ABSTRACT #105

CONTROL OF NGF AND MMP-9 EXPRESSION BY P75NTR ANTAGONIST THX-B IN BLADDER CELLS IN VITRO

Aya Hajj[1], Aalya Hamouda[1], Stephanie Sirmakesyan[1], Philippe Cammisotto[1], Lysanne Campeau[1,2]

[1]Lady Davis Institute for Medical Research, Montreal, Canada, [2]Lady Davis Institute for MEdical Research, Urology Department, Jewish General Hospital, Montreal, Canada

1.INTRODUCTION

Overactive bladder syndrome (OAB) was reported to be characterized by low levels of the neurotrophin NGF in the urine of female OAB patients, with stable levels of proNGF and a decrease in the ratio NGF over proNGF. This unbalance seemed to result from a high activity of the proteolytic enzyme matrix metalloproteinase-9 (MMP-9) found in the same samples. On the other hand, THX-B, an inhibitor of p75NTR receptor, increases NGF synthesis in a model of diabetic voiding dysfunction. The aim of the present study is to determine 1/ if NGF and MMP-9 are synthesized by cells of the bladder, 2/ if MMP-9 is central in the secretion of NGF and proNGF by the same cells and 3/ how NGF secretion might be affected by THX-B.

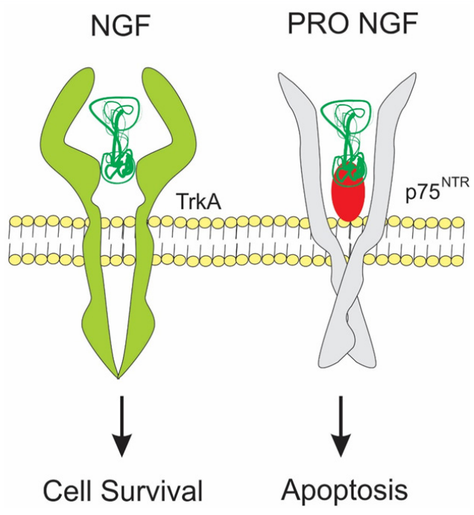

Figure 1. NGF binds TrkA receptor to activate cell survival pathways. ProNGF binds p75NTR receptor to activate apoptotic and inflammatory pathways.

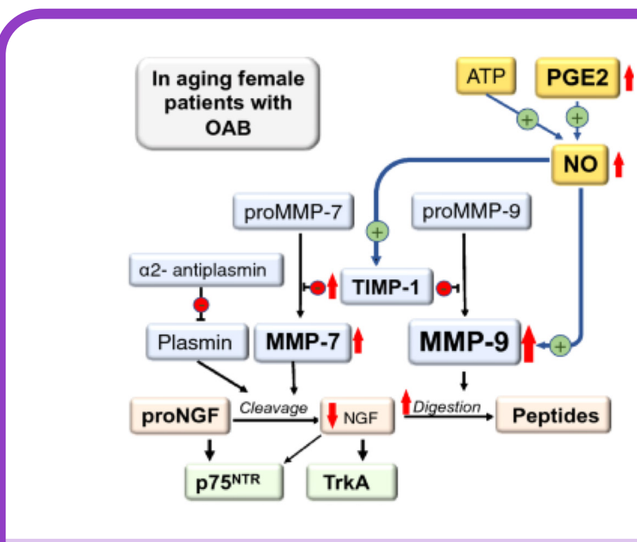

Figure 2. Urinary levels of NGF and related enzymes in aging female patients with OAB. A decreased NGF/proNG ratio might favor the activation of the proinflammatory p75NTR receptor.

2.METHODS

Rat bladders were digested by collagenase type IV to generate primary cultures of urothelial and smooth muscle cells.The expression of NGF, proNGF and MMP-9 were assessed by RT-qPCR and by immunoblotting. Cellular localisation of proteins was obtained by immunohistochemistry. Knock-down of MMP-9 was achieved using Crispr-Cas9. Levels of NGF and proNGF were measured by ELISA kits and enzyme activities by specific enzymatic kits.

3.RESULTS

Both urothelial and smooth muscle cells were significant sources of NGF, proNGF and MMP9 as revealed by RT-qPCR, immunoblotting and microscopy.

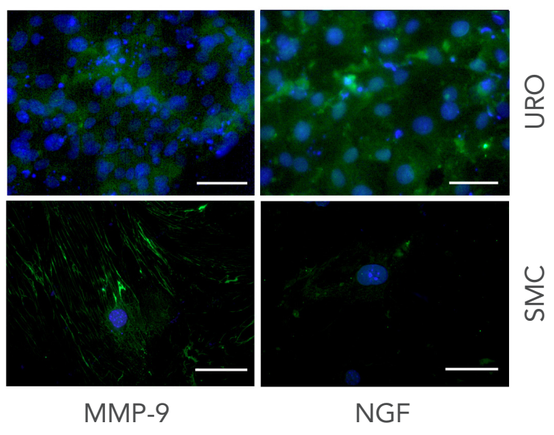

Figure 4. Immunohistochemistry: proNGF and NGF localized in the cytoplasm of cells (n=6)P-value of <0.001(***).

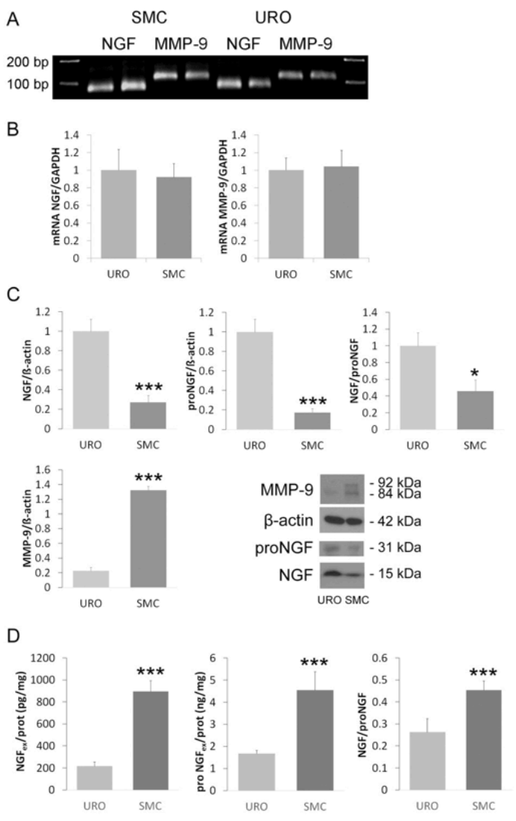

Figure 3. Urothelial (URO) and smooth muscle cells (SMC) are source of NGF and MMP-9. (A) Messenger RNA for NGF and MMP-9 were expressed in both cell types. (B) Quantification by RTqPCR revealed similar levels of expression. (C) Immunoblotting was used to semi-quantified NGF, proNGF and MMP-9 in cell content. (D) Extracellular concentrations of NGF, proNGF were assessed. (n=6),student t- test(*P<0.05, ***P<0.001).

4.CONCLUSION

Bladder cells express, synthesize and release NGF, proNGF and MMP-9.
The enzyme MMP-9 is a major regulator of NGF survival. THX-B increases NGF secretion by downregulation MMP-9 and increasing MMP-7 activities in urothelial cells.

3.RESULTS

Crispr-cas9 on NGF and proNGF secretion in (A) smooth muscle and (B) urothelial cells. Transfection was carried out using an empty plasmid (Ctl) or a plasmid containing sg primers targeting specific sequence of MMP-9 gene (MMP-9 KO). Efficiency of the MMP-9 knockout was confirmed by measuring the expression of MMP-9 mRNA by RT-qPCR, the intracellular MMP-9 relative content by immunoblotting and extracellular MMP-9 activity by an enzymatic kit. In parallel, NGF and proNGF concentrations and their ratio were assessed in extracellular culture medium. (n=6), student t-test (*P<0.05, ***P<0.001).

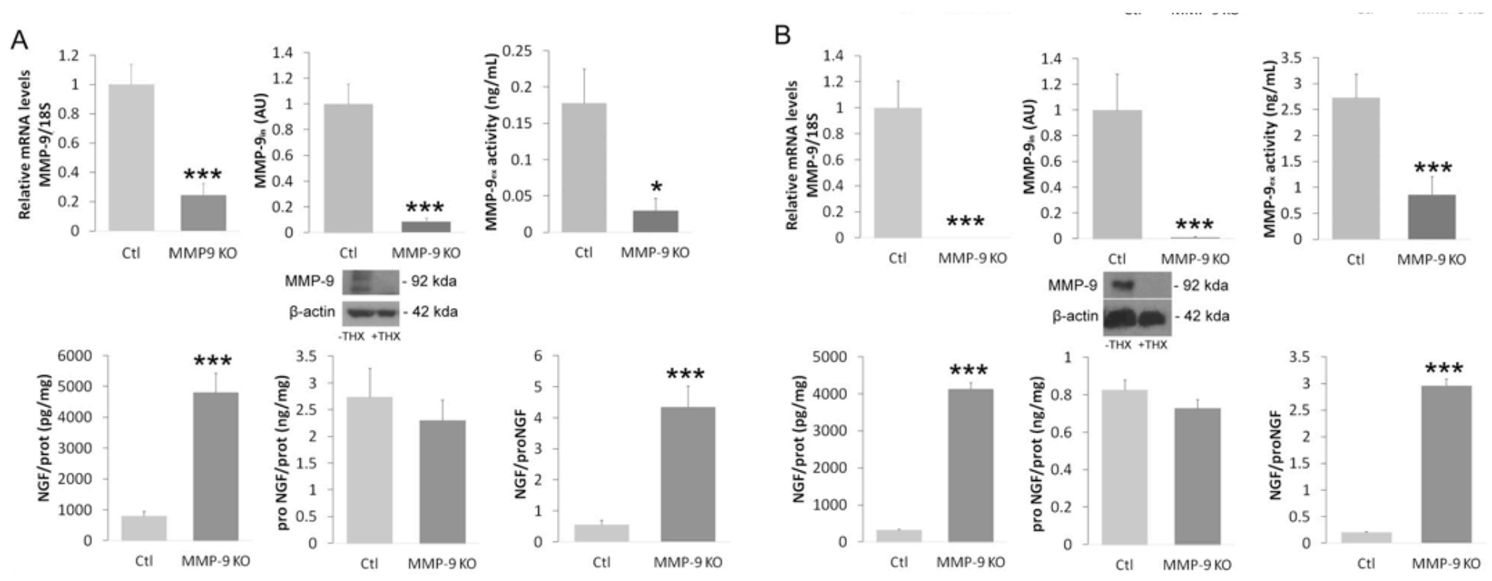

Figure 5. Crispr-cas9 on NGF and proNGF secretion in (A) smooth muscle and (B) urothelial cells. Transfection was carried out using an empty plasmid (Ctl) or a plasmid containing sg primers targeting specific sequence of MMP-9 gene. Efficiency of the MMP-9 knockout was checked by measuring MMP9/18S ratio by RT-qPCR, relative content by immunoblotting and extracellular MMP-9 activity. In parallel, NGF, proNGF and their ratio were assessed in extracellular medium. (n=6), student t-test (*P<0.05, ***P<0.001).

3.RESULTS

In urothelial cells, pathways associated to p75NTR, namely cyclic AMP, p38MAPK, erk and jnk were not affected by THX-B treatment.

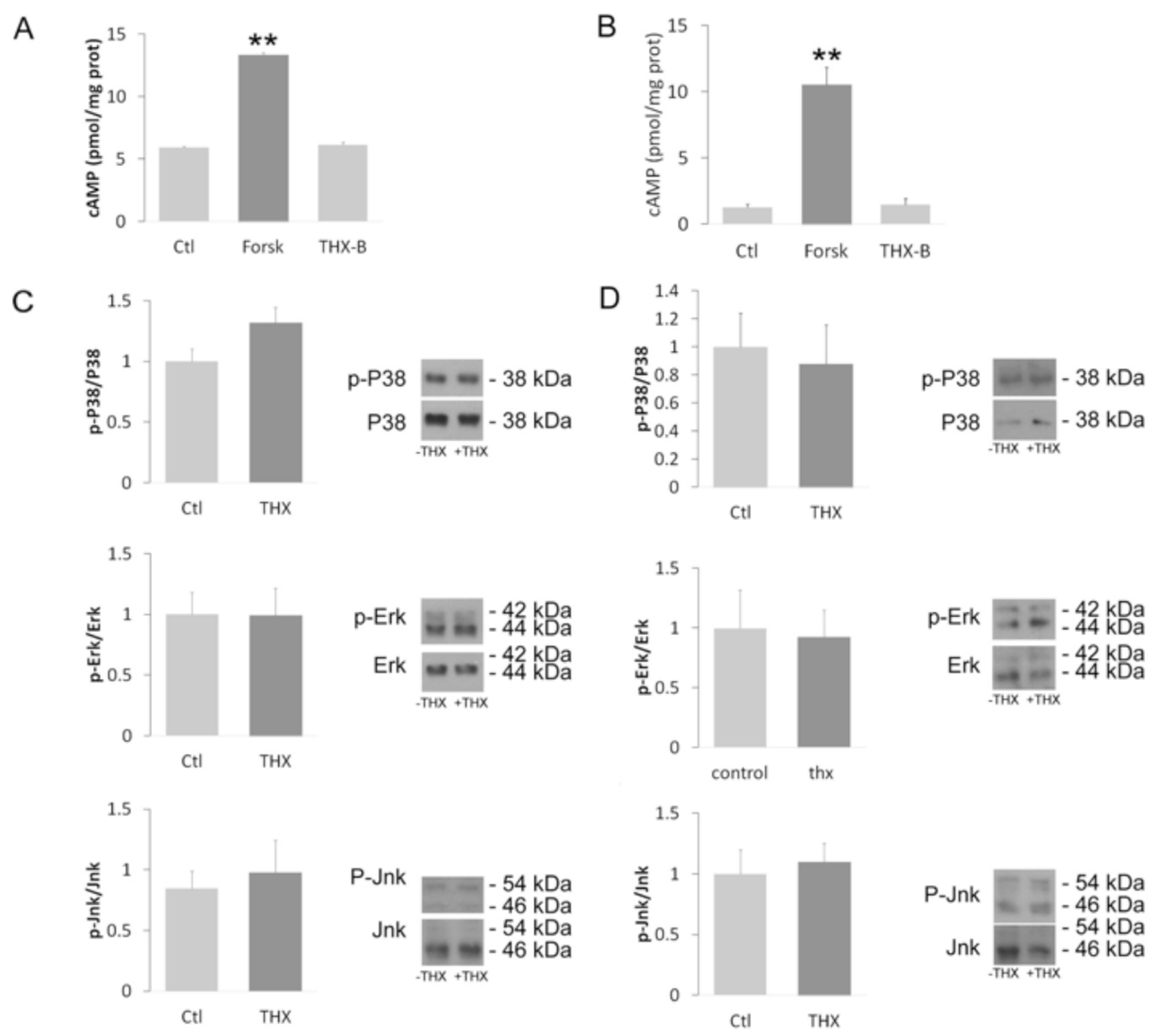

Figure 6. Measure of intracellular pathway activation by THX-B. In (A) urothelial and (B) SMCs, increase in cyclic AMP content in the presence of THX-B (5 µg/mL) for 24 hours was compared to forskolin (10 µM). (C) Urothelial and (D) SMCs were also assesed for p38MAPK, Erk and Jnk. (n=6), student t-test (**P<0.01).