Minireview

The increasingly complex regulation of adipocyte differentiation

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Abstract

Adipose (AD) tissue development and function relies on the ability of adipocytes to proliferate and differentiate into lipid-containing cells that also have endocrine function. Research suggests that certain conditions can induce AD tissue stem cells to differentiate into various cell types and that the microenvironment of the cell, including the extracellular matrix (ECM), is essential in maintaining cell and tissue function. This review provides an overview of factors involved in the proliferation and differentiation of adipocytes. A brief review of the numerous factors that influence PPARγ, the transcription factor thought to be the master regulator of adipocyte differentiation, provides context of established pathways that regulate adipogenesis. Thought provoking findings from research with hypoxia that is supported by earlier research that vascular development is related to adipogenesis are reviewed. Finally, our understanding of the critical role of the ECM and environment in adipogenesis is discussed and compared with studies that suggest that adipocytes may dedifferentiate and can convert into other cell types.

Keywords: Adipocyte, preadipocyte, stromal vascular, differentiation, hypoxia, extracellular matrix

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Introduction

Viable animal tissue development involves the commitment of pluripotent stem cells to lineages with a restricted capability to form other cell types, differentiation that includes a cell's commitment to a specific cell type, morphogenesis, maturation, and senescence. Adipose (AD) tissue originates in the embryonic mesoderm and contains a variety of cells, including mesenchymal cells, preadipocytes, fibroblasts, and adipocytes. Research conducted on AD tissue and its various cell types has provided insights into the regulation of AD tissue growth, metabolism, endocrine function,[1,2] and determination of adipocyte stem cells fate. It is well established that peroxisome proliferator-activated receptor γ (PPARγ), the master regulator of adipocyte differentiation, and other transcription factors such as CAAT enhancer binding protein (C/EBP) α play critical roles in the cell's commitment to the adipocyte phenotype and function. Early work showed that the co-expression of C/EBPα and PPARγ was essential for a differentiated and functional adipocyte. For example, insulin response in cultured fibroblasts from C/EBPα-deficient mice was wholly dependent on the presence of the C/*EBPα* gene despite the presence of PPARγ. Our current understanding of adipocyte development suggests that (1) AD tissue contains stem cells that may differentiate into other cell types as well as a variety of other differentiated cell types, (2) adipocytes may dedifferentiate and evidence, *in vitro*, suggests that they can be induced to form other cell types when exposed to specific conditions, and (3) the microenvironment of the cell, including the extracellular matrix (ECM), is essential in maintaining cell and tissue function. Our knowledge of the macroenvironment within AD tissue is also increasing. This review will focus on the most recent knowledge, from both *in vitro* and *in vivo* studies, of the regulation of adipocyte and AD tissue differentiation.

AD tissue cellularity

AD tissue contains several cell types, one of which is the lipid-laden adipocyte. When tissue is isolated and enzymatically digested, lipid-filled adipocytes tend to float while various other cells, collectively termed the stromal vascular (SV) fraction, sink. It has been suggested that SV cells can be identified phenotypically as cluster-differentiation protein (CD) 45−, CD235a−, CD31−, and CD34+ whereas SV and AD-derived stem cells (ASC) retain reactivity for CD90, CD73, CD105, and CD44 but are not reactive for CD45 and CD31 markers.[3]

A number of studies that generated monoclonal antiadipocyte antibodies (MAB) in pigs[4–8] provided insights into the identification of preadipocyte progenitors and the regulation of preadipocyte growth and development.[4,9] Antibody development included the immunization of

mice with porcine adipocyte plasma membranes,[4] iodination of adipocyte plasma membrane proteins, followed by immunoprecipitation with MAB to demonstrate protein antigens, and detection of antigens against MAB on mature adipocytes and a proportion of non-lipid-containing cells in SV cultures. Treatment of SV cultures with a combination of MAB and complement before adipogenesis demonstrated that adipocyte lineage cells were eliminated by MAB treatment.[5] Immunoreactive SV cells in primary cultures and in AD tissue markedly increased in the number between 60 d fetuses and newborn pigs,[5] which was established as a time of AD tissue expansion in this species. These data suggest that cells progressing along the adipogenic lineage possess cell surface antigens that may be unique to adipogenic cells and exhibit differential expression with age within AD tissues.

To assess proliferation of primary porcine preadipocytes, a technique was developed for measuring the proliferation of preadipocytes in cultures of SV cells from subcutaneous AD tissue using flow cytometry of propidium iodide DNA-labeled cell cultures.[7] Among other factors, the proportion of replicating cells was dependent on serum concentration and cell density. Using the AD 1 MAB, the preadipocyte subpopulation within the SV cells was 8–10% of the total whereas the proportion of replicating preadipocytes (AD-1+) was 2–6%. Insulin-like growth factor-1 (IGF-1) is critical for mitotic activity of preadipocyte cell lines and primary SV cells. Treatment with IGF-1 had several results. It increased the proportion of preadipocytes at all densities although the effect was greatest in the cultures with the greatest densities. Fat cell cluster development was also increased with IGF-1 treatment and higher densities. The proportion of replicating cells decreased with increasing density and IGF-1 significantly increased replication at all densities. These results provide direct evidence of hormonal regulation of primary preadipocyte replication.

More recently, preadipocyte factor 1 (Pref-1), also known as Delta-like 1 homolog (Dlk1), has been shown to inhibit preadipocyte proliferation and adipocyte differentiation by regulating the cell's entry into G1/S-phase and the molecular switch causing cell differentiation.[10] Established as a transmembrane protein that is a member of epidermal growth factor-like protein family, Pref-1 acts in an autocrine/paracrine manner to inhibit adipogenesis by interacting with fibronectin.[11] Pref-1 may have a potential role in early commitment stage whereby stem-like cells commit to the adipocyte lineage.[12] Pref-1 blood concentrations are higher in newborn small for gestational age infants compared with appropriate for gestational age infants or late-gestational women, but the differences in Pref-1 concentration were no longer apparent by 4 months of age.[13] This may provide insight to the previous descriptions that small for gestational age increases adiposity.[14–17]

Adipogenesis

Numerous adipogenic stimulators have been described and more continue to be identified. To date, PPAR γ, IGF-1, macrophage colony stimulating factor, fatty acids, prostaglandins (PGs), and glucocorticoids appear to mediate these adipogenic stimulators.[18] Of these, PPARγ is considered a master regulator of adipocyte differentiation. This transcription factor is a clinical target for the insulin-sensitizing agents known as thiazolidinediones.[19,20] Almost a decade of research was conducted before two groups independently reported that PPARγ was predominantly expressed in adipocytes and induced during adipogenesis[21,22] and, in 1995, a third group determined that PPARγ was the ligand for thiazolidinediones.[23] Since then, numerous studies have evaluated both direct and indirect effects of PPARγ on adipogenesis. *In vitro*, PPARγ-binding studies using differentiated primary murine adipocytes isolated from epididymal, inguinal, and brown AD tissues suggest that depot-selective binding of PPARγ may regulate depot-specific induction of gene expression.[24] One class of compounds associated with the regulation of PPARγ function in adipocytes is PG. Prostacyclin promotes adipocyte-precursor cell differentiation to AD cells by activating the expression of C/EBPβ and δ. Preceding adipocyte maturation, these proteins activate the expression of PPARγ. PG E-2 and PGF-2α inhibit the early phase of adipocyte differentiation by upregulating their own production and suppressing PPARγ function. In contrast, PGD-2 and its non-enzymatic metabolite Δ(12)-PGJ(2) appear to induce the middle-late phase of adipocyte differentiation through both DP2 receptors and PPARγ.[25] In other studies, PG metabolism was positively implicated in AD tissue development since the upregulation of PG reductase (*PTGR*) 1 gene expression was associated with porcine subcutaneous AD tissue accretion.[26] While the association between *PTGR1* gene expression and adipogenesis was novel, previous research had identified biological activity of PTGR1. In swine tissues, PTGR1 catalyzes reduction of the D13 double bond of 15-ketoprostaglandins to yield 15-keto-13,14-dihydroprostaglandins which result in a further reduction of the biological activities of PGs.[27] Enzyme assays of PTGR1 activity found that AD tissue contained the highest PTGR1-specific activity in pigs.[27] It is important to consider PG reductase, PTGR2 which is also a 15-oxoprostaglandin 13-reductase[28], since it is also predominantly distributed in AD tissue and, like PTGR1, also results in a further reduction of the biological activities of PGs. Overexpression of PTGR2 represses transcriptional activity of PPARγ and inhibits 3T3-L1 adipocyte differentiation.[28] These observations may indicate that PTGR1 has a role in adipogenesis in porcine AD tissue. Additionally, a link between PGE2 catabolism and regulation of ligand-induced PPARγ activation of adipogenesis has been established.[28]

There are several reports that suggest that there are interactions of several known adipogenic mediators of regulatory pathways that all ultimately lead to changes in PPARγ. Regulatory T (Treg) cells found in visceral AD tissue were recently implicated in controlling the inflammatory state of AD tissue. PPAR-γ expression by Treg cells was required for complete restoration of thiazolidinedione-induced insulin sensitivity in obese mice,[29] suggesting that PPARγ activity in AD tissue may also involve cell types other than adipocytes. While the critical role of the nuclear PPAR receptor during adipogenesis and for adipocyte functions has been

well established, an additional role in inducing the IGF signaling pathway through constitutive enhancers has been described.[30] Additionally, the endogenously produced PPARγ antagonist, 2,3-cyclic phosphatidic acid (cPA), appears to regulate PPARγ function by stabilizing the binding of the corepressor protein, silencing mediator of retinoic acid and thyroid hormone receptor resulting in suppressed adipocyte differentiation and lipid accumulation.[31] There are also significant roles attributed to the other PPAR subtypes. For example, it is well established that C/EBPα is most important for insulin response, the activation of PPARγ causes insulin sensitization and enhances glucose metabolism. However, PPARβ/δ play important roles in energy metabolism including fatty acid metabolism and regulation and PPARα activation has as role in reducing triglycerides and regulating energy homeostasis.[32]

More recently, research using stem cells and interest in cell survival after implantation has resulted in significant findings about the effects of hypoxia on adipogenesis. For example, research using human mesenchymal stem cells (MSC) showed that both mitochondrial biogenesis and oxygen consumption increase during adipocyte differentiation.[33] Fluorescently tagged SV cells co-implanted with minced AD tissue into nude mice showed approximately 17% of the original fluorescent tag signal even after 56 d post-implantation. The authors suggest that some cells differentiated into adipocytes while others were incorporated into new blood vessels, and it appears that SV cells may not only survive in an ischemic microenvironment but also participate in both adipogenesis and angiogenesis.[34] This notion is supported by the expanding research in the area of hypoxia-induced adipogenesis and the interrelationship of adipogenesis and angiogenesis *in vivo*,[35,36] and research showing that adipogenesis and angiogenesis appear to be spatially and temporally associated.[35] For example, it has been suggested that hypoxia causes AD tissue dysfunction with extensive effects including increased glucose metabolism and concomitant lactate production, insulin resistance, inflammation and fibrosis, and increased cell differentiation and adipokine secretion in AD tissue.[36,37] Experiments with varying oxygen levels have shown that the fate of differentiating stem cells is impacted by hypoxia. For example, reducing mitochondrial respiration induced by hypoxic growing conditions for human MSC reduced adipocyte differentiation.[33] Experimentally reducing mitochondrial transcription factor A by siRNA-based knockdown reduced both mitochondrial respiration and adipocyte differentiation.[33] Others have shown that hypoxic conditions increase adipocyte differentiation in human AD tissue MSC[38] and in the myogenic cell lines, C2C12 and G8.[39] Hypoxic conditions appear to increase vascular endothelial growth factor (VEGF) and basic fibroblast growth factor expression, which can both promote angiogenesis,[40] and leptin transcripts in human AD tissue-derived stem cells.[41] Furthermore, conditioned media collected from cells grown in hypoxic conditions increased the viability of human umbilical vein endothelial cells.[40]

Given the apparent importance of oxygenation and the spatial relationship of adipogenesis and vascular development, it is tempting to postulate potential factors that are essential for adipogenesis. Nuclear factor erythroid-derived 2-like 2 regulates key aspects of the antioxidant defense pathway and has been implicated in regulating adipocyte differentiation and oxidative stress in adipocytes.[42] Another factor that may be involved is vascular endothelial growth factor (VEGF), a key factor in angiogenesis, bone formation and chondrocyte viability, and osteoblast and adipocyte differentiation *in vitro*. VEGF may induce adipocyte differentiation by regulating the levels of Runx2 and PPARγ.[43] Research evaluating hypoxia, *in vivo*, suggests that changes in blood flow and oxygenation are not limited to those observed conditions *in vitro*. Subcutaneous AD tissue appears to have relatively high blood flow which is further increased after eating or exercise.[44] Blood flow to exercising skeletal muscle increases primarily through vasodilation in the contracting muscles thereby achieving a relatively constant flow of oxygen to the muscle.[45]

Recent interest in intramuscular adipogenesis and its roles in muscle function, disease, and exercise performance has shown that the interaction between cell types is more complex than what has been suggested by prior studies of AD tissue endocrine, autocrine, and paracrine function. One study has shown that conditioned media and co-culture of adipocytes or preadipocytes affect myotube formation *in vitro* differently suggesting that circulating factors from preadipocytes promote myogenesis while the factors from adipocytes negatively affect myogenesis and may result in muscle deterioration and pathologies.[46] This line of research has significant potential for health outcomes and interventions related to physical activity, obesity, skeletal muscle disorders, and dietary interventions.

ECM

ECM remodeling and development is critical to AD tissue maintenance by helping to regulate the development, expansion, and phenotypic fate of MSC. In a study of human MSC *in vitro*, ECM matrices for osteogenesis and adipogenesis were designed to mimic the stages of differentiation of the two cell types.[47] Osteogenesis marked by increased RUNX2 and decreased PPARγ expression was evident in MSC on matrices that mimicked early osteogenesis ECM, whereas adipogenesis was evident on matrices which mimicked early adipogenesis ECM resulting in decreased expression of RUNX2, MSX2, and TAZ. Mimicking tissue- and differentiation stage-specific ECM regulated the expression of transcription factors which, in turn, controlled the balance of osteogenesis and adipogenesis of MSC.[47]

Tension and force on the cell also appear to mediate the ultimate fate of mesenchymal stem cells[48] by modifying the ECM. Changes to ECM include actin and myosin fiber formation, activity of both the matrix metalloproteinase (MMP) family peptidases and the tissue inhibitors of MMPs (TIMPs).[48] One bioinformatic network analysis suggests that crosstalk between ECM components and transcription factors, including PPARγ, influences adipocyte differentiation. These pathways appear to be relevant in maintaining the adipogenic potential of human dedifferentiated fat (DFAT) cells.[49] Several other studies also offer

evidence in support of the importance of the ECM for adipocyte development. The plasminogen activation system, including the urokinase-type plasminogen activator (uPA), tissue-type plasminogen activator (tPA) proteases, and their inhibitor plasminogen activator inhibitor-1 (PAI-1), is a major contributor to extracellular proteolytic activity with a role in tissue remodeling. Additionally, PAI-1(−/−)-induced pluripotent stem cells form adipocytes spontaneously.[50] One research team suggests that ascorbic acid may alter adipocyte differentiation by altering collagen profiles after observing that 50 μg/mL ascorbic acid reduced type I collagen and α1 (V) procollagen while mature collagen α1 (V) protein was not detectable by western blotting proteins from 3T3-L1 cells 8 d after being induced to differentiate.[51] As a major component of the basal lamina, laminin proteins are important for AD tissue maintenance. A comprehensive study showed that laminin (LM)-α4, LM-β1, and LM-γ1 mRNAs increased during adipogenesis of human bone marrow-derived MSC. Initial basement membrane formation was apparent after 14 d of culture with basement membrane (BM)-like structures surrounding fat droplet-containing cells after 28 d. Immunoreactivity suggested that LM-411 and LM-421 are present in the BM around adipocytes implying that LM-411 may function as a structural scaffold during adipogenesis, since it is also expressed in mature human subcutaneous fat tissue *in vivo*.[52] Earlier studies assessing the influence of the ECM and ECM components on adipogenesis included *in vivo* studies and studies of primary porcine AD tissue SV cell cultures.[53] In primary AD tissue SV cell cultures, immunoreactivity for two major ECM components, type IV collagen and laminin, was coincidental with lipid deposition in preadipocytes but lagged behind immunoreactivity for the AD-3 preadipocyte marker.[53] Furthermore, immunoreactivity for type IV collagen and laminin was coincidental with the transition to a round shape and lipid deposition. Therefore, ECM expression may be associated with the morphological transition of preadipocytes to adipocytes. Studies of laminin also showed that preadipocytes just converting into adipocytes (possessing small lipid droplets) in SV cultures had a high affinity for a laminin substratum. Furthermore, laminin substratum markedly alters the morphology of preadipocytes with lipid, whereas other substrata of different ECM components had no such influence on preadipocytes. Imunocytochemistry for laminin in fetal AD tissue indicated that laminin may play a critical role in morphological aspects of preadipocyte development *in vivo* and *in vitro*. While causal relationships have not been well established, there is evidence that ECM components may be clinically relevant. Young, healthy children appear to have less total collagen in the subcutaneous AD tissue depot when overweight compared with normal weight children. In addition, adipocyte size was negatively correlated with the percentage of total and peri-cellular collagen[54] supporting the vast *in vitro* data that suggest that the ECM plays a critical role in AD tissue development. While many of the findings reported here are focused on the role of ECM components on adipogenesis, it is important to note that many of the adipokines secreted are ECM proteins.[55]

With increasing evidence of the influence of the ECM on adipogenesis, one can hypothesize that the physiology of cells and tissues being evaluated may be critically important. A number of studies have evaluated the mechanical properties of cells as well as various mechanical stress procedures during differentiation *in vitro*. One study reports that the mechanical properties of AD tissue-derived stem cells, including elasticity, viscoelasticity, and cell height, change with passage number.[56] The phospholipid monolayer surrounding the lipid droplet in 3T3-L1 adipocytes appears to increase in monounsaturated and polyunsaturated fatty acids while the saturated fatty acid content in the monolayer is decreased during differentiation suggesting that the fluid nature of cells may be altered by incorporating fatty acids that differ in physical characteristics.[57] In this regard, several studies have described an increase in cell stiffness with adipocyte differentiation. A study of differentiating 3T3L1 cells indicated that lipid droplets are stiffer than cytoplasm and the accumulation of lipid increases adipocyte stiffness,[58] additionally, MSC that are induced to differentiate into adipocytes also show similar increases in cytoskeletal stiffness.[59,60] It is conceivable that the mechanical property changes described with increasing passage number may also be relevant to two-dimensional (2-D) compared with three-dimensional (3-D) cell cultures compared with *in vivo* conditions. One example of differences between 2-D and 3-D cultures has been described in the requirements for MMP14.[61] The collagenase MMP14, also known as MT1-MMP, initially appeared to be unnecessary for differentiation in a 2-D culture. However, *in vivo* and 3-D conditions demonstrated that MMP14 plays a critical role in adipogenesis.[61] Human ASC grown on gels whose stiffness was modified using decellularized human lipoaspirate showed that increasing substrate resulted in cells that had increased spread and decreased rounded morphology and also failed to upregulate adipogenic markers.[62] As more 3-D study methods are published, it is likely that these methods will provide valuable insight about AD development, the ECM, and communication of cells that may be different from what has been described in 2-D systems. One newly described method suggests that AD tissue-derived MSC seeded as ~270 μm diameter spheroids had improved scaffold vascularization and microvessel density compared with non-seeded scaffolds and scaffolds seeded with individual MSC implanted in mice.[63] Another method modified from chondrogenic differentiation of MSC has been developed using cultures of 3-D cell aggregates and may be useful in high-throughput screening of adipocyte differentiation agents.[64]

The influence of dense type 1 collagen deposition in fetal pig subcutaneous AD tissue helps to define the structure of mesenchymal tissues by inhibiting fetal adipogenesis which can be considered mechanical stress or physical restraint on adipogenesis. The thickest collagen septa are present in the fetal pig inner subcutaneous layer and, by the end of fetal development, the densest collagen layer is in a layer below the inner subcutaneous layer. Collagen deposition in the dense layer may physically restrict local fat cell cluster growth since collagen deposition is greatly reduced and fat cell cluster development enhanced after removing the

fetal pig hypothalamus at 45 d (examined at 110 d of fetal life). Collectively, small and large type 1 collagen bundles provide the major ECM framework necessary to establish and sustain the structure and the function of fetal pig subcutaneous mesenchymal tissues. In fetal perirenal AD tissue, dense collagen is also associated with restricted fat cell cluster development but the structure and the function of fetal pig perirenal mesenchymal tissues are less discrete than in subcutaneous tissues. Collagen XIV has also been hypothesized to play a role in dedifferentiation since it reduced *de novo* DNA synthesis in primary human fibroblasts, mouse 3T3 fibroblasts, and 3T3-L1 preadipocytes.[65]

A comprehensive ECM study included AD tissue depot ECM gene profiling and examination of ECM remodeling of C57BL1/6J mice AD tissue MSC from two depots.[66] The gene expressions of adhesion and ECM molecules distinguished subcutaneous from visceral fat-derived MSC. Characteristics of collagenolysis distinguished subcutaneous from visceral AD tissue-derived SV cells with higher expressions of secreted collagens in visceral MSC than in subcutaneous MSC.[66] Therefore, in addition to membrane-associated ECM collagen, turnover of collagen septa also distinguishes AD tissue depots.

Several studies have addressed the potential of mechanical force on adipogenesis. Cyclic mechanical stretch of primary rat ASC,[67] MSC,[60] and C3H10T1/2 pluripotent MSC[68] inhibits adipogenesis. These studies report decreased PPARγ mRNA and protein level,[67,69,70] increased Runx2 mRNA and protein levels,[67] increased Pref-1 mRNA level,[67] and induced phosphorylation of ECM signal-regulated protein kinases 1 and 2 (ERK1/2) during the mechanical stretch period.[67] Chung et al.[71] observed changes in the tubulin cytoskeletal distribution that were positively correlated with marker of pericondensation (Sox9 alone), negatively correlated with chondrogenesis (Colllal), and positively correlated with adipogenesis. Exposure of MSC to stressors that change volume and shape was critical since they resulted in developing anisotropy of cytoskeletal architecture (structure), which can impact the emergent cell fate and function. Therefore, the volume and shape changing stress induced spatiotemporal organization of cytoskeleton changes that may mirror those encountered during development.[71]

The mTOR complex defined by its binding partner rictor, mTORC2, is activated by mechanical force and has been implicated in cytoskeletal architecture. Mechanical activation of mTORC2 signaling participates in mesenchymal stem cell lineage selection, preventing adipogenesis by preserving β-catenin.[60] During strain, mTORC2 becomes associated with vinculin and mTORC2 and Akt co-localize with newly assembled focal adhesions (FA).[60] Disrupting mTORC2 or Akt function prevented mechanically induced F-actin stress fiber development.[60] Knockdown of vinculin prevents mTORC2 activation while rictor-deficiency accelerated adipogenesis in MSCs.[60]

Transient knockdown of the membrane-cytoskeleton linker proteins ezrin, radixin, and moesin (ERM) by RNAi caused disassembly of actin fiber and focal adhesions and decreased stiffness resulting in impaired adipogenesis.[72] Experimentally silencing focal adhesion kinase (FAK) suppressed BMP4 and downregulated Smad1/5/8 and p38 while inducing lipid accumulation and expression of C/EBPα, PPARγ, aP2.[73] Another example of a potential mechanism by which external forces may affect an adipogenic nuclear transcription factor is C/EBPβ, which appears to be a mechanically responsive transcription factor. C/EBPβ regulated the adipogenesis of C3H10T1/2 pluripotent MSC cultured in adipogenic media with daily mechanical strain indicating that C/EBPβ is a mechanical target in MSC cells.[68] However, C/EBPβ is not the primary site at which adipogenesis is regulated since C/EBPβ overexpression did not override mechanical strain's inhibition of adipogenesis.[68]

A proteomic analysis suggests that the nucleoskeletal protein lamin-A and ECM collagens correlate with tissue elasticity and experimentally induced differentiation of stem cells into adipocytes was enhanced by low lamin-A levels. Lamin-A transcription was regulated by the vitamin A/retinoic acid (RA) pathway with broad roles in development, nuclear entry of RA receptors was modulated by lamin-A protein. Tissue stiffness and stress thus increase lamin-A levels, which stabilize the nucleus while also contributing to lineage determination.[74] Moreover, human MSC cultured in the presence of various inhibitors suggest that p38 and ERK1/2 played crucial positive roles in adipogenesis, whereas FAK, RhoA/ROCK, and cytoskeleton played negative roles. Furthermore, FAK, RhoA/ROCK, and cytoskeleton affected adipogenesis by regulating the activities of p38 and ERK1/2 which interacted with each other in the process of adipogenesis.[75]

Low-intensity pulsed ultrasound (LIPUS) suppressed adipogenic differentiation of adipogenic progenitor cell and MSC lines and also impaired lipid droplet appearance and decreased gene expression of PPARγ2 and fatty acid-binding protein 4.[76] Also, murine AD-derived stem cells induced to differentiate then treated with compressive force (2000 $\mu\varepsilon$, 1 Hz) also showed that adipocyte differentiation is inhibited as evidenced by reduced numbers of oil droplet-filled cells, down-regulated mRNA levels of both PPARγ1 and APN, and protein level of PPARγ.[70]

Cells originating in non-AD tissue

One elegant study used freshly isolated bone marrow-derived MSC that had differentiated into adipogenic lineage cells and then dedifferentiated to analyze the adipogenic differentiation of mesenchymal stem cells while assessing adipogenic-specific marker genes. This study described gene clusters including cluster 1 containing 307 genes whose expression was upregulated during adipogenesis, 198 cluster 2 genes that were downregulated during adipogenesis but upregulated during dedifferentiation, cluster 3 containing 277 genes that were downregulated during adipogenesis but upregulated in dedifferentiated cells, and 209 genes in cluster 4 that were downregulated during early adipogenesis and in differentiated cells.[77] This approach not only supports previous evidence of genes involved in adipogenesis but also provides new data about gene regulation during dedifferentiation that may be critical if dedifferentiated cells are used therapeutically

or in mechanistic studies. However, these results may differ from mesenchymal stem cells isolated from AD tissue or from adipocytes isolated from AD tissue and dedifferentiated in an *in vitro* model.

There is also growing information about liposarcomas (soft tissue, cancerous tumors that develop from fat). Dedifferentiated liposarcoma (DDLS) cells underexpressed C/EBPα and PPARγ compared with well-differentiated liposarcoma and normal adipocytes.[78] Cells from DDLS cell lines did not induce C/EBPα expression, but when expression was induced, both PPARγ expression and apoptosis increased.[78] These results raise the interesting possibility that two proteins well known for their roles in differentiation, C/EBPα and PPARγ, may be involved in maintaining differentiation or dedifferentiation of adipocytes. Retinoblastoma protein regulates the cellular G1/S transition of the cell cycle and to regulate proliferation, development, and differentiation of adipocytes and several other cell types.

Summary

While our understanding of the dedifferentiation process of various cells, including adipocytes, is limited, the research to date shows that cell regulation is much more complex than previously thought. Nonetheless, common themes regulating adipogenesis are apparent. For example, hypoxia appears to induce AD tissue dysfunction while inhibiting adipocyte differentiation. Interestingly, hypoxia is considered to be a main factor involved in the dedifferentiation of several cell types including DFATs, chondrocytes, and smooth muscle cells.[79] The clear importance of ECM components in adipocyte development and function is also paralleled by observations in DFAT cells.

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