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Shortcuts to a functional adipose tissue: The role of small non-coding RNAs

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ABSTRACT

Metabolic diseases such as type 2 diabetes are a major public health issue worldwide. These diseases are often linked to a dysfunctional adipose tissue. Fat is a large, heterogenic, pleiotropic and rather complex tissue. It is found in virtually all cavities of the human body, shows unique plasticity among tissues, and harbors many cell types in addition to its main functional unit – the adipocyte. Adipose tissue function varies depending on the localization of the fat depot, the cell composition of the tissue and the energy status of the organism. While the white adipose tissue (WAT) serves as the main site for triglyceride storage and acts as an important endocrine organ, the brown adipose tissue (BAT) is responsible for thermogenesis. Beige adipocytes can also appear in WAT depots to sustain heat production upon certain conditions, and it is becoming clear that adipose tissue depots can switch phenotypes depending on cell autonomous and non-autonomous stimuli. To maintain such degree of plasticity and respond adequately to changes in the energy balance, three basic processes need to be properly functioning in the adipose tissue: i) adipogenesis and adipocyte turnover, ii) metabolism, and iii) signaling. Here we review the fundamental role of small non-coding RNAs (sncRNAs) in these processes, with focus on microRNAs, and demonstrate their importance in adipose tissue function and whole body metabolic control in mammals.

1. Metabolic diseases

Obesity statistics are alarming. According to the World Health Organization (WHO), more than 1.9 billion adults were overweight in 2014, and more than half a billion were obese. Once an exclusive issue of high-income countries, obesity is now prevalent in the developing world and has been considered a global epidemic [1]. This is extremely worrisome given that obesity represents one of the most important risk factors for chronic diseases, including type 2 diabetes (T2D), cardiovascular diseases and cancer – the leading causes of mortality and morbidity worldwide [2]. When appearing together, these diseases are classified as a broader syndrome often referred to as the “metabolic syndrome” [3].

The increase in the prevalence of obesity is due to many factors including genetics, diet, sedentarism and issues concerning the psychological, socioeconomic, or educational status of the individual [4].

Abbreviations: 3'-UTR, 3' untranslated region; 4-HNE, 4-hydroxynonenal; AC, acetylation; ADGCR8KO, fat-specific DGCR8 knockout; ADicerKO, fat-specific Dicer knockout; ADSC, adipose-derived stem cell; AGO, Argonaute; ATGL, adipose triglyceride lipase; ATP, adenosine triphosphate; BAT, brown adipose tissue; BMI, body mass index; BMP, bone morphogenetic proteins; BMSC, bone marrow stromal cell; bp, base pair; C/EBP, CCAAT-enhancer-binding protein; CGI58, comparative gene identification-58; DGCR8, DiGeorge syndrome chromosomal [or critical] region 8; dsRNA, double stranded RNA; FFA, free fatty acid; FGF, fibroblast growth factor; FOXO1, forkhead box protein O1; GDF5, growth differentiation factor 5; GLUT4, glucose transporter type 4; GPC, G protein coupled; GPX, glutathione peroxidase; HFD, high fat diet; HIV, human immunodeficiency virus; HMGA2, high-mobility group AT-hook 2; IKK, IκB kinase; IL, interleukin; IRS1, insulin receptor substrate 1; JNK, c-Jun N-terminal kinase; KO, knockout; KSRP, KH-type splicing regulatory protein; LDL, low-density lipoprotein; miRNA, microRNA; mRNA, messenger RNA; MSC, mesenchymal stem cell; MYF5, myogenic factor 5; ncRNA, non-coding RNA; nt, nucleotide; OXPHOS, oxidative phosphorylation; PACT, Protein ACTivator of the interferon-induced protein kinase; PCOS, polycystic ovarian syndrome; PGC-1, peroxisome proliferator-activated receptor gamma coactivator 1; piRNA, piwi-interacting RNA; PKC, protein kinase C; PPARγ, peroxisome proliferator-activated receptor gamma; PRDM16, PR domain containing 16; pre-miRNA, precursor miRNA; pri-miRNA, primary miRNA; Rb, retinoblastoma protein; Rb2/p130, retinoblastoma-like protein 2; RISC, RNA-induced silencing complex; RNAi, RNA interference; ROS, reactive oxygen species; RT-qPCR, reverse transcription – quantitative polymerase chain reaction; RUNX2, Runt-related transcription factor 2; SCD-1, stearoyl-CoA desaturase-1; SGBS, Simpson-Golabi-Behmel syndrome; siRNA, small interference RNA; sncRNA, small non-coding RNA; SOD, superoxide dismutase; SREBP-1, sterol regulatory element-binding transcription factor 1; sWAT, subcutaneous white adipose tissue; T2D, Type 2 Diabetes; TCA, tricarboxylic acid; TG, triglyceride; TGFβ, transforming growth factor beta; TLR4, toll like receptor 4; TNF-α, tumor necrosis factor alpha; TRBP, HIV-1 TAR RNA binding protein; UCP1, uncoupling protein 1; VEGF, vascular endothelial growth factor; vHPA, human visceral preadipocytes; vWAT, visceral white adipose tissue; WAT, white adipose tissue; WHO, World Health Organization

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Simplistically, obesity appears when there is a positive energetic balance, *i.e.* when energy consumption (food intake) overcomes energy expenditure [5,6]. The definition and classification of obesity is still a subject of debate. The WHO divides individuals into four categories based on their body mass index (BMI, kg/m^2): underweight BMI < 18.50, normal weight BMI=18.50–24.99, overweight=BMI 25.00–29.99, and obese BMI≥30.00 [7]. This sole parameter often creates confusion regarding the risk of metabolic diseases, since individuals can be classified as overweight or obese but do not display increased risk of mortality nor any metabolic alteration [8,9]. Moreover, in some cases even a negative association between BMI and mortality - in particular cardiovascular mortality – is found [10]. To avoid misdiagnosis, supporting clinical information needs to be taken into consideration, such as patient's life history (*e.g.* addictive behavior or body weight oscillation) and localization of excessive fat mass (*e.g.* upper body or lower body) [8,11,12]. When considering the latter, obesity can be further classified into an android syndrome (*i.e.* male-type or apple-shaped), where fat is deposited preferentially in intra-abdominal regions (*i.e.* visceral white adipose tissue - vWAT), and a gynoid syndrome (*i.e.* female-type or pear-shaped), where fat is deposited over the gluteofemoral region (*i.e.* subcutaneous white adipose tissue - sWAT) [13,14]. The first has been more commonly linked to comorbidities such as hypertension, dyslipidemia, and T2D [15–17], while the second confers a neutral or even protective effect against metabolic diseases [17,18]. To account for these differences, parameters like waist-to-hip ratio, magnetic resonance imaging or dual-energy X-ray absorptiometry [19,20] have been used as bona fide predictors of metabolic diseases and as useful parameters for researchers to understand how fat accumulation determines the risk of these diseases [12,21,22].

The pleiotropic nature of fat depots has been extensively explored in mice. For example, transplantation of sWAT from donor mice into the intra-abdominal region of a host mouse improves metabolism by reducing body weight and overall fat mass, increasing insulin sensitivity and whole body glucose uptake [23], and alleviating diet-induced glucose intolerance and inflammation [24,25]. Interestingly, these effects are minor when sWAT is transplanted into the subcutaneous cavity and no effect is observed when vWAT is transplanted to the intra-abdominal area [23–25], indicating that the differences between depots are determined by intrinsic characteristics as well as by anatomical localization. This is particularly highlighted in patients with lipodystrophy, where the pattern of fat accumulation differs from normal. These patients exhibit a degree of adipose tissue atrophy (whereas some depots can be hypertrophic), and often end up accumulating fat in non-adipose organs such as liver, heart, and skeletal muscle – a phenomenon that is causally linked to the metabolic syndrome [26–28]. Lipodystrophy can be classified according to fat loss topography (*e.g.* general or partial) or cause (*e.g.* congenital or acquired) [29,30]. The most prevalent form of lipodystrophy is acquired by patients with HIV undergoing antiretroviral treatment [31,32]. For instance, the highly active antiretroviral treatment (*i.e.* a combination of at least three antiretroviral drugs) inhibits mitochondrial DNA polymerase-γ causing mitochondrial toxicity, and the use of protease inhibitors up-regulates genes that inhibit adipocyte differentiation and down-regulates pro-adipogenic transcriptional factors such as PPAR-γ, C/EBP-α and SREBP-1, as well as genes involved in lipid metabolism [26,33,34]. These alterations are observed in white adipocytes, mainly in the subcutaneous depot either from the upper (*e.g.* face and shoulder) and/or lower body (*e.g.* gluteal and femoral). Additionally, HIV patients can also exhibit increased fat accumulation in breast, cervical, dorsocervical and visceral depots [35,36]. Importantly, these patients have a higher risk to develop insulin resistance and cardiovascular disease, and appear to age prematurely [37–39]. Therefore, abnormal changes in adipose tissue accumulation, independently of fat gain or loss, impact on the risk of metabolic diseases, a feature that highlights the importance of adipose tissue to whole body metabolic control.

2. The etiopathogenesis of metabolic diseases

Insulin resistance is a common feature of metabolic diseases and represents the main cause of T2D [40]. Like for obesity, the prevalence of T2D in the world is extremely high (8.8% of the world's population) and is rapidly growing [41]. In 2015, T2D killed approximately 5 million people [42]. The causes of T2D involve both genetic [43] and environmental factors that progressively lead to insulin resistance, glucose intolerance, and ultimately beta-cell failure [44,45]. Several reports support the notion that excess lipids continuously released from visceral fat into the portal vein in obese patients is a major trigger of T2D [46,47]. These lipids expose the liver to incremental amounts of free fat acids (FFAs), inducing hepatic insulin resistance and affecting gluconeogenesis [46,48]. Interestingly, like mentioned above, despite the association between T2D and obesity, some morbidly obese individuals as classified by BMI are significantly more glucose tolerant than leaner individuals [49]. Again, this apparent paradox might be explained by changes in the pattern of fat accumulation, since increased adipose tissue expandability and plasticity, particularly of subcutaneous depots, is linked to metabolic health [50,51]. In essence, the proposed model evokes that every individual has a limited capacity for fat storage, and when this limit is reached, either by obesity or impaired adipocyte function, excess lipids accumulate ectopically, causing lipotoxicity, inflammation, tissue dysfunction and disease [52–54].

At the molecular level, FFAs are usually taken up by cells and esterified into long chain acyl CoA molecules [55]. The bulk of these metabolites are directed to beta-oxidation, but a small portion can be converted into two lipid intermediates: diacylglycerols and ceramides [56]. In excess, these intermediates can activate kinases such as Jun amino-terminal kinase (JNK), IκB kinase (IKK), and protein kinase C (PKC) [57–60], which in turn phosphorylate the insulin receptor substrate-1 (IRS1) in serine and inhibit insulin signaling downstream of the insulin receptor [60–62]. Furthermore, lipids can signal through toll-like receptors or G protein-coupled receptors to cause insulin resistance or affect insulin secretion [63,64]. Additionally, excessive adipocyte hypertrophy without proper neovascularization creates a pseudohypoxic state in the adipose tissue [65,66]. Altogether, these stimuli lead to the infiltration of immune cells into the adipose tissue [67,68]. Within the tissue, monocytes differentiate into either anti-inflammatory M2 or pro-inflammatory M1 macrophages depending on the niche [69]. M1 macrophages are more commonly present in adipose tissue under obesogenic conditions, when they are activated by fatty acids and adipocyte-derived factors, and release a variety of pro-inflammatory cytokines that signal locally or systemically to cause insulin resistance through the activation of the same group of kinases and similar mechanisms as mentioned above [70,71].

Interleukin 1 beta (IL-1β) is one such pro-inflammatory cytokine synthesized and released from M1 macrophages in the context of obesity [72,73]. IL-1β production and secretion is controlled by the inflammasome and contributes directly to establishment of insulin resistance [74–76]. Supporting this notion, Ehses et al. treated type 2 diabetic Goto-Kakizaki rats with IL-1 receptor antagonist and observed an improvement in insulin sensitivity and glucose tolerance [77]. M1 macrophages also produce tumor necrosis factor alpha (TNF-α) [78,79]. TNF-α acts in cells within the adipose tissue and in non-adipose tissues to inhibit the tyrosine phosphorylation of IRS1 and therefore its activation [80,81]. Moreover, it modifies the pattern of adipocyte differentiation [82,83] while inducing lipolysis, which in turn increases the levels of FFAs and creates a “pro-inflammatory vicious cycle” [84,85]. In addition to causing lipotoxicity, as mentioned before, FFAs stimulate the synthesis of pro-inflammatory cytokines through the toll-like receptor-4 (TLR4) pathway [86,87]. IL-6 is also involved in the pathophysiology of obesity, but its role is more complex. It is