Pharmacological blockade of histone methyltransferase SETD7 restores angiogenic response in experimental diabetes

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Background: Peripheral artery disease (PAD) is highly prevalent in patients with diabetes and associates with a high rate of limb amputation and poor prognosis. Surgical and catheter-based revascularization have failed to improve outcome in diabetic patients with PAD. Hence, a need exists to develop new treatment strategies able to promote blood vessel growth in the ischemic limb of diabetic patients. Mono-methylation of histone 3 at lysine 4 (H3K4me1) - a specific epigenetic signature induced by the methyltransferase SETD7 - favours a chromatin active and open state thus enabling the gene transcription.

Purpose: To investigate whether SETD7-dependent epigenetic changes modulate post-ischemic vascularization in experimental diabetes.

Methodology: Primary human aortic endothelial cells (HAECs) were exposed to normal glucose (NG, 5 mM) or high glucose (HG, 20 mM) concentrations for 48 hours. Unbiased gene expression profiling was performed by RNA sequencing (RNA-seq) followed by Ingenuity Pathway Analysis (IPA). In vitro angiogenic assays like migration assay & tube formation assay were performed. Pharmacological blockade of SETD7 was achieved by using the highly selective inhibitor called (R)-PFI-2. T1D mice (streptozotocin-induced diabetes) was orally treated with (R)-PFI-2 and with vehicle for 21 days and followed by induction of hindlimb ischemia. Blood flow recovery was analyzed at 30 minutes, 7 and 14 days by laser doppler imaging. Gastrocnemius muscle samples from patients with and without T2D were employed to translate our experimental findings.

Results: RNA-seq in HG-treated HAECs revealed a profound upregulation of the methyltransferase SETD7, an enzyme involved in mono-methylation of lysine 4 at histone 3 (H3K4me1). SETD7 upregulation in HG-treated HAECs was associated with an increase of H3K4-mono-methylation levels as well as with impaired endothelial cell migration and tube formation. Of interest, both gene silencing (SETD7-siRNA) and pharmacological blockade of SETD7 by (R)-PFI-2 rescued hyperglycemia-induced impairment of angiogenic properties in HAECs. RNA-seq in HG-treated HAECs with and without SETD7 depletion unveiled an array of differentially expressed genes, which were mainly involved in blood vessel growth and angiogenic response. Among dysregulated genes, Chromatin immunoprecipitation (ChIP) assays showed that SETD7 specifically mono-methylates H3K4m1 in proximity of Semaphorin-3G (SEMA3G) promoter, thus regulating its expression. Treatment of T1D mice with (R)-PFI-2 improved blood flow reperfusion at 14 days as compared to vehicle-treated animals. Finally, SETD7/SEMA3G axis was upregulated in muscle specimens from T2D patients.

Conclusion: Targeting SETD7 represents a novel epigenetic-based therapy to boost neovascularization in diabetic patients with PAD.

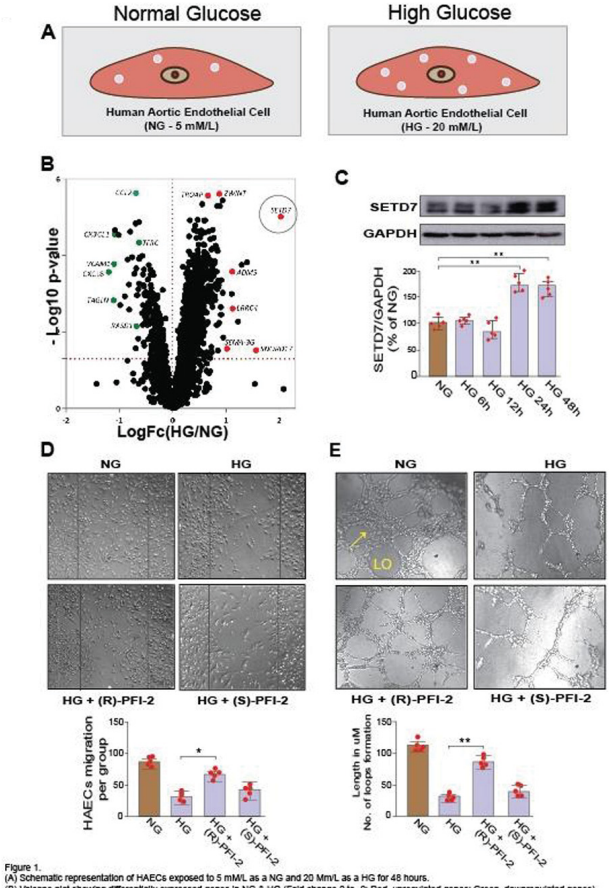

Figure 1.
(A) Schematic representation of HAECs exposed to 5 mM/L as a NG and 20 Mm/L as a HG for 48 hours.
(B) Volcano plot showing differentially expressed genes in NG & HG (Fold change 2 to -2; Red, upregulated genes; Green, downregulated genes).
(C) Western blot and relative quantification showing time dependent SETD7 protein levels in HAECs cultured in NG & HG.
(D) Scratch assay showing migration of HAECs exposed to NG, HG in presence of (R)-PFI-2 & (S)-PFI-2.
(E) Representation of Images and quantification of Matrigel-based tube formation assay in the 4 experimental groups.
The yellow arrow indicates length of the tubule and LO indicates the loop formation. Data are presented as mean + SEM and shown as percentage of control.
*, p <0.05; **,p<0.01; ***, p<0.001 multiple comparison were performed by 2-way analysis of variance (ANOVA) followed by Bonferroni correction.

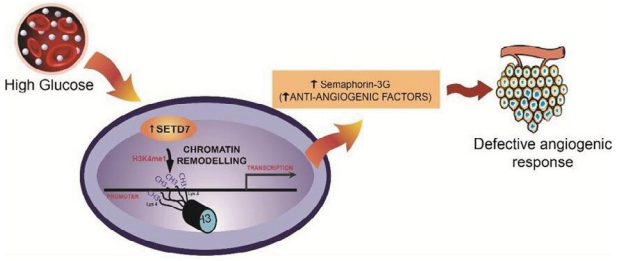

Figure 2.
Schematic showing main study findings