Long non-coding RNA PANDA drives diabetic vascular dysfunction by promoting endothelial senescence and oxidative damage

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Background: Accumulation of reactive oxygen species and inflammation are major features of diabetic vasculopathy, yet the underlying mechanisms remain elusive. Long non-coding RNAs (lncRNAs) are emerging as important players in the pathogenesis of cardiovascular disease. Recent work has shown that PANDA, a newly identified lncRNA, is a key regulator of cellular senescence and apoptosis.

Purpose: To investigate the role of lncRNA PANDA in diabetic vascular disease.

Methods: Human aortic endothelial cells (HAECs) were exposed to normal (NG, 5 mmol/L) and high glucose concentrations (HG, 25 mmol/L). PANDA depletion in HG-treated HAECs was obtained by siRNA transfection while a scrambled RNA was used as a negative control. Expression of PANDA was assessed by real-time PCR. PANDA RNA immunoprecipitation (RIP) was performed to check its binding to relevant transcriptional factors (NF-YA). Beta-galactosidase staining was used to detect endothelial senescence, while migration and tube formation were employed to evaluate angiogenic properties of HAECs. Finally, RNA sequencing (RNA-seq) and bioinformatic analysis (network perturbation amplitude, NPA) were leveraged to unveil transcriptional changes upon PANDA depletion. Finally, the ex vivo effect of PANDA siRNA on endothelial function was assessed in aortic rings from diabetic mice.

Results: PANDA expression was significantly increased in HAECs exposed to HG as compared to NG (Fig. 1A). We found that under HG conditions PANDA sequesters the transcription factor NF-YA, thus inhibiting the expression of NF-YA-dependent pro-survival and anti-aging genes (Fig. 1B). Indeed, silencing of PANDA in HG-treated HAECs restored the expression of anti-apoptotic genes Bcl-X(L) and Bcl-2 while blunting senescence-related genes such as p53 and p16INK (Fig. 1C). PANDA depletion also abolished HG-induced cellular apoptosis and senescence, as assessed by caspase-3 activity assay and b-gal staining (Fig. 1D-E), while improving endothelial migration and tube formation (Fig. 1F-G). Transcriptomic analysis revealed dysregulation of several genes upon PANDA silencing with the antioxidant gene heme oxygenase-1 (HMOX1) as the top-ranking transcript in HG-treated cells (Fig. 1H). NPA analysis showed a strong involvement of PANDA in senescence, DNA damage, NRF2 signaling, hypoxic stress response, and proliferation (Fig. 1I). Of interest, PANDA levels were increased in aortas from diabetic mice (Fig. 2A) while depletion of PANDA rescued endothelial dysfunction (Fig. 2B).

Conclusions: Hyperglycemia-induced upregulation of PANDA drives endothelial senescence while impairing angiogenic properties. PANDA depletion in HG-treated HAECs rescues maladaptive transcriptional changes by restoring expression of the antioxidant gene HMOX1. Of note, targeting PANDA in the diabetic vasculature was able to rescue endothelial dysfunction. Our results indicate PANDA as a novel molecular target in the setting of diabetic vascular disease.

Figure 1. A) Real-time PCR showing PANDA expression in HAECs cultured in normal (NG, 5mM) or high glucose (HG, 25 mM) concentrations. B) RNA immunoprecipitation showing the interaction of PANDA with the transcription factor NF-YA in HAECs exposed to NG and HG. C) Real-time PCR showing gene expression of anti-apoptotic and senescence genes in NG and HG-treated HAECs. D) PANDA depletion in HG-treated HAECs restores expression of anti-apoptotic genes Bcl-XL and Bcl-2. E) Beta-galactosidase staining showing senescence in HG-treated HAECs. F) Scratch assays showing migration of HAECs exposed to HG and HG, in the presence or absence of PANDA depletion. G) Representative images and quantification of Matrigel-based tube formation assay in different experimental groups. H) Volcano plot displaying differential gene expression in HAECs exposed to HG + PANDA-siRNA vs HG + Scr-siRNA. I) NPA analysis showing the pathway associated with PANDA dysregulation. Data are presented as mean ± SEM and shown as percentage of control. **; p<0.01; ***; p<0.001; #; p<0.05 vs. HG. Comparisons were performed by 1-way analysis of variance (ANOVA) followed by Bonferroni correction.
Figure 2. A) Real time PCR showing PANDA expression in aortas from control and diabetic mice. B) Endothelium-dependent vasorelaxation in aortic rings isolated from diabetic mice, in the presence or in the absence of PANDA siRNA. *P < 0.01, n = 7/group. Data are expressed as mean ± standard deviation. ACh, acetylcholine.