Epigenetic Repression of eNOS in Scleroderma (SSc) Microvascular Endothelial Cells (MVECs) is Related to the Downregulation of MicroRNA-152 by Enhanced DNA Methyltransferase 1 (Dnmt1) Expression.

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Objectives: Alteration in Scleroderma (SSc)-microvascular endothelial cells (MVEC) is related to epigenetic influences on gene expression level. Nitric oxide synthase gene (NOS3) repression is a prime example of epigenetic alteration of SSc-MVEC phenotype. The underlying mechanism of epigenetic imprinting in SSc-MVEC remains unknown. MicroRNAs (miRNAs), which are noncoding RNAs that regulate gene expression, are involved in diverse biological functions, including epigenetics regulation. It has been reported that downregulation of microRNA-152 induces aberrant DNA methylation by targeting the maintenance methyl transferase Dnmt1. In this study, we investigated miRNA-152 expression levels in SSc-MVEC and whether it is involved in the regulation of epigenetic imprinting in SSc.

Methods: MVEC cells were isolated from skin biopsies of SSc patients and matched control subjects. The NOS3, Dnmt1, and miR-152 expression levels in normal and SSc-MVEC were checked by real-time PCR. The epigenetic regulation of NOS3 was examined by the addition of DNA methyltransferase and histone deacetylase inhibitors to MVEC cultures and by analysis of CpG site methylation in the NOS3 promotor region. The effect of Dnmt1 on NOS3 mRNA expression was examined by transfecting SSc- MVEC with Dnmt1-specific siRNA and irrelevant control siRNA. The effect of miR-152 on Dnmt1 mRNA and NOS3 expression was examined by transfecting hsa-miR-152 into SSc-MVEC and transfecting miR-152 inhibitor into control- MVEC.

Results: A significant increase in Dnmt1 expression levels and a significant decrease in NOS3 expression levels were noted in SSc-MVEC. The addition of 2-deoxy-5-azacytidine and Trichostatin A to SSc-MVEC cultures normalized NOS3 expression levels. CpG sites in the NOS3 promoter were methylated in SSc-MVEC but not in control-MVEC. Transfection of SSc-MVEC with siRNA specific

for Dnmt1 resulted in an 80% decrease in the expression levels and an increase in the NOS3 expression level. Since DNMT1 is one of the predicted direct targets of miR-152, we investigated the expression levels of miR-152 in SSc and control MVEC. Levels were significantly down-regulated in SSc-MVEC and were inversely correlated to DNMT1 expression levels. Forced expression of miR-152 in SSc-MVEC led to a reduction in DNMT1 expression at the mRNA level in comparison with the negative control, while inhibition of miR-152 expression in control-MVEC enhanced DNMT1 expression levels in association with reduced NOS3 expression level.

Conclusion: *NOS3* expression level is down-regulated in SSc-MVEC and correlated with its promoter methylation. Dnmt1 expression is up-regulated in SSc-MVEC and inversely correlated to *NOS3* expression levels. miR-152 expression is downregulated in SSc-MVEC and inversely correlates with DNMT1 and relative correlates with *NOS3* expression levels. miR-152 may play a causal role in DNA methylation changes in SSc-MVEC through targeting Dnmt1.

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