Investigating the Roles of miR-148a-3p and miR-126-5p in Mouse and *in vitro* Models of Diabetic Retinopathy

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Purpose: Diabetic retinopathy (DR) is the leading cause of blindness in American adults. Several studies have highlighted the role of microRNAs (miRNAs) in the pathophysiology of DR. Previous studies from our lab showed that long-standing diabetes affects the expression profiles of various miRNAs, including miR-148a-3p and miR-126-5p, in mouse hematopoietic stem cells. However, these miRNAs were not investigated in retinal cells. Therefore, in the present study, we explored the expression profiles of these miRNAs using both animal models and human retinal endothelial cells (HRECs).

Methods: For animal studies, retinas were isolated from 6-month-old diabetic db/db mice and age-matched control db/m mice. qRT-PCR was used to analyze the mouse retina miRNA and mRNA targets. For *in vitro* studies, HRECs were cultured in three conditions: normal glucose [5 mM], high glucose [15 mM + NG], and mannitol osmotic control [15 mM + NG]. After 24-hour treatment, RNA was extracted, and qRT-PCR was performed to determine miRNA expression. miRDB (MicroRNA Target Prediction Database; mirdb.org) was used to identify mRNA targets regulated by these miRNAs.

Results: qRT-PCR analysis of db/db mouse retinas showed significant upregulation of miR-148a-3p compared to db/m control; miR-126-5p was upregulated but not statistically significant. Known targets of miR-148a-3p, ITGA5 and ITGA9, were significantly upregulated in db/db mouse retinas. qRT-PCR analysis of HG-treated HRECs showed an upregulation of miR-148a-3p and miR-126-5p; however, the results were not statistically significant.

Conclusions: In the present study, we found that high-glucose conditions alter the expression profiles of miR-148a-3p and miR-126-5p in db/db mice and human retinal endothelial cells. We also found upregulation of integrins ITGA5 and ITGA9 in mouse retinas, potentially implicating the interaction between miR-148a-3p and these extracellular matrix proteins in the pathophysiology of DR. Further experiments to validate these miRNAs and their mRNA targets are required to strengthen the current findings.

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