中文題目: 微小核糖核酸 146a-5p 和 O-連接的 N-乙酰葡糖胺轉移酶透過非典型調控來增強高糖刺激下的內皮細胞發炎反應

英文題目：A Non-canonical Regulation Between O-linked N-Acetylglucosamine Transferase and miR-146a-5p Sustain High Glucose-Induced Endothelial Inflammation

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ABSTRACT

Background and Aims: Increased O-GlcNAc transferase (OGT) –induced O-linked N-acetylglucosamine (O-GlcNAc) post-translational modification is associated with diabetic complications. As an anti-inflammatory microRNA (miR), miR-146a-5p is dysregulated in diabetes. Notable, high glucose (HG) stimulation persistently downregulated miR-146a-5p expression levels in endothelial cells. We previously reported that miR-200a/200b regulate HG-induced endothelial inflammation via a canonical interactions between miR-200a/200b and OGT. In this study, we reported that a non-canonical interaction between OGT and miR-146a-5p as one of mechanisms to sustain diabetic vascular complications.

Methods: Human aortic endothelial cells (HAECs) were stimulated with high glucose (25 mM) and glucosamine (25 mM) for 24 h. Western blot, real time PCR, bioinformatics analysis, luciferase reporter assay, miR-146a-5p mimic or inhibitor transfection, siRNA OGT transfection and were performed. The aortic tissues from miR-146a-5p-treated db/db diabetic mice were examined by immunohistochemistry staining.

Results: HG and glucosamine increased OGT mRNA and protein expression, and protein O-GlcNAcylation (RL2 antibody) in HAECs, and they were associated with increased IL-6 gene expression; and IL6 expression. Real time PCR analysis identified that miR-146a-5p was significantly decreased in either HG- or glucosamine-stimulated HAECs. This suggests that OGT-induced protein O-GlcNAcylation as a potential mechanism to downregulate miR-146a-5p. Bioinformatic miR target analysis identified only partial seed sequence match between miR-146a-5p and the 3′-UTR of human OGT mRNA. Based on the different web-based microRNA predication tools, partial seed sequence match between miR-146a-5p and the 3′-UTR of human OGT mRNA precluded miR-146a-5p as a post-translational regulator of OGT expression. However, a luciferase reporter assay confirmed that miR-146a-5p mimic could potently bind to 3′-UTR of human OGT mRNA, indicating that OGT is a non-canonical target of miR-146a-5p. Transfection with miR-146a-5p mimic significantly inhibited HG-induced OGT mRNA expression, OGT protein expression; protein O-GlcNAcylation level; IL6 gene expression; and IL6 expression. In contrast, miR-146a-5p inhibitor significantly increased HG-induced OGT mRNA expression, OGT protein

expression; protein O-GlcNAcylation level; IL6 gene expression; and IL6 expression. These data confirmed that miR-146a-5p can post-translationally modulated OGT/protein O-GlcNAcylation expression levels. Additionally, siRNA-mediated OGT depletion and the miR-200a/miR-200b mimic (i.e, a canonical OGT post-translation regulator) increased HG-induced miR-146a-5p expression levels, indicating that HG-induced miR-146a-5p downregulation is partially mediated through OGT-induced protein O-GlcNAcylation. These results were supported in vivo: intravenous injections of miR-146a mimic prevented endothelial OGT and IL6 expression in db/db mice.

Conclusion: A non-canonical reciprocal interaction between miR-146a-5p and OGT is involved in a vicious cycle to sustain HG-induced diabetic vascular complications.