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Role of microRNAs in the modulation of diabetic retinopathy

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ABSTRACT

Diabetic retinopathy (DR) is the leading cause of vision loss in the working-age adults. It affects a third of diabetics. Diabetic macular edema, an advanced complication of DR, develops in nearly 7% of diabetic patients. MicroRNAs (miRNAs) are a novel group of non-coding small RNAs that post-transcriptionally control gene expression by promoting either degradation or translational repression of target messenger RNA. They are implicated in a large variety of physiological and pathophysiological processes, including glucose homeostasis, angiogenesis and modulation of inflammatory response. MiRNAs also play a critical role in the pathogenesis of diabetes and the related micro- and macrovascular complications. The purpose of this review is to describe the potential role of miRNAs in diabetes and evaluate their implication in DR. MiRNAs involved in the modulation of glucose metabolism (insulin secretion and sensitivity) and MiRNAs playing a role in the pathogenesis of DR with their potential target genes are reviewed. Understanding MiRNAs implication in DR could be helpful for developing new gain- or loss- of -function strategies in order to establish effective treatments and reduce the rate of visual disability due to progression of retinopathy.

Contents

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1. Introduction

In economically developed countries, diabetic retinopathy (DR) is the leading cause of visual disability and blindness in working-age adults (20–65 years). It is caused by abnormal retinal blood vessels that are either proliferative (proliferative diabetic retinopathy, PDR) or functionally incompetent, leaking fluid and lipid into the retina. Visual impairment occurs when edema affects the central retina or macula (diabetic macular edema, or DME). PDR is the most common vision-threatening complication of type 1 diabetes mellitus (T1DM). The prevalence of DME is higher in type 2 diabetes mellitus (T2DM) and is the primary cause of visual loss in diabetic patients.

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) reported that after 15 years of known diabetes, the prevalence of diabetic macular edema is approximately 20% in patients with T1DM, 25% in patients with T2DM who require insulin, and 14% in T2DM patients who do not require insulin (The Early Treatment Diabetic Retinopathy Study Research Group, 1987). The worldwide prevalence of DR from a pooled meta-analysis of population studies conducted in USA, Australia, Europe and Asia reported an overall prevalence of 35% for any type of DR, 7.5% for PDR and 7.5% for DME among 20 to 79 year-old individuals with diabetes (Yau et al., 2012). In the coming years the prevalence of DR will rapidly increase with the increasing number and lifespan of people with diabetes. The prevalence of DR increases with duration of diabetes, hyperglycemia and hypertension, which are among the principle risk factors for onset and progression of DR. Moreover, in some patients, microvascular complications of diabetes seem to progress despite glycemic control. This phenomenon is called metabolic memory (Villeneuve et al., 2011). For these reasons it is mandatory to fully understand the molecular mechanisms underlying microvascular complications in diabetes in order to develop more effective therapies. The aim of this review is to discuss the role of microRNAs in diabetes and in particular in the development of DR.

2. MicroRNAs: synthesis and mechanism of action

MicroRNAs (miRNAs) are short (about 22 nucleotides long) and highly conserved sequences of endogenous RNA that do not encode for any protein, and represent a new powerful class of gene modulators. They are expressed in all human cell-types and are involved in the main biological processes including cell growth, differentiation, and apoptosis (Bartel, 2004). To date, it is estimated that the entire human genome encode for about 1100 miRNAs able to modulate the expression of about 60% of the protein-coding genes in mammalians (Friedman et al., 2009).

The miRNAs synthesis is a complex biological process involving several enzymes located in both the nuclear and cytoplasmic compartment of cells (Fig. 1). The gene encoding for miRNAs may be located within introns of both coding and non-coding genes, within exons, or within intergenic regions. Commonly, miRNAs located in the introns are transcribed together with the host gene, and both miRNA and protein encoded by the host gene are usually (but not always) involved in the regulation of the same biological

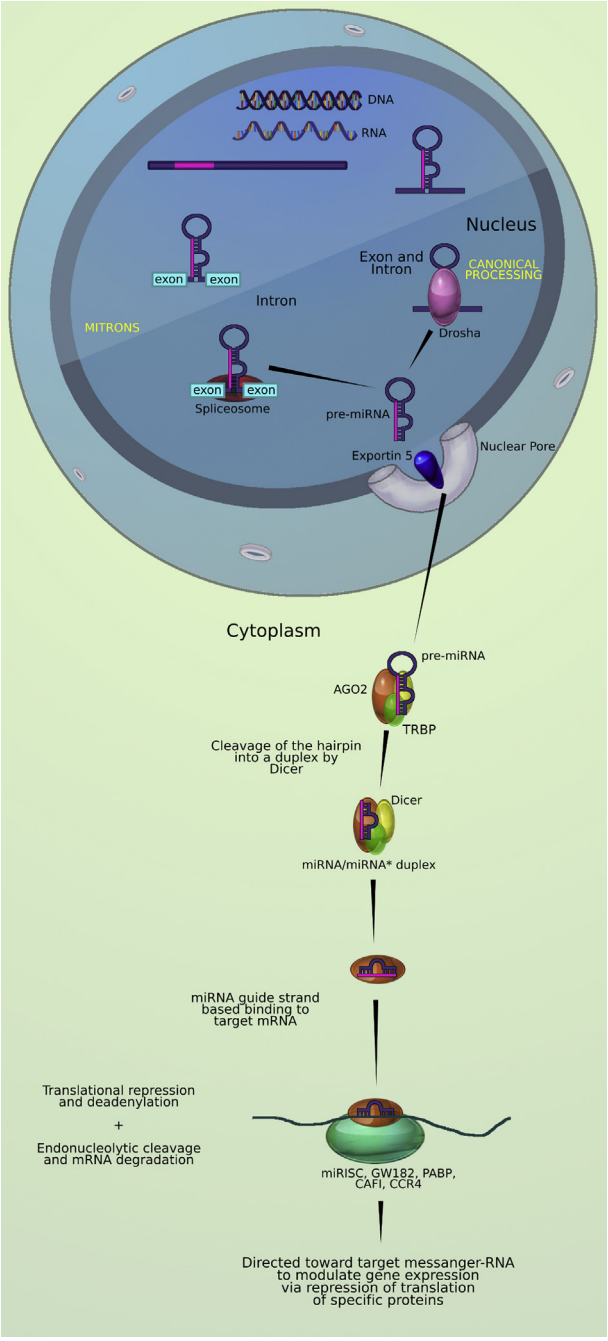

Fig. 1. MicroRNAs biogenesis. MicroRNA (miRNA) biogenesis begins in the nucleus. In the canonical pathway, RNA polymerase II processes specific transcripts in order to obtain a primary precursor (pri-miRNA) that will be further processed by Drosha in order to obtain pre-miRNAs. Pre-miRNAs are then transferred into the cytoplasm by the shuttle protein Exportin-5. Once in the cytoplasm, pre-miRNAs are further processed by Dicer and included in the RISC complex where they play a role in regulating gene expression (see text for further details).

pathway. On the other hand, miRNAs encoded in exons usually have their own promoter, and thus are usually autonomously transcribed (Lagos-Quintana et al., 2001). As messenger RNAs, the transcription process occurs in the nucleus, and is usually mediated by RNA-polymerase II. This step leads to generation of "primary miRNAs" (pri-miRNAs) that are long precursors of miRNAs (longer than 1000 nucleotides). Pri-miRNAs are then processed by capping, splicing, and polyadenylation. A typical feature of pri-miRNAs is the presence of specific loop-stem structures called hairpins. These structures are needed for recognition by the protein complex Drosha/DGCR8 (Di George syndrome Critical Region gene 8). Indeed, DGCR8 is able to bind the pri-miRNA, then *Drosha*, a nuclear endonuclease, crops the distal stem portion of the pri-miRNAs in order to obtain shorter nucleotide sequences (70–100 nucleotides) known as "pre-miRNAs". The above-described process represents the "canonical" biosynthetic pathway, however an alternative biogenesis pathway has been described (Fig. 1). In this pathway, pre-miRNAs are directly produced by the transcription of specific introns (called "miRtrons") that are exactly sized for producing pre-miRNAs, thus skipping the *Drosha* cropping step (Okamura et al., 2007). Once synthesized, pre-miRNAs are recognized by the nuclear-cytoplasmic shuttle protein Exportin-5 and are transferred to cytoplasm in a RanGTP-dependent manner. Then, pre-miRNAs are processed by another RNase III protein called "*Dicer*" in order to obtain shorter (19–25 nucleotides) double-stranded RNA sequences. These sequences contain the mature miRNA and its passenger strand (also known as "star strand", miRNA*). Some studies assessed the relationship between mature miRNAs and their precursors within different cell types. The results suggest that although some miRNA precursors may be constitutively expressed in all cell types. This does not imply the concomitant presence of their derivative mature miRNAs. For example, both miR-145 and miR-143 precursors are expressed in health colorectal tissue and cancer, whereas the mature sequences of these miRNAs are detected only in normal tissue (Michael et al., 2003). Based on these observations, it may be assumed that each cell type is characterized by a distinctive miRNAs profile (the so-called "miRNome") with a number of miRNAs that are expressed ubiquitously while other miRNAs are selectively and/or preferentially expressed in specific cell type or only under specific physiological or pathological stimuli.

After complete biogenesis, the mature miRNAs are loaded into the RNA-induced silencing complex (RISC). This is a multi-protein complex that includes, among other proteins, GW182 and Argonaute-2 (Ago-2) (Peters and Meister, 2007). According to the common view, the passenger strand is rapidly degraded. But the passenger strand of some miRNAs (i.e., miR-126) are reported to be as functional as their mature miRNA strand, and both strands may contribute to the final effect on cell biology (Guo and Lu, 2010; Schober et al., 2014; Zhang et al., 2013). Once loaded in the RISC complex, miRNAs are directed toward target messenger-RNA (mRNA) in order to exert their activities as modulators of gene expression. The so-called "seed region", spanning between nucleotides 2 and 8 in the 5′-terminus of the mature miRNA, binds a complementary sequence in the 3′ untranslated region (UTR) of target mRNAs. The complementary sequence between seed region and mRNA binding sites on 3′UTR was necessary for target recognition (Bartel, 2009). However, several other determinants are involved in the interaction between miRNA and target mRNA. These include: the number of target sites in the 3′UTR of mRNAs, the distance between miRNA-binding site and the coding sequence of mRNAs, and the secondary structure of the sequence near the seed region (Didiano and Hobert, 2006). An important aspect of miRNA research is the identification of the targeted mRNAs. Indeed, it is clear that the ultimate biological effect on cell biology is due to the repression of the translation of specific proteins involved in a particular biological pathway. Target identification may be obtained by *in vitro* experiments. The most frequently used test is the luciferase reporter in which 3′UTR is cloned by the mRNA of the target gene. However, this test is usually used in order to confirm targets that have been previously identified by *in silico* prediction tools. Indeed, a numbers of free on-line resources that are able to predict miRNA targets by using algorithms based on: sequence conservation, structural complementarity between miRNA seed and target 3′UTR sequences, thermodynamic stability calculations, and evolutionary conservation among species. The most used algorithms include PicTar, TargetScan, Miranda, and miRBase with different degrees of sensitivity and specificity in target prediction (Witkos et al., 2011).

Although the detailed mechanisms of translation repression and degradation of mRNA targets are not completely elucidated, the RISC-complex may modulate gene expression by promoting deadenylation of target mRNAs, with consequent decapping and degradation by blocking the translation initiation, by blocking protein elongation after translation initiation or by inducing a proteolytic cleavage by a protease that might be involved in the process (Filipowicz et al., 2008). Even though some protein targets are repressed without detectable changes in the levels of mRNA, in mammals miRNAs modulate protein expression by inducing a concomitant mRNA degradation in 87% of cases (Guo et al., 2010). A single miRNA may modulate the expression of several messenger RNA and, conversely, more than 60% of mRNAs have predicted binding sites for multiple miRNAs, thus allowing concomitant interaction with multiple miRNAs (Brennecke et al., 2005). After its activity, miRNAs are not rapidly degraded and thus they can recognize and modulate many other mRNAs (Hutvagner and Zamore, 2002). Indeed, the current view suggests that mature miRNAs are highly stable molecules with very long half-lives. Even though the mechanisms of miRNA decay are still far from being fully understood, recent studies demonstrated the involvement of two proteins in the regulation of their degradation and stability: namely XRN-2 and PAPD4. (Kai and Pasquinelli, 2010) In particular, the 5′-to-3′ exonuclease XRN-2 was shown to catalyze the degradation of single-stranded mature miRNAs, thus promoting the decay of miRNAs that are not incorporated in the RISC complex. *In vitro* experiments suggest that XRN-2 is able to both catalyze the disassociation of miRNAs from Argonaute proteins and their degradation when target mRNAs are not available. On the other hand, PAPD4 is an atypical poly(A)-polymerase (also known as GLD-2) which is able to add a single adenine residue to the 3′end of miRNAs thus modulating their ability to be included into the RISC complex. This in turn could modify the stability and the activity as gene regulators of miRNAs. This process appears to be highly selective for specific miRNAs. In human fibroblast and liver cells, PAPD4 is able to promote monoadenylation of miR-122 and its stabilization, despite this effect was not showed for other miRNAs. More recently, a study on human fibroblast showed that monoadenylation stabilizes and prolongs the activity of some (but not all) miRNAs and found that this effect also depends on the nucleotides at the 3′end of specific miRNAs. (D'Ambrogio et al., 2012; Kai and Pasquinelli, 2010) Although the mechanisms regulating miRNA stability and decay are the results of complex processes, the equilibrium between miRNA stabilization (by PAPD4) and degradation (by XRN-2) was recently proposed as a potential mechanism of the regulation of mature miRNA levels in the nervous system (Kinjo et al., 2013). Furthermore, both PAPD4 and XRN-2 are clearly expressed in adult rodent retina: PAPD4 is more expressed in the cytoplasm, whereas XRN-2 is localized in the nucleus of horizontal cells. Moreover, the observation that the expression of PAPD4 (but not XRN-2) was regulated by ambient light levels and dark adaptation, further supports a

potential role of this protein in regulation of retinal miRNA levels in response to ambient light (de Sousa et al., 2013).

Although the above-described pathways reflect most of the evidence on miRNAs activities, some exceptions were reported. In example, despite the majority of the studies suggesting miRNAs primarily act as post-translational modulators, miR-29b holds a hexanucleotide motif and can be re-directed into the nucleus where it might regulate gene transcription and/or splicing by binding specific nuclear targets (Hwang et al., 2007). The transport of miRNAs within the nucleus was also described for other miRNAs and appears to be mediated by the interaction between Ago-2 (which is bound to the mature miRNA) and the nuclear transporter Importin-8 (Wei et al., 2014). Moreover, although miRNAs are classified as negative modulators of gene expression, some exceptions were reported where miRNAs stimulate translation instead of repression in coordination with the cell cycle (Vasudevan et al., 2007). These observations further emphasize the complexity of miRNA biology. Future studies will be needed to improve our knowledge in this field.

3. Circulating microRNAs

Although the main biological activities of miRNAs occurs in the intracellular space, these molecules have been found to be stable in many biological fluids, including human serum, plasma, urine, saliva, tears, aqueous humor and vitreous humor (Andreeva and Cooper, 2014; Dunmire et al., 2013; Ragusa et al., 2013; Tuo et al., 2014). Circulating miRNAs are not only stable in standard conditions but also after undergoing several freeze-thawing cycles, strong variation in pH, and long exposure to room temperature (Mitchell et al., 2008). The remarkable stability of circulating miRNAs may be due to their packaging into vesicles or by the formation of stable RNA-protein complex (Table 1). In the bloodstream, miRNAs may be vehiculated by apoptotic bodies, exosomes, and microparticles. The classification of extracellular vesicles (either found in conditioned culture medium as well as in body fluids) was debated for some time. According to the most recent view (Table 1A), apoptotic bodies are 1–5 μm vesicles that are released during late steps of apoptosis, and may contain cellular organelles, protein, DNA, RNA, and microRNA. Conversely, microparticles are smaller in size (0.1 μm–1 μm), are actively released under stress conditions, retain surface molecules from the originating cell, and contain proteins, RNA, and miRNA. Exosomes are even smaller (40–100 nm), derive from a multivesicular body (MVB) and are released in extracellular space after fusion of a MVB with the plasma membrane (Loyer et al., 2014). Circulating miRNA is also bound to lipoproteins (i.e. HDL) or to RNA-binding proteins (mainly Ago-2) (Arroyo et al., 2011; Vickers et al., 2011).

Due to their stability, circulating miRNAs were proposed as biomarkers in human pathology, and reports in literature have evaluated the expression profile of circulating miRNAs in several diseases, including diabetes (see below). An interesting aspect of extracellular circulating miRNAs that has recently emerged is the possibility that they actually represent a paracrine and endocrine communication system. For example, miR-126-3p was reported to be highly expressed in endothelial cells and to be released in apoptotic bodies following apoptosis induction with different stimuli. Other endothelial cells may uptake these apoptotic bodies via an active process that requires cytoskeleton rearrangement, and miR-126-3p-promoted biological effects (such as the production of the anti-atherosclerotic chemokine CXCL12) in recipient cells (Zernecke et al., 2009). Furthermore, miR-126-3p is also released within microparticles. Once inside recipient endothelial cells, miR-126-3p targets SPRED-1 and promotes endothelial cells proliferation and migration (Jansen et al., 2013). The same miRNA may also be released in a vesicle-free manner bound to Ago-2 protein and affect the biology of recipient vascular smooth muscle cells (Zhou et al., 2013). Interestingly, circulating levels of miR-126-3p were found to be lower in patients with type 2 diabetes (Jansen et al., 2013; Ortega et al., 2014; Zampetaki et al., 2010). Other miRNAs were also shown to play a role in extracellular communication. For example, miR-105 is highly expressed in High Density Lipoprotein (HDL) from patients affected by familiar hypercholesterolemia. HDL collected from these patients was able to reduce the expression of several putative target genes in hepatocytes (Vickers et al., 2011). Finally, it was also demonstrated that miR-150 may be vehiculated by microparticles from monocytes and transferred to endothelial cells, where they can modulate the expression of target proteins (including c-Myb) (Zhang et al., 2010).

4. MicroRNA and human disease: focus on diabetes

Calin and colleagues first reported the role of miRNAs in human diseases in 2002. They highlight the reduced expression of miR-15 and miR-16 in most patients (68%) affected by chronic lymphocytic leukemia (Calin et al., 2002). Since this seminal study, numerous studies corroborated the role of miRNAs in various human pathologies, including cancer (Kong et al., 2012), liver disease (Kerr et al., 2011), inflammatory disease (Dai and Ahmed, 2011), and cardiovascular disease (Santovito et al., 2012).

Several works investigated the role of miRNA in the pathogenesis of diabetes and its complications. Diabetes is a metabolic disorder characterized by the presence of hyperglycemia due to either a deficit in insulin secretion (type 1 diabetes) or to an impaired response to insulin in peripheral tissue (type 2 diabetes). This complex disease presents numerous phenotypes based on the consequence of interaction between several factors, including: genetics, diet, obesity and environmental factors. The prevalence and mortality of diabetes (especially type 2 diabetes) is dramatically rising. In 2010 about 1.3 million deaths worldwide were caused by diabetes (Lozano et al., 2012). Although cardiovascular disease accounts for most of these deaths (about 60%), diabetes is

Table 1
Circulating microRNAs: characteristics of the different transport way in extracellular space.

Type of vesicle	Mechanism of formation	Content	Markers	Size
A. Intravesicular transport				
Exosomes	Fusion of the multivesicular bodies with plasma membrane	RNA (including miRNAs); proteins	CD63, LAMP1, TSG101, Heat Shock Proteins	40–100 nm
Microparticles	Bleeding of plasma membrane	RNA (including miRNAs); proteins; lipids	Annexin V and markers of originating cell	100–1000 nm
Apoptotic bodies	Released during apoptotic cell death	DNA; RNA (including miRNAs); cell organelles; proteins	Annexin V	1000–5000 nm
B. Extravesicular transport				
RNA–protein complexes		Argonaute-2 Nucleophosmin-1		
Lipoproteins		High Density Lipoproteins (HDLs)		

also a leading cause of end-stage kidney disease, lower extremity ischemia, and adult blindness due to the development of diabetic retinopathy.

One of the key demonstrations of miRNAs involvement in regulation of glucose metabolism was that a selective *Dicer* knockout in developing pancreatic cells hampered the pancreatic islet formation and insulin-secreting β-cell differentiation, ultimately resulting in growth failure and premature death after birth (Lynn et al., 2007). Several other publications investigated the role of miRNAs in the modulation (insulin secretion and sensitivity) of crucial pathways for glucose metabolism (Fig. 2). Among the involved miRNAs, miR-375 is highly expressed in pancreatic β-cell and was able to directly reduce insulin secretion (Poy et al., 2004). Notably, this effect was mediated by both a reduction in insulin gene expression after hyperglycemia due to direct targeting of Phosphoinositide-Dependent Protein Kinase-1 (PDK1) and by an impairment of insulin exocytosis due to the miR-375-induced myotrophin repression (El Ouaamari et al., 2008; Poy et al., 2004). The miR-375 levels were found to be higher in serum and pancreatic islets of type 2 diabetic patients, thus supporting a potential role also in humans (Kong et al., 2011; Zhao et al., 2010a). The role of miR-9 was also investigated in the regulation of insulin secretion from pancreatic β-cells. In fact, this miRNA was clearly expressed in insulin-secreting cells and was able to repress insulin release under hyperglycemia and cAMP-raising stimuli. The mechanism underlying this effect appeared to be the repression of the transcription factor Onecut-2 (OC2) which usually inhibits the expression of granuphilin, a key protein that exerts a negative control on insulin secretion (Plaisance et al., 2006).

On the other hand, miRNAs were also involved in modulation of insulin sensitivity. In particular, the miRNA family miR-103/miR-107 expression was increased in liver of obese mice and may impair insulin sensitivity (Trajkovski et al., 2011). In detail, *in vivo* miR-107 overexpression in rodents resulted in hyperglycemia, an increase in hepatic gluconeogenesis, and hyperinsulinemia. Conversely, pharmacological inhibition of miR-103/107 (by miR-103 antagomir) resulted in improvement in glycemic status and reduction in adipose tissue in obese mice. These effects could be dependent on the modulation of the insulin-receptor stabilizing protein caveolin-1. Indeed, inhibition of miR-103 expression resulted in an increase of caveolin-1 and consequently increased insulin receptor stability (Trajkovski et al., 2011). miR-143/145 were among the other miRNAs deregulated in liver of obese mice. These miRNAs are transcribed as bicistonic unit, and liver-specific knockout of miR-143/145 in mice resulted in reduced insulin-resistance induced by obesity. This effect may be mediated by direct inhibition of ORP8 (oxysterol related protein-like 8), a regulator of AKT pathway (Jordan et al., 2011). Interestingly, these miRNAs were also able to impair insulin signaling in vascular smooth muscle cells. Due to the role of these miRNAs on vascular biology, this observation may represent a new link between diabetes and vascular biology (Blumensatt et al., 2014; Cordes et al., 2009; Santovito et al., 2013). Furthermore, miR-144 was found to be up-regulated in pancreas, adipose tissue, and liver of diabetic rats (Karolina et al., 2011). It was able to directly target Insulin Receptor Substrate (IRS)-1, whose expression is concomitantly reduced in diabetes (Karolina et al., 2011). Finally, an important role in insulin-sensitivity was also demonstrated for let-7 family. A potential connection between this miRNA family and diabetes was suggested by the observation in a large genome-wide association study that several targets of let-7 were associated with type 2 diabetes (Zhu et al., 2010). Furthermore, experimental evidence showed that overexpression of let-7 in skeletal muscle resulted in impairment of insulin sensitivity and in impaired glucose tolerance. This effect was mediated by the repression of several components of the insulin transduction pathway, including Insulin Receptor (INSR), IRS-2, and insulin-like growth factor 1 receptor (IGF1R) (Zhu et al., 2011). Conversely, transgenic overexpression of Lin28a and Lin28b, negative regulators of let-7 biogenesis, significantly reduced high-fat-induced obesity and improved glucose tolerance in mice (Zhu et al., 2011). Finally, the inhibition of let-7 expression by LNA-modified anti-miR resulted in improvement of insulin resistance and blood glucose levels (Frost and Olson, 2011).

As previously mentioned, miRNAs were found to be extremely stable in body fluids and their expression profile was modified under pathological conditions. In this setting, several publications

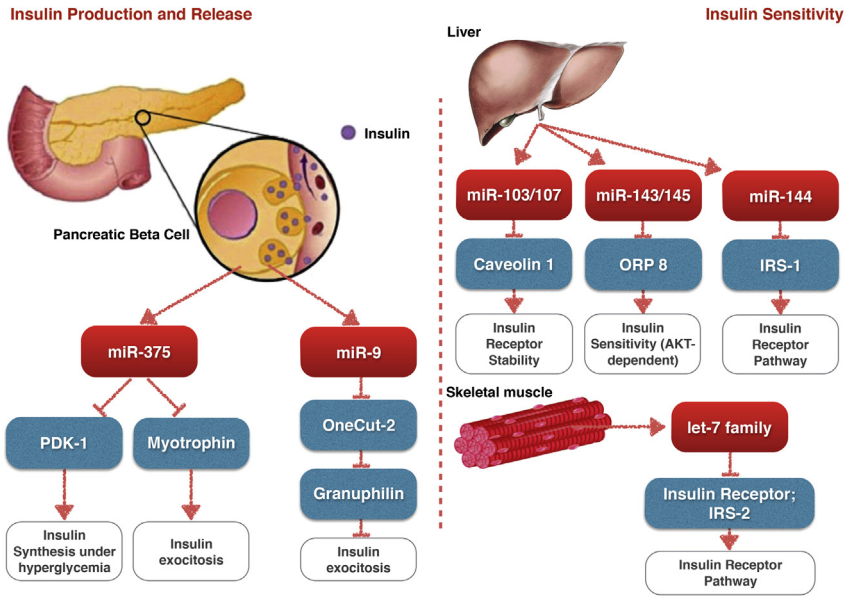

Fig. 2. The principal microRNAs involved in diabetes pathophysiology. *In vitro* and *in vivo* studies found these miRNAs to be involved in pathophysiology of diabetes. In particular, evidence supports the role of miR-375 and miR-9 in insulin synthesis and release by pancreatic beta-cells. On the other hand, miRNAs (including let-7, miR-103, miR-107, miR-143, miR-144, and miR-145) were also able to modulate peripheral insulin-sensitivity in liver tissue and skeletal muscle by regulating several steps of the insulin receptor pathway. (See text for further details).

identified circulating miRNAs as reliable biomarkers in several human diseases ranging from cancer to cardiovascular diseases (Creemers et al., 2012; Egea et al., 2012). The profile of expression of circulating miRNAs was also evaluated in diabetic patients (Table 2). A large population study found that circulating levels of miR-126-3p were reduced in patients with type 2 diabetes (Zampetaki et al., 2010). This finding is particularly interesting due to the potential effects of this miRNA on vascular biology and angiogenic proprieties that are affected in diabetic patients (Fish et al., 2008; Harris et al., 2008; Jansen et al., 2013; Nicoli et al., 2010; Zernecke et al., 2009). Moreover, circulating levels of miRNAs involved in glucose homeostasis and in insulin resistance were found to be misregulated. In fact, studies found a dysregulation in circulating levels of miR-144, miR-375, and members of let-7 family (Karolina et al., 2011; Kong et al., 2011; Santovito et al., 2014; Yang et al., 2014; Zampetaki et al., 2010). Interestingly, circulating levels of miR-146a were also found to be higher in patients with type 2 diabetes (Kong et al., 2011; Rong et al., 2013). Due to the important role of this miRNA in the modulation of inflammatory process, and considering the importance of inflammation in the pathophysiology of diabetes and its complications (including retinopathy), this finding may provide another molecular player in these processes. Finally, recent evidence suggested that the profile of circulating miRNAs were modulated, at least in part, by 3 and 12 months of pharmacological anti-diabetic treatment (Ortega et al., 2014; Santovito et al., 2014).

5. MicroRNA and angiogenesis

The importance of miRNA in regulating endothelial cells (ECs) function and particularly angiogenesis was revealed by *in vitro* and *in vivo* studies (Wang and Olson, 2009). The endothelium is a cellular monolayer that represents an internal barrier between the bloodstream and the rest of the blood vessel wall. ECs play a fundamental role in vascular development and disease. During vasculogenesis, ECs differentiate from angioblast precursors, proliferate and migrate to form the vascular network. In adult tissues, ECs are in a quiescent state. Nevertheless they retain considerable growth potential in response to physiological and pathological events such as wound healing, ocular and inflammatory disorders and cancer.

Angiogenic factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), were shown to be important for angiogenesis.

The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PlGF) (Takahashi and Shibuya, 2005). Amongst the various VEGF family members, VEGF-A, is a critical regulator of ocular angiogenesis and vascular permeability (Shams and Ianchulev, 2006). Activation of VEGF-A pathway is achieved through the binding of VEGF-A to the receptors in the cell membranes: VEGF-A receptor-1 (fms-like tyrosine kinase-1, FLT-1) and VEGF-A receptor-2 (fetal liver kinase-1, FLK-1) are activated by VEGF-A.

The FGF family is known to contain at least 20 factors, which are ~30–70% identical in their primary amino acid sequences. The biological effects of FGFs are mediated by four structurally related receptor tyrosine kinases, denoted FGFR-1, -2, -3 and -4.

Binding of VEGF and FGF to their receptors activated mitogen-activated protein kinase (MAPK), phosphinositide 3-kinase (PI3K), and other pathways, which promoted EC cell proliferation, migration and survival (Cross and Claesson-Welsh, 2001).

MiRNAs are known to be important for vascular development, physiology, and disease. The first evidence supporting the key role of miRNAs in angiogenesis and endothelial functions was obtained by disrupting the function of Dicer and Drosha - two key enzymes for miRNA biogenesis (see above). Knockdown of Dicer or Drosha *in vitro* in human ECs resulted in a decrease in angiogenesis. Moreover, *in vivo* EC-specific inactivation of Dicer in mice resulted in reduced postnatal angiogenic responses to a variety of stimuli, including exogenous VEGF, tumors, ischemia, and wound healing (Kuehbacher et al., 2007; Shilo et al., 2008). MiRNA profiling mainly carried out on human umbilical vein ECs (HUVECs) revealed that miR-126, the miR-17–92 cluster, miR-221/222, miR-21, the let-7 family, and the miRNA-23–24 cluster were highly expressed in vascular ECs (Fasanaro et al., 2008; Harris et al., 2008; Kane et al., 2014; Kuehbacher et al., 2007; Poliseno et al., 2006). Nevertheless, the number of miRNAs involved in angiogenesis might be underestimated because of differences in the parameters (such as

Table 2
Circulating miRNAs in diabetes: main findings from literature.

Author	Study design	Major findings in diabetes	miRNA source	Multivariate analysis?
Karolina et al., 2011	21 pts with diabetes; 14 pts with IGT; 15 controls	Increased miR-144, miR-150, miR-192, miR-29a, miR-320; Reduced miR-146a, miR-182	Whole blood	No
Kong et al., 2011	18 pts with type 2 diabetes 19 pts with IGT 19 controls	Increased miR-9, miR-29a, miR-30d, miR-34, miR-146, miR-124, miR-375	Serum	No
Ortega et al., 2014	48 pts with diabetes; 45 controls	Increased miR-140-5p, miR-142-3p, miR-222; Decreased miR-423-5p, miR-125b, miR-192, miR-195, miR-130b, miR-532-5p, miR-126	Plasma	Yes
Rong et al., 2013	90 pts with diabetes; 90 matched controls	Increased miR-146a	Plasma	No
Santovito et al., 2014	18 pts with type 2 diabetes; 12 controls	Increased miR-326; Decreased let-7a, let-7f	Plasma	Yes
Yang et al., 2014	24 pts with type 2 diabetes; 20 pts with pre-diabetes; 20 controls	Reduced let-7i, miR-23a, miR-96, miR-146a, miR-186, miR-191, miR-192, miR-486	Serum	No
Zampetaki et al., 2010	80 pts with diabetes; 80 matched controls	Reduced miR-20b, miR-21, miR-24, miR-15a, miR-191, miR-197, miR-223, miR-320, miR-486, miR-126	Plasma	Yes
Zampetaki et al., 2010	80 pts with diabetes 162 pts with pre-diabetes; 580 controls	Reduced miR-126	Plasma	Yes

IGT: impaired glucose tolerance.

different source of ECs, experiments performed on ECs in different conditions such as under stress conditions, or whether ECs are derived from diseased organisms) used for analysis.

Based on the evidence found in the literature regarding miRNAs expression and function in ECs, it is possible to divide the endothelial miRNAs in two classes: 1) miRNAs that target genes involved in angiogenesis (angiomiRs) and 2) miRNAs whose expression can be modulated by pro-angiogenic or anti-angiogenic stimuli (Table 3). AngiomiRs promote angiogenesis by targeting negative regulators of the angiogenesis signaling pathways, while anti-angiomiRs inhibit angiogenesis by targeting positive regulators of angiogenesis. Representative angiomiRs are miR-126, miR 221/222, miR-23/27, and the miR-17–92 cluster, miR-17-5p, miR-20a, miR-92a.

MicroRNA-126 is the only miRNA considered to be specifically expressed in ECs and hematopoietic progenitor cells. Studies in mice with a targeted deletion of miR-126 demonstrated the prominent role of miR-126 in controlling vascular integrity and angiogenesis (Fitch et al., 2004; Wang et al., 2008). MiR-126 was shown to play a significant role in vascular regeneration in response to injury and deletion of miR-126 in mice with myocardial infarction was associated with a defective cardiac neovascularization (Wang et al., 2008). The angiogenic actions of miR-126 were mediated, at least in part, by promoting MAPK and PI3K signaling in response to VEGF and FGF, by targeting negative regulators of these signaling pathways such as SPRED1 and PIK3R2 (Fish et al., 2008; Kuhnert et al., 2008; Wang et al., 2008). MiR-126 was found to be significantly decreased in the retina from oxygen-induced retinopathy (OIR) mice. Restoration of miR-126 levels in the retina inhibited the angiogenic growth factors, such VEGF, IGF, and hypoxia-inducible factor-1 alpha (HIF-1α), via down-regulating molecules of the MAPK signaling pathway, and resulted in the reduction of retinal NV in ischemic condition (Bai et al., 2011). Recently, Ye et al. showed a down-regulation of miR-126 in hypoxic cultures of monkey chorioretinal vessels ECs and in diabetic retinas.

Table 3
MicroRNA regulating angiogenesis.

MicroRNAs involved in angiogenesis		
MicroRNAs	Target/stimuli	Author
AngiomiR		
miR-126	SPRED1-PIK3R2	Fish et al. (2008)
miR-221/222	c-kit	Poliseno et al. (2006)
miR-23-27	Sprouty2-Sema6A	Zhou et al. (2011)
miR-17–92	Tsp1	Dews et al. (2006)
miR 17-5 p	TIMP-1	Otsuka et al. (2008)
miR-92a	ITGB 5	Bonauer et al. (2009)
miR-20a	VEGF	Hua et al. (2006)
MicroRNAs modulated by proangiogenic stimuli		
miR-296	HGS/VEGF	Wurdinger et al. (2008)
miR-210	EFNA3/hypoxia	Fasanaro et al. (2008)
miR-130 a	HOXA5-GAX/VEGF-bFGF	Chen and Gorski (2008)
miR-132	p120RasGAP/VEGF-bFGF	Anand et al. (2010)
miR-101	EZHZ/VEGF	Smits et al. (2011)
miR-424	CUL2/hypoxia	Ghosh et al. (2010)
miR-200b	ETS-1/hypoxia	Chan et al. (2011)
miR-191	VEGF	Suarez et al. (2008)
miR-155	VEGF	Suarez et al. (2008)
miR-31	VEGF	Suarez et al. (2008)
miR-17-5p	VEGF	Suarez et al. (2008)
miR-18a	VEGF	Suarez et al. (2008)
miR-20a	VEGF	Suarez et al. (2008)
MicroRNAs modulated by anti-angiogenic stimuli		
miR-217	SIRT-1/aging	Menghini et al. (2009)
miR-34	SIRT-1/aging	Zhao et al. (2010)
miR-200 c	ZEB1/oxidative stress	Magenta et al. (2011)
miR-503	CCNE1-cdc25A/diabetes	Caporali et al. (2011)
miR-93	VEGF-A/diabetes	Long et al. (2010)

A correlated increased expression of VEGF and matrix metalloproteinase-9 (MMP-9) was observed in monkey hypoxic chorioretinal blood vessel EC. These two factors are closely related to neovascularization (Ye et al., 2014). MMPs are the main critical proteins that take part into the migration of ECs during angiogenesis. MMPs contribute to degradation of the extracellular matrix surrounding the ECs. MMP-9, a member of the MMP family, can degrade substrates such as collagen IV, laminin, and fibronectin, which are major components of vascular lamina (Nguyen et al., 2001; Xu et al., 2012). MiR-126 also targets vascular cell adhesion molecule-1 (VCAM-1), thereby regulating the adhesion of leukocytes to the endothelium, suggesting a role of miR-126 in the modulation of vascular inflammation. MiR-126 was also reported to inhibit tumorigenesis and to be downregulated in many cancer lines (Guo et al., 2008; Harris et al., 2008; Zhang et al., 2013).

MicroRNA-221/222 are believed to be anti-angiomiRs, and their over-expression was demonstrated to impair stem cell factor (SCF)-induced angiogenesis and wound healing targeting the SCF receptor c-kit (Poliseno et al., 2006). MiR-23 and miR-27 enhance angiogenesis by suppression of Sprouty2 and Semaphorin6A (Sema6A) proteins, which exert antiangiogenic activity by negative regulation of MAPK and VEGFR2 signaling in response to angiogenic factors. The miR-17–92 cluster was the first identified tumor promoting miRNA. This gene cluster encodes miR-17, miR-18, miR-19a, 20a, miR-19b-1 and miR-92-1. MiR-17–92 promoted tumor angiogenesis by targeting anti-angiogenic proteins thrombospondin-1 (Tsp1) and connective tissue growth factor (CTGF). The proangiogenic functions of miR-17–92 was ascribed to direct repression of the secreted, antiangiogenic molecule thrombospondin-1 (TSP-1) and connective tissue growth factor (CTGF) within tumor cells, thereby promoting angiogenesis in the adjacent tumor endothelium by a paracrine, cell-nonautonomous mechanism (Dews et al., 2006; Suarez et al., 2008). MiR-17-5p seems to modulate EC migration and proliferation by targeting tissue inhibitor of metalloproteinase-1 (TIMP-1) (Otsuka et al., 2008). MiR-20a and miR-92a have a role in anti-angiogenesis activity, targeting VEGF-A and integrin α5 (ITGA5) transcripts, respectively (Bonauer et al., 2009; Hua et al., 2006).

Representative miRNAs whose expression can be modulated by pro-angiogenic stimuli are miR-296, miR-210, miR-130a, miR-31, miR-132, miR-101, miR-42, miR-200b, miR-191, miR-155, miR-31, miR-17-5p, miR-18a, and miR-20a (Anand et al., 2010; Caporali and Emanueli, 2011; Suarez et al., 2008).

MiR-296 is a pro-angiomiR targeting hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) that mediates an angiogenic function. HGS is involved in the sorting of the VEGF and PDGF receptors for degradation. MiR-296 was found upregulated in tumor ECs from human gliomas supporting a role for miR-296 in promoting angiogenesis in tumors (Wurdinger et al., 2008). Hypoxia increases the expression of miR-210, a crucial regulator of angiogenesis and EC survival in response to hypoxia. MiR-210 overexpression in normoxic ECs stimulates angiogenesis and VEGF-induced cell migration. Ephrin-A3 (EFNA3) was shown to be a direct target of miR-210. Studies demonstrated that EFNA3 down-modulation was a necessary event for miR-210-mediated stimulation of capillary-like formation and ECs chemotaxis in response to VEGF (Fasanaro et al., 2008). MiR-130a, a miRNA induced by serum, VEGF-A, and basic fibroblast growth factor (bFGF) in ECs, was able to inhibit the activity of the antiangiogenic homeobox genes GAX and HOXA5, thus regulating the angiogenic phenotype of vascular ECs (Chen and Gorski, 2008). Angiogenic growth factors, such as VEGF-A and bFGF, upregulated miR-132 in ECs. Over-expression of miR-132 increased EC proliferation and *in vitro* networking by suppressing endothelial p120RasGAP, a GTPase-activating protein (Anand et al., 2010). VEGF-A down-regulation of miR-101 promoted

angiogenesis by enhancing the expression of histone-methyltransferase enhancer of zeste 2 (EZH2), a member of the polycomb group family (Smits et al., 2011). Polycomb group proteins function as transcriptional repressors that silence specific sets of genes through chromatin modification thus regulating gene expression.

Hypoxia increases the expression of miR-424that in turn promoted angiogenesis inhibiting cullin-2 (CUL2) and, thus, increasing HIF-1α levels (Ghosh et al., 2010). CUL 2 is a scaffolding protein critical to the assembly of the ubiquitin ligase system and thereby to the stabilization of HIF-α isoforms.

Hypoxia induced miR-200b down-regulation and derepressed ETS-1 expression, a key transcription factor of angiogenesis, promoting angiogenesis in human microvascular ECs (Chan et al., 2011). VEGF-A induces time-dependent expression of miR-191, miR-155, miR-31, miR-17-5p, miR-18a, and miR-20a (Hua et al., 2006). Anti-angiogenic stimuli modulate several MiRNAs: miR-200c, miR-217, miR-34, miR-93, and miR-503. The effect of oxidative stress on ECs proliferation, death and senescence was linked to up-regulation of miR-200c and, consequently, down-modulation of its target protein zinc finger E-box-binding homeobox 1 (ZEB1), an E-box-binding transcription factor, therefore an important modulator in the network of transcriptional repressors (Magenta et al., 2011).

MiR-217 was identified in aging ECs. MiR-217 regulated the expression of silent information regulator 1 (SirT1). Silent information regulator 2 proteins or sirtuins (SIRTs) belong to Class III histone deacetylases (HDACs). They represent a small gene family with seven members designated as SIRT1–7 that regulate epigenetic gene silencing and suppress recombination of rDNA. In mammals, SIRTs have a range of molecular functions and have emerged as important proteins in aging and metabolic regulation that are known to be modulated by oxidative stress.

Inhibition of miR-217 in old ECs reduced senescence and increased angiogenic activity via an increase in SirT1 activity (Menghini et al., 2009). Similarly to miR-217, miR-34 expression was also increased in senescent HUVECs and in endothelial progenitor cells. The increased levels of this miRNA impaired angiogenesis by induction of senescence via SirT1 inhibition (Zhao et al., 2010b). MiR-93 was found to be a direct regulator of VEGF-A in diabetic environment in kidney ECs (Long et al., 2010). MiR-503 expression in ECs was observed to be upregulated under conditions mimicking diabetes mellitus (high D-glucose) and ischemia-associated starvation (low growth factors) (Caporali et al., 2011).

Cyclin (CCN) E1 and phosphatase A of the cell division cycle 25 family (cdc25A) are the direct miR-503 targets that are down-regulated by high glucose/low growth factors in ECs. Cyclin-dependent kinases (cdks), their binding partners, CCNs, and associated regulatory proteins modulate entry into each phases of the cell cycle. Cdc25 are critical for timely Cdk activation, thus regulating G1-S and G2-M transitions of the cell cycle.

6. Diabetic retinopathy and classification

Diabetic retinopathy (DR) is a progressive disease with microvascular alterations leading to retinal permeability, retinal ischemia, retinal neovascularization and macular edema. It is a duration-dependent disease that develops in stages of increasing severity. The natural history of the DR presents five stages of increasing risk of retinopathy based on the status of the retinal vasculature: no apparent retinopathy. The first level is a mild non-proliferative retinopathy (NPDR). The second level is characterized by the presence of a few microaneurysms, moderate NPDR. The third level has more than just microaneurysms but less than severe non-proliferative diabetic retinopathy, severe NPDR. In the fourth level any of the following must be present with PDR: more than twenty intraretinal hemorrhages in each of quadrants, definite venous beading in two quadrants, prominent intraretinal microvascular abnormalities in one quadrant and no signs of proliferative retinopathy. In the fifth level neovascularization and vitreous/pre-retinal hemorrhages are present (Wilkinson et al., 2003) .

DME can be associated with either NPDR or PDR stages. If present it should be further distinguished with respect to the distance of thickening and the presence of lipid exudates from the center of the fovea (Early Treatment Diabetic Retinopathy Study Research Group, 1991; Wilkinson et al., 2003).

The Early Treatment of Diabetic Retinopathy Study (ETDRS) introduced the term clinically significant macular edema (CSME). CSME was defined upon fundus biomicroscopy assessment as (1) thickening of the retina at or within 500 μm of the center of the macula; (2) hard exudates at or within 500 μm of the center of the macula associated with thickening of adjacent retina; or (3) a zone of retinal thickening one disc area or larger, any part of which is within one disc diameter of the center of the macula (Early Treatment Diabetic Retinopathy Study Research Group, 1991).

Fluorescein angiograms (FA), performed with infusion of a fluorescent dye (fluorescein), is used as an additional diagnostic tool to study areas of capillary non-perfusion that are not detectable clinically and to provide a qualitative characterization of fluid leakage in the macular area leading to DME (Early Treatment Diabetic Retinopathy Study Research Group, 1991).

7. Pathophysiology of diabetic retinopathy

The pathogenesis of diabetic retinopathy is multifactorial, and a range of hyperglycemia-linked pathways was implicated in the development and progression of this condition (Antonetti et al., 2012; Frank, 2004). Initially, retinopathy was characterized solely by microvascular abnormalities, including endothelial cell dysfunction, vessel leakage, and vascular occlusion and degeneration (Curtis et al., 2009). Recently, there is growing evidence that retinal complications of diabetes are the result of functional and structural alterations in both the microvascular and neuroglial compartments, and DR is widely recognized to be a neuro-vascular disease (Barber et al., 2011; Villarroel et al., 2010). Such neuronal and glial dysfunction occurs together with blood flow abnormalities and often before the appearance of evident microvascular damage (Antonetti et al., 2006; Ola et al., 2013). Modifications of astrocytes were observed in a diabetic mice model early in the progression of diabetes. These modifications were accompanied by inner retinal hypoxia and ganglion cell dysfunction. Muller cell gliosis and more extensive changes in neuronal function occurred after the astrocyte alterations (Ly et al., 2011). Feng et al. demonstrated that neurodegeneration and glial activation initiated vaso-regression in transgenic mice (Feng et al., 2009).

The exact mechanisms by which hyperglycemia initiated vascular or neuronal alterations in retinopathy have not been completely clarified (Curtis et al., 2009; Villarroel et al., 2010). The cellular damage in the retina was hypothesized to be caused by several mechanisms, including increased flux through the polyol pathway, production of advanced glycation end-products (AGE), increased oxidative stress and activation of the protein kinase C (PKC) pathway (Antonetti et al., 2012; Cheung et al., 2005; Frank, 2004). Nevertheless, human studies or clinical trial are needed to validate many of these hypotheses (Antonetti et al., 2012; Cheung et al., 2005; Frank, 2004).

DR has numerous similarities with chronic inflammatory diseases since it is related to increased vascular permeability, edema, inflammatory cell infiltration, tissue destruction, neovascularization, and the expression of pro-inflammatory cytokines

and chemokines in the retina. Increased expression of vasoactive factors and cytokines was suggested to play an important role in mediating the structural and functional changes in the retina (Khan and Chakrabarti, 2007; Wirostko et al., 2008). Recent studies yielded strong evidence suggesting that inflammation was also important in the pathogenesis of early stages of experimental DR. Several inflammatory molecules were shown to contribute to structural or functional alterations that are characteristic of retinopathy (Lutty, 2013).

7.1. Pathophysiology of diabetic macular edema

Diabetic macular edema is characterized by a thickening of the macular region caused by a breakdown of the blood-retinal barrier (BRB). Clinically, DME consists of detectable accumulation of extracellular fluid within and beneath the layers of the retina.

The BRB compartmentalizes the neurosensory retina from the vascular component of the eye and consists of two major components: an outer barrier and an inner barrier. The inner BRB is formed primarily by tight junctional complexes between retinal vascular ECs and a network of glial cells (astrocytes and Müller cells) that are involved in maintaining a low permeability environment. The outer BRB is formed by tight junctions between retinal pigment epithelium (RPE) cells and includes zonula occludens (Bhagat et al., 2009).

The pathophysiology of DME is a complex multifactorial process where several vascular events occur as a result of hyperglycemia and culminate in the BRB breakdown (Fig. 3) (Ciulla et al., 2003; Ehrlich et al., 2010).

The initial insult of hyperglycemia results in endothelial damage and altered leucocytes function and recruitment. The retinal blood vessels are altered with diffuse thickening of the capillary basement membrane. Increase in shear stress and decoupling of endothelial cell tight junctions as a result of intracellular dysfunction allow the leakage of proteins into the surrounding tissue. This extravasation leads to an alteration of the extracellular matrix (ECM). During this process an inflammatory response and secretion of inflammatory mediators is initiated. This further facilitates cellular hypoxia, with the secretion of growth factors such as VEGF and activation of oxidative stress reactants (Bhagat et al., 2009).

The macrovascular factors causing DME is increased retinal vascular permeability leading to the leakage of serum proteins and lipids into the intraretinal space (Knudsen et al., 2002). Hydrostatic pressure, oncotic pressure, shear stress and vasoregulation are contributing factors.

On a molecular microvascular level, hyperglycemia leads to endothelial cellular dysfunction, hypoxia and the activation of numerous mediators from the angiogenic, inflammatory and oxidative stress pathways. Hyperglycemia leads to formation of AGEs that may be the primary factor contributing to diabetic microvasculopathy (Chen et al., 2013). AGEs cause upregulation of ICAM-1, which mediates retinal capillary leukocyte adherence and inner BRB breakdown. Leukocyte adhesion to the diabetic retinal vessel wall causes apoptosis of pericytes and endothelial cells, vascular obstruction, subsequent non-perfusion, and release of cytokines that increase vascular permeability (Kaji et al., 2007; Miyamoto et al., 1999).

Activation of PKC pathway by AGEs is associated with increased vasopermeability (Brownlee, 2001, 2005). AGEs can also modify basement membrane components inducing detrimental effects on the diabetic retina by disrupting normal vascular cell function. AGE adduct effects on the basement membrane can lead to impaired cell–matrix interactions and growth factor depletion in endothelial cells and pericytes (Bhatwadekar et al., 2008). AGEs-induced alterations of glial cells result in induction of VEGF and increased vasopermeability. AGEs also lead to reduced expression of glial-cell derived neurotropic factor (GDNF) in glial cells. GDNF plays an important role in regulating the vascular permeability of the BRB (Nishikiori et al., 2007).

Hyperglycemia also stimulates the expression of some growth factor, among which VEGF-A that was implicated in the pathogenesis of DR and particularly of DME. VEGF-A was implicated in RD pathogenesis due to its capacity to promote vascular leakage.

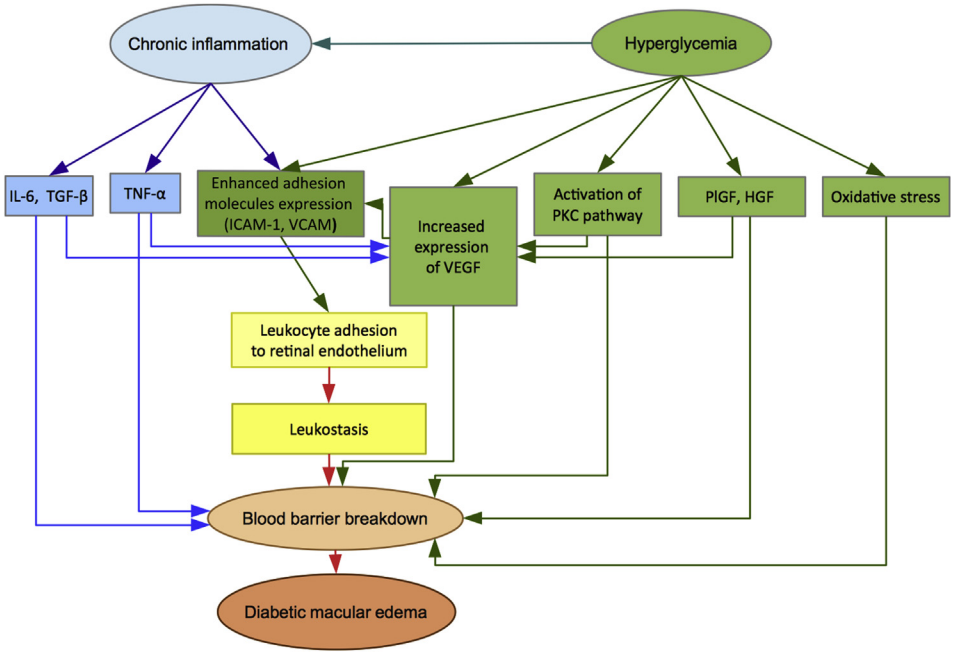

Fig. 3. Pathogenesis of diabetic macular edema. HGF = Hepatocyte growth factor; ICAM-1 = intracellular adhesion molecule-1; IL-6 = interleukin-6; PlGF = placental growth factor; PKC = protein kinase C; TGF-β = Transforming growth factor-β; TNF-α = tumor necrosis factor- α; VCAM-1 = vascular cell adhesion molecule-1; and VEGF = vascular endothelial growth factor.

Several hypotheses have been proposed to explain the mechanism by which VEGF-A contributes to BRB dysfunction (Zhang et al., 2009). Firstly, VEGF-A is a critical effector for several pro-inflammatory mediators in diabetic retinopathy, including cytokines, chemokines, and vascular cell adhesion molecules such as ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) (Kim et al., 2001). Secondly, VEGF-A has direct effects on tight junction-associated proteins that are the fundamental components of BRB. The tight junction-associated proteins, such as β-catenin, occludin, claudin-5, zonula occludens-1, and connexin, were showed to be phosphorylated in response to VEGF-A. Moreover, induction of pericytes degeneration and depletion, a hallmark of early diabetic retinopathy, is mediated by the upregulation of VEGF-A and FLK-1 in the retinal vascular wall.

PlGF, a member of the VEGF family, was also shown to increase vascular permeability (Otrock et al., 2007). Increased PlGF levels in the vitreous fluid of rat eyes can induce RPE cell tight junction alterations and can subsequently lead to subretinal fluid accumulation and retinal edema (Miyamoto et al., 2007). PlGF mainly activates VEGFR-1. Both hypoxia and insulin induce upregulation of PlGF. Hepatocyte growth factor (HGF) is an endothelium-specific growth factor with highly potent mitogenic activity that plays a role in vascular permeability. HGF and its receptors are expressed in retinal ECs and their effect on vascular permeability appears to be mediated by PI-3 kinase and MAPK. HGF can lead to endothelial cell separation and increased leakage by reducing expression of claudin, occludin, cellular adhesion molecules and cadherin (Clermont et al., 2006).

Oxidative stress also contributes to DME. Hyperglycemia was shown to increase superoxide, nitric oxide (NO) and peroxynitrite formation and VEGF expression, thus leading to the breakdown of the BRB and increased vascular permeability (El-Remessy et al., 2003).

Inflammatory stimulation was also shown to play an important role in the pathogenesis of DME (Miyamoto et al., 1999). Leucocytes naturally adhering to vascular endothelium (leukostasis) can directly increase vascular permeability and damage endothelial cells by inducing the release of free radicals, enzymes and cytokines (Schroder et al., 1991). Moreover, inflammatory stimulation can increase occludin phosphorylation, resulting in increased vascular permeability (Hirase et al., 2001). It was shown that diabetic vascular leakage and non-perfusion were associated with retinal leukostasis in diabetic rats models (Miyamoto et al., 1999). Among inflammatory mediators, tumor necrosis factor (TNF)-α, a pro-inflammatory cytokine, causes BRB breakdown, probably acting through the stimulation of leukostasis (Penfold et al., 2002). TNF-a activity was also associated with increased expression of VEGF (Penfold et al., 2002).

Interleukin-6 (IL-6), another proinflammatory cytokine, was reported to cause enhanced expression of VEGF and to increase vascular permeability. IL-6 was found to be higher in patients with diabetic macular edema in vitreous samples and epiretinal membranes (Funatsu et al., 2002, 2003).

In diabetic conditions an activation of Nuclear Factor-kappa-B (NF-κB), a proinflammatory transcription factor, was reported in retinal endothelial cells and pericytes leading to synthesis of many cytokines (VEGF), chemokines, acute phase proteins, and pro-inflammatory molecules [Inducible NO synthase (iNOS) ICAM]. Both increase in inflammatory cytokines, high glucose levels and oxidants were associated with NF-κB activation.

7.2. Pathophysiology of proliferative diabetic retinopathy

The angiogenic process in the eye has to be considered as a pathologic phenomenon. The proliferative stage of DR is one of the ocular conditions that leads to the formation of abnormal neovascularization (Rezzola et al., 2013).

A number of reports suggested that VEGF was a mediator of pre-retinal angiogenesis in proliferative diabetic retinopathy. Upregulation of VEGF-A was detected in plasma and vitreous in PDR patients, and was correlated with disease severity. Over-expression of VEGF-A was also found in excised proliferative vascular membranes from eyes with PDR (Kroll et al., 2007). Hyperglycemia-induced ischemia is regarded as the primary inducer of pre-retinal angiogenesis and VEGF-A was shown to be its critical mediator by three mechanisms. First, VEGF promotes proliferation, differentiation, division and migration of the vascular endothelial cells. Second, it induces tube formation. Third, it maintains and stabilizes the newly formed blood vessels. High levels of PlGF, another member of the VEGF family, were reported in the vitreous of patients with proliferative DR (Khaliq et al., 1998; Mitamura et al., 2002).

There is evidence that inflammatory stimulation plays an important role in the pathogenesis of PDR. Activity of TNF-α, a pro-inflammatory cytokine activity, was associated with increased expression of VEGF and intercellular adhesion molecule-1 (ICAM-1) in choroidal endothelial cells (Penfold et al., 2002). VEGF induced TNF-α and, in turn TNF-α, also increased VEGF levels. TNF-α was found to be elevated in the vitreous of patients with proliferative DR (Ben-Mahmud et al., 2006).

8. MicroRNA and diabetic retinopathy

As described above, microRNA plays a key role in embryonic development of organisms. It is not surprising that microRNAs were reported to play an important role in regulating eye development and function (Arora et al., 2010). Remarkably, retina specific deletion of the Dicer protein, a required component for mature microRNA biosynthesis within cells, resulted in the loss of photoreceptor-mediated responses to light and extensive retinal degeneration (Damiani et al., 2008).

Recent studies also indicated that miRNAs were involved in retinal and choroidal neovascularization (NV). VEGF, an important stimulator of NV in several tissue types including ocular NV, was shown to be reduced after injection of precursor of some miRNA, and was found to be downregulated in retina of postnatal mice with ischemic retinopathy. Thus suggesting that a decrease of specific miRNAs (miRNA-31, -150, and -184) that occurs in an ischemic retina may contribute, along with transcriptional regulation mechanisms, to the disease phenotype (Shen et al., 2008).

DR is a vascular retinal disease that is a very common microvascular complication of diabetes and the leading cause of blindness and visual disability in working age adults in developed countries (Ding and Wong, 2012). The altered expression of growth factors and vasoactive factors, including VEGF, in sustained hyperglycemia, results in both structural and functional changes in the retina of diabetic patients (Brownlee, 2001; Khan et al., 2006).

However, to date few studies have investigated the role of miRNAs in DR (Table 4, Fig. 4). Kovacs et al. provided the first insight into the roles of miRNAs in the pathogenesis of DR by identifying a series of miRNAs whose expression is modified in the retinas and in retinal endothelial cells (RECs) of Streptozotocin (STZ) induced diabetic rat models three months after diabetes onset (Kovacs et al., 2011). It was found that 80 miRNAs were significantly increased ($P < 0.01$) whereas six miRNAs were significantly decreased ($P < 0.01$) in retinas of diabetic rats compared with controls and 16 miRNAs were significantly upregulated ($P < 0.01$), whereas 104 miRNAs were downregulated in RECs of diabetic rats compared with controls ($P < 0.01$).

Interestingly, NF-kB-responsive miRNAs (including miR-146a, miR-146b, miR-155, miR-132, and miR-21) were upregulated in

Table 4
MiRNA involved in diabetic retinopathy.

Author	Major findings/Targets	Cell types and animal model
Kovacs et al., 2011	**MiRNA-expression profiling** **In rat retinas:** a 14 of the 80 most upregulated miRNA (miR-31, miR-31*, miR-34b-3p, miR-34c, miR-184, miR-199a, miR-200a, miR-200b, miR-205, miR-223, miR-335–3p, miR-378*, miR-488, miR-574–3p, miR-497, miR-685) b 6 miRNA downregulated (miR-20b, miR-499, miR-690; miR-375, miR-431; miR-872) **In RECs:** a 11 of the 15 most upregulated miRNA (miR-15b, miR-19b, miR-21, miR-31, miR-132, miR-142–3p, miR-146a, miR-155, miR-339–5p, miR-342–3p, and miR-450a) b 5 of the 104 most downregulated miRNA (miR-20b-5p, miR-29c, miR-181c, miR-136*, and miR-376c) NF-kB responsive miRNA upregulated in RECs (miR-146a, miR-146b, miR-155, miR-132, and miR-21): modulation of NF-kB activation pathway VEGF responsive miRNA upregulated in RECs and rat retinas (miR-17-5p, miR-18a, miR-20a, miR-21, miR-31, miR-155) p53-responsive (miRNA-34 family) **MiRNA targets** MiRNA 146: IRAK1 and TRAF6	Retinas and RECs of STZ induced diabetic rat models 3 months after diabetes onset
Wu et al., 2012	**MiRNA-expression profiling** 11 miRNAs significantly up-regulated (miR-182, miR-96, miR-183, miR-211, miR-204, miR-124, miR-135b, miR-592, miR-190b, miR-363, and miR-29c-5p) 6 miRNAs significantly downregulated (miR-10b, miR-10a, miR-219-2-3p, miR-144, miR-338, and miR-199a-3p)	Retinas of STZ induced diabetic rat models 10 weeks after diabetes onset
McArthur et al., 2011	**MiRNA expression profiling** Downregulation of MiRNA 200-b **MiRNA targets** MiRNA 200-b: VEGF	In HGRECs and in retinas of STZ-induced diabetic rats 1 month after diabetes onset
Murray et al., 2013	**MiRNA expression profiling** Upregulation of MiRNA 200-b **MiRNA targets** OXR1	8-Month old diabetic rat (Akita mice)
Feng et al., 2011	**MiRNA expression profiling** Downregulation of MiRNA 146a **MiRNA targets** Fibronectin	In HGRECs of STZ-induced diabetic rats 1 month after diabetes onset
Silva et al., 2011	**MiRNA expression profiling** Upregulation of MiRNA-29b **MiRNA targets** RAX	RGCs and the cells of the INL of the retinas from STZ-induced diabetic rats within 35 h from STZ injection
Bai et al., 2011	**MiRNA expression profiling** Downregulation of MiRNA 126 **MiRNA targets** VEGF, IGF-2, up stream regulator of HIF-1α	Oxygen-induced retinopathy model in mice (Akita mice)
Ye et al., 2014	**MiRNA expression profiling** Downregulation of MiRNA 126	Retinas of STZ-induced diabetic rats within 24 h from STZ injection
Mortuza et al., 2014	**MiRNA expression profiling** Upregulation of MiRNA 195 **MiRNA targets** SIRT1	Human retinal microvascular endothelial cells exposed to high glucose

the RECs of diabetic rats compared with controls. Notably, NF-kB is a ubiquitous inducible transcription factor and is a key modulator of cellular inflammatory pathways playing a role in the early phases of DR development (Kowluru et al., 2003). NF-κB is activated under hypoxic conditions and in retinal endothelial cells and pericytes exposed to hyperglycemia *in vitro* and *in vivo*. Known NF-kB downstream genes, such as ICAM-1 and monocyte chemotactic protein-1 (MCP-1) were also found to be upregulated in rat retinas confirming the role of inflammation in the early phases of diabetes (Kovacs et al., 2011).

Moreover, it was found that miR-146 is not only transactivated by NF-_B but also exerts a negative feedback on NF-kB activation in endothelial cells by targeting two key adapter molecules: IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) of the myeloid differentiation primary-response protein-88 (MyD88)-dependent IL-1R/Toll-like receptor (TLR)-mediated NF-kB activation pathway in monocytes. TLRs (Toll-like receptors) are transmembrane proteins expressed by cells of the innate immune system, which recognize pathogen molecules and activate signaling pathways that launch immune and inflammatory responses. All TLRs can trigger intracellular signals because of the presence of Toll and IL-1 receptor (TIR) domains in their cytoplasmic tails. The signaling cascade of TLR4, the first discovered member of the family and a sensor of Lipopolysaccharide (LPS), is initiated when adapter proteins MyD88 and TIR domain-containing adapter-inducing IFN-β (TRIF) are recruited to the receptor, activating two independent branches of TLR signaling. MyD88 serves as a bridge between TLR4 and IRAK1 that then recruits into the complex TRAF6. This chain of events triggers activation of IkB kinase and c-Jun Kinase (JNK) and, in turn, the downstream NF-kB transcription (Taganov et al., 2006).

The negative feedback on NF-kB activation suggests that miR-146 may be an alternative therapeutic target for the treatment of DR through NF-kB inhibition. In addition, VEGF-responsive miRNAs (miR-17-5p, miR-18a, miR-20a, miR-21, miR-31, and miR-155) were upregulated in both retinas and RECs of the diabetic rats (Kovacs et al., 2011). The p53-responsive miR-34 family was also found to be upregulated, consistent with p53 activation in the diabetic

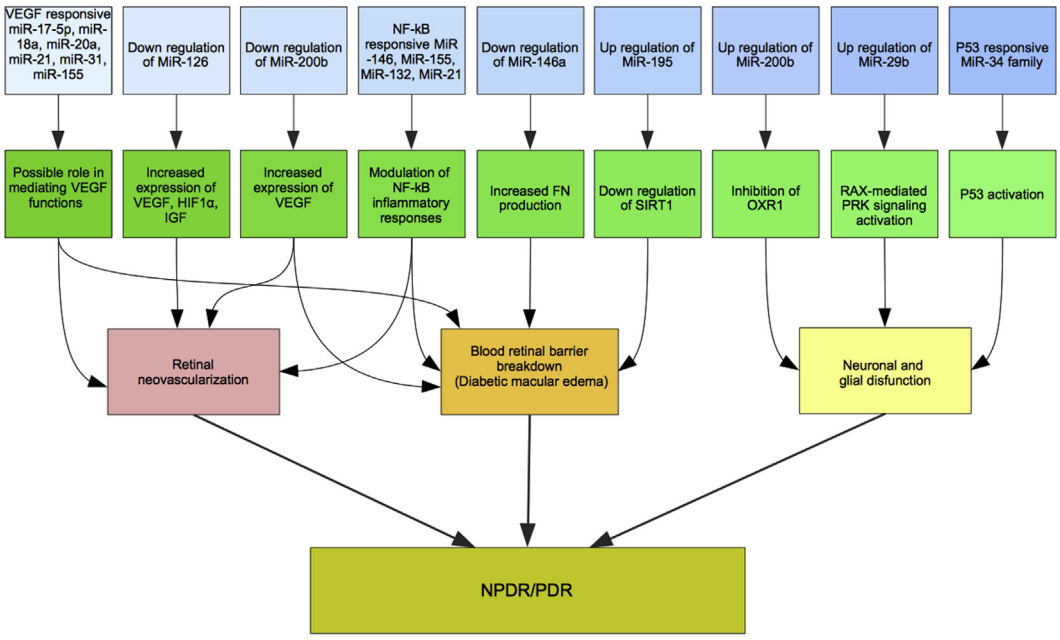

Fig. 4. Main microRNAs involved in diabetic retinopathy pathophysiology. The main miRNAs reported to be involved in pathophysiology of diabetic retinopathy (NPDR and PDR) in *in vitro* and *in vivo* studies are shown. In particular the roles of miR-200b (targets: VEGF, OXR1), miR 146 a (target: fibronectin), miR-29b (RAX), miR-126 (targets: VEGF, IGF-2, up stream regulator of HIF-1α) and miR-195 (SIRT1) were demonstrated. (See text for further details).

retina, suggesting a role of miR-34 family in p-53-induced apoptosis of neuoretinal and endothelial cells contributing to the development of DR (Kovacs et al., 2011).

Recently, Wu et al. (2012) reported a significant abnormal expression of miRNA in rat retinas of STZ induced diabetic rat models 10 weeks after STZ injection compared to normal controls: 11 miRNAs significantly upregulated (miR-182, miR-96, miR-183, miR-211, miR-204, miR-124, miR-135b, miR-592, miR-190b, miR-363, and miR-29c-5p) and six miRNAs notably downregulated (miR-10b, miR-10a, miR-219-2-3p, miR-144, miR-338, and miR-199a-3p). Moreover, changes in retinal miRNA expression levels of some miRNA paralleled the course of DR suggesting an association of miRNA disregulation and the development of DR (Wu et al., 2012).

McArthur et al. (2011) described down-regulation of miR-200b in endothelial cells treated with high glucose and in retinas of STZ-induced diabetic rats one month after the onset of diabetes. In parallel VEGF (a validated target of miR-200b) expression was elevated at both mRNA and protein levels (McArthur et al., 2011). Moreover, *in vitro* transfection of ECs or *in vivo* intravitreal injection of a miR-200b-mimic prevented diabetes-induced increase in VEGF mRNA and protein levels and also prevented glucose-induced increased permeability and angiogenesis. On the other hand, miR-200b antagonist could increase VEGF production, thus further demonstrating the role of this miRNA in the pathogenesis of DR.

Another study in a genetic model of type 1 diabetes (Akita mice) found significantly increased miR-200b expression in 8-month-old diabetic rats compared to controls (Murray et al., 2013). In this model, increased miR-200b inhibited Oxr1, a protective gene involved in resistance to oxidative stress. In their study, transfection with miR-200b-mimic in retinal Müller cells resulted in a remarkable increase in miR-200b levels and a related augmented apoptosis induced by 4-hydroxynonenal (4-HNE), an oxidative stressor. The disparities between different studies in miR-200b levels in the retina of diabetic models may be ascribed to different factors, such as the durations of diabetes, different models of diabetes (genetic model of diabetes versus STZ-induced diabetes) or cultured cell types (Müller cells vs. retinal endothelial cells) (Murray et al., 2013).

Recently, Feng et al. (2011) observed a decrease in miR-146a in high-glucose-treated retinal endothelial cells from type 1 diabetic STZ rat model at one month of diabetes. MiR-146a regulates fibronectin expression (Feng et al., 2011). One of the major glucose-induced dysfunctions includes increased production of fibronectin, an extracellular matrix (ECM) protein, and occurs in several diabetes complications, including diabetic retinopathy and nephropathy. MiR-146a down-regulation could be a key mechanism for increased extracellular matrix protein production in diabetes. Furthermore, intravitreal injection of miR-146a mimics decreased fibronectin levels in diabetes.

Silva et al. (2011) demonstrated that the involvement of miR-29b and its potential target RAX [an activator of the pro-apoptotic RNA-dependent protein kinase (PKR) signaling pathway] in the apoptosis of retinal neurons, a phenomenon playing a crucial role in the pathogenesis of DR. PKR is a stress-responsive kinase and has been described that apoptosis in mammalian cells occurs in response to stress. It was suggested that RAX may be a direct stress-sensitive activator of PKR and could induce apoptosis by activating the PKR-signaling pathway under stress conditions (Ito et al., 1999). De Silva et al. observed that miR-29b and RAX were localized in the retinal ganglion cells (RGCs) and the cells of the inner nuclear layer (INL) of the retinas in normal and STZ-induced diabetic rats. They speculated that that RAX expression may be indirectly regulated by miR-29b, and the up-regulation of this miRNA in the early stage of STZ-induced diabetes may have a protective effect against the apoptosis of RGCs and cells of the INL by the pro-apoptotic PKR signaling pathway. These results raise the hope that an over-expression of miR-29b (i.e., by intra-vitreal injection of a miR-29b mimic) may represent a new powerful therapeutic strategy in patients with DR (Silva et al., 2011).

Bai et al. (2011) found a down-regulation of miR-126 in oxygen-induced retinopathy (OIR) model in mice and angiogenesis *via* regulating the signaling of angiogenic growth factors (Fish et al., 2008; Wang et al., 2008). Retinal NV, is a key pathological

alteration of PDR leading to dramatic visual loss and, as mentioned above, can be regulated by many angiogenic factors such as VEGF, HIF-1α and IGF. MiR-126 was found to be reduced in OIR mice and the restoration of MiR-126 levels inhibits the expression of vaso-active factors, such as VEGF, IGF, and HIF-1α expression resulting in reduction of retinal NV in ischemic condition. Bai et al. (2011) showed that the production of angiogenic factors regulated by miR-126 may depend on p38 and extracellular signal-regulated kinase (ERK), both enzymes of the MAPK pathway. It was suggested that in ischemia retina, reduction of miR-126 level could stimulate the activation of p38 and ERK pathway, and increase the expression of angiogenic factors down-stream. Recently, Ye et al. confirmed the downregulation of miR-126 in diabetic retinas of STZ-induced diabetic rats 24 h after diabetes onset (Ye et al., 2014). Mortuza et al. (2014) demonstrated an upregulation of miR-195 in human retinal microvascular ECs exposed to high glucose levels and a related downregulation of silent information regulator protein 1 (SIRT1).

SIRT1, the leading enzyme in the SIRT family is reported to play a crucial role in regulating cell cycle, survival, metabolism and development in mammals. Glucose-induced increased oxidative stress was shown to cause rapid aging in ECs and retinas in diabetes and these processes were mediated through alteration of SIRTs. SIRT1 downregulation in ECs, which also mediates aging-like changes, vascular permeability and FN upregulation in diabetes (Mortuza et al., 2013).

Our preliminary data on microarray analysis suggest a deregulation expression of circulating miRNAs potentially involved in key steps of disease progression. A relationship was detected between grade of DR and different expression of several miRNA (Poster presented at 13th International AMD and retinal congress, 2013, Dublin, Ireland).

9. Conclusion

The worldwide prevalence of diabetes is constantly increasing, exceeding previous estimates, and is associated with an increased incidence of diabetes-related complications. Among these, diabetic retinopathy still represents the first cause of blindness in westernized countries. A thorough understanding of the pathophysiological mechanisms underlying the development of these complications represents a key hurdle for improving the standard of care. MicroRNAs represent a new powerful class of modulator of gene expression and their involvement in pathologies such as diabetes mellitus and in particular in diabetic retinopathy are now starting to become clear.

The involvement of some MicroRNA in the pathogenetic mechanism of DR was reported by means of expression profiling studies and the specific role of few of these compounds were elucidated. The possibility of a single miRNA to regulate several target genes, thus influencing multiple molecular pathways has also been demonstrated. Although the number of studies is growing, the role of miRNAs in a complex biological event such as diabetic retinopathy needs to be fully understood and other miRNA molecules with their target genes involved in this pathological event still need to be discovered.

Understanding their role in retinopathy could be helpful for developing new gain- or loss-of-function strategies to effectively treat this disease and reduce the rate of patients developing blindness due to progression of retinopathy. MiRNA antagonists or mimics could be used as a new class of drugs to modulate the occurrence and progression of pathological alterations of DR.

Future direction

Understanding the role of microRNAs in retinopathy could be helpful for developing new gain- or loss-of-function strategies to effectively treat diabetic retinopathy and reduce the rate of patients developing blindness due to progression of retinopathy.

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References

Anand, S., Majeti, B.K., Acevedo, L.M., Murphy, E.A., Mukthavaram, R., Scheppke, L., Huang, M., Shields, D.J., Lindquist, J.N., Lapinski, P.E., King, P.D., Weis, S.M., Cheresh, D.A., 2010. MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. Nat. Med. 16, 909–914.

Andreeva, K., Cooper, N.G., 2014. MicroRNAs in the neural retina. Int. J. Genom. 2014, 165897.

Antonetti, D.A., Barber, A.J., Bronson, S.K., Freeman, W.M., Gardner, T.W., Jefferson, L.S., Kester, M., Kimball, S.R., Krady, J.K., LaNoue, K.F., Norbury, C.C., Quinn, P.G., Sandirasegarane, L., Simpson, I.A., Group, J.D.R.C., 2006. Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. Diabetes 55, 2401–2411.

Antonetti, D.A., Klein, R., Gardner, T.W., 2012. Diabetic retinopathy. N. Engl. J. Med. 366, 1227–1239.

Arora, A., Guduric-Fuchs, J., Harwood, L., Dellett, M., Cogliati, T., Simpson, D.A., 2010. Prediction of microRNAs affecting mRNA expression during retinal development. BMC Dev. Biol. 10, 1.

Arroyo, J.D., Chevillet, J.R., Kroh, E.M., Ruf, I.K., Pritchard, C.C., Gibson, D.F., Mitchell, P.S., Bennett, C.F., Pogosova-Agadjanyan, E.L., Stirewalt, D.L., Tait, J.F., Tewari, M., 2011. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. U. S. A. 108, 5003–5008.

Bai, Y., Bai, X., Wang, Z., Zhang, X., Ruan, C., Miao, J., 2011. MicroRNA-126 inhibits ischemia-induced retinal neovascularization via regulating angiogenic growth factors. Exp. Mol. Pathol. 91, 471–477.

Barber, A.J., Gardner, T.W., Abcouwer, S.F., 2011. The significance of vascular and neural apoptosis to the pathology of diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 52, 1156–1163.

Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–297.

Bartel, D.P., 2009. MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233.

Ben-Mahmud, B.M., Chan, W.H., Abdulahad, R.M., Datti, A., Orlacchio, A., Kohner, E.M., Chibber, R., 2006. Clinical validation of a link between TNF-alpha and the glycosylation enzyme core 2 GlcNAc-T and the relationship of this link to diabetic retinopathy. Diabetologia 49, 2185–2191.

Bhatwadekar, A.D., Glenn, J.V., Li, G., Curtis, T.M., Gardiner, T.A., Stitt, A.W., 2008. Advanced glycation of fibronectin impairs vascular repair by endothelial progenitor cells: implications for vasodegeneration in diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 49, 1232–1241.

Blumensatt, M., Wronkowitz, N., Wiza, C., Cramer, A., Mueller, H., Rabelink, M.J., Hoeben, R.C., Eckel, J., Sell, H., Ouwens, D.M., 2014. Adipocyte-derived factors impair insulin signaling in differentiated human vascular smooth muscle cells via the upregulation of miR-143. Biochim. Biophys. Acta 1842, 275–283.

Bonauer, A., Carmona, G., Iwasaki, M., Mione, M., Koyanagi, M., Fischer, A., Burchfield, J., Fox, H., Doebele, C., Ohtani, K., Chavakis, E., Potente, M., Tjwa, M., Urbich, C., Zeiher, A.M., Dimmeler, S., 2009. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 324, 1710–1713.

Brennecke, J., Stark, A., Russell, R.B., Cohen, S.M., 2005. Principles of microRNA-target recognition. PLoS Biol. 3, e85.

Brownlee, M., 2001. Biochemistry and molecular cell biology of diabetic complications. Nature 414, 813–820.

Brownlee, M., 2005. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54, 1615–1625.

Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., Rassenti, L., Kipps, T., Negrini, M., Bullrich, F., Croce, C.M., 2002. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. U. S. A. 99, 15524–15529.

Caporali, A., Emanueli, C., 2011. MicroRNA regulation in angiogenesis. Vasc. Pharmacol. 55, 79–86.

Caporali, A., Meloni, M., Vollenkle, C., Bonci, D., Sala-Newby, G.B., Addis, R., Spinetti, G., Losa, S., Masson, R., Baker, A.H., Agami, R., le Sage, C., Condorelli, G., Madeddu, P., Martelli, F., Emanueli, C., 2011. Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia. Circulation 123, 282–291.

Chan, Y.C., Khanna, S., Roy, S., Sen, C.K., 2011. miR-200b targets Ets-1 and is down-regulated by hypoxia to induce angiogenic response of endothelial cells. J. Biol. Chem. 286, 2047–2056.

Chen, M., Curtis, T.M., Stitt, A.W., 2013. Advanced glycation end products and diabetic retinopathy. Curr. Med. Chem. 20, 3234–3240.
Chen, Y., Gorski, D.H., 2008. Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5. Blood 111, 1217–1226.
Cheung, A.K., Fung, M.K., Lo, A.C., Lam, T.T., So, K.F., Chung, S.S., Chung, S.K., 2005. Aldose reductase deficiency prevents diabetes-induced blood-retinal barrier breakdown, apoptosis, and glial reactivation in the retina of db/db mice. Diabetes 54, 3119–3125.
Ciulla, T.A., Amador, A.G., Zinman, B., 2003. Diabetic retinopathy and diabetic macular edema: pathophysiology, screening, and novel therapies. Diabetes Care 26, 2653–2664.
Clermont, A.C., Cahill, M., Salti, H., Rook, S.L., Rask-Madsen, C., Goddard, L., Wong, J.S., Bursell, D., Bursell, S.E., Aiello, L.P., 2006. Hepatocyte growth factor induces retinal vascular permeability via MAP-kinase and PI-3 kinase without altering retinal hemodynamics. Invest. Ophthalmol. Vis. Sci. 47, 2701–2708.
Cordes, K.R., Sheehy, N.T., White, M.P., Berry, E.C., Morton, S.U., Muth, A.N., Lee, T.H., Miano, J.M., Ivey, K.N., Srivastava, D., 2009. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature 460, 705–710.
Creemers, E.E., Tijsen, A.J., Pinto, Y.M., 2012. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? Circ. Res. 110, 483–495.
Cross, M.J., Claesson-Welsh, L., 2001. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. Trends Pharmacol. Sci. 22, 201–207.
Curtis, T.M., Gardiner, T.A., Stitt, A.W., 2009. Microvascular lesions of diabetic retinopathy: clues towards understanding pathogenesis? Eye (Lond) 23, 1496–1508.
D'Ambrogio, A., Gu, W., Udagawa, T., Mello, C.C., Richter, J.D., 2012. Specific miRNA stabilization by Gld2-catalyzed monoadenylation. Cell. Rep. 2, 1537–1545.
Dai, R., Ahmed, S.A., 2011. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Transl. Res. 157, 163–179.
Damiani, D., Alexander, J.J., O'Rourke, J.R., McManus, M., Jadhav, A.P., Cepko, C.L., Hauswirth, W.W., Harfe, B.D., Strettoi, E., 2008. Dicer inactivation leads to progressive functional and structural degeneration of the mouse retina. J. Neurosci. 28, 4878–4887.
de Sousa, E., Walter, L.T., Higa, G.S., Casado, O.A., Kihara, A.H., 2013. Developmental and functional expression of miRNA-stability related genes in the nervous system. PLoS One 8, e56908.
Dews, M., Homayouni, A., Yu, D., Murphy, D., Sevignani, C., Wentzel, E., Furth, E.E., Lee, W.M., Enders, G.H., Mendell, J.T., Thomas-Tikhonenko, A., 2006. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat. Genet. 38, 1060–1065.
Didiano, D., Hobert, O., 2006. Perfect seed pairing is not a generally reliable predictor for miRNA-target interactions. Nat. Struct. Mol. Biol. 13, 849–851.
Ding, J., Wong, T.Y., 2012. Current epidemiology of diabetic retinopathy and diabetic macular edema. Curr. Diab. Rep. 12, 346–354.
Dunmire, J.J., Lagouros, E., Bouhenni, R.A., Jones, M., Edward, D.P., 2013. MicroRNA in aqueous humor from patients with cataract. Exp. Eye Res. 108, 68–71.
Early Treatment Diabetic Retinopathy Study Research Group, 1991. Grading diabetic retinopathy from stereoscopic color fundus photographs – an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group Ophthalmology 98, 786–806.
Egea, V., Schober, A., Weber, C., 2012. Circulating miRNAs: messengers on the move in cardiovascular disease. Thromb. Haemost. 108, 590–591.
Ehrlich, R., Harris, A., Ciulla, T.A., Kheradiya, N., Winston, D.M., Wirostko, B., 2010. Diabetic macular oedema: physical, physiological and molecular factors contribute to this pathological process. Acta Ophthalmol. 88, 279–291.
El Ouaamari, A., Baroukh, N., Martens, G.A., Lebrun, P., Pipeleers, D., van Obberghen, E., 2008. miR-375 targets 3′-phosphoinositide-dependent protein kinase-1 and regulates glucose-induced biological responses in pancreatic beta-cells. Diabetes 57, 2708–2717.
El-Remessy, A.B., Abou-Mohamed, G., Caldwell, R.W., Caldwell, R.B., 2003. High glucose-induced tyrosine nitration in endothelial cells: role of eNOS uncoupling and aldose reductase activation. Invest. Ophthalmol. Vis. Sci. 44, 3135–3143.
Fasanaro, P., D'Alessandra, Y., Di Stefano, V., Melchionna, R., Romani, S., Pompilio, G., Capogrossi, M.C., Martelli, F., 2008. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J. Biol. Chem. 283, 15878–15883.
Feng, B., Chen, S., McArthur, K., Wu, Y., Sen, S., Ding, Q., Feldman, R.D., Chakrabarti, S., 2011. miR-146a-Mediated extracellular matrix protein production in chronic diabetes complications. Diabetes 60, 2975–2984.
Feng, Y., Wang, Y., Stock, O., Pfister, F., Tanimoto, N., Seeliger, M.W., Hillebrands, J.L., Hoffmann, S., Wolburg, H., Gretz, N., Hammes, H.P., 2009. Vasoregression linked to neuronal damage in the rat with defect of polycystin-2. PLoS One 4, e7328.
Filipowicz, W., Bhattacharyya, S.N., Sonenberg, N., 2008. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat. Rev. Genet. 9, 102–114.
Fish, J.E., Santoro, M.M., Morton, S.U., Yu, S., Yeh, R.F., Wythe, J.D., Ivey, K.N., Bruneau, B.G., Stainier, D.Y., Srivastava, D., 2008. miR-126 regulates angiogenic signaling and vascular integrity. Dev. Cell. 15, 272–284.
Fitch, M.J., Campagnolo, L., Kuhnert, F., Stuhlmann, H., 2004. Egfl7, a novel epidermal growth factor-domain gene expressed in endothelial cells. Dev. Dyn.: Off. Publ. Am. Assoc. Anat. 230, 316–324.
Frank, R.N., 2004. Diabetic retinopathy. N. Engl. J. Med. 350, 48–58.
Friedman, R.C., Farh, K.K., Burge, C.B., Bartel, D.P., 2009. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 19, 92–105.
Frost, R.J., Olson, E.N., 2011. Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs. Proc. Natl. Acad. Sci. U. S. A. 108, 21075–21080.
Funatsu, H., Yamashita, H., Ikeda, T., Mimura, T., Eguchi, S., Hori, S., 2003. Vitreous levels of interleukin-6 and vascular endothelial growth factor are related to diabetic macular edema. Ophthalmology 110, 1690–1696.
Funatsu, H., Yamashita, H., Noma, H., Mimura, T., Yamashita, T., Hori, S., 2002. Increased levels of vascular endothelial growth factor and interleukin-6 in the aqueous humor of diabetics with macular edema. Am. J. Ophthalmol. 133, 70–77.
Ghosh, G., Subramanian, I.V., Adhikari, N., Zhang, X., Joshi, H.P., Basi, D., Chandrashekhar, Y.S., Hall, J.L., Roy, S., Zeng, Y., Ramakrishnan, S., 2010. Hypoxia-induced microRNA-424 expression in human endothelial cells regulates HIF-alpha isoforms and promotes angiogenesis. J. Clin. Invest. 120, 4141–4154.
Guo, C., Sah, J.F., Beard, L., Willson, J.K., Markowitz, S.D., Guda, K., 2008. The noncoding RNA, miR-126, suppresses the growth of neoplastic cells by targeting phosphatidylinositol 3-kinase signaling and is frequently lost in colon cancers. Genes. Chromosom. Cancer 47, 939–946.
Guo, H., Ingolia, N.T., Weissman, J.S., Bartel, D.P., 2010. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466, 835–840.
Guo, L., Lu, Z., 2010. The fate of miRNA* strand through evolutionary analysis: implication for degradation as merely carrier strand or potential regulatory molecule? PLoS One 5, e11387.
Harris, T.A., Yamakuchi, M., Ferlito, M., Mendell, J.T., Lowenstein, C.J., 2008. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc. Natl. Acad. Sci. U. S. A. 105, 1516–1521.
Hirase, T., Kawashima, S., Wong, E.Y., Ueyama, T., Rikitake, Y., Tsukita, S., Yokoyama, M., Staddon, J.M., 2001. Regulation of tight junction permeability and occludin phosphorylation by Rhoa-p160ROCK-dependent and -independent mechanisms. J. Biol. Chem. 276, 10423–10431.
Hua, Z., Lv, Q., Ye, W., Wong, C.K., Cai, G., Gu, D., Ji, Y., Zhao, C., Wang, J., Yang, B.B., Zhang, Y., 2006. MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. PLoS One 1, e116.
Hutvagner, G., Zamore, P.D., 2002. A microRNA in a multiple-turnover RNAi enzyme complex. Science 297, 2056–2060.
Hwang, H.W., Wentzel, E.A., Mendell, J.T., 2007. A hexanucleotide element directs microRNA nuclear import. Science 315, 97–100.
Ito, T., Yang, M., May, W.S., 1999. RAX, a cellular activator for double-stranded RNA-dependent protein kinase during stress signaling. J. Biol. Chem. 274, 15427–15432.
Jansen, F., Yang, X., Hoelscher, M., Cattelan, A., Schmitz, T., Proebsting, S., Wenzel, D., Vosen, S., Franklin, B.S., Fleischmann, B.K., Nickenig, G., Werner, N., 2013. Endothelial microparticle-mediated transfer of MicroRNA-126 promotes vascular endothelial cell repair via SPRED1 and is abrogated in glucose-damaged endothelial microparticles. Circulation 128, 2026–2038.
Jordan, S.D., Kruger, M., Willmes, D.M., Redemann, N., Wunderlich, F.T., Bronneke, H.S., Merkwirth, C., Kashkar, H., Olkkonen, V.M., Bottger, T., Braun, T., Seibler, J., Bruning, J.C., 2011. Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. Nat. Cell. Biol. 13, 434–446.
Kai, Z.S., Pasquinelli, A.E., 2010. MicroRNA assassins: factors that regulate the disappearance of miRNAs. Nat. Struct. Mol. Biol. 17, 5–10.
Kaji, Y., Usui, T., Ishida, S., Yamashiro, K., Moore, T.C., Moore, J., Yamamoto, Y., Yamamoto, H., Adamis, A.P., 2007. Inhibition of diabetic leukostasis and blood-retinal barrier breakdown with a soluble form of a receptor for advanced glycation end products. Invest. Ophthalmol. Vis. Sci. 48, 858–865.
Kane, N.M., Thrasher, A.J., Angelini, G.D., Emanueli, C., 2014. MicroRNAs as modulators of stem cells and angiogenesis. Stem Cells 32, 1059–1066.
Karolina, D.S., Armugam, A., Tavintharan, S., Wong, M.T., Lim, S.C., Sum, C.F., Jeyaseelan, K., 2011. MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. PLoS One 6, e22839.
Kerr, T.A., Korenblat, K.M., Davidson, N.O., 2011. MicroRNAs and liver disease. Transl. Res. 157, 241–252.
Khaliq, A., Foreman, D., Ahmed, A., Weich, H., Gregor, Z., McLeod, D., Boulton, M., 1998. Increased expression of placenta growth factor in proliferative diabetic retinopathy. Lab. Invest. 78, 109–116.
Khan, Z.A., Chakrabarti, S., 2007. Cellular signaling and potential new treatment targets in diabetic retinopathy. Exp. Diab. Res. 2007, 31867.
Khan, Z.A., Farhangkhoee, H., Chakrabarti, S., 2006. Towards newer molecular targets for chronic diabetic complications. Curr. Vasc. Pharmacol. 4, 45–57.
Kim, I., Moon, S.O., Kim, S.H., Kim, H.J., Koh, Y.S., Koh, G.Y., 2001. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. J. Biol. Chem. 276, 7614–7620.
Kinjo, E.R., Higa, G.S., de Sousa, E., Casado, O.A., Damico, M.V., Britto, L.R., Kihara, A.H., 2013. A possible new mechanism for the control of miRNA expression in neurons. Exp. Neurol. 248, 546–558.
Knudsen, S.T., Bek, T., Poulsen, P.L., Hove, M.N., Rehling, M., Mogensen, C.E., 2002. Macular edema reflects generalized vascular hyperpermeability in type 2 diabetic patients with retinopathy. Diabetes Care 25, 2328–2334.
Kong, L., Zhu, J., Han, W., Jiang, X., Xu, M., Zhao, Y., Dong, Q., Pang, Z., Guan, Q., Gao, L., Zhao, J., Zhao, L., 2011. Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study. Acta Diabetol. 48, 61–69.

Kong, Y.W., Ferland-McCollough, D., Jackson, T.J., Bushell, M., 2012. microRNAs in cancer management. Lancet Oncol. 13, e249–258.
Kovacs, B., Lumayag, S., Cowan, C., Xu, S., 2011. MicroRNAs in early diabetic retinopathy in streptozotocin-induced diabetic rats. Invest. Ophthalmol. Vis. Sci. 52, 4402–4409.
Kowluru, R.A., Koppolu, P., Chakrabarti, S., Chen, S., 2003. Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. Free Radic. Res. 37, 1169–1180.
Kroll, P., Rodrigues, E.B., Hoerle, S., 2007. Pathogenesis and classification of proliferative diabetic vitreoretinopathy. Ophthalmologica 221, 78–94.
Kuehbacher, A., Urbich, C., Zeiher, A.M., Dimmeler, S., 2007. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ. Res. 101, 59–68.
Kuhnert, F., Mancuso, M.R., Hampton, J., Stankunas, K., Asano, T., Chen, C.Z., Kuo, C.J., 2008. Attribution of vascular phenotypes of the murine Egfl7 locus to the microRNA miR-126. Development 135, 3989–3993.
Lagos-Quintana, M., Rauhut, R., Lendeckel, W., Tuschl, T., 2001. Identification of novel genes coding for small expressed RNAs. Science 294, 853–858.
Long, J., Wang, Y., Wang, W., Chang, B.H., Danesh, F.R., 2010. Identification of microRNA-93 as a novel regulator of vascular endothelial growth factor in hyperglycemic conditions. J. Biol. Chem. 285, 23457–23465.
Loyer, X., Vion, A.C., Tedgui, A., Boulanger, C.M., 2014. Microvesicles as cell-cell messengers in cardiovascular diseases. Circ. Res. 114, 345–353.
Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S.Y., Alvarado, M., Anderson, H.R., Anderson, L.M., Andrews, K.G., Atkinson, C., Baddour, L.M., Barker-Collo, S., Bartels, D.H., Bell, M.L., Benjamin, E.J., Bennett, D., Bhalla, K., Bikbov, B., Bin Abdulhak, A., Birbeck, G., Blyth, F., Bolliger, I., Boufous, S., Bucello, C., Burch, M., Burney, P., Carapetis, J., Chen, H., Chou, D., Chugh, S.S., Coffeng, L.E., Colan, S.D., Colquhoun, S., Colson, K.E., Condon, J., Connor, M.D., Cooper, L.T., Corriere, M., Cortinovis, M., de Vaccaro, K.C., Couser, W., Cowie, B.C., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahodwala, N., De Leo, D., Degenhardt, L., Delossantos, A., Denenberg, J., Des Jarlais, D.C., Dharmaratne, S.D., Dorsey, E.R., Driscoll, T., Duber, H., Ebel, B., Erwin, P.J., Espindola, P., Ezzati, M., Feigin, V., Flaxman, A.D., Forouzanfar, M.H., Fowkes, F.G., Franklin, R., Fransen, M., Freeman, M.K., Gabriel, S.E., Gakidou, E., Gaspari, F., Gillum, R.F., Gonzalez-Medina, D., Halasa, Y.A., Haring, D., Harrison, J.E., Havmoeller, R., Hay, R.J., Hoen, B., Hotez, P.J., Hoy, D., Jacobsen, K.H., James, S.L., Jasrasaria, R., Jayaraman, S., Johns, N., Karthikeyan, G., Kassebaum, N., Keren, A., Khoo, J.P., Knowlton, L.M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Lipnick, M., Lipshultz, S.E., Ohno, S.L., Mabweijano, J., MacIntyre, M.F., Mallinger, L., March, L., Marks, G.B., Marks, R., Matsumori, A., Matzopoulos, R., Mayosi, B.M., McAnulty, J.H., McDermott, M.M., McGrath, J., Mensah, G.A., Merriman, T.R., Michaud, C., Miller, M., Miller, T.R., Mock, C., Mocumbi, A.O., Mokdad, A.A., Moran, A., Mulholland, K., Nair, M.N., Naldi, L., Narayan, K.M., Nasseri, K., Norman, P., O'Donnell, M., Omer, S.B., Ortblad, K., Osborne, R., Ozgediz, D., Pahari, B., Pandian, J.D., Rivero, A.P., Padilla, R.P., Perez-Ruiz, F., Perico, N., Phillips, D., Pierce, K., Pope 3rd, C.A., Porrini, E., Pourmalek, F., Raju, M., Ranganathan, D., Rehm, J.T., Rein, D.B., Remuzzi, G., Rivara, F.P., Roberts, T., De Leon, F.R., Rosenfeld, L.C., Rushton, L., Sacco, R.L., Salomon, J.A., Sampson, U., Sanman, E., Schwebel, D.C., Segui-Gomez, M., Shepard, D.S., Singh, D., Singleton, J., Sliwa, K., Smith, E., Steer, A., Taylor, J.A., Thomas, B., Tleyjeh, I.M., Towbin, J.A., Truelsen, T., Undurraga, E.A., Venketasubramanian, N., Vijayakumar, L., Vos, T., Wagner, G.R., Wang, M., Wang, W., Watt, K., Weinstock, M.A., Weintraub, R., Wilkinson, J.D., Woolf, A.D., Wulf, S., Yeh, P.H., Yip, P., Zabetian, A., Zheng, Z.J., Lopez, A.D., Murray, C.J., AlMazroa, M.A., Memish, Z.A., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2095–2128.
Lutty, G.A., 2013. Effects of diabetes on the eye. Invest. Ophthalmol. Vis. Sci. 54, ORSF81–87.
Ly, A., Yee, P., Vessey, K.A., Phipps, J.A., Jobling, A.I., Fletcher, E.L., 2011. Early inner retinal astrocyte dysfunction during diabetes and development of hypoxia, retinal stress, and neuronal functional loss. Invest. Ophthalmol. Vis. Sci. 52, 9316–9326.
Lynn, F.C., Skewes-Cox, P., Kosaka, Y., McManus, M.T., Harfe, B.D., German, M.S., 2007. MicroRNA expression is required for pancreatic islet cell genesis in the mouse. Diabetes 56, 2938–2945.
Magenta, A., Cencioni, C., Fasanaro, P., Zaccagnini, G., Greco, S., Sarra-Ferraris, G., Antonini, A., Martelli, F., Capogrossi, M.C., 2011. miR-200c is upregulated by oxidative stress and induces endothelial cell apoptosis and senescence via ZEB1 inhibition. Cell. Death Differ. 18, 1628–1639.
McArthur, K., Feng, B., Wu, Y., Chen, S., Chakrabarti, S., 2011. MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. Diabetes 60, 1314–1323.
Menghini, R., Casagrande, V., Cardellini, M., Martelli, E., Terrinoni, A., Amati, F., Vasa-Nicotera, M., Ippoliti, A., Novelli, G., Melino, G., Lauro, R., Federici, M., 2009. MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. Circulation 120, 1524–1532.
Michael, M.Z., O' Connor, S.M., van Holst Pellekaan, N.G., Young, G.P., James, R.J., 2003. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol. Cancer Res. 1, 882–891.
Mitamura, Y., Tashimo, A., Nakamura, Y., Tagawa, H., Ohtsuka, K., Mizue, Y., Nishihira, J., 2002. Vitreous levels of placenta growth factor and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. Diabetes Care 25, 2352.
Mitchell, P.S., Parkin, R.K., Kroh, E.M., Fritz, B.R., Wyman, S.K., Pogosova-Agadjanyan, E.L., Peterson, A., Noteboom, J., O'Briant, K.C., Allen, A., Lin, D.W., Urban, N., Drescher, C.W., Knudsen, B.S., Stirewalt, D.L., Gentleman, R., Vessella, R.L., Nelson, P.S., Martin, D.B., Tewari, M., 2008. Circulating microRNAs as stable blood-based markers for cancer detection. Proc. Natl. Acad. Sci. U. S. A. 105, 10513–10518.
Miyamoto, K., Khosrof, S., Bursell, S.E., Rohan, R., Murata, T., Clermont, A.C., Aiello, L.P., Ogura, Y., Adamis, A.P., 1999. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. Proc. Natl. Acad. Sci. U. S. A. 96, 10836–10841.
Miyamoto, N., de Kozak, Y., Jeanny, J.C., Glotin, A., Mascarelli, F., Massin, P., BenEzra, D., Behar-Cohen, F., 2007. Placental growth factor-1 and epithelial haemato-retinal barrier breakdown: potential implication in the pathogenesis of diabetic retinopathy. Diabetologia 50, 461–470.
Mortuza, R., Chen, S., Feng, B., Sen, S., Chakrabarti, S., 2013. High glucose induced alteration of SIRTs in endothelial cells causes rapid aging in a p300 and FOXO regulated pathway. PLoS One 8, e54514.
Mortuza, R., Feng, B., Chakrabarti, S., 2014. miR-195 regulates SIRT1-mediated changes in diabetic retinopathy. Diabetologia 57, 1037–1046.
Murray, A.R., Chen, Q., Takahashi, Y., Zhou, K.K., Park, K., Ma, J.X., 2013. MicroRNA-200b downregulates oxidation resistance 1 (Oxr1) expression in the retina of type 1 diabetes model. Invest. Ophthalmol. Vis. Sci. 54, 1689–1697.
Nguyen, M., Arkell, J., Jackson, C.J., 2001. Human endothelial gelatinases and angiogenesis. Int. J. Biochem. Cell Biol. 33, 960–970.
Nicoli, S., Standley, C., Walker, P., Hurlstone, A., Fogarty, K.E., Lawson, N.D., 2010. MicroRNA-mediated integration of haemodynamics and Vegf signalling during angiogenesis. Nature 464, 1196–1200.
Nishikiori, N., Osanai, M., Chiba, H., Kojima, T., Mitamura, Y., Ohguro, H., Sawada, N., 2007. Glial cell-derived cytokines attenuate the breakdown of vascular integrity in diabetic retinopathy. Diabetes 56, 1333–1340.
Okamura, K., Hagen, J.W., Duan, H., Tyler, D.M., Lai, E.C., 2007. The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. Cell 130, 89–100.
Ola, M.S., Nawaz, M.I., Khan, H.A., Alhomida, A.S., 2013. Neurodegeneration and neuroprotection in diabetic retinopathy. Int. J. Mol. Sci. 14, 2559–2572.
Ortega, F.J., Mercader, J.M., Moreno-Navarrete, J.M., Rovira, O., Guerra, E., Esteve, E., Xifra, G., Martinez, C., Ricart, W., Rieusset, J., Rome, S., Karczewska-Kupczewska, M., Straczkowski, M., Fernandez-Real, J.M., 2014. Profiling of circulating MicroRNAs reveals common MicroRNAs linked to type 2 diabetes that change with insulin sensitization. Diabetes Care 37, 1375–1383.
Otrock, Z.K., Makarem, J.A., Shamseddine, A.I., 2007. Vascular endothelial growth factor family of ligands and receptors: review. Blood Cells Mol. Dis. 38, 258–268.
Otsuka, M., Zheng, M., Hayashi, M., Lee, J.D., Yoshino, O., Lin, S., Han, J., 2008. Impaired microRNA processing causes corpus luteum insufficiency and infertility in mice. J. Clin. Invest. 118, 1944–1954.
Penfold, P.L., Wen, L., Madigan, M.C., King, N.J., Provis, J.M., 2002. Modulation of permeability and adhesion molecule expression by human choroidal endothelial cells. Invest. Ophthalmol. Vis. Sci. 43, 3125–3130.
Peters, L., Meister, G., 2007. Argonaute proteins: mediators of RNA silencing. Mol. Cell. 26, 611–623.
Plaisance, V., Abderrahmani, A., Perret-Menoud, V., Jacquemin, P., Lemaigre, F., Regazzi, R., 2006. MicroRNA-9 controls the expression of Granuphilin/Slp4 and the secretory response of insulin-producing cells. J. Biol. Chem. 281, 26932–26942.
Poliseno, L., Tuccoli, A., Mariani, L., Evangelista, M., Citti, L., Woods, K., Mercatanti, A., Hammond, S., Rainaldi, G., 2006. MicroRNAs modulate the angiogenic properties of HUVECs. Blood 108, 3068–3071.
Poy, M.N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P.E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P., Stoffel, M., 2004. A pancreatic islet-specific microRNA regulates insulin secretion. Nature 432, 226–230.
Ragusa, M., Caltabiano, R., Russo, A., Puzzo, L., Avitabile, T., Longo, A., Toro, M.D., Di Pietro, C., Purrello, M., Reibaldi, M., 2013. MicroRNAs in vitreus humor from patients with ocular diseases. Mol. Vis. 19, 430–440.
Rezzola, S., Belleri, M., Gariano, G., Ribatti, D., Costagliola, C., Semeraro, F., Presta, M., 2013. In vitro and ex vivo retina angiogenesis assays. Angiogenesis 17, 429–442.
Rong, Y., Bao, W., Shan, Z., Liu, J., Yu, X., Xia, S., Gao, H., Wang, X., Yao, P., Hu, F.B., Liu, L., 2013. Increased microRNA-146a levels in plasma of patients with newly diagnosed type 2 diabetes mellitus. PLoS One 8, e73272.
Santovito, D., De Nardis, V., Marcantonio, P., Mandolini, C., Paganelli, C., Vitale, E., Buttitta, F., Bucci, M., Mezzetti, A., Consoli, A., Cipollone, F., 2014. Plasma exosome microRNA profiling unravels a new potential modulator of adiponectin pathway in Diabetes: effect of glycemic control. J. Clin. Endocrinol. Metab., jc20133843.
Santovito, D., Mandolini, C., Marcantonio, P., De Nardis, V., Bucci, M., Paganelli, C., Magnacca, F., Ucchino, S., Mastroiacovo, D., Desideri, G., Mezzetti, A., Cipollone, F., 2013. Overexpression of microRNA-145 in atherosclerotic plaques from hypertensive patients. Expert Opin. Ther. Targets 17, 217–223.
Santovito, D., Mezzetti, A., Cipollone, F., 2012. MicroRNAs and atherosclerosis: new actors for an old movie. Nutr. Metab. Cardiovasc. Dis. 22, 937–943.
Schober, A., Nazari-Jahantigh, M., Wei, Y., Bidzhekov, K., Gremse, F., Grommes, J., Megens, R.T., Heyll, K., Noels, H., Hristov, M., Wang, S., Kiessling, F., Olson, E.N., Weber, C., 2014. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat. Med. 20, 368–376.
Schroder, S., Palinski, W., Schmid-Schonbein, G.W., 1991. Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. Am. J. Pathol. 139, 81–100.

Shams, N., Ianchulev, T., 2006. Role of vascular endothelial growth factor in ocular angiogenesis. Ophthalmol. Clin. North. Am. 19, 335–344.
Shen, J., Yang, X., Xie, B., Chen, Y., Swaim, M., Hackett, S.F., Campochiaro, P.A., 2008. MicroRNAs regulate ocular neovascularization. Mol. Ther. 16, 1208–1216.
Shilo, S., Roy, S., Khanna, S., Sen, C.K., 2008. Evidence for the involvement of miRNA in redox regulated angiogenic response of human microvascular endothelial cells. Arterioscler. Thromb. Vasc. Biol. 28, 471–477.
Silva, V.A., Polesskaya, A., Sousa, T.A., Correa, V.M., Andre, N.D., Reis, R.I., Kettelhut, I.C., Harel-Bellan, A., De Lucca, F.L., 2011. Expression and cellular localization of microRNA-29b and RAX, an activator of the RNA-dependent protein kinase (PKR), in the retina of streptozotocin-induced diabetic rats. Mol. Vis. 17, 2228–2240.
Smits, M., Mir, S.E., Nilsson, R.J., van der Stoop, P.M., Niers, J.M., Marquez, V.E., Cloos, J., Breakefield, X.O., Krichevsky, A.M., Noske, D.P., Tannous, B.A., Wurdinger, T., 2011. Down-regulation of miR-101 in endothelial cells promotes blood vessel formation through reduced repression of EZH2. PLoS One 6, e16282.
Suarez, Y., Fernandez-Hernando, C., Yu, J., Gerber, S.A., Harrison, K.D., Pober, J.S., Iruela-Arispe, M.L., Merkenschlager, M., Sessa, W.C., 2008. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc. Natl. Acad. Sci. U. S. A. 105, 14082–14087.
Taganov, K.D., Boldin, M.P., Chang, K.J., Baltimore, D., 2006. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc. Natl. Acad. Sci. U. S. A. 103, 12481–12486.
Takahashi, H., Shibuya, M., 2005. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. Clin. Sci. (Lond) 109, 227–241.
The Early Treatment Diabetic Retinopathy Study Research Group, 1987. Photocoagulation for diabetic macular edema: Early Treatment Diabetic Retinopathy Study Report no. 4. Int. Ophthalmol. Clin. 27, 265–272.
Trajkovski, M., Hausser, J., Soutschek, J., Bhat, B., Akin, A., Zavolan, M., Heim, M.H., Stoffel, M., 2011. MicroRNAs 103 and 107 regulate insulin sensitivity. Nature 474, 649–653.
Tuo, J., Shen, D., Yang, H.H., Chan, C.C., 2014. Distinct microRNA-155 expression in the vitreous of patients with primary vitreoretinal lymphoma and uveitis. Am. J. Ophthalmol. 157, 728–734.
Vasudevan, S., Tong, Y., Steitz, J.A., 2007. Switching from repression to activation: microRNAs can up-regulate translation. Science 318, 1931–1934.
Vickers, K.C., Palmisano, B.T., Shoucri, B.M., Shamburek, R.D., Remaley, A.T., 2011. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat. Cell. Biol. 13, 423–433.
Villarroel, M., Ciudin, A., Hernandez, C., Simo, R., 2010. Neurodegeneration: an early event of diabetic retinopathy. World J. Diab. 1, 57–64.
Villeneuve, L.M., Reddy, M.A., Natarajan, R., 2011. Epigenetics: deciphering its role in diabetes and its chronic complications. Clin. Exp. Pharmacol. Physiol. 38, 451–459.
Wang, S., Aurora, A.B., Johnson, B.A., Qi, X., McAnally, J., Hill, J.A., Richardson, J.A., Bassel-Duby, R., Olson, E.N., 2008. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev. Cell. 15, 261–271.
Wang, S., Olson, E.N., 2009. AngiomiRs–key regulators of angiogenesis. Curr. Opin. Genet. Dev. 19, 205–211.
Wei, Y., Li, L., Wang, D., Zhang, C.Y., Zen, K., 2014. Importin 8 regulates the transport of mature microRNAs into the cell nucleus. J. Biol. Chem. 289, 10270–10275.
Wilkinson, C.P., Ferris 3rd, F.L., Klein, R.E., Lee, P.P., Agardh, C.D., Davis, M., Dills, D., Kampik, A., Pararajasegaram, R., Verdaguer, J.T., Global Diabetic Retinopathy Project, 2003. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 110, 1677–1682.
Wirostko, B., Wong, T.Y., Simo, R., 2008. Vascular endothelial growth factor and diabetic complications. Prog. Retin. Eye Res. 27, 608–621.
Witkos, T.M., Koscianska, E., Krzyzosiak, W.J., 2011. Practical aspects of microRNA target prediction. Curr. Mol. Med. 11, 93–109.
Wu, J.H., Gao, Y., Ren, A.J., Zhao, S.H., Zhong, M., Peng, Y.J., Shen, W., Jing, M., Liu, L., 2012. Altered microRNA expression profiles in retinas with diabetic retinopathy. Ophthal. Res. 47, 195–201.
Wurdinger, T., Tannous, B.A., Saydam, O., Skog, J., Grau, S., Soutschek, J., Weissleder, R., Breakefield, X.O., Krichevsky, A.M., 2008. miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. Cancer Cell 14, 382–393.
Xu, J., Zhu, D., Sonoda, S., He, S., Spee, C., Ryan, S.J., Hinton, D.R., 2012. Overexpression of BMP4 inhibits experimental choroidal neovascularization by modulating VEGF and MMP-9. Angiogenesis 15, 213–227.
Yang, Z., Chen, H., Si, H., Li, X., Ding, X., Sheng, Q., Chen, P., Zhang, H., 2014. Serum miR-23a, a potential biomarker for diagnosis of pre-diabetes and type 2 diabetes. Acta Diabetol. Jul 1. [Epub ahead of print].
Yau, J.W., Rogers, S.L., Kawasaki, R., Lamoureux, E.L., Kowalski, J.W., Bek, T., Chen, S.J., Dekker, J.M., Fletcher, A., Grauslund, J., Haffner, S., Hamman, R.F., Ikram, M.K., Kayama, T., Klein, B.E., Klein, R., Krishnaiah, S., Mayurasakorn, K., O'Hare, J.P., Orchard, T.J., Porta, M., Rema, M., Roy, M.S., Sharma, T., Shaw, J., Taylor, H., Tielsch, J.M., Varma, R., Wang, J.J., Wang, N., West, S., Xu, L., Yasuda, M., Zhang, X., Mitchell, P., Wong, T.Y., Meta-Analysis for Eye Disease Study Group, 2012. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care 35, 556–564.
Ye, P., Liu, J., He, F., Xu, W., Yao, K., 2014. Hypoxia-induced deregulation of miR-126 and its regulative effect on VEGF and MMP-9 expression. Int. J. Med. Sci. 11, 17–23.
Zampetaki, A., Kiechl, S., Drozdov, I., Willeit, P., Mayr, U., Prokopi, M., Mayr, A., Weger, S., Oberhollenzer, F., Bonora, E., Shah, A., Willeit, J., Mayr, M., 2010. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ. Res. 107, 810–817.
Zernecke, A., Bidzhekov, K., Noels, H., Shagdarsuren, E., Gan, L., Denecke, B., Hristov, M., Koppel, T., Jahantigh, M.N., Lutgens, E., Wang, S., Olson, E.N., Schober, A., Weber, C., 2009. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci. Signal 2, ra81.
Zhang, X., Bao, S., Hambly, B.D., Gillies, M.C., 2009. Vascular endothelial growth factor-A: a multifunctional molecular player in diabetic retinopathy. Int. J. Biochem. Cell Biol. 41, 2368–2371.
Zhang, Y., Liu, D., Chen, X., Li, J., Li, L., Bian, Z., Sun, F., Lu, J., Yin, Y., Cai, X., Sun, Q., Wang, K., Ba, Y., Wang, Q., Wang, D., Yang, J., Liu, P., Xu, T., Yan, Q., Zhang, J., Zen, K., Zhang, C.Y., 2010. Secreted monocytic miR-150 enhances targeted endothelial cell migration. Mol. Cell. 39, 133–144.
Zhang, Y., Yang, P., Sun, T., Li, D., Xu, X., Rui, Y., Li, C., Chong, M., Ibrahim, T., Mercatali, L., Amadori, D., Lu, X., Xie, D., Li, Q.J., Wang, X.F., 2013. miR-126 and miR-126* repress recruitment of mesenchymal stem cells and inflammatory monocytes to inhibit breast cancer metastasis. Nat. Cell. Biol. 15, 284–294.
Zhao, H., Guan, J., Lee, H.M., Sui, Y., He, L., Siu, J.J., Tse, P.P., Tong, P.C., Lai, F.M., Chan, J.C., 2010a. Upregulated pancreatic tissue microRNA-375 associates with human type 2 diabetes through beta-cell deficit and islet amyloid deposition. Pancreas 39, 843–846.
Zhao, T., Li, J., Chen, A.F., 2010b. MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1. Am. J. Physiol. Endocrinol. Metab. 299, E110–E116.
Zhou, Q., Gallagher, R., Ufret-Vincenty, R., Li, X., Olson, E.N., Wang, S., 2011. Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23~27~24 clusters. Proc. Natl. Acad. Sci. U. S. A. 108, 8287–8292.
Zhou, J., Li, Y.S., Nguyen, P., Wang, K.C., Weiss, A., Kuo, Y.C., Chiu, J.J., Shyy, J.Y., Chien, S., 2013. Regulation of vascular smooth muscle cell turnover by endothelial cell-secreted microRNA-126: role of shear stress. Circ. Res. 113, 40–51.
Zhu, H., Shah, S., Shyh-Chang, N., Shinoda, G., Einhorn, W.S., Viswanathan, S.R., Takeuchi, A., Grasemann, C., Rinn, J.L., Lopez, M.F., Hirschhorn, J.N., Palmert, M.R., Daley, G.Q., 2010. Lin28a transgenic mice manifest size and puberty phenotypes identified in human genetic association studies. Nat. Genet. 42, 626–630.
Zhu, H., Shyh-Chang, N., Segre, A.V., Shinoda, G., Shah, S.P., Einhorn, W.S., Takeuchi, A., Engreitz, J.M., Hagan, J.P., Kharas, M.G., Urbach, A., Thornton, J.E., Triboulet, R., Gregory, R.I., Consortium, D., Investigators, M., Altshuler, D., Daley, G.Q., 2011. The Lin28/let-7 axis regulates glucose metabolism. Cell 147, 81–94.