中文題目:微核糖核酸146a透過對第四型NADPH氧化酶的抑制可減少受高糖和凝血酶協同刺激下的內皮細胞發炎反應

英文題目: MicroRNA-146a Decreases High Glucose/Thrombin-induced Endothelial Inflammation by Inhibiting NAPDH Oxidase 4 Expression

作者: 王黃舟[a,b], 羅婉瑜[c]

[a] 中國醫藥大學附設醫院內科部心臟內科

[b] 中國醫藥大學醫學系

[c] 中國醫藥大學中西醫結合研究所

ABSTRACT

Background: The metabolic abnormality of diabetes is associated with hyperglycemia and increased thrombin generation. Both high glucose and thrombin can increase reactive oxygen species (ROS) generation, which is responsible for accelerated atherosclerosis and thrombotic complications in diabetic patients. MicroRNA (miR) plays a key role in modulating gene expression and cellular function. Among multiple microRNAs, miR-146a is an important feedback regulator of inflammation by targeting NFκB signaling pathway. Several evidences indicate that impaired miR-146a expression contributes to the proinflammatory state in diabetes. It is unknown whether a combination of high glucose and thrombin can modulate the expression of NAPDH oxidase (Nox) subtypes in human aortic endothelial cells (HAECs). Moreover, we investigated the role of miR-146a in a diabetic atherothrombosis model.

Materials and Methods: Methods: HAECs were treated with high D-glucose (25 mmol/l, HG) and thrombin (2 U/ml). Real-time PCR, western blot, ROS assay kit, Inflammatory cytokine kit, THP-1 adhesion assay, bioinformatics prediction, luciferase reporter assay and miR-146a mimic transfection were performed.

Results: We showed that high glucose (HG) exerted a synergistic effect with thrombin to induce a 10.69-fold increase in Nox4 mRNA level in HAECs. Increased Nox4 mRNA expression was associated with increased Nox4 protein expression and ROS production. Inflammatory cytokine kit identified that the treatment increased IL-8 and IL-6 levels. Moreover, HG/thrombin treatment caused an 11.43-fold increase of THP-1 adhesion to HAECs. *In-silico* analysis identified the homology between miR-146a and the 3′-untranslated region of the Nox4 mRNA, and a luciferase reporter assay confirmed that the miR-146a mimic bound to this Nox4 regulatory region. Additionally, miR-146a expression was decreased to 58% of that in the control, indicating impaired feedback restraint of HG/thrombin-induced endothelial inflammation. In contrast, miR-146a mimic transfection attenuated

HG/thrombin-induced upregulation of Nox4 expression, ROS generation, and inflammatory phenotypes.

Conculsion: MiR-146a is involved in the regulation of endothelial inflammation via modulation of Nox4 expression in a diabetic atherothrombosis model.