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

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PURPOSE

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 [1-2]. 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. patients

METHODS

Primary human aortic endothelial cells (HAECs) were exposed to normal glucose (NG, 5 mM) or high glucose (HG, 20 mM) concentrations for 48 hours. *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 analysed 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

CONCLUSIONS

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

REFERENCES

1. Fadini GP et al. Arterioscler Thromb Vasc Biol. 2020;40(1):34-44
2. Caporali et al. Cardiovasc Res. 2018;114(11):1411-1421.

RESULTS

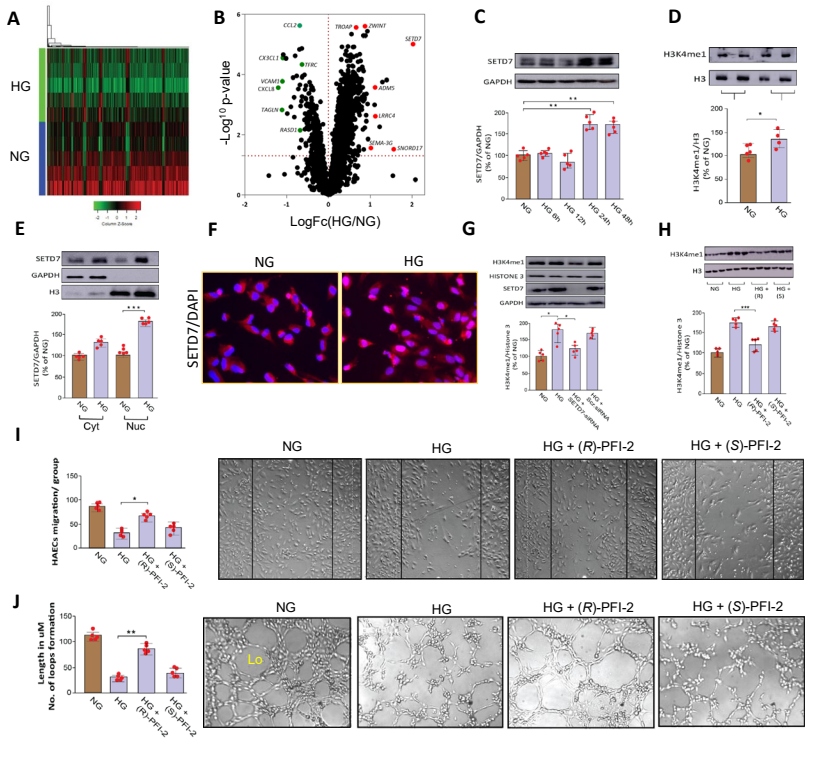

Figure 1. HG increases SETD7 expression and H3K4me1 levels in HAECs and thus impairing endothelial migration and tube formation. A-B) Heat map and volcano plot showing differentially expressed genes in HAECs exposed to normal glucose (NG, 5mm/L) and high glucose (HG, 20 mm/L). Red, upregulated genes; Green, down regulated genes. Cells were cultured in growth factor-free medium to mimic a condition of diabetic ischemia. **C)** Western blot showing time-dependent SETD7 protein levels in HAECs cultured in NG and HG. **D)** Western blot showing H3K4me1 levels in HAECs treated with NG and HG. **E-F)** Western blot and immunofluorescence showing SETD7 translocation in HAECs treated with NG and HG. **G)** Western blot showing H3K4me1 levels in HAECs treated with NG and HG in presence of SETD7-siRNA and Scr.siRNA. **H)** Western blot showing H3K4me1 levels in HAECs treated with NG and HG in presence of SETD7 selective inhibitor called (*R*)-PFI-2 and its inactive enantiomer (*S*)-PFI-2. **I)** Scratch assay showing migration of HAECs exposed to NG and HG in presence of (*R*)-PFI-2 and (*S*)-PFI-2. **J)** Representative images and quantification of Matrigel-based tube formation assay in HAECs exposed to NG and HG in the presence of *R*)-PFI-2 and (*S*)-PFI-2.The yellow arrow indicates tubule length, and Lo indicates Loop formation in NG treated cells. Data are presented as mean ± SEM and shown as % of control **, p<0.01.

RESULTS

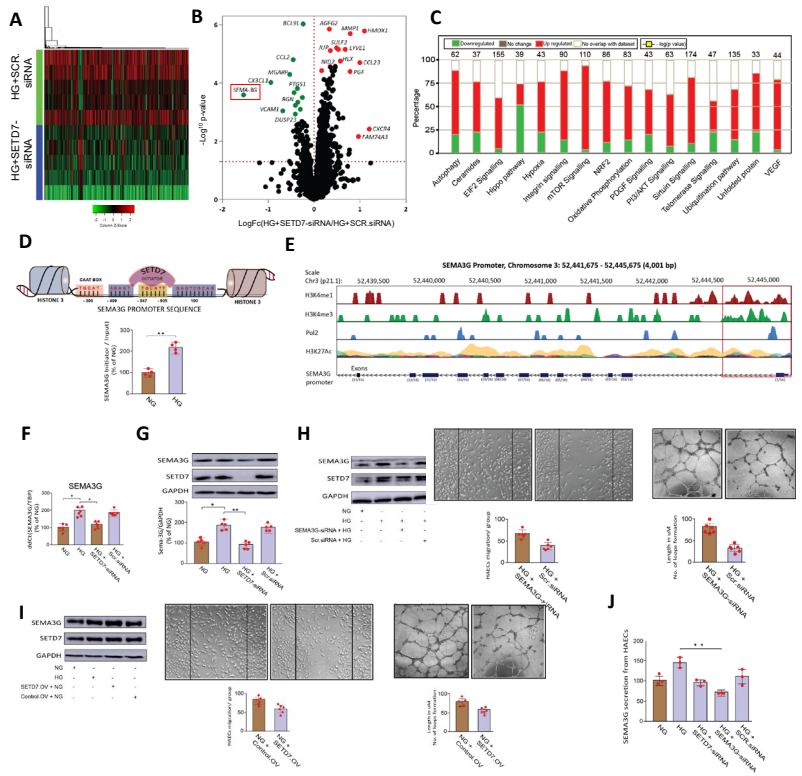

Figure 2. SETD7-dependent chromatin changes regulate Semaphorin-3G transcription. A-B) Heat map and volcano plot showing differentially expressed genes in HAECs exposed to HG, in the presence of SETD7-siRNA and Scr.siRNA. **C)** Ingenuity pathway analysis show that SETD7 depletion affects transcriptional programs implicated in cell migration, angiogenesis and vasculogenesis. **D)** ChIP assay shows SETD7 enrichment on SEMA3G promoter in HG as compared to NG-treated HAECs. **E)** ChIP-seq showing enrichment of H3K4me1 on SEMA3G promoter. **F)** Real time PCR showing SEMA3G gene expression in NG and HG-treated HAECs, in the presence of SETD7-siRNA or Scr.siRNA. **G)** representative Western blot and relative quantification showing SEMA3G downregulation at protein level in SETD7-depleted cells **H)** Representative Western blot showing knockdown of SEMA3G in HAECs exposed to HG and NG and its effect on cell migration and tube formation. **I)** Representative Western blot showing overexpression of SETD7 in HAECs treated with NG and HG and its effect on SEMA3G expression, cell migration and tube formation. **J)** SEMA3G secretion in HAECs exposed to HG and NG, in presence of SETD7-siRNA and SEMA3G-siRNA. Data are presented as mean ± SEM and shown as percentage of control **, p<0.01

RESULTS

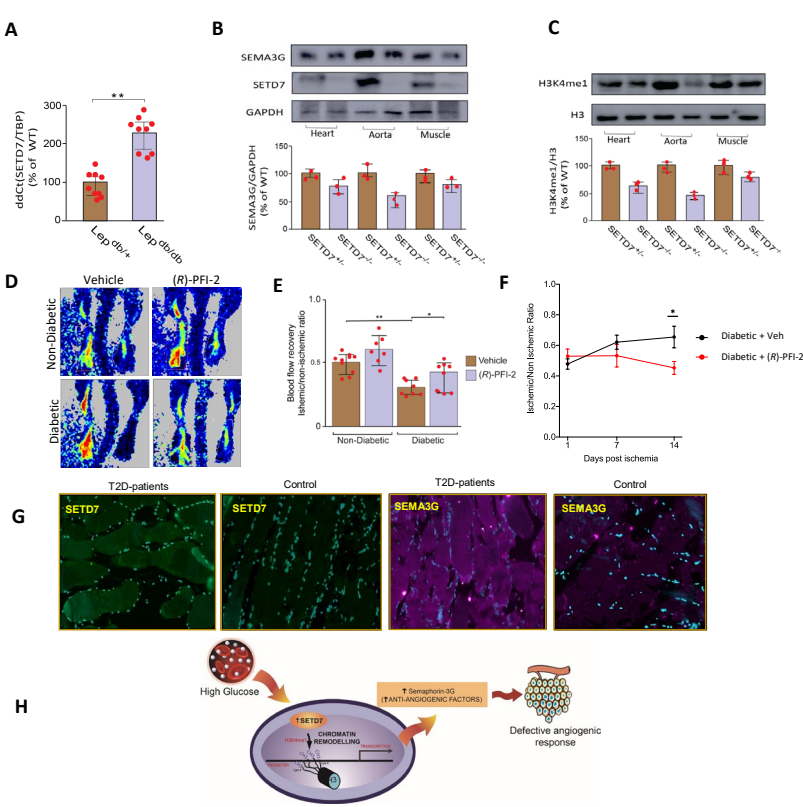

Figure 3. (*R*)-PFI-2 improves post-ischemic vascularization and limb perfusion in diabetic mice. A) Real time PCR shows SETD7 gene expression in ischemic muscular specimens from WT and *db/db* mice. **, $p<0.01$. B) Western blot showing SEMA3G protein levels in SETD7 knockout mice in heart, aorta and gastrocnemius muscle. C) Western blot showing H3K4me1 levels in SETD7 knockout mice in heart, aorta and gastrocnemius muscle. D) Laser Doppler perfusion imaging was serially performed to determine blood flow recovery after hindlimb ischemia. E) Bar graphs represent blood flow recovery in the 4 experimental groups at 14 days. F) Time-dependent changes of blood flow recovery (expressed as the ratio of blood perfusion in the ischemic versus non-ischemic hindlimb) in diabetic mice treated with (*R*)-PFI-2 or vehicle. G) Immunofluorescence showing staining for SETD7 and SEMA3G in Gastrocnemius muscle in T2D patients and healthy controls. H) Schematic representing main study findings.