

# Vasopressin Modulates Endothelial and Fibroblast Gene Expression

Yongqing Wang, PhD<sup>1\*</sup>, and Bashar Kahaleh, MD<sup>2</sup>

<sup>1</sup>Division of Pulmonology, Department of Medicine, The University of Toledo, Toledo, OH 43614

<sup>2</sup>Division of Rheumatology, Department of Medicine, The University of Toledo, Toledo, OH 43614

\*Corresponding author: [yongqing.wang2@utoledo.edu](mailto:yongqing.wang2@utoledo.edu)

Published: 05 May 2023

**Introduction:** SSc vasculopathy is characterized by deficient endothelial nitric oxide (eNOS) and enhanced platelet adhesion to endothelial cells. In this study, we examined the epigenetic regulation involved in enhanced platelet adhesion, deficient eNOS expression, and the role of miRNA-126 in this process.

**Methods:** Platelet adhesion to MVECs was determined by the Calcein AM method. eNOS, NOS3, miR-126, DNA methyltransferase-1 (Dnmt1) expression were measured by qPCR and WB. L-NAME was used as NO antagonist. MiR-126 expression was inhibited by hsa-miR-126 inhibitor and enhanced by hsa-miR-126 Mimic.

**Results:** MiR-126 expression levels were significantly downregulated in SSc-MVECs. SSc MVECs supported platelet adhesion at a higher level than control cells (10.16 $\pm$ 2.8 platelet/EC vs. 3.3  $\pm$ 0.94 in control cells, mean  $\pm$ SD, P<0.001). Addition of L-NAME to control MVECs resulted in enhanced platelet adhesion in a dose-dependent fashion. NOS3 expression levels were significantly reduced in SSc cells, Dnmt1 expression levels were significantly higher in SSc cells. NOS3 under expression in SSc cells was related to heavy DNA methylation of the promoter CpG islands as shown by promoter sequence analysis of DNA after bisulfite modification. Upregulation of MiR126 in SSc MVECs resulted in the reduction of Dnmt1 and upregulation of NOS3 expression levels, while the inhibition of MiR 126 expression levels in control MVECs resulted in decreased NOS3 levels and enhanced Dmnt1 levels.

**Conclusion:** The data demonstrate that defective miR-126 expression in SSc MVES leads to upregulation of Dmnt1 expression and downregulation of NOS3 expression that is associated with defective NO release and enhanced platelet/endothelial interaction.