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INHIBITION OF PRONGF PATHWAY RESTORES ERECTILE DYSFUNCTION THROUGH DUAL ANGIOGENIC AND NEUROTROPHIC EFFECTS IN THE DIABETIC MOUSE

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Introduction and Objective: Patients with diabetic erectile dysfunction (ED) often respond poorly to oral PDE5 inhibitors due to a lack of bioavailable nitric oxide from severe endothelial and neural dysfunction. ProNGF and its receptor $p75^{NTR}$ are known to be up-regulated in diabetic condition and to induce endothelial cell apoptosis and neuronal degeneration in the retina. The aim of this study was to investigate the role of proNGF-$p75^{NTR}$ signal pathway and effectiveness of proNGF-neutralizing antibody (proNGFab) in restoring erectile function in streptozotocin-induced diabetic mouse.

Methods: Diabetes was induced by intraperitoneal injection of streptozotocin (50 mg/kg) into 8-week-old C57BL/6 male mice for 5 consecutive days. At 8 weeks after the induction of diabetes, the animals were distributed into 3 groups: controls, streptozotocin-induced diabetic mice receiving repeated intracavernous injections of PBS (days -3 and 0; 20 μL) or proNGFab (days -3 and 0; 20 μg in 20 μL of PBS). The penis was harvested for histological and biochemical studies. We also examined the effect of proNGFab and $p75^{NTR}$ siRNA in primary cultured mouse cavernous endothelial cells, pericytes and major pelvic ganglion.

Results: The cavernous expression of proNGF and p75NTR was up-regulated in diabetic patients and STZ-induced diabetic mouse. Intracavernous injection of proNGF-Ab successfully restored erectile function in diabetic mice, which reach up to 90-100% of control values. ProNGFab significantly increased cavernous endothelial cell content and endothelial cell-cell junction proteins; decreased endothelial cell apoptosis, and restored neuronal cell content in the cavernous tissue of diabetic mice. Under the high glucose condition, proNGFab and $p75^{NTR}$ siRNA also promoted tube formation in mouse cavernous endothelial cells and enhanced neurite sprouting in major pelvic ganglion culture.

Conclusions: Our findings suggest that proNGF-$p75^{NTR}$ signal pathway played an important role in pathophysiology of diabetic erectile dysfunction and inhibition of proNGF-$p75^{NTR}$ pathway is a promising therapeutic strategy for diabetic ED.

Keywords: erectile dysfunction, diabetes mellitus, proNGF, $p75^{NTR}$

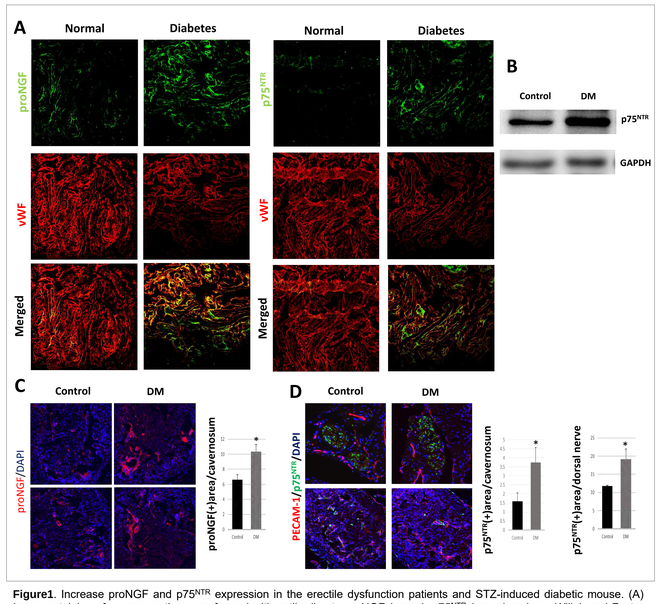

Figure1. Increase proNGF and $p75^{NTR}$ expression in the erectile dysfunction patients and STZ-induced diabetic mouse. (A) Immunostaining of cavernous tissue performed with antibodies to proNGF (green), $p75^{NTR}$ (green) and von Willebrand Factor (vWF; red) in non-erectile dysfunction patient and diabetic erectile dysfunction patient. (C) DAPI (blue) and proNGF (red) immunostaining in cavernous tissue from age-matched control (C) and diabetic (DM) mice. The proNGF-immunopositive area was quantified by ImageJ (n = 6). *P < 0.01 vs. control group. (D) $p75^{NTR}$ (green), PECAME-1 (red) and DAPI (blue) immunostaining in each group. The $p75^{NTR}$ immunopositive area in the dorsal nerve bundle and cavernous tissue was quantified by ImageJ (n = 6). *P < 0.001 vs. control group. (B) Representative Western blot analysis for $p75^{NTR}$ in mouse cavernous tissue.

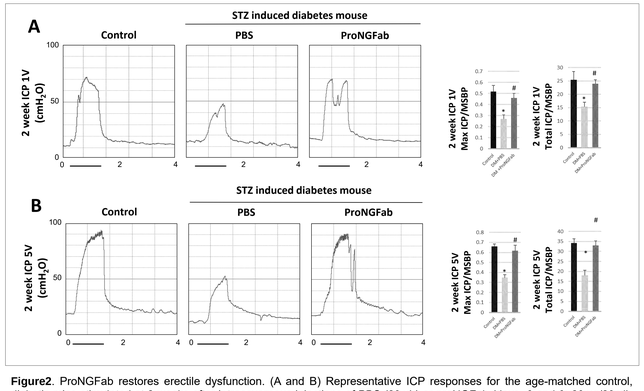

Figure2. ProNGFab restores erectile dysfunction. (A and B) Representative ICP responses for the age-matched control, diabetic mice stimulated at 2 weeks after intracavernous injections of PBS (20 μL) or proNGFab (days -3 and 0; 20 μg/20 μl). The cavernous nerve was stimulated at 1V and 5V. The stimulus interval is indicated by a solid bar. Ratio of mean maximal ICP and total ICP (area under the curve) to MSBP at 1V (n = 6). *P < 0.001 vs. control group; #P < 0.001 vs. PBS treat DM groups. Ratio of mean maximal ICP and total ICP (area under the curve) to MSBP at 5V (n = 6). *P < 0.001 vs. control group; #P < 0.05 vs. PBS treated DM groups.

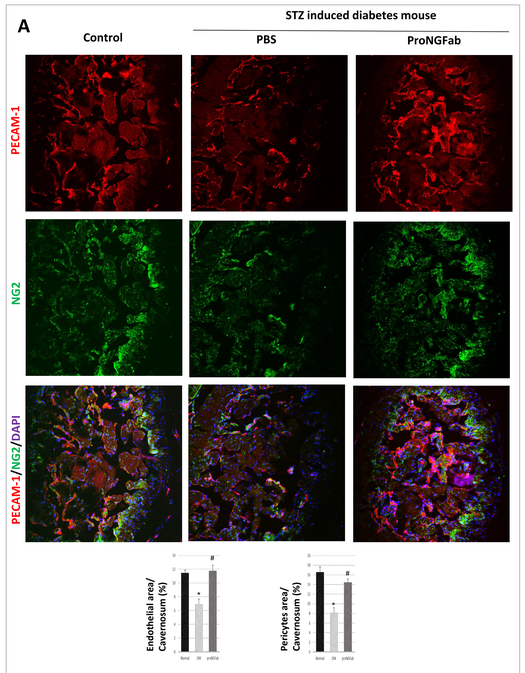

Figure 3. ProNGFab restores cavernous endothelial cell, pericyte content and enhances tube formation. (A) PECAM-1 (red) and NG2 (green) immunostaining in cavernous tissue from age-matched control, diabetic mice at 2 weeks after intracavernous injections of PBS (20 μL) or proNGFab (days -3 and 0; 20 μg/20 μl). Nuclei were labeled with DAPI (blue).Quantification of cavernous endothelial cell and pericyte content by ImageJ (n = 6). *P < 0.05 vs. control group, # P < 0.05 vs. PBS treated DM group. (B) Tube formation assay in primary cultured mouse cavernous endothelial cells (MCEC) exposed to normal and high glucose condition, those were treated with either mutant-proNGF or combination mutant-proNGF and siRNA$p75^{NTR}$.

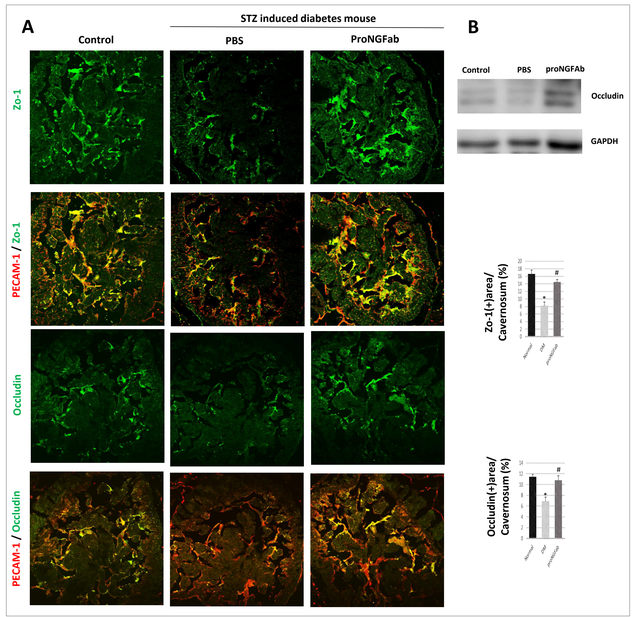

Figure 4. ProNGFab restored cavernous endothelial cells junction proteins. (A) Immunohistochemical staining of cavernous tissue with antibodies to Occludin (green), Zo-1 (green) and PECAM-1 (red) 2 weeks after treatment. Quantitative analysis of Occludin and Z0-1 positive area in 6 mice per group. *P < 0.05 vs. control group, # P < 0.05 vs. PBS treated DM group. (B) Representative Western blot analysis for $p75^{NTR}$ in mouse cavernous tissue.

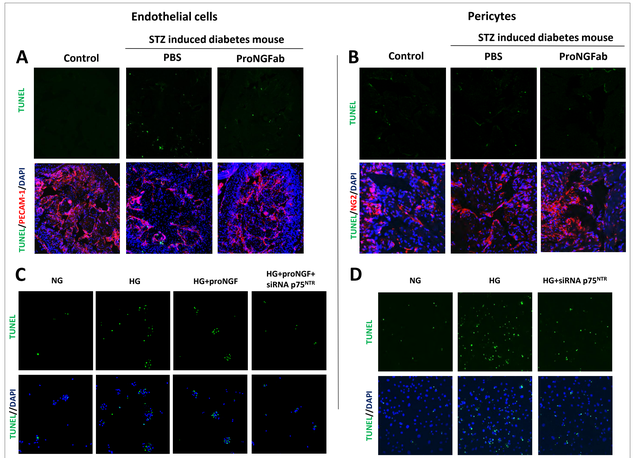

Figure 5. ProNGFab siRNA $p75^{NTR}$ decreases apoptosis of both endothelial cells and pericytes in vivo and vitro study.
(A and B) Immunofluorescent double staining of cavernous tissue were performed with TUNEL (green) and PECAM-1 (red) or NG2 (red).
(C and D) TUNEL assay in endothelial cells and pericytes exposed to a normal glucose and high glucose condition, those were treated with mutant-proNGF, combination mutant-proNGF and siRNA $p75^{NTR}$ or siRNA $p75^{NTR}$ only.

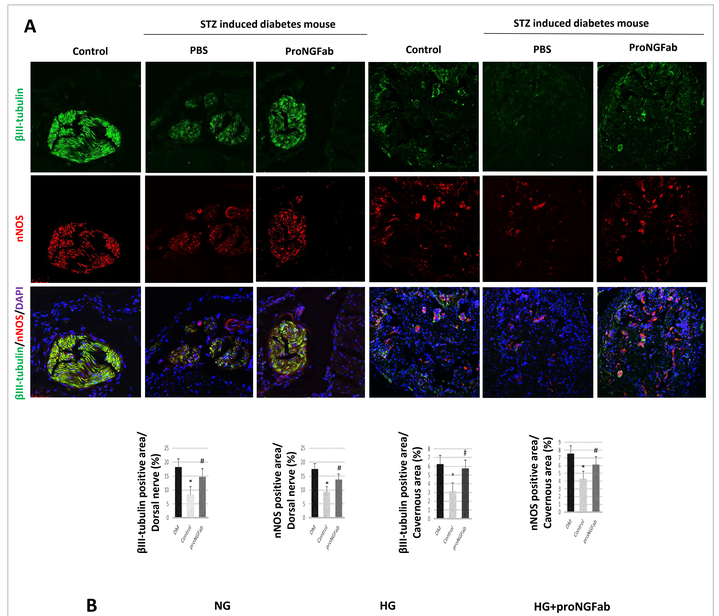

Figure 6. ProNGFab induces neural regeneration. (A) nNOS (red) and βIII-tubulin (green) staining of penis tissue from age-matched control, diabetic mice at 2 weeks after intracavernous injections of PBS (20 μL) or proNGFab (days -3 and 0; 20 μg/20 μl). Nuclei were labeled with DAPI (blue). Quantitative analysis of cavernous nNOS- and βIII-tubulin -immunopositive area in cavernous tissue or dorsal nerve bundle (DNB) was performed by an image analyzer (n=6 per group). *P < 0.05 vs. control group, # P < 0.05 vs. PBS-treated DM group. (B) βIII-tubulin (red) staining in mouse major pelvic ganglion (MPG) tissue, which were treated with PBS or proNGFab (1μL/mL).