PERSPECTIVE

Regulatory role of microRNA on inflammatory responses of diabetic retinopathy

The prevalence of diabetes has been increasing in the U.S., with diabetes as a significant concern for patients' physical and financial health. Diabetic retinopathy is the leading cause of visual loss in working-age of adults and is characterized by retinal neurodegeneration and microvascular abnormalities in the eye. Hyperglycemia is one significant risk factor for diabetic retinopathy and can result in increased inflammatory responses and vascular dysfunction. However, the molecular mechanisms underlying these pathologies are not fully understood. Although treatments are currently available for the patients with proliferative diabetic retinopathy or macular edema, including laser photocoagulation, steroids, or anti-vascular endothelial growth factor (VEGF) injections, many patients fail to respond to these therapies. Therefore, it is imperative to develop additional novel therapeutics for diabetic retinopathy.

As an intriguing and potent mediator in the pathological mechanisms of diabetic retinopathy, microRNA (miRNA) have been investigated for the expression profile and regulatory networks during the last decade. miRNA are small non-coding molecules, with ubiquitous expression in the body. The level of miRNA expression differs between different types of cells and disease states. miRNA have been implicated as a promising candidate of molecular therapeutics to protect damaged and injured retina, as they have the capacity for gene regulation and can be biomarkers for many human diseases.

The relevance of miRNA on the pathological mechanisms of diabetic retinopathy was first shown approximately a decade ago. We still have little knowledge which miRNA are involved in the onset and progression of diabetic retinopathy. A small number of studies have identified the expression and functions of specific miRNA in diabetic retinopathy (Kovacs et al., 2011; McClelland and Kantharidis, 2014). Computational analyses have been done to find regulatory networks of miRNA, but the majority of miRNA targets have not been experimentally tested. Moreover, little has been shown for the regulatory networks of miRNA in diabetic retinopathy.

We reported that high glucose culturing conditions decreased the levels of miR-15a/16 expression in human retinal endothelial cells (REC). Altered expression of miR-15a in different diabetic conditions have been reported in a small number of other studies, as shown in human umbilical vein endothelial cells (HUVEC), under the conditions of high glucose and/or calcitriol deficiency (Zitman-Gal et al., 2014), in the plasma of patients with prevalent diabetes mellitus (Zampetaki et al., 2010), and in the vitreous humor from patients with proliferative diabetic retinopathy (Hirota et al., 2015). The changes in miR-15a expression were differential and appeared to be cell/tissue type- and condition-specific changes.

Activation of pro-inflammatory signaling is a key pathological response of diabetic retinopathy and contributes to endothelial dysfunction. Further, levels of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNFα), are increased in the pathogenesis of early diabetic retinopathy, which can mediate the progression of diabetic retinopathy to later stage disease. Nuclear factor-kappa B (NF-κB) is a transcription factor and key mediator of inflammatory signaling, with the activation of NF-κB inducing the upregulation of many pro-inflammatory molecules, including TNFα and IL-1β. NF-κBp65 is a potent activator of gene expression, and our previous study showed elevated levels of NF-κBp65 phosphorylation in REC under high glucose conditions (Ye and Steinle, 2016). We demonstrated that miR-15a/16 played a role in reducing pro-inflammatory signaling of IL-1β, TNFα, and the phosphorylation of NF-κBp65 (ser536) in cultured REC. In addition to NF-κB, we have also reported that miR-15b/16 reduced the levels of TNFα and suppressor of cytokine signaling 3 (SOCS3) to inhibit insulin resistance in REC (Ye and Steinle, 2015). It is anticipated that miR-15a and -15b have functional similarity as they share the same seed sequence. Another study has shown a role for miR-15a on inflammatory pathways of diabetic retinopathy, whereby miR-15a played a role in suppressing pro-inflammatory pathway through inhibition of sphingomyelinase in sphingolipid metabolism. Other than miR-15a and -16, only a small number of miRNAs has been tested for their regulatory roles in pro-inflammatory pathways of diabetic retinopathy.

In addition to miR15a/16, our group and others have examined the effects of miR-146a on key pro-inflammatory signaling pathways. We previously reported that miR-146a decreased levels of toll-like receptor 4 (TLR4), as well as both myeloid differentiation primary response gene 88 (MyD88)-dependent and -independent pathways. Levels of NF-κB and TNFα were reduced in REC when miR-146a was overexpressed in REC under high glucose conditions (Ye and Steinle, 2016). Other groups have reported negative regulatory roles of miR-146 in NF-κB activation and subsequent leukocyte adhesion to human REC, adenosine deaminase and TNFα release in human macrophages, as well as intercellular adhesion molecule 1 (ICAM-1) levels in human REC. In addition to miR15a/16 and miR146a, work has shown that miR-200b can regulate VEGF and permeability and oxidation resistance 1 (Oxr1), miR-152 regulation of (pro)renin receptor, VEGF, and transforming growth factor beta1 (TGFbeta1), miR-106a regulation of ICAM-1, miR-195 regulation of sirtuin 1 (SIRT1), miR-23b-3p regulation of acetylated-NF-κB expression, miR-184 regulation of Wnt signaling and ischemia-induced retinal neovascularization, and miR-21 regulation of RhoB and choroidal neovascularization, miR-92a regulation of IL-1beta in $CD34^+$ cells from patients with diabetic retinopathy, and miR-126 regulation of high motility group box 1 (HMGB1).

Pro-inflammatory cytokines, such as TNFα and NF-κB, and adhesion molecules mediate leukocyte adhesion to endothelium under diabetic conditions. Additionally, high glucose conditions and hyperglycemia can initiate leukostasis. Leukostasis is a key inflammatory change, and may represent the first step in diabetic retinopathy. Retinal leukostasis is regarded as a histological indication of retinal inflammation. We generated conditional knockout mice in which miR-15a/16 was eliminated in vascular endothelial cells and found that retinal leukostasis could be attenuated by miR-15a/16. Specifically, we showed that miR-15a/16 played a role in reducing the influx of $CD45^+$ leukocytes in the retina, with our outcome excluding the possibility that the regulatory effects were induced by potential changes due to the number of circulating pool of leukocytes (Ye et al., 2016). In addition to our work on miR15a/16 on retinal leukostasis, miR-126 was shown to regulate vascular cell adhesion molecule

1 (VCAM-1) expression and inhibit leukocyte adhesion in the retinas of diabetic rats. Additionally, an inhibitory role of miR-146 in thrombin-induced increased leukocyte adhesion to human REC and the relation of miR-335 to leukocyte activation were found in the retina of streptozotocin (STZ)-diabetic rats.

There may be other potential mechanisms through which miRNA directly target signaling molecules that are related to pathological pathways of diabetic retinopathy, such as mitogen-activated protein kinase (MAPK) family, matrix metallopeptidases (MMPs), integrin, and others (targetscan.org). Further investigations will be required to gain a better understanding of the regulatory functions of miR-15a/16 in diabetic retinopathy.

The ultimate goal of studying miRNA in association with diabetic retinopathy would be developing novel therapeutic strategies using potent miRNA for the treatment of the diabetic retina. *In vivo* research must be performed to prove the protective effects, safety, and efficiency of miRNA-based therapy. A small number of studies have been done for *in vivo* delivery of miRNA in animal models, in which an intravitreal injection was done to deliver miRNA into the eyes of diabetic mice and rats. Those studies have shown inhibitory effects of miRNA on retinal neovascularization (miR-31, -150, and -184, miR-126, anti-miR-155, miR-184, miR-218), fibronectin (miR-146a), VEGF (miR-200b), and SIRT1(miR-23b-3p and miR-195) (Shen et al., 2008; Mortuza et al., 2014).

To date, there are no available miRNA in clinical use for the treatment of diabetic retinopathy. Less than a dozen of miRNAs have been tested in preclinical, Phase I, or II trials for other types of diseases, including myocardial infarction, liver cancer, and hepatitis C virus (Christopher et al., 2016). For the applications of RNAi therapeutics, there is a necessity to overcome challenges of miRNA use. As a single miRNA can affect hundreds of target genes and cause random regulation of a wide range of mRNAs, specific targeting of diabetic retinopathy-related transcripts is needed to prevent potential deleterious side effects to patients. Extensive research on genome wide analysis for mRNA/miRNA expression and pathway enrichment analysis may be able to resolve the issue. Additionally, further studies on the manipulation of miRNA for more efficient, specific targeting and increased safety are required to accelerate clinical trials of RNAi therapeutics.

Determination of effective routes of miRNA-administration is another key interest for developing therapeutic strategies for diabetic retinopathy. To date, only a few studies have been conducted on miRNA-delivery *in vivo* and suggested potential delivery vehicles, such as liposome-nanoparticles (miR-184) and lentiviral vectors (anti-miR-155), which were examined in murine models of retinal neovascularization. Other types of miRNA-delivery technique have been studied in many diseases, which include AAV- and lentiviral-vectors, bacteriophage MS2 virus-like particles (MS2 VLPs), nanohydroxyapatite particles combined with collagen-nanohydroxyapatite scaffolds, and nanoparticles. Interestingly, a potential availability of plant-based dietary miRNA has been suggested (Hirschi et al., 2015), which may represent a novel avenue providing patients more convenient and non-invasive way of RNAi treatment.

Taken together, recent findings suggest that increasing numbers of miRNA, including miR-15a/16 and miR146a, have been identified and shown to play a role in the regulation of inflammatory responses of diabetic retinopathy. These miRNAs offer novel molecular targets for anti-inflammatory therapies for diabetic retinopathy in clinical applications. Further research should be conducted for a better understanding on the profile of miRNA expression, regulatory networks of miRNA in pathological mechanisms of diabetic retinopathy, and more effective methods of miRNA delivery. That would increase therapeutic usefulness of miRNA as a potent RNAi drug for patients with diabetic retinopathy.

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References

Christopher AF, Kaur RP, Kaur G, Kaur A, Gupta V, Bansal P (2016) MicroRNA therapeutics: Discovering novel targets and developing specific therapy. Perspect Clin Res 7:68-74.

Hirota K, Keino H, Inoue M, Ishida H, Hirakata A (2015) Comparisons of microRNA expression profiles in vitreous humor between eyes with macular hole and eyes with proliferative diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol 253:335-342.

Hirschi KD, Pruss GJ, Vance V (2015) Dietary delivery: a new avenue for microRNA therapeutics? Trends Biotechnol 33:431-432.

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.

McClelland AD, Kantharidis P (2014) microRNA in the development of diabetic complications. Clin Sci 126:95-110.

Mortuza R, Feng B, Chakrabarti S (2014) miR-195 regulates SIRT1-mediated changes in diabetic retinopathy. Diabetologia 57:1037-1046.

Shen J, Yang X, Xie B, Chen Y, Swaim M, Hackett SF, Campochiaro PA (2008) MicroRNAs regulate ocular neovascularization. Mol Ther 16:1208-1216.

Ye EA, Steinle JJ (2015) miR-15b/16 protects primary human retinal microvascular endothelial cells against hyperglycemia-induced increases in tumor necrosis factor alpha and suppressor of cytokine signaling 3. J Neuroinflammation 12:44.

Ye EA, Steinle JJ (2016) miR-146a attenuates inflammatory pathways mediated by TLR4/NF-kappaB and TNFalpha to protect primary human retinal microvascular endothelial cells grown in high glucose. Mediators Inflamm 2016:3958453.

Ye EA, Liu L, Jiang Y, Jan J, Gaddipati S, Suvas S, Steinle JJ (2016) miR-15a/16 reduces retinal leukostasis through decreased pro-inflammatory signaling. J Neuroinflammation 13:305.

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.

Zitman-Gal T, Green J, Pasmanik-Chor M, Golan E, Bernheim J, Benchetrit S (2014) Vitamin D manipulates miR-181c, miR-20b and miR-15a in human umbilical vein endothelial cells exposed to a diabetic-like environment. Cardiovasc Diabetol 13:8.