中文題目: MicroRNA-203 透過 Wnt/β-catenin 途徑減少醛固酮腺瘤患者製造醛固酮

英文題目: MicroRNA-203 Mitigated Aldosterone Production in Aldosterone-Producing Adenomas by Targeting Wnt/β-catenin Pathway

作　　者: 陳泓維[1], 吳允升[1], 彭康詠[2]

服務單位: 台大醫院內科部腎臟科[1], 台大醫院內科部[2]

Background: Primary aldosteronism (PA), characterized by an inappropriate production of aldosterone, is the most common form of secondary hypertension. The two major PA subtypes are unilateral aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia. To date, the molecular mechanisms underlying APA are still largely unknown. Increasing evidence shows that aberrant expression of microRNAs (miRNAs) is linked to the pathogenesis of many human diseases, including cancers. They can post-transcriptionally regulate the expression of their target genes and function as tumor suppressors or oncogenes. Furthermore, several studies have demonstrated the potential of circulating miRNAs in peripheral blood to act as novel noninvasive biomarkers for the early diagnosis of various cancers and other diseases. miR-203 was reported to act as a tumor suppressor, and its expression was downregulated in various cancers. Circulating miR-203 has also been implicated as a biomarker in some human disease. However, its status and role in APA is still unclear. In this study, we investigate the role and mechanism of miR-203 in the pathogenesis of APA.

Method: The expression level of miR-203 in plasma, adrenal adenoma, and cultured adrenal cells were validated by using quantitative reverse transcriptase polymerase chain reaction (qPCR). Radioimmunoassay (RIA) was used to measure the aldosterone concentration in the culture supernatants and mouse serum. Liposome-mediated transfection and intra-adrenal injection with miR-203 inhibitor, mimicking hyperaldosteronism were constructed into HAC15 human adrenocortical cells and C57BL/6 mice. Using synthetic miR-203 mimics to restore miR-203 expression in primary human APA cells. The expression of Wnt/β-catenin signaling pathway, targeted by miR-203 were assessed by immunoblotting and qPCR. The change of proliferation ability of HAC15 cells and primary APA cells was detected by Cell Counting Kit-8 (CCK-8) assay.

Result: We found that miR-203 was down-regulated in APA when compared to the adjacent non-tumor tissues ($p= 0.037$). Besides, the miR-203 expression levels in APA was negative correlated with the serum aldosterone level of APA patients

(R□=□-0.5975, P□<□0.0042). In mechanism study, miR-203 inhibitor significantly increase mRNA expression level of Wnt4, aldosterone synthase (CYP11B2), protein expression of active form of β-catenin protein and promoted aldosterone production in HAC15 human adrenocortical cells. Furthermore, β-catenin small interfering RNA (siRNA) abolished the effect of miR-203 on activation of Wnt pathway and aldosterone production. Restoration of miR-203 in primary APA cells significantly inhibited cell growth, the mRNA expression level of Wnt4, CYP11B2, the protein expression level of active form of β-catenin and aldosterone production were down-regulated as compared with negative control transfection. Importantly, *in vivo* adrenal gland selective inhibition of miR-203 led to significant upregulation of systolic blood pressure in mice (125±4 vs. 105±2 mmHg in controls, p=0.006). MiR-203 reinforced these effects by targeting Wnt/β-catenin signaling pathway, which promoted aldosterone production in adrenal cells. In APA patients, abrogated serum level of miR-203 restored at 6 months after adrenalectomy (p=0.023).

Conclusion: Our findings suggest that miR-203 may mediate some of hyperaldosterone in aldosteronism, in part via Wnt/β-catenin signaling pathway. The novel results add another dimension to accumulating evidence regarding further development of new therapies and diagnosis in APA.