An Update on Microrna’s and Metabolic Regulation with Future Therapeutic Potentials Regarding Diagnosis and Treatment of Obesity, Metabolic Syndrome and Other Related Disorders

Kulvinder Kochar Kaur*, Gautam Nand Allahbadia and Mandeep Singh
Dr Kulvinder Kaur Centre for Human Reproduction, Jalandhar, Punjab, India

Introduction

Micro RNAs is a family of highly conserved, single stranded 19-23 nucleotide long noncoding endogenous RNAs which negatively regulate gene expression, either by inhibiting translation or by degrading largest mRNAs [1]. Over 2000 unique miRNAs have been identified in humans [2]. The miRNAs are initially transcribed as long RNA precursors termed primary miRNAs (pri-miRNAs) that require the RNAse III enzyme Drosha in the nucleus to trim them into premiRNAs. The premiRNAs are characterized by a stemloop or hairpin structure of 70-100 nucleotides and are exported to the cytoplasm by the nuclear export factor exportin 5. The pre-miRNAs are subsequently cropped to become mature miRNA by another RNAse enzyme Dicer in the cytoplasm [3]. Although our understanding of the specific roles of miRNAs in cellular function is only beginning, recent studies revealed that miRNAs play a pivotal role in the most critical biological events such as development, proliferation, differentiation, apoptosis, transduction and carcinogenesis [4]. The mature miRNA’s are conserved about 2 nucleotides noncoding RNAs that anneal to inexact complementary sequences in the 3’ untranslated regions (3’UTR) of target mRNA of protein coding genes, resulting in silencing of the target gene. While the mechanism of action of miRNAs is yet to be fully understood, the widely known mode of gene regulation by miRNAs occurs at the post transcriptional level by either specific inhibition of translation or induction of mRNA cleavage [5,6], resulting in a reduction in protein levels of their targets (Figure 1) [7]. Alternatively, if the expression of miRNA’s is inhibited, then increased protein expression may be seen in animal species. miRNAs are also capable of modifying chromatin (Figure 1) [6]. dsRNA miRNA complex which is composed of the mature miRNA and its passenger strand (miR*) is unwound by helicase activities of the Argonaute (Ago) multiprotein complex, globally known as the RNA-induced silencing complex (RISC) [8-10]. Determination of the active (guide) strands based upon simple energetics between strands at the 3’ ends of the dsRNA complex [11]. The preferred guide strand is subsequently incorporated into the RISC complex by directly binding to the key component of Ago [12]. miRNA target sites generally occur within the coding frame of mRNA and the 3’ UTR [13]. We can regulate cancer, obesity and viral induced diseases by using miRNAs. Further work is going on in developing potential RNAi (miRNAand siRNA) molecules to get insights for the chemical synthesis of antiviral RNA molecule for the treatment of middle east respiratory syndrome (MERS) corona virus (CV), at genomic level [14], or development of antiviral RNA molecule for the treatment of Merkel cell polymyxoma virus (MCC) which causes 80% Merkel cell carcinomas (MCC), for T antigen silencing at genome level [15]. Similarly in HSV-1, siRNA were designed against ICP22 (US1) gene (intermediate gene) responsible for genomic replication of HSV. This approach may help us in synthesizing antiviral RNA molecules for treatment of HSV-1, at genomic level [16]. Zhou et al. have further reviewed the role of nanoparticle based delivery of RNA interference (RNAi) therapeutics [17] along with different delivery systems for various cancers e.g. role of (ALN-VSP), a lipid formation delivering siRNAs against two important cancer genes, kinesin spindle protein (KSP) and vascular endothelial growth factor (VEGF), for the treatment of liver cancers in phase 1 trials [18]. Further doxorubicin combined with polyethyleneimine (PEI) along with Pin X1-si RNA is delivered to knock out PIN2 interacting protein (PINX1) gene in C6 glioma cells. Pin X1 is anucleolar protein associated with telomere and telomerase. Thus Pin X1-siRNA-mPEG (monomethoxy poly ethyleneglycol)-PEI super paramagnetic ironoxide nanoparticles (SPION) in combination with doxorubicin maybe a more effective treatment of gliomas with negligible side effects [19]. Similarly ScFv decorated Poly ethylene glycol (PEG)-block poly D,L-Lactide (PLA)-based nanoparticles show greater potential for targeted RNAi therapy of Her2 (+) breast cancer [20].

The incidence of obesity defined as BMI ≥ 30 kg/m² increased dramatically worldwide during recent decades. Obesity associated with a cluster of metabolic disorders including risk of T2DM, hypertension, dyslipidemia, atherosclerosis, cardiovascular diseases and obesity per se constitute a series of threat known as metabolic syndrome [21]. This review aims to emphasize the role and recent advances of miR’s in the aetiopathogenesis of obesity only and how further it can be utilized in prevention and management of obesity and its associated complications like atherosclerosis, IR, and other components of the MS.

miRNAs in Adipose Tissue, Insulin Resistance (IR), Obesity and Diabetes

Aetiopathogenesis of obesity

Adipose tissue (AT) is increasingly being recognized as a key regulator of whole body energy homeostasis and consequently as a prime therapeutic target for metabolic syndrome (MS). A substantial continuing growing body of evidence supports the concept that chronic low grade inflammation is a central characteristic of obesity contributing to development of insulin resistance (IR) in AT and other target organs including muscle, liver and the vasculature [22-24].

miRNAs in WAT differentiation: Adipocyte differentiation occurs in several stages, involving several many signaling pathways and this progress depends on various stimuli like nutrients and hormones. The most important of this cascade of transcription factors

*Corresponding author: Kulvinder Kochar Kaur, Dr Kulvinder Kaur Centre for Human Reproduction, Jalandhar, Punjab, India, Tel: 91-181-4613422; E-mail: kulvinder.dr@gmail.com

Received January 28, 2015; Accepted March 19, 2015; Published March 26, 2015

Citation: Kaur KK, Allahbadia GN, Singh M (2015) An Update on Microrna’s and Metabolic Regulation with Future Therapeutic Potentials Regarding Diagnosis and Treatment of Obesity, Metabolic Syndrome and Other Related Disorders. J Health Med Informat 6: 184. doi:10.4172/2157-7420.1000184

Copyright: © 2015 Kaur KK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
controlling adipogenesis tightly is CCAAT/enhancer binding protein (C/EBP) and peroxisome proliferator activated receptor γ (PPARγ). Besides these several signaling molecules including wingless and INT 1 proteins (Wnt) and insulin modulate adipogenesis [25,26]. Adipogenic marker genes, such as CCAAT/enhancer binding protein α (Cebpα), peroxisome proliferator activated receptor γ (Pparγ), adipocyte fatty acid binding protein (Ap2), fatty acid synthase (Fas), are regarded as the essential transcriptional regulators of preadipocyte differentiation and lipid storage in mature adipocytes. Canonical Wnt/β catenin signaling is recognized as a negative molecular switch during adipogenesis. Chen et al. found miR-135a-5p is markedly downregulated during the process of 3T3L1 preadipocyte differentiation. Overexpression of miR135a-5p impairs the expression of adipogenic marker genes as well as lipid droplet accumulation and adipogenesis. Further studies show that miR135a-5p directly targets adenomatous polyposis coli (Apc), contributes to the translocation of β-Catenin from cytoplasm to the nucleus, and then activates the expression of Cyclin D1 (CyclinD1) and Cmyc, indicating the induction of canonical Wnt/β-catenin signaling. In addition, inhibition of APC and siRNA exhibits the same effects as overexpression of miR135a-5p. Thus concluding that miR135a-5p suppresses 3T-L1 preadipocyte differentiation and adipogenesis through the activation of canonical Wnt/β-Catenin signaling by directly targeting Apc, giving profound insight into adipogenesis and development of adipose tissue [27]. Yang et al. demonstrated that the levels miR1908 increases during adipogenesis of human multipotent adipose derived stem cells (hADAMS) and human visceral preadipocytes. Over expression of miR1908 in hADAMS cells inhibited adipogenic differentiation and increased cell proliferation, suggesting that miR1908 is involved in the regulation of adipocyte cell differentiation and metabolism and thus may have an effect on human obesity [28].

**MiR in brown adipocyte differentiation:** Thermitic adipocytes are distinct from white adipocytes in that they have more mitochondria, and in that uncoupling protein 1 (UCP1) is highly enriched in these mitochondria. UCP1 uncouples substrate oxidation from ATP production so that heat is generated instead [29]. In addition to thermogenic brown adipocytes which are located in BAT upon cold exposure, UCP1 – expressing thermogenic adipocytes can be recruited in WAT, so called brite (brown in white) or beige adipocytes resulting in WAT browning [30-33]. Thermogenic adipocytes have a substantial impact on the energy balance, as UCP1 -mediated adipocyte differentiation, concomitant with PPARγ protein expression. Thus overexpression of miR27a and miR130a evidently suppresses adipocyte differentiation-while miR17-92 were found to promote white adipocyte differentiation of 3T3L1 preadipocytes. Wu et al. described a negative effect of miR106b and miR 93 on brown adipogenesis [40]. While miR26a/b induced and promoted human brite adipocyte differentiation, mechanistically the combination of transcriptomics, an RNAi screen and reporter assays revealed that the effects of miR26a/b are largely mediated via its target ADAM metallo pepidase17 (ADAM17/TACE), a gene that was previously described to negatively regulate nonshivering thermogenesis [41,42].

**Role of PPARγ:** Further PPARγ has been shown to be the major driver of the accumulation of phenotype of AT T reg cell. This was in keeping with that PPARγ expression by VAT Treg cells was necessary for complex restoration of insulin sensitivity in obese mice by the thiazolidenedone drug pioglitazone [43]. Ortega et al. [44] have found that transducin like enhancer split 3 (TLE3) in AT is increased in situations characterized by decreased PPARγ gene expression, like type2 diabetes mellitus (T2DM) and suggest TLE3 maybe a homeostatic linchpin in IR and defective PPAR-γ [44]. It has been reported that both miR27a and miR130a suppress adipocyte differentiation through PPARγ downregulation [45,46]. In 3T3-L1 cells, the levels of miR27a and miR130a are gradually decreased during adipogenesis, which is inversely correlated with the expression levels of PPARγ. Furthermore overexpression of miR27a and miR130a evidently suppresses adipocyte differentiation, concomitant with PPARγ protein expression. Thus these 2 miRs negatively regulate PPARγ expression [26,45,46].

**Obesity in Infancy and Early Childhood**

Both allergy and obesity development have been traced back to accelerated growth early in life

The nutrient sensitive kinase mTORC 1 is the master regulator of cell growth which is predominantly activated by amino acids. In contrast to breastfeeding, artificial infant formula feeding bears the risk of uncontrolled excessive protein intake overactivating the infant’s mTORC 1 signaling pathways. Over activated mTORC1 enhances S-6-K-1 mediated adipocyte differentiation, but negatively regulates growth and differentiation of Fox P3 regulatory T cells [Treg] which are deficient in atopic individuals. Thus the ‘early protein hypothesis’ not only explains the mTORC1 mediated increased infant growth but also the development of mTORC1 driven diseases such as allergy and obesity due to a postnatal deviation from the appropriate mTORC1 axis driven metabolic and immunologic programming. Intake of fresh unpasturized cow’s milk exhibit an allergy preventive effect in farm children associated with increased Fox3+ Treg cell numbers. Human and bovine milk contain a substantial amounts of exosomal miRNA’s which have been postulated to be involved in postnatal immune regulation [47-50]. Milk miRNA’s are transported by membranous vesicles called exosomes which play a pivotal role for horizontal miRNA transfer [50]. Unidirectional transfer of miRNA loaded exosome from Tcell to antigen presenting cells (APC) has recently been confirmed. Human and bovine milk contain high amounts of exosomal miRNA155 [48,49,51,52]. Admyra et al. [50] showed that incubation of human peripheral mononuclear cells with which isolated human
milk exosomes increased the number of CD4+CD25+FoxP3+Treg cells in a dose dependent fashion. It has been shown that the ancient immune regulatory miRNA155 is required for the development of Treg cells [53]. miRNA 155 deficient mice have a reduced number of Treg cells both in thymus and in the periphery [54]. FoxP3 binding to the promoter of bic, the gene which encodes miRNA155 [54-56]. T cell receptor (TCR) and notch signaling upregulates IL-2Ra chain (CD25) rendering thymocytes receptive to subsequent cytokine signals that foster their Treg as determined into fully functional FoxP3+Treg [57-60]. IL-2 is capable of traducing signals in CD4+FoxP3+Treg as determined by phosphorylation of signal transducer and activator of transcription5 (STAT5) [60]. Deletion of miRNA155 results in limited IL-2/STAT5 signalling, which reduced Treg numbers [61]. Thus miR 155 is an important negative regulator of IL-2R/STAT5 signalling by which it enhances FoxP3 expression [61,62].

Obesity in childhood

Landgraf et al. 2015 showed that an increase in adipocyte size and number occurred, examining 171 AT samples in early childhood obese vs lean children (0-18yrs) where decreased basal lipolytic activity and significantly enhanced stromal vascular cell proliferation in vitro, potentially explained the AT cell hypertrophy and hydroplesia seen in obese children respectively. Macrophage infiltration underlying the formation of crown like structures (CLS) was increased in AT of obese children from 6yrs associated with higher hsCRP serum levels along with IR [63]. With the idea to define the circulating miRNAs in childhood obesity, genome wide circulating miRNA profile was assessed by RTPCR in 10 boys (5 lean and 5 obese children) and further most relevant miRNAs were cross validated in 85 lean vs 40 obese children and longitudinally evaluated in samples from same children when they were 7 and 10yrs old. 1Specific miRNAs were significantly deregulated in prepubertal obesity including the decreased miR 221 and miR28-3p and increased concentration in plasma of miR486-5p, 486-3p, miR 142-3p, miR130b and miR423-5b. The circulating concentrations of these miRNAs was significantly associated with BMI, HOMA-IR and high molecular weight adiponectin, CRP and circulating lipids in concordance with anthropometric associations. Plasma concentration of 10 of these circulating miRNAs changed significantly and differently during the 3-year follow up in children who decreased or increased their normalized weight. Thus Prats-Puig et al. [64] concluded that this is the first study which provides evidence that circulating miRNAs are deranged in prepubertal children. Thus the very early detection of an abnormal circulating miRNA profiles maybe a promising strategy to identify obese children who may suffer from metabolic abnormalities [64].

Obesity in adulthood: Effects of miR in WAT inflammation and IR

**Role of miRNA 125 a:** While one miR can target several miRNAs, one transcript can be targeted by several miRNAs. Such interactions regulate various aspects of metabolism through pancreatic development, insulin biosynthesis, secretion and signaling, adipocyte differentiation and glucose uptake [65,66]. Dawara et al. studying the role of miRNA 125a in pathogenesis of insulin resistance (IR) in both men and mice studied BALB/c and C57BL/6 mice in response to high fat diet. miRNA 125a expression was downregulated in vitro in IR 3T-L1 adipocytes and exvivo in adipose tissue of obese patients.In vitro modulation of miRNA 125a expression in 3T-L1 adipocytes did not affect glucose uptake.Gene set enrichment analysis (GSEA) identified significantly altered expression pattern of predicted miRNA125a gene targets in transcriptomic datasets of adipose tissue from HFD-fed mice and obese patients. Among genes that contributed to global enrichment of altered expression of miRNA125a targets, thyrotriph embryonic factor (Tef), Mannan-binding lectin serine peptidase 1, Reticulon 2, and Ubiquitin-conjugating enzyme E2L3 were significantly differentially expressed in adipose tissue in these groups. They showed that Tef expression is reduced in adipose tissue of obese patient’s following gastric bypass surgery. Hence they concluded that miRNA 125a expression in adipose tissue adapts to IR and may play a role in the development of obesity, both in mice and obese subjects coupled through uncoupled regulation of the expression of miRNA125a and its targets [67].

**miR in adult obese vslean reflecting WAT inflammation:** Ortega et al. performed miRNA array on human subcut Ortega et al. [68] performed miRNA array on human subcutaneous AT. 50 of the 799 miRNA tested (6.2%) significantly differed between obese (n=9) and lean (n=6) subjects (17). Among these 50 miRNAs 17 were highly correlated with BMI and metabolic parameters (fasting glucose, and or triglycerides). These data were concordant with those obtained in overweight and obese patients by Kloting et al. [69]. They showed significant correlations between the expression of selected miRNA and both AT morphology, key metabolic parameters including visceral fat area, HbA1c, fasting plasma glucose and circulating Lep, Adiponectin (Apn) and IL-6 [18]. Another 2 miRNAs, miRNA 17-5p, and miR122 were significantly decreased in the omental fat and circulation of obese subjects [69].

**Effects of proinflammatory cytokines:** Adipocytes are continuously stimulated by proinflammatory cytokines such as TNF-α which cause adipocyte dysfunction by facilitating the inflammatory response. Although miR 130 was reported to be an important regulator of adipogenesis by targeting PPAR-y mRNA, little is known about the mechanisms regulating miR130 expression during the proinflammatory response. Kim et al. examined miR 130 levels in white adipose tissue (WAT) from high fed fat diet (HFD) mice and TNF-α stimulated adipocyte [70]. Primary microtranscripts of miR130 were increased after TNF-α stimulation indicating that induction of miR 130 during the post inflammatory response is regulated by a transcriptional event. A chromatin immunoprecipitation assay showed that p65 binding to the promoter regions of the miR 130 was enhanced after TNF-α treatment. These findings suggest that induction of miR130 by TNF-α is responsible for adipocyte dysfunction [70]. Obesity is associated with low grade inflammation of WAT which can subsequently lead to IR, impaired glucose tolerance and even DM. miRNAs have been implicated as negative regulators, controlling diverse biological processes at the level of post transcriptional repression. miR 146b is an intergenic miRNA, that can regulate the inflammatory process by attenuating cytokine signaling via the nuclear KB pathway. Shi et al. focused on the expression of miR 146b in mature human adipocytes and their response to proinflammatory cytokines. They found miRNA 146b was highly expressed in the mature adipocytes. The mature human adipocytes responded to proinflammatory cytokines (TNFα and IL-6) by highly increasing the expression of miR146b. They cloned and identified a potential promoter of the transcriptional regulator of miR146b. Interestingly they found a fragment about 950bp length upstream sequences of miR146 b had apparent transcriptional activity. In addition the increase in miR 146b promoter activity by TNF-α and IL-6 was also effectively elevated. Thus these results indicated a novel role for miR 146b in AT inflammation and miR146b may be an important mediator in the process of obesity complications via its own transcription mechanisms [71].

**Role of miR 26b:** Subsequently the same group investigated the effects of energy source material and hormones associated with obesity.
on miR26b, which is an obesity related intronic miRNA located in the intron of the carboxy terminal domain, RNA polymerase II, polypeptide A, small phosphatase I gene. miR26b is abundantly expressed in mice and mature human adipocytes. They demonstrated that free fatty acids (FFA) glucose, glucocorticoids and growth hormone (GH) downregulate the expression of miR26 b in human adipocytes. These results indicate that the expression of miR26b is affected by a variety of factors that are correlated with obesity and insulin sensitivity. Therefore miR26b may be an important mediator of the development of obesity associated IR [72].

Effects of bariatric surgery: Obesity is considered a multifactorial disease, with epigenetic alterations and modifications described in expression of some miRNAs and proteins. Adipose tissue (AT) is formed by different cell types and has an endocrine function, since it secretes substances such as leptin (LEP), insulin like growth factor 1 (ILF1), interleukin-10 (IL-10) [73-77]. Collares et al in an attempt to study the correlation of obese patients studied the gene expression of LEP, LEP receptor (LEPR), IGF1 and IL-10 and of miR 27a, miR27b, miR143, miR145. RNA was extracted from biopsies of subcutaneous tissue and visceral fat of 15 obese subjects submitted to bariatric surgery and of 15 non obese subjects submitted to cholecystectomy for CDNA synthesis and for RTPCR. The miRNA were chosen using the Target Scan software. An increased expression of LEP and IGF1 was identified in the subcutaneous fat of the obese group as compared to control, while the expression of IGF1 was higher in the control group than in obese one. miRNA 27a had a higher expression in the omentum of the obese patients. There was a positive correlation in the expression of miRNA145 and LEPR in the omentum of the group [78].

Interrelation of hypoxia and cytokines in FA storage: Recent findings in adipose tissue (AT) have uncovered negative interactions among obesity, lipogenesis and fatty acid (FA) storage, perhaps in response to the increased production of proinflammatory cytokines and transcription factors. Emerging evidence highlights that local hypoxia, generation of reactive oxygen and nitrogen species, increases immune cell infiltration and activation, senescence, inflammation, energy consumption and decreased lipogenesis in AT are interrelated and may lead to impaired cytokine and hormonal secretion by adipocytes, and ectopic fat deposition in obesity that strengthen the increased risk of suffering metabolic disorders in obese subjects. That “inflamed” and “dysfunctional” AT are synonymous when referring to obesity, is defended by Ortega et al. and they tried to explain how it may happen in severe obese subjects having a large and longtime fat excess, when fat deposits have reached a point when excessive fat storage, cell density, and diminished oxygen availability promote decreased lipoogenesis and increased lipolysis and FA release. This response maybe an important inflammatory component that promotes angiogenesis and IR, but also by leptin and increase of T3 in hyperplastic AT [78,79].

Role of hypoxia: Hypoxia induces a complex and still incompletely understood adaptations that influence cell survival and function. Many of these adaptations are directly controlled by a master transcription factor, hypoxia inducible factor alpha (HIF-α). Role of hypoxia and miR 210: In response to hypoxia, HIF-α levels increase and directly induce the transcription of >100 genes, influencing function ranging from metabolism, survival, proliferation, migration angiogenesis among others. Recently, it has been demonstrated that a specific set of miRNA molecules are upregulated in hypoxia, which are denoted as “hypoxamirs”. Specially the HIF-responsive hypoxamir miRNA 210 is a unique miRNA that is evolutionally conserved and ubiquitously expressed in hypoxic cell and tissue types. A number of direct targets of miR210 have been identified in silico, transcriptional and biochemical methods, a subset of which has been extensively validated. As a result miR 210 has been mechanistically linked to the control of wide range of cellular responses known to influence normal developmental physiology as well as a number of hypoxia dependent disease states including tissue ischaemia, inflammation and tumorigenesis. Thus reflecting the pleiotropic actions of HIF-α, miR210 appears to function as a “master microRNA” relevant to the control of diverse functions of hypoxic state [80].

Role of miR 322 in ER stress: The disruption of the energy or nutrient balance triggers endoplasmic reticulum (ER) stress, apress that mobilizes various strategies collectively called the unfolded protein response (UPR) which reestablishes homeostasis of the cell [81]. Activation of the UPR stress senses IRE-1α (Inositol requiring enzyme 1α) stimulates the endoribonuclease activity leading to the generation of mRNA encoding the transcription factor XBP-1 (X-box binding protein-1) which requires the transcription of genes encoding factors involved in controlling the quality and folding of proteins . Groenendijk et al. [81] found the activity of IRE 1α was regulated by the ER oxireductase PDA16 (Protein disulfide isomerase A6) and the miR 322 in response to disruption of ER Ca2+ homeostasis. PDA16 interacted with IRE1-α and enhanced IRE-1α and XBP1mRNA splicing but PDA16 did not substantially effect the activity of other pathways that mediate responses to ER stress. ER Ca2+ depletion and activation of storeoperated Ca2+ entry reduced the abundance of the miR322 which increases PDA16 mRNA stability and consequently IRE-1α to activity during the ER stress. Responses in vivo experiments with mice and worms showed that the induction of ER stress correlated with increased miR322 abundance, increased PDA16 mRNA abundance or both. This combined findings demonstrate that ER Ca2+ PDA16, IRE-1α, and miR322 function in a dynamic feedback loop, modulating the UPR under conditions of disrupted ER Ca2+ homeostasis [82].

ER stress and the UPR lead to obesity induced inflammation and metabolic abnormalities by several distinct mediators including the activation of JNK, AP-1 (Jun N terminal kinase-activator protein 1) and IKK (IkB kinase) NFκB pathway, the induction of the acute phase response and the production of reactive oxygen species (ROS) [83,84].

MiR’s regulating WAT inflammation: Expression of miR 221 and miR 222 has been positively correlated to TNF-α and negatively correlated to adiponectin (ApN) expression in WAT of mice [85]. miR 132, which was downregulated in human obese omental fat [86], has been reported to activate NF-κB and the transcription of IL-8 and MCP 1 in primary human preadipocytes and in vitro differentiated adipocytes. Zhuang et al. [87] demonstrated that miR 232 played a crucial role in modulating macrophage polarization in a pattern that protects mice from diet induced AT inflammation and systemic IR. Consequently miR 223 suppressed the infiltration of proinflammatory M1 classically activated macrophages by targeting Pknox1 in vitro, while miR 223 deficient mice fed a HFD exhibited increased AT inflammation characterized by enhanced proinflammatory activation of macrophages [88]. In macrophages functional polarization is associated with the upregulation of distinct set of miR’s [89,90]. TLR4 mediated activation of NF-KB induces a negative feedback loop by upregulating miR’s such as miR 21, miR 147, miR 210, miR34a and miR 146 which dampen TLR induced signaling and cytokine expression [90-92]. In contrast miR 155 shows both anti and proinflammatory effects by regulating TAB2a and SOCS 1 respectively.

Ge et al. reported several miR’s which were regulated by adiponectin in WAT in vivo. The miR 883b-5p, which was upregulated by ApN and down regulated in obesity, repressed the LPS binding protein (LBP) and TLR4 signaling, acting therefore as a major mediator.
of the anti-inflammatory action of ApN. miR 883b-silencing in the denovo AT formed in vivo differentiation of preadipocytes also induced LBP production and tissue inflammation [92]. Further Ishida et al. found levels of premiR 378 to modulate ApN expression via the 3'UTR sequence binding site. They found levels of ApN mRNA and protein were decreased in 3T3-L1 cells overexpressing the mimic of miR378 both in silico study and confirmed by luciferase assay [93]. To understand how increased chemokine motif ligand 2 (CCL2) secretion may initiate adipocyte inflammation Arner et al. studied miRNA's which are dysregulated in obesity. Of these they overexpressed 9 individual miRNA's which have been defined dysregulated in AT in human obesity in human adipocyte differentiated in vitro. Of the 10 they found 9 significantly reduced the CCL2 which is an initiator of AT inflammation by attracting the migration of inflammatory cells into the tissue. Among these 10 affected adipocyte CCL2 secretion in vitro and for 2 miR’s miR 126 and 193 b regulatory circuits were defined. Among these miR’s only miR 126 was predicted by in silico analysis and confirmed by luciferase transcription assays in 3 T3-L1 cells to bind directly to 3’UTR of CCL2 whereas miR 193 b affected indirectly CCL2 production through downregulating transcription factors (TF’s) of CCL2 (RELB, STAT6, and ETSl). The levels of 2 miR’s in subcutaneous WAT were significantly associated with CCL2 secretion of integrin α-X mRNA levels (a gene specific for proinflammatory (M1) macrophage marker ). Thus they concluded that miR’s regulate AT inflammation through their effects on CCL2 release from human adipocytes and macrophages [94]. Thus miR’s may mediate AT inflammation by regulating either the activation of macrophages or the production of adipokines.

Role of Circulating MicroRNAs in Obesity

Circulating miR’s as biomarkers

Based on Genome wide studies having yielded important insights into the pathogenesis of obesity and that circulating miRNA’s have been stable biomarkers of systemic diseases and potential therapeutic targets, Ortega et al. assessed genome wide circulating miRNAs profile cross sectioning 22 men and after surgery induced weight loss in 6 morbidly obese patients. The most relevant miRNAs were cross sectionally validated in 80 men and longitudinally in 22 patients (after surgery induced weight loss). They evaluated the effects of diet induced weight loss in 9 obese patients. 36 miRNAs were associated with anthropometric variables on the initial sample. In the validation study morbidly obese patients showed a marked increase of 37 miR 140-5p,miR 142-3p, and miR 222 and decreased levels of 39 miR 532-5p, miR125b, miR130b, miR221, miR15a, miR423-3p, and miR520c-3p. Interestingly in silico targets were significantly associated with in silico targets of ApN. Moreover a discriminant function of three miRNAs, miR15a, miR520c-3p, and miR423-3p was specific for morbid obesity with an accuracy of 93.5%. Surgery induced (but not diet induced) weight loss led to a marked decrease of miR140-5p, miR122, miR193-a-5p and miR 16-1 and upregulation of miR221 and miR199a-3p. Thus they concluded that circulating miRNAs are deregulated in severe obesity. Weight loss induces changes in the profile and of the study in silico targets supports this observation and suggests a potential mechanistic relevance [95].

Since miRNAs changed in relation to different modes of treatment, this finding suggests that miRNA are related to the process by which the body weight is lost rather than an effect arising for example from shrinking adipose organs [96]. Further although Ortega et al. identified changing serum miRNA profiles they do not speculate on how these miRNAs are transported, whether they are protein or HDL bound or contained within exosomes maybe of mechanistic relevance. Extracellular vesicles are well described to mediate cell-cell communication by fusing with remote cells and transferring bioactive contents such as DNA, RNA and cytokines. The findings of miRNA within circulating vesicles raises the possibility that miRNA may also be transferred to recipient cells [95,97].

Micro RNAs as biomarkers for DM in obesity

Biomarkers in medical care are bench mark in the body preferably in blood circulation that can reliably be used to indicate the physiological and metabolic disturbances [98]. Several components associated with the pathophysiology of type 2 diabetes mellitus (T2DM) have been uncovered in the last decades, being the result of alteration in insulin secretion coupled with changes in insulin action in insulin sensitive tissue (i.e. muscle, liver and AT) and modulated by complex multifactorial webs of relationships [99]. In this new scenario, virtually all tissues and systems are active players modulating both insulin action and response. The existence of these complex systems aimed at regulating energy balance, in close association with the immune system and chronic inflammation, calls for a broader view of the paradigm [99]. In this context it has been postulated that circulating miRNAs could act as a new model of communication between insulin sensitive tissues from obese or T2DM patients suggesting a potential role for these small RNA molecules in the complications associated with the metabolic complications [100,101]. During last 5years it has been demonstrated that miRNAs are not only intracellular molecules, since they are also detectable outside the cell s in body fluids (e.g., in serum, plasma, saliva, urine and milk) [102]. They are protected from degradation by RNAses because they are contained in small membranous vesicles (e.g., exosomes, exosome like vesicles, microparticles and apoptotic bodies [103], packaged within HDL [104] and linked to RNA binding proteins (e.g., argonaute 2 and nucleoplasmin 1 [105]. It has been suggested that extracellular miRNAs have specific physiological functions depending on their cellular origin and regulating immune function, cell migration, differentiation and other aspects of cell-cell communication [101,105].

Hence the concept that extracellular miRNAs contained in body fluids could be used as biomarkers for the detection and classification of disease has been proposed [106]. Their presence in plasma and the possibility of detecting and small interindividual variations make circulating miRNAs excellent potential biomarkers for complex systemic diseases eg T2DM [102,107,108]. Thereby body fluids miRNAs could provide an integrated view of the metabolic profile of T2DM patients [109]. Hence by means of both cross sectional and longitudinal analysis in men which included placebo controlled with metformin, acute insulin-hyperinsulinemic-euglycemic clamp) and insulin infusion and intralipid/heparin known to induce IR [110], this study by Ortega et al. aimed to identify circulating miR’s associated with T2DM and their responses to insulin sensitivity. In an attempt to identify the profile of circulating miRNA’s in T2DM and its response to changes in insulin sensitivity, Ortega et al. assessed in 12 men, 6 with normal glucose tolerance (NGT) and 6 T2DM patients [111]. The Association of 10 circulating miRNAs with T2DM was cross validated in an extended sample of 45NGT vs 48T2DM subjects (65 nonobese and 25 obese men) and longitudinally in 35T2DM patients who were recruited in a randomized double blind and placebo controlled 3 month trial of metformin treatment. Circulating miRNAs were also measured in 7 healthy volunteers before and after a 6 h hyperinsulinemic-euglycemic clamp and insulin plus intralipid/heparin infusion. Amarked increase
of miR140-5p, miR142-3p and miR222 was disclosed by crosssectional studies along with decreased miR 423-5p, miR 125-b, miR192, miR 195, miR130b, miR 532-5p, and miR126 in T2DM patients. Multiple linear regression analysis revealed that miR140-5p and miR423-5p, contributed independently to explain 49.5% of fasting glucose variance after controlling for confounders. A discriminant function of four miRNA (miR140-5p, miR423-5p, miR195 and miR126) was specific for T2DM. With an accuracy of 89.2% Metformin (but not placebo) led to significant changes in circulating miR 192 (49.5%), miR140-5p (-15.8%), and miR222 (-47.2%) in parallel to decrease fasting glucose and HbA1C. Furthermore while insulin infusion during clamp decreased miR 222 (-62%), the intralipid/heparin infusion mixture increased circulating miR222 (163%) and miR140-5p (67.5%). Thus concluding the close association between variations in circulating miRNAs and T2DM and their potential relevance in insulin sensitivity [112].

Leroith commented that the strength of study of Ortega et al. lies in identifying miRNAs, that are associated with obesity, IR, and T2DM as well as response to metformin therapy. But weakness relates to the relatively small sample size with some aspects of their study and absence of the effects of other medications [111,113].

Further questions posed are 1) do circulating miRNAs represent biological markers for various metabolic disorders [114] 2) Do miRNAs truly function as intercellular regulators? 3) How are miRNA levels controlled at the cell of origin? 4) How do lifestyle and other pharmaceutical agents affect miRNA expression and levels in the circulation thermogenic adipocytes?

Role of chromium deficiency and miR375/30d

Chromium deficiency leads to impaired glucose tolerance, due to IR and hyperglycemia [115]. Trivalent chromium is a complex known as glucose tolerance factor, such as chromium picolinate, is considered the biologically active form. To study the antidiabetic effect of TIAN MAI (TM) Xiaoake tablet commonly used in China to treat DM which contains chromium picolinate (1.6 mg/tab = 200 mg snake gourd root, dwarf alyr urf tuber and Chinese magnolia fruit in ratio of 1.66:2.5:62.5:25) and is found to be effective in reducing HbA1c Zhang et al. studied diabetic mice to study the mechanism of action using low dose TM L and high dose of TM groups before and after oral glucose administration and studied fasting insulin HOMA-IR. Eight weeks regimen significantly reduced in TM treated before and after oral glucose administration and HOMA1R insulin were suppressed in TM treated groups. miR448, let 7b, miR540, miR296, miR880, miR200a, miR-500, miR10b, miR336, miR30d, miR208, miR501, and miR188 were upregulated, while miR10b, miR134 and miR652 were downregulated. Through target gene analysis and real time PCR verification they found these miRNAs especially, miRNA375 and miRNA30d can simulate insulin secretion in islets. Hence they concluded that TM improves blood glucose in diabetic rats by involving expression of miRNA375 and miRNA30d to activate insulin synthesis in islets and possibly that is how it had been working in Chinese patients treated with TM [115,116].

Regulation of Cholesterol and Fatty Acid Metabolism and miRNAs

Introduction: Lipid homeostasis is regulated by a family of membrane bound transcription factors called sterol regulatory element binding proteins (SREBP’s) [117-119] which directly activate the expression of >30 genes involved in synthesis and uptake of cholesterol, FA’s, triglycerides and phospholipids as well as the NADPH cofactor required to synthesize these molecules. Of the SREBP isoforms SREBP1 is selectively involved in activating genes involved in FA metabolism and denovo lipogenesis, whereas SREBP2 is more selective for genes involved directly in cholesterol homeostasis [117-119]. Aberrant expression of SREBP’s in mice leads to metabolic syndrome (MS) with physiological effects similar to specific disorders of lipid metabolism in humans [117-119]. Beside SREBP’s the liver X receptor (LXR’s) are also important transcriptional regulators of cholesterol metabolism [120,121]. LXRα (NR1H3) and LXRβ (NR1H2) nuclear receptors form heterodimers with retinoid X receptors and are activated by a variety of sterol including oxysterol intermediates that form during cholesterol biosynthesis [121]. LXR’s activate the transcription of genes involved in cellular cholesterol efflux, including Abca1 and Abcg1 [122]. LXR’s also impact SREBP 1transcription, thereby mediating crosstalk between these pathways to increase FA synthesis [122]. Mice with targeted deletion in the LXRα gene were noted to be deficient in expression of SREBP1c, fatty acid synthase (FAS), SCID1 (steroyl CoA desaturase 1) and ACC (Acyl coA Carboxylase) [122,123].

Micro RNAs and lipid metabolism

Of the several miRNAs highlighted till date miR122, miR370, miR378/378’, miR335, miR125a-5p and miR33 are the important regulators of lipid metabolism.

Role of miR122: miR122 was initially identified as a highly abundant miRNA in the liver, accounting for 70% of initial miRNA expression [124]. MiR122 has been associated with the regulation of liver metabolism as well as hepatitis C infection (HCV), and has been shown to be downregulated in hepatocellular carcinoma (HCC) [125-128]. Astudy employing cholesterol conjugated antisense oligonucleotides (termed antagoniRs showed that injection of antagoniRs that target miR122 into mice resulted in altered hepatic expression of a number of genes including some involved in cholesterol biosynthesis such as 3hydroxy -3methyl glutaryl-coenzyme A Reductase (Hmgcr), 3-hydroxy-3-methylglilaryl-CoA-synthase 1 (Hmgcs1) and 7-dehydrocholesterolreductase (Dhcr7) [126]. The use of antisense miR122 down regulated several genes implicated in liver metabolism and produced an increase in expression of hundreds of genes that are normally repressed in hepatocytes suggesting that miR122 functions to maintain the liver phenotype [125,126]. In addition several genes involved in FA synthesis and oxidation were altered in mice treated with antimiR122 including FAS, ACC1 and ACC2 [125,126]. Silencing of miR122 in high fat fed mice induced hepatic steatosis which was linked to a reduction in cholesterol synthesis and stimulation of FA oxidation [126]. In contrast, two independent studies have recently shown that mice lacking miR 122 in the liver or in the germline develop steatohepatitis by increasing the expression of genes involved in TG synthesis, including 1-acyl glycerol-3-phosphate acyl transferase alpha (Apgat1), Apgat3, Apgat9, Monoacetyl glycerol-O-acyl transferas (Mogat1), phosphatidic acid phosphatase-2a (Ppap2a and Ppap2c) [129,130]. Further studies are needed in order to explain the differences observed between miR silencing using ASO and miR knockdown.

Role of miR370: MiR370 targets carnitine palmitoyl transferase (Cpt1a), a mitochondrial enzyme that mediates the transport of long chain FAs across the membrane by binding them to carnitine, thereby reducing FFA oxidation [131]. miR 370 transfection of human hepatic cell lines Hep G2 upregulates the expression of miR122 leading to increased expression of lipogenic genes including SREBP1c and DGAT2 which suggests that miR370 provides an additional point of regulation of this pathway [131].
Role of miR378/378*: MiR378/378* is an intronic miRNA located within the PPAR-γ coactivator1 alpha (PGC1-a) genomic sequence. Overexpression of miR378/378 during adipogenesis increases triacylglycerol accumulation [132]. Transfection with miR378/378 of adipocytic ST2 cells leads to increased expression of FA metabolism genes including FAS, fatty acid binding protein4 (FABP4), and sterol-coenzyme-A desaturase 1 (SCD1) [132].

Role of MiR33 as a key regulator of lipid metabolism: The group of Marquart TJ et al., Najafi-Shoushtari et al. and others identified a highly conserved miRNA family, miRNA 33, within the intronic sequences of the Srebp genes [132-135]. Two miR33 genes are present in human miR33b, which is encoded within intron 17 of Srebp 1 gene on chromosome 17 and miR33a which is expressed in intron 16 of the Srebp-2 gene on chromosome 22. In mice however, there is only one miR33 gene which is the ortholog of human miR33a and is located within intron 15 of the mouse Srebp-2 gene. miR33a and miR33b are cotranscribed with its host genes, like many intronic miRNAs and they target genes involved in regulating cholesterol homeostasis (Abca1, Abcg, and Npc1) [135-138] and fatty acid metabolism (Ampk, Cpt1a, Crot, Hadhb, and Sir6) [136,137]. During miR biogenesis premiR33 (as well as other premiR's) is cleaved with dicer which leads to the creation of a miR33 duplex composed of mature miR33 and passage miR33 (miR33*) strands. As shown by Goedecke et al. in 2013, miR33 is one of the examples where both strands are loaded into RISC and consecutively execute their tightly related function, thereby affecting cholesterol metabolism [133-139], fatty acid metabolism (136, 138g) glucose metabolism/insulin signaling [136,138,140]. Within the cholesterol metabolism, both miR33 and miR33* target ABCA1 and Niemann-Pick disease C1 (NPC1), that is, molecules important for loading cholesterol into HDL particles and for cholesterol transport from the lysosomal compartment within the cell respectively [133-138]. miR33 itself was further shown to target ABCG1 in mice, which has a similar function to ABCA1, that is enabling HDL formation and reverse cholesterol transport (e.g., cholesterol transport from atherosclerotic plaque macrophages back to the liver); however this target was not confirmed in humans [133,135]. Interestingly both ABCA1 and ABCG1 are under the transcriptional control of LXR [133] and miR33 thus provides the connecting link between SREBP induced cholesterol synthesis and retention and LXRx mediated cholesterol efflux and reverse cholesterol transport [141]. In addition to above, miR33 targets ABCB11 and ATP5B1, both of which are important molecules in cholesterol efflux into biliary ducts; this targeting thus supports cholesterolretention caused by the upregulation of SREBP and miR33 in hepatocytes [139] (Figure 2). This upregulation may induced by extracellular signals including low c irculating cholesterol levels or statin therapy [139]. Confirmed targets for both miR33 and miR33* in the fatty acid metabolism include carnitine palmitoyltransferase 1A (cpt1a) and carnitine O- octaniltransferase (CROT) [136-138]. Furthermore, miR33 also target hydroxyl acyl-Co-A-dehydrogenase (HADHH)[136,137], sirtuin 6 (SIRT6), and AMPKα [136], while miR33* targets sterol receptor coactivator1 (SRC1), SRC3, Nuclear transcription factor Y (NFYC), and receptor interacting protein 140 (RIP140). By regulating all of these molecules post-transcriptionally, both miR33 and miR33* reduce fatty acid oxidation when upregulated and vice versa, that is stimulating the process when downregulated [136,138]. MiR 33 also influences insulin signalling by targeting insulin receptor substrate 2 (IRS2), thereby affecting both lipid and glucose metabolism [136], with the glucose metabolism also being affected by glucose-6-phosphatase (G6PC) and phosphoenolpyruvatecarboxykinase targeting [140]. Thus therapeutic downregulation of their signalling may be beneficial in patients suffering from atherosclerosis, as it would result in an increase of HDL levels and a decrease in fatty acids and glucose levels.

These findings suggest that Srebp genomic loci, which encode transcription factors and miR 33a/b may cooperate to regulate lipid metabolism [141,142]. Inhibition of miR33 using different strategies increases plasma high density lipoproteins (HDL) in mouse and promotes the expression of atherosclerosis in mice [133-135,143,144].

Altogether these observations suggest that intronic miRNAs work in conjunction with host genes to regulate similar cellular processes. Since SREBP’s regulate cellular proliferation and cell cycle progression, Cirera-Salinas et al. tested the role of miR33 in regulating these cellular functions. They identified putative binding sites for miR33 in the 3’UTR of cycles dependent kinases (Cd6k, Cd8k and Cd19), Cnd1, and d2, p53, Pten, Myc and mitogen activated protein kinase inhibitor (Map3k1, Map3k7, Map3k, Mapk6, Mapk10, Mapk14). They showed that overexpression of miR33 inhibits CDK6, and cyclin D1 expression, reduces cell proliferation in different human cell lines and leads to cell cycle arrest in G1 phase. Conversely endogenous inhibition of miR33 expression using 2fluoromethoxyl ethyl-modified (2T/MOE-modified) phosphorothioate backbone antisense oligonucleotides improves liver regeneration after partial hepatectomy in mice. Altogether concluding that SREBP-Mir33 locus may cooperate to regulate cell proliferation and cell cycle progression and may also be relevant to human liver regeneration [144,145].

Role of miR 143/145

The miR143/145 cluster regulates vascular smooth muscle cell (VSMC) specific gene expression, thus controlling differentiation, plasticity and contractile function, and promoting the VSMC phenotypic switch from a contractile/non proliferative to a migratory proliferative state [146-148]. More recently increased miR145 expressions were observed in human carotid atherosclerotic plaques from symptomatic patients [149]. Sala et al. showed that miR143/145 deficiency attenuates the progression of atherosclerosis in Lsc mice. This event could be the consequence of multiple mechanisms including changes in VSMC function and increased vascular and hepatic ABCA1 expression. miR143 has been shown to regulate adipocyte differentiation as well as a regulator of hepatic insulin signaling [150,151]. The expression of miR143/145 cluster is upregulated in the liver of genetic and dietary models of obesity [151]. Specifically, over expression of miR143, but not miR145, impairs insulin stimulated Akt activation and glucose homeostasis. Conversely mice deficient for the miR143/145 cluster are protected for the development of obesity associated IR [151]. Altogether these observations suggest that the effect of miR145 and miR143 in controlling lipid and glucose metabolism, might be mediated by the direct regulation of ABCA1 by miR145 and the regulatory role of miR143 on hepatic insulin signaling and adipocyte differentiation. Thus Sala et al. concluded that their results, that miR 145 inhibits ABCA1 coupled to the identification of increased miR145 levels to human carotid plaques from symptomatic patients [150], supports the relevance of evaluating anti miR145 strategies in the context of novel approaches under investigation for dyslipidemia [152,153] and cardiovascular related diseases, including atherosclerosis [154]. Based on these Muthiah et al designed a method to coat the stent surface with miR145 to suppress the overgrowth of smooth muscle cell (SMC) and used polysorbol osmotically active transporter (PSOAT) for effective miR145 delivery and found a drastic reduction in SMC proliferation after PSOAT/miR145 nanoparticle (PEM) treatment and miR 145 target proteins were downregulated upon miR145 replacement. Hence PEM is useful for patients with restenosis where
Role of miR 27b

MiR 33 which is encoded within an intron of SREBP2 is cotranscribed with SREBP2 and regulates the expression of ATP-binding cassette transfer proteins (ABCA1), a critical player of reverse cholesterol transport and in hepatic cell lines [133-135]. miR 33 has also been shown to regulate fatty acid oxidation in hepatic cell lines [136]. But despite knowing that miR are post transcriptional regulators has also shown to regulate fatty acid oxidation in hepatic cell lines. miR 33 binding cassette transfer proteins (ABCA1), is a critical player of lipid metabolism. Based on Monte Carlo simulations, miR27 b was predicted to target significantly more lipid associated genes than expected by chance and more than any other other hepatic miRNAs. Of the other miRNAs predicted to be regulatory hubs in lipid metabolism miR365 miR223 and this is the first example of extracellular miR regulating gene expression in cells where it is not transcribed.In this study by Wagners et al. also showed that naïve HDL particlesare able to promote miRNA export to exosomes [159160], therefore the possibility of miR being transferred from HDL to target cells [160]. Interestingly, Wagner et al. also showed that naïve HDL particles are able to cause transient downregulation of some miR’s in the recipient cells, which is most likely caused by pumping of miR’s into the HDL [160]. None of the miRNA’s known to modulate lipid metabolism are among those prevalent in human HDL from healthy subjects or patients with familial hypercholesteremia. This raises the possibility that miRNAs carried by HDL could influence pathways other than those strictly related to lipid metabolism. For e.g., HDL is emerging as a new player in immunity [161-163] and in silico analysis predicted that the most abundant miRNAs present on human HDL might affect the signaling pathways associated with innate and adaptive immunity [104].

Role of miR223

miR223 is one of the most abundant miRNAs in monocytes and macrophages and a key regulator of inflammation [164]. miR223 null mice have a significantly increased number of circulating neutrophils and enhanced systemic inflammation following lipopolysaccharide challenge [164]. Further extending their study Remaley’s group showed that HDL suppresses expression of intracellular adhesion molecule 1(ICAM 1) through the transfer of miR223 in endothelial cells. After incubation with HDL, mature miR223 levels are significantly increased in EC’s and decreased on HDL. However, miR223 is not tanscribed in EC’s and is not increased in cells treated with HDL, from miR223/- mice. HDL inhibits ICAM1 protein levels, but not in cells pretreated with miR223 inhibitors. ICAM1 is a direct target of HDL-transferred miR223 and this is the first example of extracellular miR regulating gene expression in cells where it is not transcribed.In this study they established that mature miR 223 is abundant in EC (HCAEC to transport lipids, proteins and also miRNA’s in circulation. The interaction between specific protein of Lp’s and their receptors in cell membrane ensures a selective interaction and exchange of Lp transported moieties. Although both LDL and HDL have been shown to transport miRNAs, they present a different miRNA signature with LDL presenting a miRNA profile closer to that of plasma exosomes than that of HDL [104]. HDL represents, so far the only class of Lp widely studied for its ability to retrieve, transport and deliver miRNAs to cells. HDL reconstituted with human apolipoprotein A1 (Apo-A1) and phosphatidyl choline (hHDL) is able to retrieve a large amount of miRNAs in vivo. Following injection of rHDL in mice (wild type or Apo-E deficient on chowatherogenic diet) the majority of miRNAs retrieved by the Lp’s were the same independently of mice genotype or the presence of atherogenic diet; however the presence of atherogenic diet was associated with relatively different amount of miRNAs loaded on HDL [104]. Lu D speculated that Apo-A1 might bridge the Lp to the cells, and witzerioncic, that was shown to complete small RNAs, might bind the miRNA’s. miRNA export from macrophages to HDL is favoured by the expression of ABCA1 expression, but also by the inhibition of the ceramide pathways [158]. The latter was shown to promote miRNA export to exosomes [159160], therefore the possibility that miRNAs loading on HDL or exosomes could be related to the different bioavailability of cellular lipids should be investigated. The delivery of miRNA from HDL in cells is highly dependent on scavenger receptor B1 (SR-B1), SR-B1, a scavenger receptor with a mission to transport HDL lipids, which may also lead to less degradation of miRNA’s. Indeed HDL-miR223 complex when incubated with hepatocytes releases miR223 to the cells and reduced the expression of their targeted genes in the cells [104]. Although a subsequent study by Wagners et al. confirmed results of Vickers et al. in the case of in vitro models of endothelial cells, SMC’s and macrophages, the study indicated that the miR uptake is not sufficient due to the low amounts of miR being transferred from HDL to target cells [160]. Interestingly, Wagner et al. also showed that naïve HDL particles are able to cause transient downregulation of some miR’s in the recipient cells, which is most likely caused by pumping of miR’s into the HDL. [160]. None of the miRNA’s known to modulate lipid metabolism are among those prevalent in human HDL from healthy subjects or patients with familial hypercholesteremia. This raises the possibility that miRNAs carried by HDL could influence pathways other than those strictly related to lipid metabolism. For e.g., HDL is emerging as a new player in immunity [161-163] and in silico analysis predicted that the most abundant miRNAs present on human HDL might affect the signaling pathways associated with innate and adaptive immunity [104].

Role of lipoproteins

Lipoproteins represent a highly evolutionary conserved system.
and HUVECs) where it is neither transcribed nor processed. This is explained by the transfer of mature miR223 from HDL to both HCAEC and HUVECs. HDL also transfers functional miR223 to HCAEC where it controls inflammatory gene expression. It is therefore likely that, in the context of atherosclerosis and other inflammatory diseases macrophages export miR to HDL, which in turn transfer miR223 to ECs as a feedback mechanism to antagonize inflammation [165]. The levels of extracellular miRNA, specifically HDL-miR223 levels are altered in disease states including atherosclerosis [104,166]. Further Vickers et al. identified that genetic ablation of miR223 in mice resulted in increased HDL-C levels and particle size, as well as increased hepatic and plasma total cholesterol levels. They summarized that miR223 can be a potential therapy for treating cholesterol related disorders [172].

Role of miR 30c

High plasma lipids are risk factors for various cardiovascular and metabolic disorders such as atherosclerosis, obesity and metabolic syndrome (MS). CVS disease itself is a leading cause of death. Statins reduce plasma lipids and lower the incidence for some of these disorders in 20-40% individuals, highlighting a need for new approaches to lower plasma lipids. Dietary and endogenous lipids are transported in plasma by lipoproteins made mainly by enterocytes and hepatocytes. Microsomal triglyceride transfer protein (MTP) assists the lipoprotein assembly by interacting and capacitating nascent apolipoprotein B (apoB) to form precursors of lipids [170,171]. Therefore MTP has been targeted to lower plasma lipids. But these attempts have been hindered as MTP inhibitors cause steatosis and increase plasma transaminases AST/ALT. Hence Soh et al show that miR-30c interacts with the 3’UTR of the MTP mRNA and induce degradation leading to reductions in its activity and media apoB. Further miR 30c reduces hyperlipidemia and atherosclerosis in western fed mice by decreasing lipid synthesis and secretion of triglyceride-rich apoB containing lipoproteins. Therefore miR30c coordinately reduces lipid biosynthesis and lipoprotein secretion to control hepatic and plasma lipidsand might be useful in treating hyperlipidemias and associated disorders [172].

Role of dysregulated cholesterol metabolism in brain

Dysregulation of cholesterol metabolism in the brain has been associated with many neurodegenerative disorders, such as Alzheimer’s disease, Nieman-Pick type C disease, Smith-Lemli-Opitz Syndrome, Huntington’s disease and Parkinson’s disease. Specifically genes involved in cholesterol biosynthesis (24-dehydrocholesterol reductase DHCR24) and cholesterol efflux (ATP binding cassette transporter 1(ABCA1), and apolipoprotein E (APOE), has been associated with the development of Alzheimer’s disease. Indeed APOE was the first gene variation found to increase the risk of Alzheimer’s disease and remains the risk gene with the greatest known impact. Mutations in another cholesterol biosynthetic gene, 7-dehydrocholesterol reductase (DHCR7), cause Smith-Lemli-Opitz syndrome and impairment in cellular cholesterol trafficking caused by mutations in the NPC1 protein results in Niemann-Pick type C disease. Taken together, these findings provide strong evidence that cholesterol metabolism needs to be controlled at very tight levels in the brain. Recent studies have implicated microRNAs as novel regulators of cholesterol metabolism in several tissues. Goedeke et al. reviewed how cholesterol metabolism is regulated by miRNAs and their potential implications in several neurodegenerative disorders e.g., Alzheimer’s disease and they discussed how antagonizing miRNA expression could be a potential therapy for treating cholesterol related disorders [172].

Role of miR 155

Hepatic steatosis is a globalepidemic that is thought to contribute to the pathogenesis of type2 diabetes. Hepatic expression profiling has revealed temporal changes in miR expression in human and murine nonalcoholic fatty liver disease(NASH), and identified several differentially expressed miR’s including miR 21,miR34, and miR122 [168]. In addition, it has been shown that miR155 expression is increased in murine models of NASH and HCC and its expression is correlated with disease severity. In line with these miR155 target genes, C/EBP beta (Cebpb) and Suppressor of cytokine signaling 3(Socs3) were decreased. miR155 is a multifunctional miRNA known to regulate numerous biological processes including haematopoiesis, inflammation, satherosclerosis and cancer [169]. Hence Miller et al. reported that the absence of miR155 in mice fed HFD was associated with a significantly increased hepatic steatosis and V LDL/LDL cholesterol levels and alanine transaminase (ALT) levels, as well as increased hepatic expression of genes involved in glucose regulation (Fck1,Cebpa), fatty acid uptake (CD36) and lipid metabolism (Fasn, Fabp4, Lpl, Abcd2,Pla2g7). Using miR target prediction algorithms and the microarray transcription profile of miR155 +/- livers they identified and validated liver x recetor α (LXRA) (Nrlh3) as a direct miR155 target gene that ispotentially responsible for the liver phenotype of miR155 +/- mice. Thus, their data directly implicates miR 155 in liver homeostasis and its deregulation as a pivotal factor in the pathogenesis of fatty liver diseases [169].
Potential Therapeutic Use

miRNA’s as biomarkers

With the discovery that miRNA’s are found in extracellular space, constitute a form of cell-cell communication and having been found in plasma, urine, saliva and recently been shown to be carried on lipoproteins has increased the complexity of miRNA mediated pathway. This has led to a proposal that circulating miRNA’s maybe useful biomarkers of various diseases including cardiovascular diseases, DM, and other terms of dysregulated metabolism. Although the understanding of the cellular machinery responsible for the secretion of miRNA is incomplete it has been demonstrated that miRNA’s are packaged into exosomes, microvesicles and apoptotic bodies (Figure 3) [173,174]. The excitement for miRNA’s as biomarkers is mounting as more and more evidence supports that noncoding RNA’s are actively secreted from diseased tissues, possibly before the onset of overt disease. While caution should be taken in these earlier days, there is little doubt that extracellular miRNA’s will hold tremendous potential as both diagnostic and therapeutic agents [175].

Therapeutic uses of AMO’S (antimiRNA oligonucleotides) / LNA’S (locked nucleic acids)

With recent implications of miRNA as gene regulatory molecules implicated in pathogenesis of several human diseases e.g., neurodegenerative disorders, cancer, viral and metabolic diseases. “Silecing” of key miRNAs and replacement of certain tissue specific miRNA whose expression is known to be decreased are potential therapeutic interventions. Techniques can be anti miRNA oligonucleotides (AMO’S) to deactivateand silence miRNAs. AMO’S are synthetic oligonucleotides that competitively inhibit interaction between miRNAs. Several antisense oligonucleotides that were based on first generation chemical compounds (2’-O-methyl or 2’-O-methoxyethyl) have been extensively evaluated in clinical trials for renal toxicity. While miRNAs whose expression is decreased during disease can be achieved through miRNA mimicry. Plasma viral vectors encoding miRNAs are encouraging strategies to replace miRNA in vivo with good transduction efficiency and minimal toxicity [175,176]. To date, the greatest efforts have been made in exploring the potential applications of miRNA therapeutics in cancer and liver disorders. Gain or loss of function of individual miRNAs has been reported in almost every haematological and solid cancer, with therapeutic suppressive effect in tumor cell proliferation, progression and metastasis of tumors [176]. Just as silencing of miR122, which has been proved to regulate cholesterol metabolism and HCV infection can be silenced by intraperitoneal administration of high affinity LNA has resulted in lowering of cholesterol both in mice and non human primates (monkeys) [59,177] and is almost ready for human use [128,178]. Miravirsen is a 15 nucleotide LNA-modified antisense complementary to and with a high affinity and specificity for 5’ region of mature miR122. Jansenn et al. have used miravirsen to treat patients with known HCV infection (4 groups in different doses with a placebo group) and despite low numbers and short follow up a minimal insignificant risk of renal toxicity warrants close monitoring and careful selection of patients [127].

Therapeutic potential of lipid metabolism mirnas in atherosclerosis

Hyperlipidemia is one of the most critical risk factors contributing to atherosclerosis along with other well known factors like obesity, IR and smoking. At present most therapeutic strategies focus on lowering LDL-cholesterol, for e.g., by using a strategy which has been known to lower patients’ mortality. However a more complex approach focusing
on the lowering of the level of circulating Fatty acids and increasing the levels of HDL is now being investigated. Due to their multitargeting essence, miRNA’s may thus become very powerful tools, influencing allstages of the pathogenic process of hyperlipidemia/dyslipidemia and possibly affecting the entire blood lipid spectrum of affected patients. At present, there are two main ways of using miRNAs therapy: the inhibition strategy uses antagomiRs (sequences that bind to target miR and block its function) while replacement therapy uses miRNA mimics [179].

Most of the work in the field of cardiometabolic diseases has so far focused on miR33 [180-183]. Since the effects of of this miR are mostly proatherosclerotic, as described above, antagomiR therapy against miR33 would be a reasonable choice [184-187]. Short term treatment with miR33 inhibitors markedly increased plasma HDL-C levels and chances of regression of atherosclerosis [133-137,143]. However the efficacy of antimiR therapy on the progression of atherosclerosis is controversial [180,182,184].

A number of studies have recently identified miR33 as a potential therapeutic target for treating cardiometabolic disorders including atherosclerosis and MS [143,180,183]. These reports demonstrate that miR silencing in mice results in increased circulating HDL-C and bile secretion, thereby enhancing mobilization of sterols accumulated from the peripheral tissue through the reverse cholesterol transport (RCT) pathway [139,144]. Since increased RCT correlates inversely with the incidence of coronary artery disease, several groups studied the efficacy of antimiR33 therapy during the progression and regression of atherosclerosis. In the single -regression study published, Moore’s group demonstrated that 4-week treatment with 2’F/MOE antimiR33 Oligo nucleotides accelerated the regression of atherosclerosis in Ldlr/-/- mice with established atherosclerotic plaques [143]. The atherosclerosis progression studies, however have opposite outcomes, while Baldan’s group found that a 12-week anti-miR33 therapy failed to sustain increased circulating HDL-C and prevent atherogenesis [182], they reported that miR33 ASO successfully reduced the progression of atherosclerosis, despite the insignificant alterations of HDL-C levels [183]. Thediscrepancies observed between both atherosclerosis progression studies may be explained by the different oligonucleotide chemistry and slightly different diets used. However the fact that the genetic ablation of miR33 protects against the progression of atherosclerosis in apoE/-/- mice suggests that longterm antimiR33 therapy should be beneficial for treating atherosclerotic vascular disease [180].The most remarkable difference between the miR-33 antisense therapy and genetic studies is that the ability to increase plasma HDL-C levels was lost in the two progression studies, using antimiR33 Oligos, while miR33/-/-apoE/-/-mice still had increased circulating HDL-C. These results suggest that antimiR ASO delivery may not completely inhibit miR33 activity in the liver. Goedeke et al. used a combination of bioinformatics approaches (targetscan, pictar, and mirwalk) to identify potential novel targets of miR33 to determine whether miR 33 directly regulates genes involved in fatty acid and cholesterol metabolism. They found that miR33 had predicted binding sites in the 3’UTR of SREBP1 and HMGCGR. Both predicted binding sites are conserved in mammals. On cloning the 3’UTR of HMGCGR and SREBP1 into luciferase transporter plasmids, they found miR33 fails to repress the 3’UTR of both genes, suggesting that miR 33 does not regulate their expression directly. On long term therapeutic silencing of miR33 they found increase in circulating triglycerides (TG) levels and lipid accumulation in the liver. These adverse effects were only found in mice fed HFD. Mechanistically they demonstrated that chronic inhibition of miR 33 increases the expression of genes involved in FA synthesis like ACC. FAS in the livers of mice treated withmiR 33 antisense oligonucleotides. They also reported that antimiR33 therapy enhances the expression of nuclear transcription Y subunit gamma (NFYC), a transcriptional regulator required for DNA binding and full transcriptional activation of SREBP response genes, including ACC and FAS. Taken together these results suggest that persistent inhibition of miR33 when mice are fed a HFD might cause deleterious effects such as moderate hepatic steatosis and hypertriglyceridemia. They concluded that these unexpected findings highlight the importance of assessing the chronic inhibition of miR 33 in non human primates before we can translate therapy to humans [185].

Conclusions

Thusin this review we have tried to summarize how miR’s have a role in the current known aetipathogenetic factors of obesity at different age groups starting with infancy where how bottlesfeeding has become an important cause of increased obesity in early infancy and role of miR 155 in development of Treg cells and its relation besides bifidobacteria, mTORC1 in development of allergic disorders and obesity. Then in early childhood the role of miR27a and 130a is emphasized in the PPAR gamma and role in adipocyte differentiation and Treg cell differentiation besides role of macrophages in development of inflammation and correlation with chemokine CCL2 monocytes [186,187] and importance of miR223 in inflammation and chemokine is emphasized [187], not only do miR’s affect predispose conversion but also affect macrophage conversion and then HDL’S are carriers and transport miR223 and help explaining recent involvement of HDL in innate and adaptive immunity. Further the complications are interlinked e.g., miR 143/145 cluster has a role in predispose differentiation, VSMC proliferation which is used therapeutically e.g., in cases of restenosis and further has been used for breast cancer as well [188]. Although miR33 offered a lot of promise to start with, still its use in human beings can not be offered with the results of chronic use by Goedecke [183]. The process of miR’s not only helps us to understand the pathophysiology of lipid metabolism and its correlation in the development of obesity but helps us to use circulating miR’s as biomarkers for disease process and then to further get pharmacological strategies to treat obesity and related disorders. Further two stable nucleic acid lipid particle ( SNALP)-formulated siRNA drugs were designed to treat high levels of blood cholesterol or hypercholesterolemia, though TKM-ApoB (Tekmira) targeted ApoB [189], while ALN-PCS (Alnylam) targeted proprotein convertase subtilisin/kexin type 9 (PCSK9) [188]. Both drugs were tested in Phase 1 clinical trials and were determined to be safe and well tolerated with no serious adverse events associated with drug administration. Polymer-based nanomedicine, an arena that entails the use of polymeric nanoparticles, polymer micelles, dendrimers, polymersomes, polyplexes, polymer-lipid hybrid systems and polymer-drug/protein conjugates has greatly revolutionized the therapy of cancer by surrounding the current limitations in conventional chemotherapy, which include undesirable biodistribution, cancer cell drug resistance and severe systemic side effects and in combination of various miRNA and siRNA can achieve efficient cancer chemotherapy [189].

References


