can find adipocytes, preadipocytes, fibroblasts, endothelial cells and multipotent stem cells, able to differentiate into several cell types (Fig. 1). Overall, fat tissue consists of approximately one third of mature adipocytes. The remaining two thirds are a combination of small blood vessels, nerve tissue, fibroblasts and preadipocytes in various stages of development. Preadipocytes have the ability to proliferate and differentiate into mature adipocytes, conferring adipose tissue a constant functional plasticity, which determines its ability to expand throughout the entire lifespan [Sethi and Vidal-Puig, 2007].

In addition, adipose tissue releases adipokines into the bloodstream, whose primary role is the integration of multiple functions, such as energy balance, food intake and appetite, immunity, insulin sensitivity, blood pressure, and reproduction [Caprio et al., 2001]. Dysfunctional secretion of adipokines and free fatty acids contribute to the development of an inflammatory state that is believed to underlie the insulin-resistant state of obesity [Wellen and Hotamisligil, 2005]. Importantly, these alterations are linked to the specific site of deposit, with visceral fat accumulation being the main responsible for dysfunctional adipose tissue, metabolic complications and increased cardiovascular risk, even in normal weight individuals [Berg and Scherer, 2005; Wildman et al., 2008].

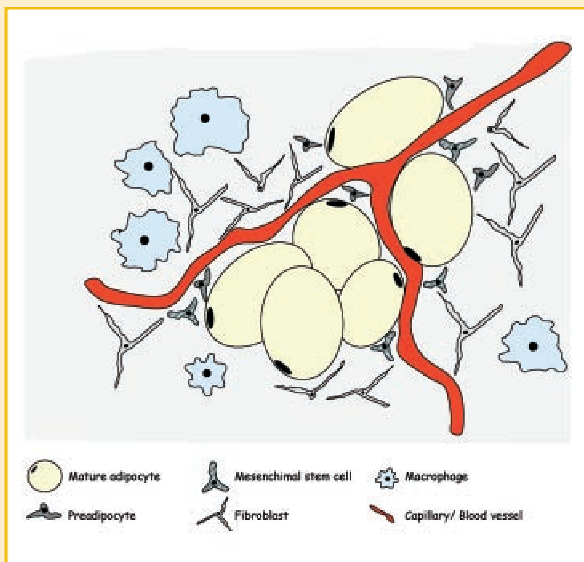


Fig. 1. Adipose tissue contains multiple cell types including mature adipocytes, pradipocytes, endothelial cells, macrophages, fibroblasts, and adipose-derived stem cells (ADSC), capable to differentiate toward the osteogenic, adipogenic, myogenic, chondrogenic, and neurogenic lineages in vitro. Preadipocytes can proliferate and differentiate into adipocytes. Macrophages are recruited into adipose tissue and, together with endothelial cells and mature adipocytes, contribute to the pro-inflammatory state observed in obesity.

Primary preadipocytes can be isolated and cultured from fat tissue explants. In particular culture conditions, they are able to proliferate, undergo adipose conversion and acquire the phenotype of mature adipocytes, whose main functions (i.e., adipokine secretion, lipolysis, etc.) can be induced and investigated in vitro [Hauner et al., 1989; Reyne et al., 1989; Litthauer and Serrero, 1992; Kirkland et al., 1994]. However, the use of primary culture suffers major drawbacks, due to technical difficulties to isolate preadipocytes from other fibroblast-like cells. Also, large amounts of fat tissue are required because preadipocytes constitute only a small percentage of total fat tissue. In addition, primary cultures have a limited life span.

## ADIPOGENESIS

Adipogenesis is a multi-step process involving a cascade of transcription factors and cell-cycle proteins regulating gene expression and leading to adipocyte development. Several coactivators and negative regulators of this network have been elucidated in the recent years [Lefterova and Lazar, 2009].

Many review articles focused on the complex network of transcription factors and pathways regulating adipogenesis have been already published [Farmer, 2006; Rosen and MacDougald, 2006]. Thus, here we just briefly resume the most important factors involved in adipocyte development.

Peroxisome proliferator-activated receptor-gamma (PPAR$\gamma$) and CCAAT/enhancer-binding proteins alfa (C/EBP$\alpha$) are two crucial molecules for driving the transcriptional cascade that leads to adipocyte differentiation and have been extensively studied. All the studies performed on PPAR$\gamma$ gain and loss of function models confirmed that PPAR$\gamma$ is both necessary and sufficient for fat formation [Farmer, 2006]. Ectopic expression of C/EBP$\alpha$ in fibroblasts can induce adipogenesis only in the presence of PPAR$\gamma$ [Freytag et al., 1994]. Accordingly, PPAR$\gamma$ ectopic expression can induce adipogenesis in mouse embryonic fibroblasts (MEFs) lacking C/EBP$\alpha$, but C/EBP$\alpha$ can not rescue adipogenesis, if PPAR$\gamma$ is not expressed, indicating that PPAR$\gamma$ is a master regulator of adipogenesis [Rosen et al., 2002].

C/EBP$\beta$ and C/EBP$\delta$ are two different members of the C/EBP family expressed in earlier phases of adipogenesis. Several studies suggest that C/EBP$\beta$ and C/EBP$\delta$ cooperate inducing expression of C/EBP$\alpha$, PPAR$\gamma$ and other genes involved in terminal differentiation [Lefterova and Lazar, 2009]. Their expression coincide with the latest phases of mitotic clonal expansion of preadipocytes, where cells re-enter the cell cycle and undergo several rounds of supplementary cell divisions [Ntambi and Young-Cheul, 2000]. These events depend on a complex coordinated cascade of cell cycle proteins, such as members of E2F and retinoblastoma protein (RB) [Puigserver et al., 1998; Fajas et al., 2002a,b], and are necessary for terminal adipocyte differentiation of murine preadipocytes. The mitosis is believed necessary to unwind DNA, allowing transcription factors access to regulatory response elements present in genes involved in adipogenesis [Cornelius et al., 1994].

The Wnt/beta-catenin pathway represents a signaling cascade which negatively regulates adipocyte differentiation [Ross et al., 2000; Moldes et al., 2003]. Such pathway has been shown to play an important role in the morphogenesis of other organs [Clevers, 2006] such as intestine, hematopoietic system, and bone. Importantly, Wnt/beta-catenin mediate the inhibitory effects of androgens on adipose conversion of 3T3-L1 cells [Singh et al., 2006]. Members of the Kruppel-like factor (KLF) family are also importantly implicated in adipogenesis and can modulate either positively or negatively adipocyte differentiation depending on the specific protein involved [Banerjee et al., 2003; Mori et al., 2005; Lefterova and Lazar, 2009]. For instance, KLF4 transactivates CEBPβ promoter initiating the adipogenic program [Birsoy et al., 2008]. KLF6 represses DLK1 which is a well established inhibitor of adipogenesis, therefore promoting adipose conversion [Li et al., 2005]. Differently KLF2 can repress PPARγ2 promoter activity, inhibiting adipogenesis when overexpressed in 3T3L1 cells [Banerjee et al., 2003; Wu et al., 2005].

## ESTABLISHED PREADIPOSE CELL LINES AND PRIMARY PREADIPOCYTE CULTURES

A large part of knowledge on the molecular pathways governing adipocyte development has been clarified thanks to available cellular models of preadipocytes. Two different kinds of cell lines are currently available: (1) preadipocyte cell lines, already committed to the adipocyte lineage, and (2) multipotent stem cell lines, able to commit to different lineages including adipose, bone and muscle lineage.

3T3-F442A and 3T3-L1 cells, isolated from the Swiss 3T3 cell line, derived from disaggregated 17- to 19- day-old Swiss 3T3 mouse embryos, are the most frequently used preadipocyte lines [Green and Meuth, 1974; Green and Kehinde, 1976]. Importantly, clonal cells lines are homogenous in terms of cellular population, and their cell types are all at the same differentiation stage. This allows a homogeneous response to treatments. In addition, these cells can be passaged indefinitely, which provides a consistent source of preadipocytes for study. For all these reasons clonal cell models are an interesting and complementary tool to animal models for the study of relevant biological questions. 3T3-F442A are generally regarded as a model with a more advanced commitment in the adipose differentiation process than 3T3-L1; for this reason they do not require early exposure to glucocorticoids to differentiate [Gregoire et al., 1998].

During proliferation, all preadipose cell models show a similar morphology to fibroblasts. Induction of differentiation triggers deep phenotipical changes of preadipocytes that become spherical and filled with lipid droplets, displaying many morphological and biochemical characteristics of adipocytes differentiated in vivo.

Ob17 cells, derived from adipose precursors present in epididymal fat pads of genetically obese (*ob*/*ob*) adult mice are employed less frequently [Negrel et al., 1978]. With respect to 3T3-F442A and 3T3-L1 cells, adult derivation of Ob17 cells represents a later preadipocyte stage. The derivation from an obese animal could also confer properties different from those of embryonic origin.

When implanted in nude mice, 3T3-F442A and Ob17 preadipocytes produce fat pads histologically identical to those of the host adipose tissue [Green and Kehinde, 1979; Vannier et al., 1985].

C3H10T1/2 cells, established in 1973 from 14- to 17-day-old C3H mouse embryos, are mesenchimal stem cells which, following treatment with 5-azacytidine, can be differentiate into cells showing morphology and biochemical features of muscle, bone, cartilage and adipose tissue [Taylor and Jones, 1979; Konieczny and Emerson, 1984]. Unlike 3T3-L1 cells, pluripotent C3H10T1/2 stem cells do not differentiate into adipocytes in the presence of adipose differentiation inducers.

Interestingly, treatment of proliferating C3H10T1/2 cells with bone morphogenetic protein 4 (BMP-4) induces commitment to adipocyte lineage cells which can differentiate into adipocytes when exposed to adipocyte differentiation inducers. This process of commitment is also functional in vivo, in fact C3H10T1/2 cells treated with BMP-4 in culture and implanted into athymic mice form adipose tissue indistinguishable from the adipose tissue of the host animal [Tang et al., 2004].

Most available models of murine preadipocyte (3T3-L1 at a greater extent, 3T3-F442A, and Ob17), once they reach confluence and growth arrest, upon opportune hormonal induction, re-enter cell cycle and undergo several rounds of post-confluent mitosis, known as mitotic clonal expansion (MCE). This is a fundamental requirement for terminal adipocyte differentiation. In fact, blocking the entry of 3T3-L1 cells into S phase at the time of MCE completely inhibits the adipose conversion program [Tang et al., 2003]. Also, inhibition of DNA synthesis in 3T3-F442A cells prevents formation of fat cells [Kuri-Harcuch and Marsch-Moreno, 1983]. However, confluent 3T3-F442A cells shifted to suspension culture maintain their ability to differentiate, suggesting that growth arrest but not confluency is required for adipocyte formation [Pairault and Green, 1979]. Similarly, C3H10T1/2 cells treated with BMP-4 that triggers commitment to adipose lineage undergo MCE in the presence of differentiation inducers [Tang et al., 2004].

Although extensive studies clarified the molecular pathways leading preadipocytes to differentiate into mature adipocytes, there is yet little knowledge about the initial processes and factors determining commitment of pluripotent stem cells towards the adipocyte lineage.

An alternative cell model for studying adipose commitment is represented by pluripotent embryonic stem (ES) cells. ES cells derive from the inner cell mass of 3.5 days old mouse blastocysts. ES cells in vitro can remain in a undifferentiated state or are able to differentiate into various lineages. ES cells cannot differentiate into adipocytes in the presence of adipogenic inducers. However, pretreatment of ES cells-derived embryoid bodies with retinoic acid (RA) allows commitment to adipose lineage and gives rise to adipocytes following induction with standard adipogenic hormones [Dani et al., 1997].

Fat cell precursors from adult WAT of various species, including humans, can be isolated and differentiated in vitro into mature adipocytes (Fig. 2). Despite the relative disadvantages of primary preadipocyte cultures, as discussed in the previous section, they may better reflect the context of adipose function in vivo, representing a

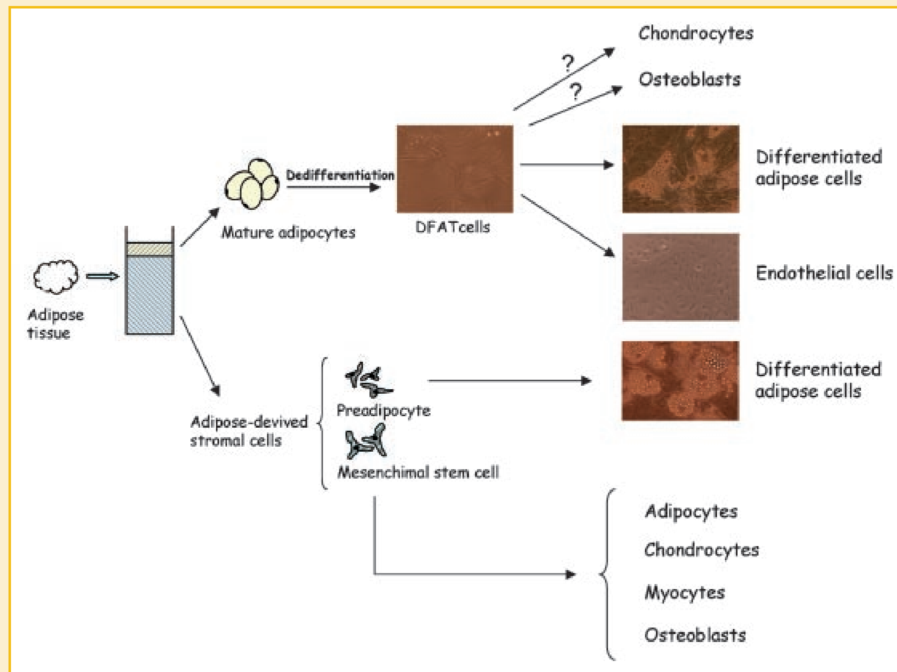


Fig. 2. Preadipocytes residing in the stromal vascular fraction of adipose tissue can be easily separated from mature adipocytes by centrifugation, followed by selective purification through appropriate culture techniques or flow cytometric cell sorting. Under opportune culture conditions, preadipocytes are capable of proliferating and differentiating in vitro. Mature adipocytes, through the ceiling culture method, can dedifferentiate into proliferating fibroblast-like cells. Such cells, known as DFAT cells, are able to differentiate again into adipocytes as well as to different cell types.

suitable cellular system to confirm data deriving from preadipocyte lines.

In addition, primary preadipocytes do not undergo continuous passages, hence they keep a diploid status, better reflecting the context in vivo. Interestingly, proliferation and differentiation of primary preadipocytes is clearly influenced by the anatomic site of the depots as well the age of the donor. In particular, aging reduces replicative ability of primary preadipocytes in cell culture [Djian et al., 1983].

However, established preadipocyte lines and primary cultures require different hormonal signals for differentiation and show a distinct responsiveness to hormones and factors regulating differentiation and metabolism. The discrepancies are mainly explained by the fact that primary preadipocytes derive from a cellular context where different cell types may affect differentiation ability and hormonal responsiveness.

The problem of cellular heterogeneity of the stromal vascular fraction (SVF), containing preadipocytes, endothelial cells, fibroblasts, monocytes, red blood cells, etc., can be overcome by selective culture techniques. Using an appropriate culture medium, not containing fetal bovine serum, but only specific factors selectively promoting adipogenesis [Hauner et al., 1989], will allow only preadipocyte to proceed towards the differentiation program. However, cellular composition of the SVF has been clarified by flow cytometric analysis. Expression of different patterns of clusters of differentiation (CD) represents a "hallmark" which enables to identify diverse cell types. For instance, cells corresponding to the adipose-derived stromal cells are defined by the following phenotype: $CD31^-$, $CD34^+$, $CD45^-$, $CD90^+$, $CD105^-$, $CD146^-$, and represent 70–90% of the total $CD45^-$ adipose cells [Sengenes et al., 2005]. SVF also includes endothelial cells, defined as $CD34^+$/$CD31^+$ cells, and machrophages, which express CD14 and CD31. Cells capable of differentiating into adipocytes are included in the $CD34^+$/$CD31^-$ cell fraction and do not express the mesenchimal stem cell marker CD105 [Sengenes et al., 2005]. For this reason, adipose-committed preadipocytes express a specific pattern of cell surface markers, allowing selective purification by immune-magnetic beads or by flow cytometric cell sorting.

## HOW TO INDUCE PREADIPOSE CELLS TO DIFFERENTIATE? IN VITRO AND EX VIVO AVAILABLE PROTOCOLS

The availability of adipose clonal cell lines and primary preadipocytes has allowed researchers to investigate the adipogenic or antiadipogenic potential of hormones, growth factors and various pharmacological compounds.

Confluent 3T3-L1 preadipocytes can be differentiated synchronously by a defined adipogenic cocktail. Maximal differentiation is achieved upon early hormonal induction for 48 h with a combination of insulin, glucocorticoids, and methylisobutylxanthine (MIX), which elevates intracellular cAMP levels, in the presence of fetal bovine serum. Dexamethasone (dxm), a synthetic glucocorticoid agonist, is traditionally used to stimulate the glucocorticoid receptor (GR), but a recent report demonstrated the prevalent involvement of

mineralocorticoid receptor (MR) in glucocorticoid-induced adipogenesis, since preadipocytes actively express MR during differentiation and glucocorticoids can bind to both GR and MR [Caprio et al., 2007]. After the first 48 h, insulin alone is required to continue the differentiation program. Interestingly, dxm is a powerful inductor of adipogenesis at early stages of differentiation, but displays antiadipogenic effects when added at later stages of adipose maturation, indicating that the effects of hormones are strictly time-dependent [Caprio et al., 2007].

Efficient maturation of 3T3-L1 cells has been also obtained by replacing IBMX and dxm with the non-steroidal antiinflammatory drug indomethacin during the first 2 days of differentiation [Norman et al., 2003]. In these conditions 3T3-L1 cells express and secrete higher levels of leptin, undergoing cell differentiation even more rapidly and completely than in the presence of dxm; such effect cannot be ascribed to inhibition of cyclo-oxygenase [Knight et al., 1987]. These data demonstrate that preadipocyte are able to undergo adipose conversion through different signals, also in the absence of active corticosteroids, leading to the activation of convergent or independent molecular pathways.

Differentiation of 3T3-F442A preadipocytes does not require early induction with glucocorticoids, since their commitment in adipogenesis is more advanced compared to 3T3-L1 cells. It is worthy to note that treatment of 3T3-442A cells with dxm represses adipogenesis, confirming what observed in 3T3-L1 cells exposed to glucocorticoid at a later stage of adipose conversion. Interestingly, MR activation is able to induce adipogenesis also in this cellular model [Caprio et al., 2007].

Adipogenesis can be influenced in a positive or negative way by many hormones, cytokines and growth factors. It is well known that insulin-like growth factor-1 (IGF-1), thyroid hormones, glucocorticoids, mineralocorticoids, PPARγ agonists promote differentiation, whereas androgens, estrogens and pro-inflammatory cytokines display an inhibitory effect. In particular, TNF-α has been shown to decrease the amount of intracytoplasmatic lipids also in mature adipocytes, conferring them the phenotype of preadipocytes, through a marked reduction of CEBPα and PPARγ expression [Torti et al., 1985, 1989; Xing et al., 1997].

Sometimes the effect of a specific substance depend upon the cellular context and origin. For example, growth hormone (GH) promotes differentiation in murine preadipose cell lines but inhibits differentiation of rat, porcine and human primary preadipocytes [Guller et al., 1989; Hausman and Martin, 1989; Corin et al., 1990; Wabitsch et al., 1995, 1996]. Other factors display different effects depending on the differentiation stage of cells. Interestingly, retinoic acid (RA) is able to influence the process of adipocyte formation in two distinct stages of the differentiation program. In addition to its role in the commitment of ES cells to adipose lineage, already discussed in a previous section, it can also influence terminal differentiation of preadipocytes to mature adipocytes, in both primary and clonal cells [Gregoire et al., 1998].

In general, the differentiation cocktail employed for primary preadipocytes is devoid of serum, but contains specific adipogenic factors (i.e., T3, insulin, glucocorticoids, IBMX, troglitazone) which allow selective culture and differentiation of preadipocytes. Interestingly, human primary preadipocytes do not require a significant degree of cell proliferation in vitro to enter the differentiation process, in fact they keep the ability to differentiate even when plated in serum-deprived condition. In line with this, blockade of cell mitosis by cytosine arabinoside does not interfere with adipocyte development [Entenmann and Hauner, 1996], suggesting that primary preadipose cells may have already undergone a critical level of cell division in vivo, necessary to enter terminal differentiation in vitro [Entenmann and Hauner, 1996; Boone et al., 2000]. In this context, allowing proliferation of primary preadipocyte in serum free medium may be useful to remove interferences with serum components during the differentiation phase and investigate, in a simplified culture setting, the effect of distinct compounds capable of modulating adipogenesis.

## MATURE ADIPOCYTE-DERIVED DEDIFFERENTIATED FAT CELLS

Recently, several authors showed that mature adipocytes derived from fat tissue retain the ability to de-differentiate in vitro into fibroblast-like cells. The culture technique developed to de-differentiate adipocytes is known as ceiling culture [Sugihara et al., 1986, 1987; Yagi et al., 2004; Fernyhough et al., 2008; Matsumoto et al., 2008; Nobusue et al., 2008]. In this protocol, floating unilocular mature adipocytes adhere to the top inner surface of a culture flask filled completely with medium. After about 7 days of culture, the adipocytes change morphology, spread and show fibroblast like shape with no lipid droplets. These cells, known as dedifferentiated fat (DFAT) cells, retain remarkable proliferative ability and are able to differentiate again into mature adipocytes both in vitro and in vivo (Fig. 2). Human DFAT cells from human subcutaneous adipocytes do not express adipocyte markers such as LPL, leptin, GLUT4 and C/EBPα, showing low levels of PPARγ, C/EBPβ and C/EBPδ transcripts. Interestingly, these cells express RUNX2 and SOX9, critical factors for osteogenesis and condrogenesis respectively, and are able to undergo osteogenic and chondrogenic differentiation in vitro in the presence of appropriate culture conditions. Moreover, they are able to form osteoid matrix when implanted in nude mice, after osteogenic induction in vitro [Matsumoto et al., 2008].

The ability of DFAT cells to proliferate and differentiate into multiple mesenchimal lineages confers to these cells the characteristics of adult stem cells. Kano et al. obtained DFAT cells from green fluorescent protein (GFP) transgenic mice. When implanted, these cells, which were easily traced, formed adipose tissue, differentiating not only into mature adipocytes but also into vascular endothelial cells [Nobusue et al., 2008]. These experiments suggest that DFAT cells represent a useful model for studying adipogenesis also in vivo.

The process of reversal of mature adipocytes to fibroblast-like cells in vitro might intriguingly occur also in vivo, raising the possibility of new unexplored sources of stem cells in adipose depots. Further investigations are required to understand whether different fat depots have a different potential of producing DFAT cells in vitro.

Adipose tissue has been shown to represent a source of multipotent stem cells, known as adipose-derived stem cells

(ADSCs), able to self-renew and differentiate along multiple lineages in vitro and in vivo [Nakagami et al., 2006; Schaffler and Buchler, 2007]. ADSCs show a cell surface antigen profile similar to that observed on mesenchymal stem cells (MSCs) in adult bone marrow, but are more simple to purify, given that their source is easily available.

MSCs and ADSCs are characterized by an heterogeneous populations, that contains also differentiated cells, contaminating the stem cells preparation. Removal of the contaminating differentiated cells requires several passages. Flow cytometer analysis show that DFAT cells are more homogeneous than ADSCs, representing an interesting cell source for cell engineering and regenerative medicine applications [Matsumoto et al., 2008]. Thanks to the adipose differentiation potential of DFAT cells, they represent a valuable cell system to study adipocyte development and metabolism, which could potentially replace conventional primary preadipocyte cultures. Table I summarize all adipose cell lines above discussed, including also DFAT cells.

## CO-CULTURES

Adipose tissue is a source of molecules that are secreted into the blood stream and modulate the function of other organs and tissues. Conversely, it receives signals from distant, different tissues, influencing its metabolism. Co-cultures of adipocytes and other cell types (i.e., endothelial cells, macrophages, muscle cells) have shed light on the complexity of the cross-talk occurring between adipose tissue and other organs [Turtzo et al., 2001; Aoki et al., 2003; Wang et al., 2006; Lumeng et al., 2007; Vu et al., 2007]. In fact, in the last years particular culture methods have been set up, which allow to culture separately two different cell types, thanks to specific devices with separated chambers and permeable barriers that permit secreted factors in the medium to pass between the two cultures, without any direct cell–cell contact. These systems have clarified several important interactions occurring between different tissues and cell types, as we briefly discuss in this section.

In adipocytes, cathecolamines interacting with β-adrenergic receptors are able to trigger lipolysis. Cathecolamines are released from sympathetic neurons that innervate WAT; co-cultures of 3T3-L1 adipocytes with primary sympathetic neurons isolated from newborn rats allow investigation of the interactions between the two cell types [Turtzo et al., 2001]. In such co-culture system, both adipocytes and neurons show appropriate morphology and cell-type specific markers. Moreover, leptin secretion and lipolysis induced by β-agonists are downregulated; such effects are mediated by the increased production of NPY by the sympathetic neurons, demonstrating a functional cross-talk between these cells and adipocytes [Turtzo et al., 2001].

Obesity and insulin resistance are highly correlated. In obesity, an increase of macrophages infiltration in visceral adipose tissue has been observed [Weisberg et al., 2003]. These cells represent an important local source of cytokines such as TNF-α and plasminogen activator inhibitor (PAI-1), together with C-reactive protein (CRP) and IL-6. Different systems of co-culturing macrophages and adipocytes have shown that macrophages-specific factors, as well as direct cell–cell contact, are able to induce an inflammatory response and insulin resistance in adipocytes [Lumeng et al., 2007].

Co-culture of primary hepatocytes and 3T3-L1 cells developed by Wang et al. [2006] has provided evidence that insulin resistance in the liver is mediated by adipokines such as IL-6 and TNF-α secreted by the adipose cells.

In diabetic and obese individuals, circulating levels of adiponectin secreted by adipose tissue are reduced [Havel, 2004; Bastard et al., 2006]. Glucose uptake of mature myocytes is increased when these cells are co-cultured with primary adipocytes, and such effect has been shown to be mediated by adiponectin deriving form co-cultured fat cells [Vu et al., 2007]. Accordingly, co-cultures of muscle cells and primary adipocytes from diabetic rats, expressing reduced levels of adiponectin, have shown a lower degree of glucose uptake in muscle cells, compared to co-cultures set up with adipocytes from normal rats [Vu et al., 2007].

Interestingly, it is known that skeletal muscle can influence adipose tissue formation by secretion/expression of interleukin-15 (IL-15) [Quinn et al., 2009], whose expression is abundant in muscle.

TABLE I. All Preadipose Cell Lines Discussed in This Review Are Summarized Here

| Cell lines | Cell type | Derivation | Characteristics | References |
|---|---|---|---|---|
| 3T3-L1 | Preadipose cells | 17- to 19-day disaggregated Swiss mouse embryos | Most frequently used cellular model of murine preadipocyte | Green and Meuth [1974] |
| 3T3-F442A | Preadipose cells | As above | Similar to 3T3-L1 cells, but more committed in the adipose differentiation process | Green and Kehinde [1976] |
| Ob17 | Preadipose cells | Epididimal fat pads of obese (*ob*/*ob*) adult mice | Low fatty acid biosynthesis and different responsivity to adipogenic and lipolytic stimuli | Negrel et al. [1978] |
| C3H10T1/2 | Stem cells | 14- to 17-day old C3H mouse embryos | Suitable for studies on the commitment of stem cells toward adipocyte lineage | Taylor and Jones [1979], Konieczny and Emerson [1984], Tang et al. [2004] |
| ES cells | Stem cells | 3.5 days old mouse blastocyst | As above | Dani et al., 1997 |
| DFAT cells[a] | Pluripotent cells | Mature adipocyte from WAT of various species, including human | Capability to proliferate and differentiate into adipocytes and other cell types | Matsumoto et al., 2008; Nobusue et al. [2008], Sugihara et al. [1986], Fernyhough et al. [2008] |

[a]DFAT cells do not represent a conventional established preadipocyte cell line but show fibroblast-like morphology and retain the ability to proliferate and differentiate into mature adipocyte as well as other cell types in vitro and in vivo. Thus, DFAT cells show differentiating potential similar to stem cells.

In line with this, the same authors have shown that skeletal muscle overexpression of IL-15 in vivo is associated to body fat reduction in transgenic mice [Quinn et al., 2009].

Adipose tissue contains in its context several different cell types including endothelial cells, which might modulate differentiation and function of adipocytes. Adipocyte-endothelial cell interaction is known to be involved in the homeostasis and maintenance of adipose mass. In fact, direct co-culture of endothelial cells and mature adipocytes has been shown to trigger an increase in proliferation of dedifferentiated cells deriving from mature adipocytes, due to direct cell contact [Aoki et al., 2003], with subsequent possible involvement in the enlargement of adipose tissue mass.

## THREE-DIMENSIONAL CELL CULTURES

In addition to conventional two-dimensional cultures, three-dimensional adipocyte cultures developed with extracellular matrix and polymer scaffolds as cell carrier [Sugihara et al., 1988; Fischbach et al., 2004; Sonoda et al., 2008; Toda et al., 2009] represent a more physiological model for the study of adipose tissue, mimicking at a higher level the context in vivo.

Sugihara et al. [1988] developed a three-dimensional system in which unilocular fat cells were embedded in collagen gel. The authors showed that fat cells keep specific functional properties together with dedifferentiation ability in these conditions [Sugihara et al., 1988]. A more detailed study on adipogenesis in a three-dimensional model has been subsequently set up [Fischbach et al., 2004], where the authors have generated constructs of 3T3-L1 preadipocytes and polymer scaffolds made from poliglycol acid. Induction of adipose differentiation has been obtained with an appropriate hormonal cocktail and adipogenesis analyzed in conditions of dynamic cultivation in an orbital shaker and in stirred bioreactors. Differentiation of the constructs has also been carried out in static conditions. Interestingly, differentiation occurring under dynamic conditions was characterized by a more physiological tissue architecture, with higher production of triglycerides and enhanced expression of adipocyte specific genes, allowing investigation in a more physiological context [Fischbach et al., 2004].

Finally, a recent method for studying regenerative processes in the context of adipose tissue has been recently developed [Sonoda et al., 2008]. This protocol requires mincing of adipose tissue and generates tissue fragments which are embedded in a three-dimensional collagen gel. Such culture system, known as adipose tissue-organotypic culture, enables to maintain viable mature adipocytes and proliferating preadipocytes as well as mesenchimal stem cells for 4 weeks in culture. Interestingly, in this peculiar cellular system, preadipocytes and mesenchimal stem cells localize at the peripheral zone of the fragment, whereas mature adipocytes reside in the central zone. Such distinct localization of proliferating cells allows an easier study of active regeneration zones in the tissue fragment, making possible a detailed investigation of regenerative processes occurring within adipose tissue [Sonoda et al., 2008].

## CONCLUSIONS

Several studies have shown that altered adipokines production plays a major role in the development of metabolic diseases such as obesity and diabetes. A detailed knowledge of the molecular pathways regulating adipocyte development and metabolism is a critical requirement for developing strategies to treat the above mentioned metabolic diseases. Employment of cellular models has provided a growing body of evidence that may elucidate the contribution of adipose tissue to energy homeostasis. In particular, three-dimensional adipocytes cultures and co-cultures of adipocytes with other cell types are crucial tools for understanding the multiple metabolic connections between fat and other tissues. The goal of these studies is to get more insights in factors and pathways that may be the target of new pharmacological interventions against obesity and diabetes.

## ACKNOWLEDGMENTS

The authors wish to thank Bruno Fève and Maria-Christina Zennaro for excellent scientific and technical support in the study of adipocyte models. This work has been supported in part by Progetti Ricerca Interesse Nazionale Ministero dell'Università e della Ricerca 2007 (PRIN 2007 to A.F.).

## REFERENCES

Aoki S, Toda S, Sakemi T, Sugihara H. 2003. Coculture of endothelial cells and mature adipocytes actively promotes immature preadipocyte development in vitro. Cell Struct Funct 28:55–60.

Banerjee SS, Feinberg MW, Watanabe M, Gray S, Haspel RL, Denkinger DJ, Kawahara R, Hauner H, Jain MK. 2003. The Kruppel-like factor KLF2 inhibits peroxisome proliferator-activated receptor-gamma expression and adipogenesis. J Biol Chem 278:2581–2584.

Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. 2006. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 17:4–12.

Berg AH, Scherer PE. 2005. Adipose tissue, inflammation, and cardiovascular disease. Circ Res 96:939–949.

Birsoy K, Chen Z, Friedman J. 2008. Transcriptional regulation of adipogenesis by KLF4. Cell Metab 7:339–347.

Boone C, Gregoire F, Remacle C. 2000. Culture of porcine stromal-vascular cells in serum-free medium: Differential action of various hormonal agents on adipose conversion. J Anim Sci 78:885–895.

Caprio M, Fabbrini E, Isidori AM, Aversa A, Fabbri A. 2001. Leptin in reproduction. Trends Endocrinol Metab 12:65–72.

Caprio M, Feve B, Claes A, Viengchareun S, Lombes M, Zennaro MC. 2007. Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis. FASEB J 21:2185–2194.

Clevers H. 2006. Wnt/beta-catenin signaling in development and disease. Cell 127:469–480.

Corin RE, Guller S, Wu KY, Sonenberg M. 1990. Growth hormone and adipose differentiation: Growth hormone-induced antimitogenic state in 3T3-F442A preadipose cells. Proc Natl Acad Sci USA 87:7507–7511.

Cornelius P, MacDougald OA, Lane MD. 1994. Regulation of adipocyte development. Annu Rev Nutr 14:99–129.

Dani C, Smith AG, Dessolin S, Leroy P, Staccini L, Villageois P, Darimont C, Ailhaud G. 1997. Differentiation of embryonic stem cells into adipocytes in vitro. J Cell Sci 110(Pt 11): 1279–1285.

Djian P, Roncari AK, Hollenberg CH. 1983. Influence of anatomic site and age on the replication and differentiation of rat adipocyte precursors in culture. J Clin Invest 72:1200–1208.

Entenmann G, Hauner H. 1996. Relationship between replication and differentiation in cultured human adipocyte precursor cells. Am J Physiol 270: C1011–C1016.

Fajas L, Egler V, Reiter R, Hansen J, Kristiansen K, Debril MB, Miard S, Auwerx J. 2002a. The retinoblastoma-histone deacetylase 3 complex inhibits PPARgamma and adipocyte differentiation. Dev Cell 3:903–910.

Fajas L, Landsberg RL, Huss-Garcia Y, Sardet C, Lees JA, Auwerx J. 2002b. E2Fs regulate adipocyte differentiation. Dev Cell 3:39–49.

Farmer SR. 2006. Transcriptional control of adipocyte formation. Cell Metab 4:263–273.

Fernyhough ME, Hausman GJ, Guan LL, Okine E, Moore SS, Dodson MV. 2008. Mature adipocytes may be a source of stem cells for tissue engineering. Biochem Biophys Res Commun 368:455–457.

Fischbach C, Seufert J, Staiger H, Hacker M, Neubauer M, Gopferich A, Blunk T. 2004. Three-dimensional in vitro model of adipogenesis: Comparison of culture conditions. Tissue Eng 10:215–229.

Freytag SO, Paielli DL, Gilbert JD. 1994. Ectopic expression of the CCAAT/enhancer-binding protein alpha promotes the adipogenic program in a variety of mouse fibroblastic cells. Genes Dev 8:1654–1663.

Green H, Kehinde O. 1976. Spontaneous heritable changes leading to increased adipose conversion in 3T3 cells. Cell 7: 105–113.

Green H, Kehinde O. 1979. Formation of normally differentiated subcutaneous fat pads by an established preadipose cell line. J Cell Physiol 101: 169–171.

Green H, Meuth M. 1974. An established pre-adipose cell line and its differentiation in culture. Cell 3:127–133.

Gregoire FM, Smas CM, Sul HS. 1998. Understanding adipocyte differentiation. Physiol Rev 78:783–809.

Guller S, Sonenberg M, Wu KY, Szabo P, Corin RE. 1989. Growth hormone-dependent events in the adipose differentiation of 3T3-F442A fibroblasts: Modulation of macromolecular synthesis. Endocrinology 125:2360–2367.

Hauner H, Entenmann G, Wabitsch M, Gaillard D, Ailhaud G, Negrel R, Pfeiffer EF. 1989. Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. J Clin Invest 84:1663–1670.

Hausman GJ, Martin RJ. 1989. The influence of human growth hormone on preadipocyte development in serum-supplemented and serum-free cultures of stromal-vascular cells from pig adipose tissue. Domest Anim Endocrinol 6:331–337.

Havel PJ. 2004. Update on adipocyte hormones: Regulation of energy balance and carbohydrate/lipid metabolism. Diabetes 53(Suppl 1): S143–S151.

Kirkland JL, Hollenberg CH, Kindler S, Gillon WS. 1994. Effects of age and anatomic site on preadipocyte number in rat fat depots. J Gerontol 49:B31–B35.

Knight DM, Chapman AB, Navre M, Drinkwater L, Bruno JJ, Ringold GM. 1987. Requirements for triggering of adipocyte differentiation by glucocorticoids and indomethacin. Mol Endocrinol 1:36–43.

Konieczny SF, Emerson CP Jr. 1984. 5-Azacytidine induction of stable mesodermal stem cell lineages from 10T1/2 cells: Evidence for regulatory genes controlling determination. Cell 38:791–800.

Kuri-Harcuch W, Marsch-Moreno M. 1983. DNA synthesis and cell division related to adipose differentiation of 3T3 cells. J Cell Physiol 114:39–44.

Lefterova MI, Lazar MA. 2009. New developments in adipogenesis. Trends Endocrinol Metab 20:107–114.

Li D, Yea S, Li S, Chen Z, Narla G, Banck M, Laborda J, Tan S, Friedman JM, Friedman SL, Walsh MJ. 2005. Kruppel-like factor-6 promotes preadipocyte differentiation through histone deacetylase 3-dependent repression of DLK1. J Biol Chem 280:26941–26952.

Litthauer D, Serrero G. 1992. The primary culture of mouse adipocyte precursor cells in defined medium. Comp Biochem Physiol A Comp Physiol 101:59–64.

Lumeng CN, Deyoung SM, Saltiel AR. 2007. Macrophages block insulin action in adipocytes by altering expression of signaling and glucose transport proteins. Am J Physiol Endocrinol Metab 292:E166–E174.

Matsumoto T, Kano K, Kondo D, Fukuda N, Iribe Y, Tanaka N, Matsubara Y, Sakuma T, Satomi A, Otaki M, Ryu J, Mugishima H. 2008. Mature adipocyte-derived dedifferentiated fat cells exhibit multilineage potential. J Cell Physiol 215:210–222.

Moldes M, Zuo Y, Morrison RF, Silva D, Park BH, Liu J, Farmer SR. 2003. Peroxisome-proliferator-activated receptor gamma suppresses Wnt/beta-catenin signalling during adipogenesis. Biochem J 376:607–613.

Mori T, Sakaue H, Iguchi H, Gomi H, Okada Y, Takashima Y, Nakamura K, Nakamura T, Yamauchi T, Kubota N, Kadowaki T, Matsuki Y, Ogawa W, Hiramatsu R, Kasuga M. 2005. Role of Kruppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. J Biol Chem 280:12867–12875.

Nakagami H, Morishita R, Maeda K, Kikuchi Y, Ogihara T, Kaneda Y. 2006. Adipose tissue-derived stromal cells as a novel option for regenerative cell therapy. J Atheroscler Thromb 13:77–81.

Negrel R, Grimaldi P, Ailhaud G. 1978. Establishment of preadipocyte clonal line from epididymal fat pad of ob/ob mouse that responds to insulin and to lipolytic hormones. Proc Natl Acad Sci USA 75:6054–6058.

Nobusue H, Endo T, Kano K. 2008. Establishment of a preadipocyte cell line derived from mature adipocytes of GFP transgenic mice and formation of adipose tissue. Cell Tissue Res 332:435–446.

Norman D, Isidori AM, Frajese V, Caprio M, Chew SL, Grossman AB, Clark AJ, Michael BG, Fabbri A. 2003. ACTH and alpha-MSH inhibit leptin expression and secretion in 3T3-L1 adipocytes: Model for a central-peripheral melanocortin-leptin pathway. Mol Cell Endocrinol 200:99–109.

Ntambi JM, Young-Cheul K. 2000. Adipocyte differentiation and gene expression. J Nutr 130:3122S–3126S.

Pairault J, Green H. 1979. A study of the adipose conversion of suspended 3T3 cells by using glycerophosphate dehydrogenase as differentiation marker. Proc Natl Acad Sci USA 76:5138–5142.

Puigserver P, Ribot J, Serra F, Gianotti M, Bonet ML, Nadal-Ginard B, Palou A. 1998. Involvement of the retinoblastoma protein in brown and white adipocyte cell differentiation: Functional and physical association with the adipogenic transcription factor C/EBPalpha. Eur J Cell Biol 77:117–123.

Quinn LS, Anderson BG, Strait-Bodey L, Stroud AM, Argiles JM. 2009. Oversecretion of interleukin-15 from skeletal muscle reduces adiposity. Am J Physiol Endocrinol Metab 296:E191–E202.

Reyne Y, Nougues J, Dulor JP. 1989. Differentiation of rabbit adipocyte precursor cells in a serum-free medium. In Vitro Cell Dev Biol 25:747–752.

Rosen ED, MacDougald OA. 2006. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7:885–896.

Rosen ED, Spiegelman BM. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 444:847–853.

Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, Spiegelman BM. 2002. C/EBPalpha induces adipogenesis through PPARgamma: A unified pathway. Genes Dev 16:22–26.

Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA. 2000. Inhibition of adipogenesis by Wnt signaling. Science 289: 950–953.

Schaffler A, Buchler C. 2007. Concise review: Adipose tissue-derived stromal cells–basic and clinical implications for novel cell-based therapies. Stem Cells 25:818–827.

Sengenes C, Lolmede K, Zakaroff-Girard A, Busse R, Bouloumie A. 2005. Preadipocytes in the human subcutaneous adipose tissue display distinct features from the adult mesenchymal and hematopoietic stem cells. J Cell Physiol 205:114–122.

Sethi JK, Vidal-Puig AJ. 2007. Thematic review series: Adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. J Lipid Res 48:1253–1262.

Singh R, Artaza JN, Taylor WE, Braga M, Yuan X, Gonzalez-Cadavid NF, Bhasin S. 2006. Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: Nuclear translocation of androgen receptor complex with beta-catenin and T-cell factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. Endocrinology 147:141–154.

Sonoda E, Aoki S, Uchihashi K, Soejima H, Kanaji S, Izuhara K, Satoh S, Fujitani N, Sugihara H, Toda S. 2008. A new organotypic culture of adipose tissue fragments maintains viable mature adipocytes for a long term, together with development of immature adipocytes and mesenchymal stem cell-like cells. Endocrinology 149:4794–4798.

Spiegelman BM, Choy L, Hotamisligil GS, Graves RA, Tontonoz P. 1993. Regulation of adipocyte gene expression in differentiation and syndromes of obesity/diabetes. J Biol Chem 268:6823–6826.

Sugihara H, Yonemitsu N, Miyabara S, Yun K. 1986. Primary cultures of unilocular fat cells: Characteristics of growth in vitro and changes in differentiation properties. Differentiation 31:42–49.

Sugihara H, Yonemitsu N, Miyabara S, Toda S. 1987. Proliferation of unilocular fat cells in the primary culture. J Lipid Res 28:1038–1045.

Sugihara H, Yonemitsu N, Toda S, Miyabara S, Funatsumaru S, Matsumoto T. 1988. Unilocular fat cells in three-dimensional collagen gel matrix culture. J Lipid Res 29:691–697.

Tang QQ, Otto TC, Lane MD. 2003. Mitotic clonal expansion: A synchronous process required for adipogenesis. Proc Natl Acad Sci USA 100:44–49.

Tang QQ, Otto TC, Lane MD. 2004. Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. Proc Natl Acad Sci USA 101:9607–9611.

Taylor SM, Jones PA. 1979. Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. Cell 17:771–779.

Toda S, Uchihashi K, Aoki S, Sonoda E, Yamasaki F, Piao M, Ootani A, Yonemitsu N, Sugihara H. 2009. Adipose tissue-organotypic culture system as a promising model for studying adipose tissue biology and regeneration. Organogenesis 5:50–56.

Torti FM, Dieckmann B, Beutler B, Cerami A, Ringold GM. 1985. A macrophage factor inhibits adipocyte gene expression: An in vitro model of cachexia. Science 229:867–869.

Torti FM, Torti SV, Larrick JW, Ringold GM. 1989. Modulation of adipocyte differentiation by tumor necrosis factor and transforming growth factor beta. J Cell Biol 108:1105–1113.

Turtzo LC, Marx R, Lane MD. 2001. Cross-talk between sympathetic neurons and adipocytes in coculture. Proc Natl Acad Sci USA 98:12385–12390.

Vannier C, Gaillard D, Grimaldi P, Amri EZ, Djian P, Cermolacce C, Forest C, Etienne J, Negrel R, Ailhaud G. 1985. Adipose conversion of ob17 cells and hormone-related events. Int J Obes 9(Suppl 1): 41–53.

Vu V, Kim W, Fang X, Liu YT, Xu A, Sweeney G. 2007. Coculture with primary visceral rat adipocytes from control but not streptozotocin-induced diabetic animals increases glucose uptake in rat skeletal muscle cells: Role of adiponectin. Endocrinology 148:4411–4419.

Wabitsch M, Hauner H, Heinze E, Teller WM. 1995. The role of growth hormone/insulin-like growth factors in adipocyte differentiation. Metabolism 44:45–49.

Wabitsch M, Heinze E, Hauner H, Shymko RM, Teller WM, De Meyts P, Ilondo MM. 1996. Biological effects of human growth hormone in rat adipocyte precursor cells and newly differentiated adipocytes in primary culture. Metabolism 45:34–42.

Wang Z, Lv J, Zhang R, Zhu Y, Zhu D, Sun Y, Zhu J, Han X. 2006. Co-culture with fat cells induces cellular insulin resistance in primary hepatocytes. Biochem Biophys Res Commun 345:976–983.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808.

Wellen KE, Hotamisligil GS. 2005. Inflammation, stress, and diabetes. J Clin Invest 115:1111–1119.

Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, Sowers MR. 2008. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: Prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch Intern Med 168:1617–1624.

Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. 2005. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation 112:3066–3072.

Wu J, Srinivasan SV, Neumann JC, Lingrel JB. 2005. The KLF2 transcription factor does not affect the formation of preadipocytes but inhibits their differentiation into adipocytes. Biochemistry 44:11098–11105.

Xing H, Northrop JP, Grove JR, Kilpatrick KE, Su JL, Ringold GM. 1997. TNF alpha-mediated inhibition and reversal of adipocyte differentiation is accompanied by suppressed expression of PPARgamma without effects on Pref-1 expression. Endocrinology 138:2776–2783.

Yagi K, Kondo D, Okazaki Y, Kano K. 2004. A novel preadipocyte cell line established from mouse adult mature adipocytes. Biochem Biophys Res Commun 321:967–974.