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MicroRNAs: A New Class of Master Regulators of Adipogenesis

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Abstract

Adipogenesis is the process by which Mesenchymal Stem cells commit to become adipocyte precursor cells, which will later differentiate into mature adipocytes following exposure to differentiation factors. The transcriptional programs involved in the differentiation process have been carefully studied. Recently, small non-coding RNAs, microRNAs, have been found to play critical roles throughout the adipogenic process. MicroRNAs have been identified that are involved in promoting or inhibiting adipogenesis by targeting anti- or pro-adipogenic factors and cell cycle regulatory proteins. Here, we will discuss the latest discoveries regarding microRNA regulation of adipocyte differentiation.

Function of Adipose Tissue

Adipose tissue, or body fat, is an essential class of connective tissue prominent throughout the body. Adipose tissue serves to regulate and maintain energy homeostasis within an organism, and is primarily composed of adipocytes, or fat cells [1]. These cells are classified into two distinct types: White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT). WAT is an active endocrine organ whose function is to store energy and to regulate the activity of insulin, lipid metabolism and satiety. BAT is located in discrete pockets, contains a large number of mitochondria and undergoes thermogenesis, where heat is expelled as chemical energy [2]. This review mainly focuses on the impact of microRNAs on WAT-related adipogenesis.

Adipocytes function to store reserves of nourishment in times of abundance, and to release these reserves in times of need. This ability of adipose tissue to maintain the necessary amount of energy for use in metabolism is essential for the survival of any organism. However, the intake of too much fat may result in dysfunctional adipose tissue, which may contribute to disease development including obesity, diabetes, and coronary artery disease [3,4]. Indeed, adipocytes are much larger in size in obese individuals compared to those in leaner counterparts [5]. As such, a thorough assessment on the regulation of adipocyte differentiation may provide a better understanding on the molecular mechanisms that give rise to adipogenic dysfunctions. These mechanisms could potentially serve as markers for therapy against metabolic disorders.

Adipogenesis

Adipocytes differentiate from mesenchymal stem cells (MSCs) in a process known as adipogenesis, and normally surround themselves with a network of other cell-types that include fibroblasts, preadipocytic cells, endothelial cells, nerves and immune cells [6]. Although much of the classic knowledge regarding adipogenic regulation delves into the existence of various regulatory proteins, recent literature has touched upon the role of microRNAs in the differentiation of MSCs to adipocytes. This review summarizes the current paradigms that support an association between numerous regulatory microRNAs and the adipogenic process.

During adipogenesis, cells from adipose tissue proliferate and differentiate into lipid assimilating cells. This is known to occur in two distinct stages: the first stage being the commitment stage, in which MSCs commit to becoming preadipocytes, and the second stage being the terminal differentiation stage [7]. During adipogenic competency and commitment, extracellular matrix (ECM) and cell shape remodeling play a role in coordinating various signaling cascades [2]. This stage involves many key regulators that are modulated during the transition between MSC to preadipocytes, including WNT family members and Rho-family GTPases [2]. WNT family members are secreted glycoproteins that are known to inhibit adipogenesis through WNT ligand-mediated signaling cascades. This WNT signaling activity is not exhibited in mouse embryonic fibroblasts lacking the WNT receptor, which in turn develop increased adipocyte differentiation [8,9].

Adipogenic Commitment

MSC commitment to preadipocytes is characterized by ECM stiffness that results in tension, which enhances actin and myosin formation and subsequent cell stretching [7,10]. This phenomenon was previously observed in 3T3-F442A cells, in which differentiation into adipocytes was inhibited by fibronectin and rescued by chemical inhibition of actin stress fiber formation [11]. Furthermore, both human MSCs and mouse preadipocytes grown on stiffer matrices with higher concentration of collagen I display reduced rates of adipocyte differentiation than those grown in softer gels with lower stiffness [12,13].

Ectopic induction of α5 integrin inhibits adipocyte differentiation by maintaining high expression of the Rho family GTPase known as Rac in its activated form (Rac bound to GTP) [14]. Rac-GTP activates Rho GTPase-Rho-associated kinase (ROCK) signaling, which facilitates actinomyosin fiber formation that blocks adipogenesis [15,16]. In order for differentiation to occur, Rac-GTP must be down-regulated and its GDP bound form (inactive) must predominate. This is experimentally shown in 3T3-L1 cells treated with adipogenic stimuli, in which α5

integrin is repressed, Rac is inactivated and terminal differentiation commences [14].

Cell-to-cell contact and cell shape are also factors that can influence adipogenesis. Preadipocytes undergo a period of mitotic clonal expansion upon the addition of adipogenic stimuli such as cAMP agonist, insulin and glucocorticoids. These stimuli further advance MSCs to peadipocytic commitment and committed preadipoctyes to confluency and a rounded morphology [2,17,18]. Differentiation of confluent preadipocytes requires cell cycle regulatory proteins such as E2Fs and p130 [19]. Once the preadipocytes are fully committed, a signal cascade is activated to induce adipocyte differentiation.

Terminal Differentiation of Adipocytes

Terminal differentiation begins with the expression of two families of genes that encode the regulatory proteins CCAAT/enhancer binding protein (C/EBP) and the adipogenic master regulator, Peroxisome proliferator-activated receptor (PPARγ). Adipogenic stimuli promote the down-regulation of adipogenesis repressor preadipocyte factor 1 (Pref-1) and the increased expression of C/EBPß and C/EBPδ, which together recruit a transcriptional activation complex that consists of transcription factors, glucocorticoid receptor, STAT5A, RXR and a co-activator complex [2]. This complex recognizes acetylated and methylated regions of the PPARG locus that are epigenetically marked for histone modification [20-22]. Previous studies have reported on the conservation of adipogenic stimulus-dependent induction of PPARγ binding and histone modification near genes involved in adipogenesis, such as fatty acid binding protein 4 (FABP4), PPARG and GLUT4 [2, 22].

C/EBPß binds to DNA when phosphorylated by the MAPK pathway and glycogen synthase kinase 3ß and synergizes with C/EBPδ to activate transcription of PPARγ [23-25]. During terminal differentiation, a shift in gene expression occurs between genes encoding transcription factors that are prominent in the preadipocyte phenotype and those that are prominent in the adipocyte phenotype. C/EBPα and PPARγ work in tandem to mediate the expression of these adipogenic gene products, which include FABP4, adipsin and lipoprotein lipase (LPL) [2, 26, 27].

Additional adipogenic transcription factors include Krüppel-like factors (KLFs) and C10orf116. The latter is a novel transcription factor that has recently been discovered to transcribe the protein, Adipogenesis Factor Rich in Obesity (AFRO), which is thought to stimulate the transcription of PPARγ and C/EBPα in the early stages of adipogenesis [25]. KLFs are zinc finger transcription factors that serve as both positive and negative adipogenic regulators, depending on the isoform. For example, KLF5, a positive adipogenic regulator, works in concert with C/EBPδ to induce the expression of PPARγ2 [28,29].

MicroRNA Regulation of Adipogenesis

Current findings report on the existence of numerous inhibitory and promoting microRNAs (miRs) involved in the regulation of adipogenesis, both in the commitment stage and in terminal differentiation. MiRs are defined as a set of non-coding RNAs ranging from about 18 to 22 nucleotides in length. Their function is to target specific mRNA transcripts for degradation or translational repression, thus playing a regulatory role in numerous biological processes such as development, reproduction and differentiation [30].

Knelangen et al. conducted a miR profile in mouse embryonic stem cells to monitor miR involvement during the entire process of adipogenic differentiation. From this profile, 129 miRs exhibited change in expression levels at distinct time points, ranging from mesodermal progenitor cells (day 5) to mature adipocytes (day 21) [31].

miR-17-92 cluster

The miR-17-92 cluster was found to accelerate adipocyte differentiation by inhibiting the tumor suppressor, Rb2/p130 during early clonal expansion of preadipocytes. The miR-17-92 cluster is abundantly expressed in embryonic stem cells (ESCs) where researchers believe that this miR cluster gets sequestered with alternative networks to maintain the pluripotency and self-renewal associated with an ESC state [31,32].

miR-138

MiR-138 knocks down the expression of EID1, which is a gene involved in adipocyte differentiation in adipose-derived MSCs. Previous research has shown that overexpression of miR-138 reduces triglyceride accumulation and inhibits the expression of adipogenic markers such as C/EBPα and PPARγ [33].

miR-155

Terminal differentiation from preadipocytes to mature adipocytes is also governed by miRs [31]. For example, tumor necrosis factor (TNFα)-mediated overexpression of miR-155 via the NFκB pathway results in the down-regulation of C/EBPß and CREB [34]. MiR-155 inhibits these proteins by targeting their 3'untranslated regions (UTRs), thereby inhibiting the transcription of later adipogenic markers, PPARγ and C/EBPα. This was shown experimentally through the use of an anti-miR-155 that reduced TNFα-induced inhibition of adipogenesis and through exogenous expression of miR-155, which inhibits adipogenesis [34].

miR-27 and miR-130

MiR-27a/b and miR-130 are known adipogenic inhibitors that target PPARγ directly [35-37]. Chan et al. performed analyses on miR-27b in tandem with a component of ginseng extract known as Ginsenoside-Rb$_1$ (Rb$_1$). This group postulated that Rb$_1$ might function to down-regulate miR-27b gene transcription and mature miR-27b activity to facilitate PPARγ expression and subsequent adipogenesis [38]. They also found that treating adipocytes with Rb$_1$ resulted in decreased expression of pri-miR-27b as well as mature miR-27b. Furthermore, suppressing PPARγ with the antagonist, GW9662 in Rb$_1$ treated adipocytes rescued the expression of mature miR-27b and pri-miR-27b, which supports that inhibition of miR-27b transcription and function is PPARγ-dependent [38]. Another study reports on a significant lower level of miR-130 expression and a markedly higher expression of PPARγ mRNA in adipose tissue obtained from obese women compared to that of lean women [37]. These studies reveal that miR-130 potently represses PPARγ expression and biosynthesis through selective targeting of PPARγ mRNA coding as well as its 3'UTR.

miR-448

MiR-448 selectively targets KLF5 to down-regulate its induction of PPARγ expression, thereby reducing adipocyte differentiation [39]. This was confirmed by results obtained from 3T3-L1 cells overexpressing miR-448, in which researchers observed reduced triglyceride accumulation. Conversely, researchers observed an increase in adipocyte differentiation in 3T3-L1 cells lacking miR-448 [39].

miR-200 family

Some examples of pro-adipogenic miRs include members of the

miR-200 family (mir-200c/141 and miR200b, a/429 clusters), which inhibit WNT singling that normally functions to down-regulate C/EBPα and PPARγ [40,41].

miR-143

There have been numerous recent findings regarding the link between regulatory miRs and adipogenesis, including the classification of miR-143 as a "fine-tuning miR" rather than an adipogenic switch. He et al. reports that Fgf7, a member of the fibroblast growth factor family, is a putative target of miR-143 [30]. Fgfy may function as a fine-tuning molecule in the adipogenic process. He et al. observed that knock down of miR-143 does not yield significant changes in phenotype with *in vivo* approaches compared to *in vitro* studies, which may be attributed to redundant functions of other related miRs [30].

miR-140

Recently, miR-140 was identified as a downstream component of BMP4 signaling that acted to promote adipocyte commitment. MiR-140 was found to directly target Osteopetrosis-associated transmembrane protein 1 (Ostm1), an anti-adipogenic factor [42].

Outstanding Questions

Recent experimental approaches that manipulate the expression levels of various miRs in cell cultures have resulted in either the inhibition or stimulation of adipocyte differentiation. These analyses support a potential regulatory function for miRs in mammalian adipogenesis, in which miRs selectively target anti- or pro-adipogenic factors. Some major discoveries in the field include the negative regulation of EID1 by miR-138 in MSCs [33]; the link between miR-27b regulation and Rb$_1$ promoting adipogenesis with PPARγ binding [38]; C/EBPß and CREB inhibition as a result of TNFα-induced overexpression of miR-155 [34]; and more recently, the classification of miR-143 as an adipogenic fine-tuner [30]. Since most of these findings rely on *in vitro* studies, further *in vivo* analyses are needed to evaluate a defined association between miRs and adipogenesis. Indeed, the scientific community has yet to completely address the complexity of post-transcriptional regulation that miRs perform in adipogenesis. This will require proteomic studies, since adipogenic regulation by miRs may be more apparent at the protein level, as well as further insight on candidate adipogenic markers that are specific for distinct stages of precursor cell development.

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