Review Article

Regulation of Adipogenesis Process by PU.1 Antisense Long-Non-Coding Ribonucleic Acid: A Review

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Earlier, long non-coding ribonucleic acid molecules were considered as a part of transcriptional noise and ignored imprudently, but gradually got revealed as potential regulators in many biological processes and their roles in gene expression influencing almost every aspect associated with genes, including epigenetic, transcriptional, and post-transcriptional regulation. Apart from their involvement in normal physiology, long-non-coding ribonucleic acid expression functions are also related to adipose biology, indirectly leading to obesity. This review discusses the beneficial role and mechanisms of action of PU.1 antisense long-non-coding ribonucleic acids in normal adipogenesis and their implications for obesity. Extensive research and identification of prominent long-non-coding ribonucleic acids in adipose biology will not only grant insights into diseases associated with obesity but also give ensure therapeutic targets for it.

Key words: Antisense long-non-coding ribonucleic acid, antisense oligonucleotides, long-non-coding ribonucleic acid, PU.1 antisense long-non-coding ribonucleic acid

In both developed and developing countries, obesity is becoming increasingly common. By 2030 the proportion of overweight and obese individuals is predicted to hit 89 and 85 percent in men and women, respectively[1,2]. Even though excessive weight gain is associated with metabolic syndrome disorders, including hyperglycemia, dyslipidemia, high blood pressure, atherosclerosis[3,4], diabetes, and cardiovascular disease, the global obesity epidemic is a significant challenge to human health[5,6]. An unusual increase in the number and size of adipocytes causes obesity due to excessive fat accumulation in White Adipose Tissue (WAT)[7]. Apart from the widely accepted high-caloric intake, genetic and epigenetic factors play roles in obesity, as evident from different studies[8,9].

Non-coding Ribonucleic Acid (ncRNA) molecules are genomic sequence transcripts not intended to be translated[10]. Short ncRNA (sncRNA equals 30 nt) and long ncRNA (lncRNA greater than 200 nt) are two ncRNAs categorized arbitrarily based on the length of RNA produced post-transcriptionally. With the discovery and functional characterization of lncRNAs, the family of regulatory Ribonucleic Acids (RNAs) has seen an explosion in the past decade. LncRNAs are a distinctive class of transcripts of more than 200 nucleotides, frequently polyadenylated and missing an active open reading frame[11-13]. LncRNAs categories are intergenic, antisense, divergent, intronic, and enhancer lncRNAs based on the relative location of the neighboring coding genes (fig. 1). The control of cellular functions by lncRNAs is by various mechanisms; they could act as scaffolds, decoys, or guides[12,14].

LncRNAs control gene expression at both the transcriptional and post-transcriptional levels, resulting in several biological processes such as tumor initiation, growth, and metastasis in various human diseases, including cancer[15-17] and in some obesity-related conditions.

LncRNAs in the adipogenesis process and its implication in obesity:

Adipocytes are peculiar cells destined to store

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Accepted 01 September 2023
Revised 04 July 2023
Received 14 September 2021
Indian J Pharm Sci 2023;85(5):1190-1197

excess energy as triglycerides and are involved in adipokine secretion that impairing systemic energy homeostasis[18]. Obesity typically happens when the volume or size of adipocytes increases[18]. WAT and Brown Adipose Tissue (BAT) are the two distinct kinds of mammalian adipose tissue. WAT is essential for storing and secretion of adipokines that affect energy homeostasis and metabolic processes, while BAT specializes in energy expenditure and thermogenesis[19,20]. Maintaining normal adiposity and optimizing lipid metabolism requires a proper balance of these processes. WAT consists of adipocytes generated by the differentiation of preadipocytes. Very high or low WAT leads to metabolic disorders such as hyperlipidemia, resistance to insulin, and type 2 diabetes[21]. For optimum health, maintenance of sufficient amounts of WAT is essential. The two primary types of white adipose tissue are Subcutaneous Adipose Tissue (SAT) below the skin and Visceral Adipose Tissue (VAT) found inside particular regions of the abdominal cavity[22]. Excessive fat accumulations relative to both SAT and VAT are responsible for the incidence of various metabolic diseases[23], especially fat accumulation in VAT, regarded as a high-risk factor for many metabolic disorders and cardiovascular diseases[24-26]. For a long time, studies on the differentiation of visceral adipocytes and their potential regulatory mechanisms have been at the forefront of obesity science. Adipose tissue also serves as an endocrine organ by secreting adipokines that impact the body's glucose and energy homeostasis[27]. A cascade of transcription factors, cofactors, and signaling intermediates from various pathways orchestrates the adipogenesis process[7]. Adipogenic differentiation is mainly monitored by the master regulator peroxisome proliferator activator receptor for adipogenesis, together with other transcription factors and cofactors like CCAAT/enhancer binding proteins (C/EBPs), Kruppel-like factors (KLFs) or Wingless proteins (Wnt)[28]. The regulation of adipogenesis happens in multidimensional ways involving components of numerous pathways in a co-ordinated manner sequentially, as seen in fig. 2.

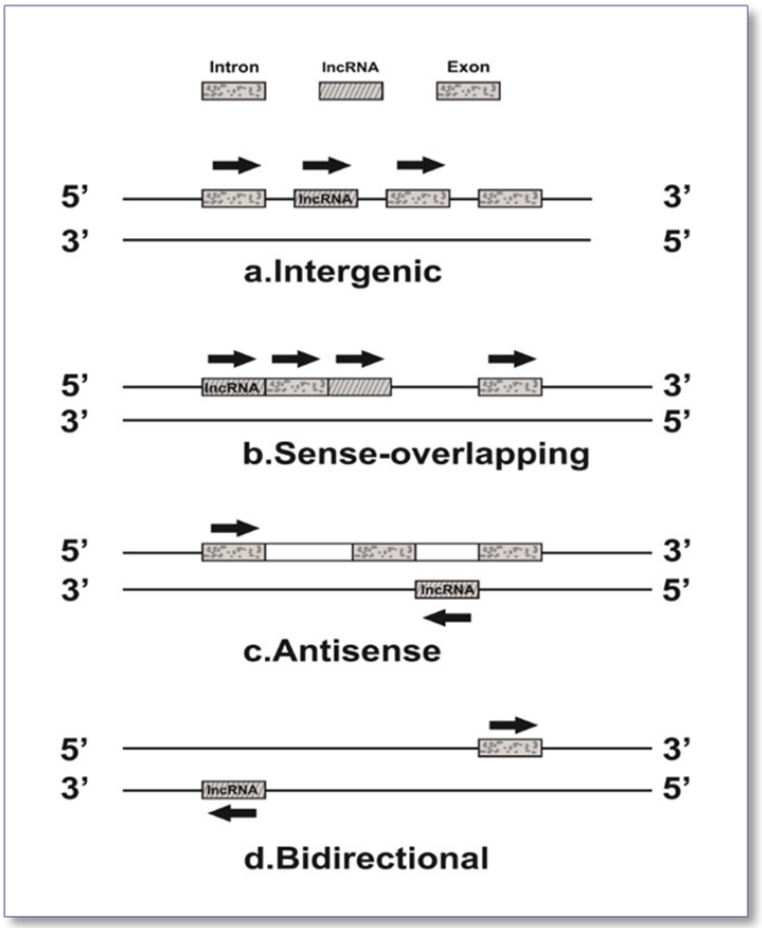

Fig. 1: Classification of lncRNA relative to its genomic location
Note: a: Intergenic lncRNAs sited between protein-coding genes; b: Bidirectional lncRNAs transcribed from the same promoter as a protein-coding gene but in the opposite direction; c: Antisense lncRNAs originate from the antisense RNA strand of a protein-coding gene and d: Sense-overlapping lncRNAs overlap with one or more introns and/or exons of a protein-coding gene in the sense RNA strand direction

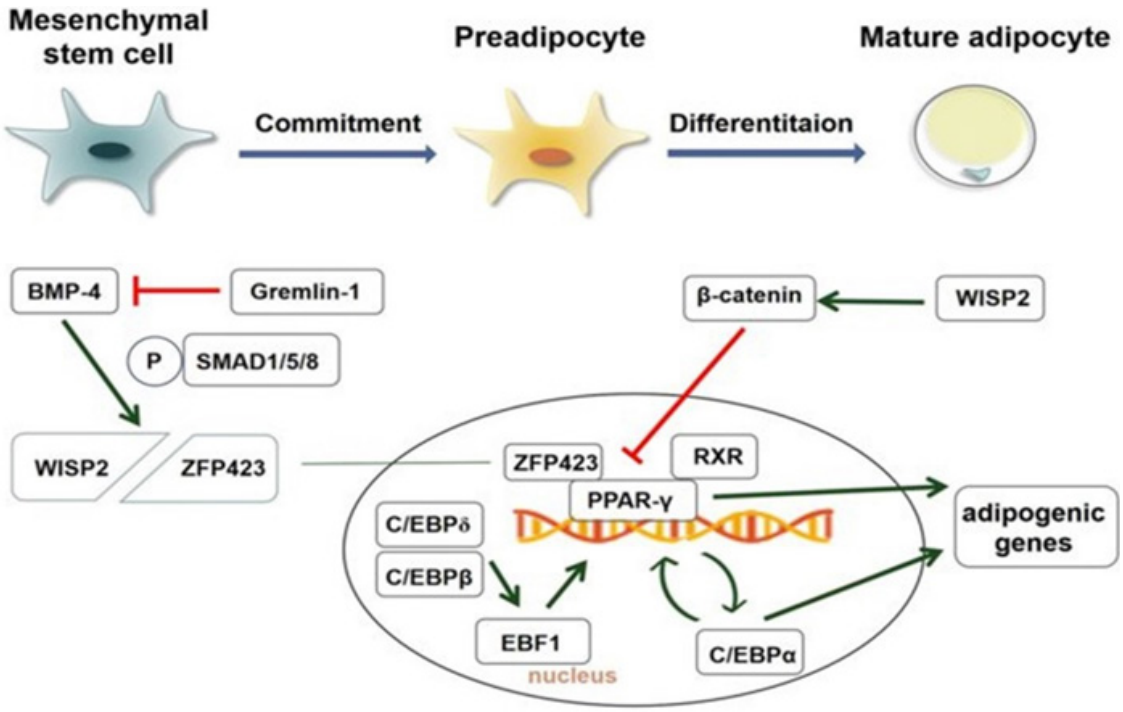

Fig. 2: Regulation of adipogenesis happens in multidimensional ways involving components of numerous pathways
Note: (→): Promote and (⊣): Inhibit

The role of RNAs, especially ncRNAs, has recently been extensively studied for their contribution to the production and function of adipose tissue. In many studies, the role of sncRNAs, including micro RNAs, in BAT and WAT biology. While the role of lncRNA in maintaining adipose tissue activities is in a few research studies only[29], after analyzing the differential expression of lncRNAs across primary WAT and BAT, preadipocytes, and cultured adipocytes, Sun *et al.*[29] characterized 175 lncRNAs regulated during adipogenesis. The group pointed out and analyzed 20 lncRNAs likely to be controlled by Peroxisome Proliferator-Activated Receptor gamma (PPARγ) and C/EBPs, the master regulators of adipogenesis. They also carried out a loss-of-function screen and demonstrated that 10 of them, including lncRAP-1 and lncRAP-2, function to modulate adipocyte differentiation[29]. Many other studies also showed definite roles of lncRNAs in adipogenesis and adipocyte biology networks. Utilizing RNA-seq analysis, Alvarez *et al.*[30] identified 1500 lncRNAs expressed in inguinal white, epididymal, and brown fat in mice. Exclusive expression of 127 lncRNAs was associated with BAT, most targeting the critical regulators of adipogenesis, including C/EBPα, C/EBPβ, and PPARγ. Table 1 describes the role of various lncRNAs involved during adipogenic differentiation. The exact role and function of lncRNAs in obesity and adipogenesis are still unknown, even if there is rapid research in this area[31-61].

Targeting RNA molecules as a promising therapeutic approach:

Identifying the potential targets modifiable therapeutically to deal with the broad clinical needs of patients with various ailments is critical. Most clinical drugs target proteins[62]; however, as they can also interact with proteins that aren't their targets, these frequently cause problems. RNA represents one class of targets, as proteins come from specific messenger RNAs (mRNAs); hence, modulations in mRNAs or pre-mRNA levels could broaden the therapeutic targets. Nucleic acids are evolving therapeutics for unmet medical needs since they might cause fewer side effects than existing therapies[63]. The drawbacks of targeting proteins using conventional small-molecule or protein-based strategies (adapter proteins, transcription factors, etc.) can be easily targeted by modulating the mRNA levels and translation to the protein. Identifying ncRNAs' unique regulatory roles and roles in normal cellular physiology is expanding, as RNAs can directly promote pathology[64]. Current strategies to modulate the RNA functions in cells include the usage of small molecules targeting RNA, genome editing, gene therapy, delivery of exogenously expressed mRNAs genome editing,

and synthetic Antisense Oligonucleotides (ASOs). ASOs are oligonucleotides artificially synthesized with a size range of 12-30 nucleotides designed to bind to RNA by Watson-Crick base pairing rules. They can bind uniquely to only one target RNA, modulating its function by several mechanisms[65]. Nowadays, antibacterial and anti-cancer therapies use drugs targeting nucleic acids. One primary approach to targeting lncRNAs for treatment is deregulating high lncRNA levels with ASOs, which block lncRNA activity, further leading to their degradation. Alternatively, the lncRNA function may be blocked by small molecules that cover the binding site of interrelating proteins or by antisense oligonucleotides that connect to the lncRNAs and restrain their protein binding capacity[66]. ASOs can target those lncRNAs that positively regulate white adipogenesis (up-regulated) instead of brown as a control method of obesity. Preliminary *in vitro* studies[67] showed that the ASO approach could be a critical tool for treating obesity. The practicability of ASO therapy for targeting lncRNAs is in some pre-clinical models; Antisense phosphorothioate oligonucleotides can target lncRNAs involved in Angelman syndrome and lung cancer in mice[68].

Natural Antisense (AS) transcripts (NATs) are RNA molecules transcribed from the opposite Deoxyribonucleic Acid (DNA) strand and partly overlap with sense mRNA. Antisense (AS) RNA is a relatively uncommon term in a physiology environment until short interfering RNAs emerged as the tool of choice to knock down the expression of specific genes[69]. Recently, NAT levels have been dysregulated in various disease states[70]. Computational studies suggest that 15-25 % of mammalian genes overlap, giving primarily to pairs of sense and antisense RNAs[71]. NATs, mostly categorized as AS lncRNAs, play notable roles in the clarified regulation of animal genes in almost all stages of gene expression, from transcription initiation to translation to RNA degradation[72]. However, we know little about their exact functions and molecular mechanisms in many biological processes, especially in animal adipogenesis. The mammalian genome contains large spans of AS lncRNAs, and recent studies have indicated that some of these AS lncRNAs might be functional[73]. The biological role of antisense lncRNAs, despite their low expression, could still be rationalized because there are two copies of DNA for any given gene in a cell; consequently, just two antisense lncRNA molecules are sufficient to interact with the two gene copies and elicit regulatory effects[74] AS lncRNAs at numerous gene loci silences sense transcription by affecting histone acetylation and methylation states and regulating mRNA dynamics at a post-transcriptional level[75]. Many studies indicated that AS lncRNA decreases mRNA levels, such as AS lncRNAs of tie-1[76], Fibroblast Growth Factor-2 (FGF-2)[77], and Multiple peptide resistance factor (MprF)[78]. The regulatory mechanism of AS lncRNA remains unclear, although there is evidence for the regulation by similar means as for protein-coding genes. AS lncRNAs play a positive or negative role in translation[79], transcription[80-82] and stabilization of mRNA[83].

TABLE 1: LncRNAs INVOLVED IN REGULATING THE ADIPOGENESIS PROCESS

lncRNAs	Functions	References
SRA	Improves white adipogenesis	[31-33]
NEAT1	Improves white adipogenesis	[34,35]
Lnc-RAP-n	Improves white adipogenesis	[29,36]
SlincRAD	Improves white adipogenesis	[37]
PU.1 AS	Improves white adipogenesis	[38,39]
ADINR	Improves white adipogenesis	[40]
Paral1	Improves white adipogenesis	[41]
lnc-leptin	Improves white adipogenesis	[42]
HOTAIR	Improves white adipogenesis	[43]
ADNCR	Represses white adipogenesis	[44]
HoxA-AS3	Improves white adipogenesis	[45]
lnc-U90926	Represses white adipogenesis	[46]

MIR31HG	Improves white adipogenesis	[47]
Gm15290	Improves white adipogenesis	[48]
TCONS_00041960	Improves white adipogenesis	[49]
HoxA11-AS1	Improves white adipogenesis	[50]
Adiponectin AS	Represses white adipogenesis	[51]
MEG3	Represses white adipogenesis	[52]
H19	Represses white adipogenesis	[53,54]
Blnc1	Improves brown adipogenesis	[55,56,57]
lnc-BATE1; lnc-BATE	Improves brown adipogenesis	[30,58]
lnc-uc.417	Represses brown adipogenesis	[59]
AK079912	Improves brown adipogenesis	[60]
GM13133	Improves brown adipogenesis	[61]

PU.1 AS lncRNA in adipogenesis:

The PU.1 gene was initially recognized as a proviral integration site for the Spleen Focus-Forming Virus (SFFV) in erythroleukemia[84]. The transcription factor Spi1/PU.1 (SFFV proviral integration oncogene/PU box binding protein) is a hematopoietic ETS family member that is influences in the immune system generation[85]. PU.1 is a critical transcription factor in biological processes, for it plays crucial roles not only in the hematopoiesis and immune system development[85] but also in cell cycle exit[86,87] and epigenetic silencing[88]. PU.1 functions solely in a cell-intrinsic manner to monitor the development of granulocytes, macrophages, and B and T lymphocytes[89]. PU.1 deficiency generally arrests lymphopoiesis and myelopoiesis in mice fatally, very recently human congenital PU.1 disorder has been recognized in six agammaglobulinemia patients with varying SPI1 mutations but shared insufficient levels of PU.1 and absence of B cells with consequently, zero antibodies and the condition got reversed on CRISPR editing of SPI1 in cord blood *in vitro*[90].

Overexpression of PU.1 downregulated essential adipogenic genes C/EBPβ and PPARγ in the C/EBPα/β-PPARγ terminal pathway of adipogenic differentiation[91]; however, the underlying mechanism that PU.1 suppressed the expression of C/EBPb and PPARc remains elusive. Two master regulators-C/EBPβ/α and PPARγ regulate adipogenesis, which in turn could be strongly inhibited by PU box-binding protein (PU.1). Proven to be expressed in the adipose tissue of humans and other animals, PU.1 could suppress the C/EBPβ/α-PPARγ pathway, significantly and negatively influence adipogenesis[91].

Recent studies validated that a novel mechanism of gene regulation was found in mouse PU.1 locus, as the locus gave rise to both mRNA and NATs, as AS lncRNAs, which originated from an intronic promoter, and PU.1 gene level regulated through coordinated expression of its mRNA and AS lncRNAs[92]. Antisense RNAs could regulate the expression of their respective gene-altering processes in which they are involved. Previous studies indicated that PU.1 AS lncRNA promoted adipogenesis in 3T3-L1 adipocytes by preventing PU.1 mRNA translation[93]. PU.1 AS-PU1 RNA duplex inhibits adipogenesis and modulation of sense gene expression by altering its protein expression and decreasing PPARγ, fatty acid synthase, and adiponectin expression in the mouse. The porcine PU.1 locus transcribed both PU.1 mRNA and PU.1 AS lncRNA, which regulates adipogenesis[93]. Antisense lncRNA overlaps PU.1 mRNA and negatively affects PU.1 protein expression *via* blocking translation without down-regulating mRNA levels. The regulatory mechanism in general for the PU.1 AS lncRNA during terminal differentiation of adipogenesis is shown in fig. 3. Knockdown of PU.1 AS lncRNA in zebrafish or mice up-regulated levels of PU.1 mRNA, causing expression changes of downstream genes[93]. These findings suggest that the same AS lncRNA showed distinct regulatory mechanisms, which is crucial because of its size and position in different species. Moreover, lncRNAs are so long and complicated that a slight disparity in the sequence may lead to a tremendous change in the secondary structure, distinctly altering their functions and mechanisms.

CONCLUSION

Therapeutic interventions in obesity depend on knowledge of molecular mechanisms that could help prevent adipogenesis. This review presents a recent approach to targeting adipogenesis utilizing antisense non-coding transcripts, indirectly, obesity. The data revealed the definite mechanism of PU.1 inhibiting adipogenesis and provided insight into the adipogenesis regulatory networks. Shortly, we will witness comprehensive and functional aspects of lncRNAs during all stages of metabolism. It is interesting to consider a combination of multiple schemes aiming at molecular mechanisms targeting adipogenesis could emerge for beneficial personalized treatment of obesity and its related complications.

Acknowledgments:

Taking account of the abundant literature on adipogenesis, we apologize if some relevant papers were not cited here due to space limitations; we thank Prof. (Dr) P. R. Sudhakaran, Prof. (Dr) G. Mohanadasan Nair and Prof. (Dr) A. Gangaprasad for stimulating discussions.

Funding:

Department of Higher Education, Govt. of Kerala, India.

Conflicts of interest:

The authors express that no conflict of interest exists.

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