MicroRNA-132/212 family promotes arteriogenesis by prolonging Ras-MAPK signaling

Z. Lei1; M. M Brandt2; A. Van Mil3; M. Smits4; B. Van Middelaar3; T. Fukao4; C. Cheng2; P. Doevendans1; J. P G Sluiter1

1University Medical Center Utrecht, cardiology, Utrecht, Netherlands; 2University Medical Center Utrecht, Nephrology & hypertension, Utrecht, Netherlands; 3University Medical Center Utrecht, cardiology, Utrecht, Netherlands; 4University Heart Centre Freiburg - Bad Krozingen, Department of Cardiology and Angiology I, freiburg, Germany; 5Max Planck Institute of Immunobiology and Epigenetics, freiburg, Germany

Arteriogenesis is a complicated process induced by local shear stress caused by local occlusion and is enhanced by growth factors such as VEGF, which is secreted by inflammatory cells and endothelial cells in response to hypoxia. To search for microRNAs involved in arteriogenesis, we performed microarray analysis on hindlimb ischemia mice tissue. We observed a dynamic temporal regulation of microRNAs expression, among which miR-132/212 were significantly upregulated 4 days after occlusion of femoral artery. The aim of our study was to unravel the role of miR-132/212 in arteriogenesis. In a pericyte-endothelial cell in vitro co-culture assay, overexpression of miR-132/212 in endothelial cells resulted in enhanced neovascularization capacity, including more tubular structures and junctions and longer total tubule length. Inhibition of miR-132/212 decreased total tubule length, and the number of junctions and tubule structures. Ex vivo aorta ring assay demonstrated more branches in wild-type than of miR-132/212 knockout mice. In line with these, microRNA132/212 Knockout mice displayed slower perfusion recovery after hind-limb ischemia than wild type mice. Immunohistochemistry studies further demonstrated that knockout mice have similar number of collateral arteries but they are smaller than wild type. Based on microRNA targets prediction and luciferase assay we found that miR-132/212 enhanced Ras-MAPK signaling by directly inhibiting Rasa1, Spred1, and Spry1 expression which are inhibitors of the Ras-MAPK signaling pathway. In summary, our results show that miR-132/212 promote arteriogenesis by prolonging VEGF initiated Ras-MAPK signaling.