中文題目：在冠狀動脈疾病患者，研究其血液中的外吐小體微小核醣核酸對冠狀動脈血管新生所扮演的角色

英文題目：The role of circulating exosomal microRNAs for coronary neovascularization in patients with coronary artery disease

作　　者：王怡智[1] 陳泊志[3] 張坤正[2] 黃瑞仁[1] 林俊立[1] 陳錦澤[2,3]

服務單位：台大醫院內科[1]　中國醫藥大學附設醫院內科[2]
中央研究院生物醫學研究所[3]

Background: Coronary artery disease (CAD) is the leading cause of death in the developed countries. Although current percutaneous coronary intervention improved the prognosis in patients with acute coronary syndrome, the fundamental therapeutic strategy of CAD patients based on the development of collateral circulation via angiogenesis beyond the stenotic lesions, functioning as " natural bypass". micro-RNAs(miRNAs), a novel class of endogenous small non-coding RNAs regulate the expression of its multiple target genes at post-transcriptional level based on sequence complementaries with their target mRNA molecules. Previous studies have identified that miRNAs, negative regulators of gene expression are highly expressed in vasculature and have been proposed to be involved in neovascularization. Recent studies revealed that exosome-carried miRNAs are able to be released into circulating blood from the ischemic tissue. We hypothesize that circulating exosomal miRNAs derived from ischemic myocardium in CAD patients play pivotal roles in genetic exchange between cells. In clinical setting, it has long been observed that there is marked inter-individual variation in the extent of collateral development in CAD patients. In this study, we attempt to (1) compare the circulating exosomal miRNA expression profiling between patients with angiographically-assessed absent and well-developed collateral vessels to elucidate the underlying molecular mechanism of collaterogenesis and (2) identify the specific miRNAs and their respective target genes responsible for angiogenesis.

Materials and Methods: We recruited 109 subjects who received coronary angiography. Among them, Forty-four CAD patients had good collateral flow (Grade 2), assessed by Rentrop scoring system, 31 patients had no visible flow (Grade 0), and the 34 remainders had patent coronary arteries served as the control group. After collecting exosomes from peripheral blood, we extracted and amplified miRNAs. Expression profiling of miRNAs in exosomes was performed with miRNA array system. Microarray hybridization results were ranked by the differential expression ratio between groups with good and no collateral flow. The top 5 miRNAs with high differential expression ratio were validated by microRNA RT-qPCR assay. We then screen angiogenic ability of specific miRNAs with overexpression in human umbilical vein endothelial cells (HUVECs) including proliferation, migration and tube

formation.

Result: Based on the array data, the miRNAs with high differential expression ratio between Grade 2 and Grade 0 collateral flow were selected. The top five candidate miRNA markers that are over-expressed in patients with good collateral perfusion were miR-300, miR-576-3p, miR-642, miR-620, and miR-1255a. By the TargetScan Human software prediction and literature search, one of the target genes of miR-620, DICER1 gene is related to angiogenesis. The top five over-expressed miRNAs in patients with no collateral flow were miR-29a, miR-592, miR-518e, miR-32, and miR-766. Among the candidate miRNAs, the target genes of miR-29a including COL3AI, ELN, and COL5A1 genes are involved in blood vessel development. Analyzing expression profiling between groups with good collateral flow and control group with patent coronary arteries, the top five differentially over-expressed miRNAs in good-flow patients were miR-300, miR-642, miR-30c miR-485-3p and miR-1253, and the top five candidate miRNAs in control group were miR-5481, miR-664*, miR-34a, miR-1255-5p and miR-658. According to the microarray data, the high-expressed miRNAs could be the candidate biomarkers for distinguishing different types of collateral circulation in CAD patients and for diagnostic/prognostic usage in high-risk patients with CAD.

Conclusion: We have successfully established the procedural pipe-line for measurement of microRNA expression profiling in circulating exosomes. It is a powerful platform technology for exploring the pathophysiological state of the cardiovascular disease. Our finding may provide a non-invasive and cost-effective way to identify high-risk CAD patients and may serve as novel therapeutic targets for vascular disease including CAD with impaired collateral angiogenesis.