Case Study
HTG EdgeSeq
miRNA Whole Transcriptome Assay

HTG EdgeSeq miRNA Whole Transcriptome Assay uncovers novel miRNAs associated with hyperglycemia in type I diabetes patients

Problem: The mechanism of hyperglycemia is unclear

Chronic hyperglycemia, the underlying symptom associated with diabetes, is considered the main risk factor for development of future and more severe complications of diabetes such as retinopathy, nephropathy, and neuropathy. The mechanisms by which hyperglycemia induces diabetes complications is unclear, though multiple factors have been proposed[1-3]. Most recently, dysregulation of miRNA expression, has been implicated in hyperglycemia-related diabetes complications[4].

Solution: Probing the mechanism of hyperglycemia with miRNA profiling

To investigate the role of miRNA in chronic hyperglycemia, researchers from Harvard Medical School employed the HTG EdgeSeq platform to profile 2,083 miRNAs from plasma samples from diabetes patients[5]. Although previous studies have investigated relationships between plasma-circulating miRNAs and hyperglycemia, these studies have been limited by a focus on specific miRNAs[6-8]. The HTG EdgeSeq miRNA Whole Transcriptome Assay circumvents these issues by profiling nearly all known circulating miRNAs without requiring potentially confounding normalization techniques.

Satake et al. used the HTG EdgeSeq miRNA Whole Transcriptome Assay to profile miRNA levels in two different cohorts of type I diabetes patients with a high degree of variability in hyperglycemic index (assessed via plasma hemoglobin A1c [HbA1c]). Analysis of plasma samples in the initial screening cohort identified 54 miRNAs that were found to be correlated to hyperglycemic index. Validation of expression for 48 of these 54 miRNAs was conducted using quantitative reverse-transcription PCR (qRT-PCR) in the second cohort. Twenty-six of these miRNAs were detected in more than half of the patients and 10 shared miRNAs correlated with glycemic index in both cohorts. After correcting for age, BMI, systolic blood pressure, and albumin-to-creatinine ratios, four miRNAs replicated with high statistical significance.

1 **2,083 miRNAs** measured via HTG EdgeSeq platform in patient cohort #1; 54 significantly correlated with HbA_{1c}

2 **26/48 of those** detected via qRT-PCR in >50% of patients in cohort #2

3 **10 miRNAs** significantly correlated with HbA_{1c} in both cohorts

4 **4 miRNAs** examined further:
miR-125b-5p
miR-365a-3p
miR-5190
miR-770-5p

Significance: HTG EdgeSeq miRNA Whole Transcriptome Assay provided the first global picture of the role of miRNA in hypoglycemia

This study, which employed the HTG EdgeSeq miRNA Whole Transcriptome Assay, provides a comprehensive analysis of nearly all known miRNAs in type I diabetes patients. The scale of the assay enabled the researchers to confirm candidate miRNA for further analysis by qRT-PCR. While miRNA activity had been implicated in hyperglycemia-related diabetes complications, this study, for the first time, identified specific miRNAs and pathways correlated with HbA1c from a comprehensive miRNA expression screen.

The nature of the most significant pathways targeted by these four miRNAs, including their ability to alter the neuronal network (i.e. axon guidance and neurotrophin) as well as other cell processes including cell crosstalk, motility, and differentiation (i.e. Rap1 and focal adhesion pathways), suggests that these pathways may indeed regulate complications associated with type I diabetes.

This work highlights the utility of the HTG EdgeSeq miRNA Whole Transcriptome Assay for assessing transcriptomic miRNA changes in diabetes and suggests that this easy-to-use platform could provide insight into other diseases associated with miRNA dysregulation.

References

1: Barrett, E. J. *et al.* Diabetic Microvascular Disease: An Endocrine Society Scientific Statement. *J. Clin. Endocrinol. Metab.* 102, 4343–4410 (2017).

2: Stratton, I. M. *et al.* Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 321, 405–412 (2000).

3: Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. Diabetes Control and Complications Trial Research Group. *J. Pediatr.* 125, 177–188 (1994).

4: Alexandru, N. *et al.* Vascular complications in diabetes: Microparticles and microparticle associated microRNAs as active players. *Biochem. Biophys. Res. Commun.* 472, 1–10 (2016).

5: Satake, E. *et al.* Circulating miRNA Profiles Associated with Hyperglycemia in Patients with Type 1 Diabetes. *Diabetes* 67, 1013–1023 (2018).

6: Zampetaki, A. *et al.* Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ. Res.* 107, 810–817 (2010).

7: Kong, L. *et al.* Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study. *Acta Diabetol.* 48, 61–69 (2011).

8: Pescador, N. *et al.* Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers. *PloS One* 8, e77251 (2013).

HTG Molecular Diagnostics, Inc.
Call 1-877-289-2615
www.htgmolecular.com

Rev 2: 29-APR-2021