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Review

MicroRNAs are key regulators of brown adipogenesis

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ABSTRACT

The recent discovery of microRNA, thousands of short, non-coding strands of RNA, that regulate gene expressions on the transcriptional level throughout the body, raises the possibility of their roles as therapeutic targets in the treatment of a diverse range of diseases including diabetes, cancer, cardiovascular disease, and obesity. Specifically, their potential as therapeutic targets in the treatment of obesity has been highlighted. Brown adipose tissue containing a large number of mitochondria and expressing Ucp-1 is metabolically active through dissipating energy as heat in cold temperatures. Brown adipose, which was previously thought to be present only in neonatal and infants, has been recently unexpectedly identified in various anatomical regions of the adult human body. Furthermore, brown adipocytes have been shown to originate from skeletal and cardiovascular myoblast progenitor cells. Several identified microRNAs participate in the regulation of brown adipocyte differentiation through pathways involving the Prdm16 and C/ebp-β program. These miRNAs are potential therapeutic targets in the induction of brown adipocyte lineage differentiation from myoblast and white adipose, through which the Ucp-1 expression is regulated to increase calorie expenditure and reduce body weight in obese individuals. This review focuses on the current understanding of miRNAs on the regulation of brown adipogenesis.

1. Introduction

1.1. Obesity and type 2 diabetes

Obesity is a major risk factor for a number of chronic diseases such as type II diabetes, stroke, and cardiovascular disease. Obesity has become a growing international concern with prevalence now doubled in the US compared to two decades ago [1]. Weight loss is known to improve or slow down the progression of type 2 diabetes and other chronic diseases associated with obesity [2,3]. Although medications for the treatment of obesity would be an ideal option for weight loss, the development of safe and effective drugs has been unsuccessful [4].

1.2. Adipose tissue

There are two types of adipose tissue in mammals, which are white adipose tissue (WAT) and brown adipose tissue (BAT). White adipocytes contain very few mitochondria and no Ucp-1 (uncoupling protein-1) expression. Brown adipocytes consist of a relatively large number of mitochondria and abundant levels of Ucp-1. White adipose tissue is sub-categorized as visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). VAT is associated with increased several metabolic risk factors, and thus identified as a pathogenic fat depot [5]. On the contrary, SAT is associated with improved metabolic profiles [5]. Brown adipocytes generate heat to maintain the core body temperature when stimulated by cold and a β-3 adrenergic agonist through a process assisted by the mitochondrial Ucp-1 [6]. Classic brown adipocytes, concentrated in BAT, identified as adipocytes expressing Ucp-1, are found specifically in interscapular, perirenal, and axillary depots. However, it was recently discovered that a small percentage of adipocytes that express Ucp-1 exist in SAT [7,8]. These clusters of Ucp-1 expressing adipocytes in SAT are defined as beige/brite adipocytes or 'browning' in white adipocytes [7,8]. However, the ultimate thermogenic function of the beige/brite adipocytes does not differ from that of classical brown adipocytes [8]. Importantly, functionally active regions of brown adipose tissue are present in adult males and females and are inversely associated with BMI, indicating its potential role in weight loss therapy [9–11].

1.3. Differentiation and development of brown adipocyte and beige fat: Prdm16 and C/ebp program

Classical brown fat is derived from *Myf-5* positive progenitor cells, which are muscle-like cellular lineage during embryonic development [12]. Interestingly, various tissues such as skin fibroblasts, myoblasts,

Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue; iBAT, intrascapular brown adipose tissue; SAT, subcutaneous fat; Mef-2, myocyte enhancer factor-2; PGC-1α, Ppar-Y co-activator; Prdm16, PR domain containing 16; C/ebpβ, CCAAT/enhancer-binding protein β; Ucp-1, uncoupling protein-1; Ppars, peroxisome proliferator-activated receptors; Rux1t1, runt-related transcription factor 1, translocate to 1; hMADS, human multipotent adipose-derived stem; Adam17, Adam metallopeptidase domain 17

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and skeletal myoblast stem cells (satellites cell) were recently found to differentiate into BAT. These stem cells are derived from the *Myf-5* positive lineage [6,13]. The "brown-like" cells or "beige" or "brite" cells within white adipose tissue are previously thought to arise from the *Myf-5* negative lineage [12,14]. Using a new lineage-tracing approach optimal for adipocyte, recent findings suggest that beige fat is derived from *Myf 5-Cre* positive or *Myf 5*-negative lineage, depending on the depot [15,16]. Importantly, classic brown as well as beige adipocytes can be activated by cold (referred to as 'browning' of adipocytes) and share similar transcriptional cascades in adipogenic differentiation [17]. Two transcription factors, Prdm16 (PR domain containing 16), a zinc-finger transcriptional factor, and C/ebp-β (CCAAT/enhancer-binding protein-β), are highly expressed in brown adipocytes compared to white adipocytes and myocytes [12,18,19]. Their expressions determine the differentiation direction of brown fat precursors to myogenic or brown fat, and the differentiation direction of white fat precursors to brown adipocytes or white fat [12,20]. Inhibition of Prdm16 expression in brown fat precursors promotes muscle differentiation. In contrast, myoblasts can differentiate into brown fat cells induced by ectopic expression of Prdm16 [12]. Prdm16 thus acts as a key control factor in the brown fat program [12,21]. Ectopic expression of C/ebp-β in white adipocytes is associated with a significant increase in brown adipocyte-specific genes and inhibition of white adipocyte-specific genes [19,22,23]. Prdm16 together with the active form of C/ebp-β forms a transcriptional complex, which plays a critical role in controlling the cell switch from myoblastic precursors to brown fat cells [19,21], and drives a full functional brown fat program in non-adipogenic cells [19]. Brown fat differentiation also requires Ppar-ϒ [17], furthermore, activation of Ppar-γ agonist in white adipocytes by can induce brown adipose specific genes [18]. C/ebp-β activates transcription of C/ebp-α and Ppar-ϒ, and induces transcription of Ucp1 [24]. Ppar-ϒ and the C/ebp peptide family function synergistically to stimulate adipogenesis and maintain the differentiated state of white and brown adipocytes by promoting cell-cycle re-entry and mitotic clonal expansion [18].

2. Role of miRNAs: inducers of brown adipocytes and beige fat

Only in recent years, it is found that several microRNAs (miRNAs) associate with these transcription factors that play important regulator roles in the switching on and off of the brown adipogenic lineage determination either as a ligand or an agonist. This review focuses on our current understanding of the role of specific miRNAs in the differentiation of various tissues to BAT, BAT development, and their potential as therapeutic targets for the treatment of obesity. Identified microRNAs that are known to regulate brown adipogenesis are listed in Table 1.

2.1. MicroRNAs

MicroRNAs (miRNAs) are a family of short, non-coding, 19–23 base pair RNA molecules that mediate gene expression by inhibiting messenger RNA translations and destabilizing messenger RNA [25]. Expressed throughout the body in various types of tissue, miRNAs regulate more than 60% of the mammalian genome [25]. At least 1000 unique miRNAs were identified in the human genome until now [26]. MiRNA transcripts, which are transcribed by RNA Polymerase II, are located in various non-coding parts of the genome; some are found in introns and others in intergenic domains. The expression of miRNA is regulated either through several transcription factors or through processing precursors [27]; on the other hand, one individual miRNA can target and regulate hundreds of genes via interaction with partially complementary sites located at the 3′UTR of mRNAs to destabilize and inhibit mRNA translation, further reducing protein production [27,28]. MiRNAs are, Q3 therefore, very important post-transcriptional regulators of gene expression. Due to their special role in mediating gene expression in various tissues, miRNAs have been shown to regulate adipocyte differentiation, insulin secretion [29], insulin signaling and glucose homeostasis [30]. MiRNAs are also proven to be key regulators of tissue development and differentiation. Several miRNAs have been found to participate in the regulation of brown adipocyte differentiation through pathways involving the Prdm16 and C/ebp-β program [26].

The abundance of these conserved miRNAs in developing adipose tissues was also studied recently. A deep sequencing approach shows that the expression levels of these miRNAs have a large range, and vary from several counts for rare miRNAs to several million reads for most abundant miRNAs. Thirty-two miRNA families are classified as abundantly expressed [31]. Among those miRNAs expressed differently in brown and white adipocytes, the miR-27 family is one of the most abundant miRNAs [31]. MiR-193b–365 cluster is another miRNA that Q4 Q5 has been demonstrated to be enriched in brown fat [32]. MiR 133a, a muscle specific miRNA, was absent from white adipocytes but highly Q6

Table 1
MiRNAs and their roles in the brown adipogenesis program.

MiRNA	Regulator	Target genes	Effects on adipogenesis	References
Mir-26a/b	Rosiglitazone Cold temperature	Ucp-1 Adam17	Promote brown fat adipogenesis Promote white fat adipogenesis	[49]
MiR-27	Cold temperature	Ppar-α Pgc1-β Prdm16	Suppress brown fat adipogenesis	[44]
MiR-106b–93		Ucp-1 Prdm16 Pparα Pgc-1α	Suppress brown fat adipogenesis	[43]
MiR-133	Forskolin Cold temperature Mef2	Prdm16	Promote myogenesis Suppress brown fat adipogenesis Suppress beige fat adipogenesis	[13,35]
MiR-155	Tgfβ-1	C/ebp-β Pgc-1α	Suppress brown fat adipogenesis Promote beige fat adipogenesis	[36]
MiR-193b/365	Prdm16	Runxltl Ppar-Y C/ebp-α	Suppress myogenesis Promote brown fat adipogenesis Promote white fat adipogenesis	[32]
MiR-196a	Forskolin Cold temperature Adrenergic	Hoxc8	Suppress WAT adipogenesis Promote beige fat adipogenesis	[45]
MiR-378	MyoD Cold temperature	MyoR C/ebp-β C/ebp-α	Promote myogenesis Promote brown adipogenesis	[41,42]
MiR-455	Norepinephrine Ppar-Y agonist	Ucp-1	Suppress myogenesis Promote brown fat adipogenesis	[33]

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