Diabetic Complications Consortium

Application Title: PTP1B, a Potential New Therapeutic Target for Diabetic Vascular Complications

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1. Project Accomplishments:

During the current budget year, we pursued the investigation of the role of PTP1B in the control of endothelial function, in the context of ER stress and diabetes. In agreement with our central hypothesis, we demonstrated that diabetes concomitantly increased the expression of PTP1B and ER stress markers in saphenous veins and aortic biopsies collected from patients undergoing cardiac bypass surgery. Using a mouse model deficient in PTP1B and primary human aortic endothelial cells, we investigated the molecular mechanisms whereby PTP1B regulates endothelial function in the context of diabetes. We reported that PTP1B deficiency in mice prevented diabetes- as well as ER stress-induced endothelial dysfunction. Separately, we showed that reduction in PTP1B expression in human aortic endothelial cells prevented high glucose-induced ER stress. As high glucose and ER stress lead to cell apoptosis we investigated the interaction between PTP1B and endothelial cell viability and reported that PTP1B deletion in primary endothelial cells protects from ER stress-mediated reduction in endothelial cell viability. In addition we reported that PTP1B deletion prevented the increase in markers of apoptosis triggered by ER stress inducers. In parallel we observed that inhibition of P38 and JNK, two main effector of the apoptosis pathway, protected aortic rings from ER stress-induced endothelium dysfunction. To further investigate the mechanisms whereby PTP1B protects from ER stress and diabetes induced endothelial dysfunction we focus our interest on AMPKα, a major regulator of cell survival. We demonstrated that reduction in PTP1B expression is associated with an increase in AMPKα expression and activity while an increase in PTP1B expression is accompanied by a reduced AMPKα expression and activity. All together these data strongly suggest that PTP1B is a new regulator of endothelial cell viability and that its deletion protects from diabetes and ER stress-induced endothelial dysfunction by preserving cell from apoptosis via AMPK dependent mechanisms.

The data generated thanks to this award constitute the basis of a R01 application submitted in June 2015 to the VCMB study section of the NHLBI that received a score of 31% upon first submission.

Specific Aims:

Aim 1: ***Test the hypothesis that diabetes induces dysfunction of the endothelium secondary to increased PTP1B and ER stress in human saphenous vein and pulmonary artery endothelial cells.***

Results: We quantified the expression of PTP1B and ER stress markers (CHOP, GRP78, XBP1 and Spliced XBP1) in human saphenous veins and aortic biopsies collected from patients undergoing cardiac bypass surgery.

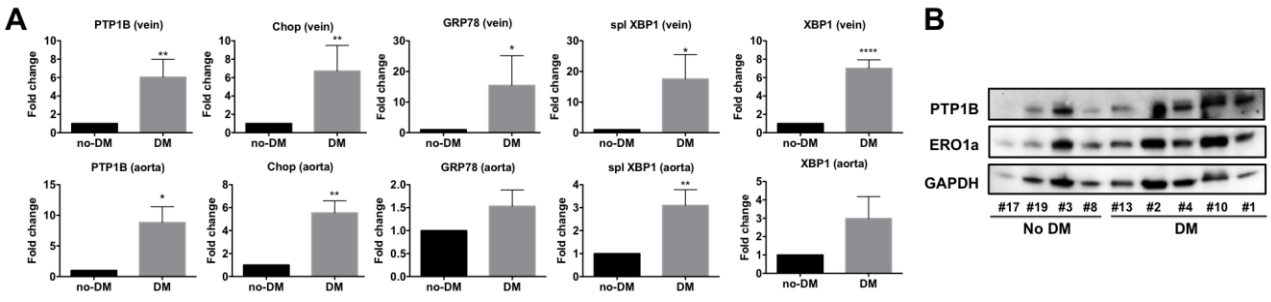

Figure 1: **Diabetes increases PTP1B and ER stress markers of apoptosis in human vessels.** (A) Quantitative real-time PCR quantification of PTP1B and ER stress markers (CHOP, GRP78, Spliced XBP1, XBP1) in saphenous veins and aortas from diabetic (DM) and non-diabetic patients (no-DM). (B) Protein expression of PTP1B and ER stress markers of apoptosis (ERO1α) in human saphenous veins. Data are mean ± sem. *p<0.05, **p<0.001 *vs.* No-DM. n=6 per group.

With quantitative real-time RT-PCR and western-blot we demonstrated that diabetes significantly increased PTP1B expression as well as ER stress markers in these two different vessels (Fig. 1).

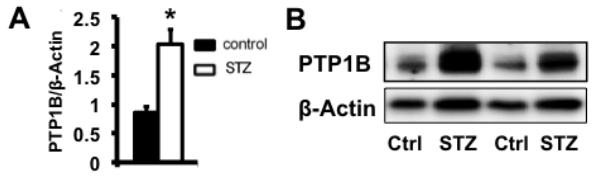

Figure 2: **Type 1 Diabetes increases PTP1B expression in mouse aorta. (**A) Western-blot quantification of aortic PTP1B expression in mice treated or not with streptozotocin (STZ). (B) Representative western-blot. Data are mean ± sem. *p<0.05 *vs.* Control. n=6 per group.

Separately we demonstrated that induction of type 1 diabetes with streptozotocin treatment increases aortic PTP1B expression in mice (Fig. 2) and showed that human aortic endothelial cell exposure to high glucose leads to an increase in PTP1B and ER stress markers expression (Figure not shown). In parallel, we reported that reduction in PTP1B expression in human aortic endothelial cells via siRNA blunted tunicamycin- (ER stress inducer) and high glucose-induced increased in ER stress marker expression (Fig. 3).

Aim 2: ***Test the hypothesis that PTP1B deletion or inhibition protects endothelial cells from diabetes-induced endothelial dysfunction by improving insulin sensitivity, and eNOS function and by reducing ER stress.***

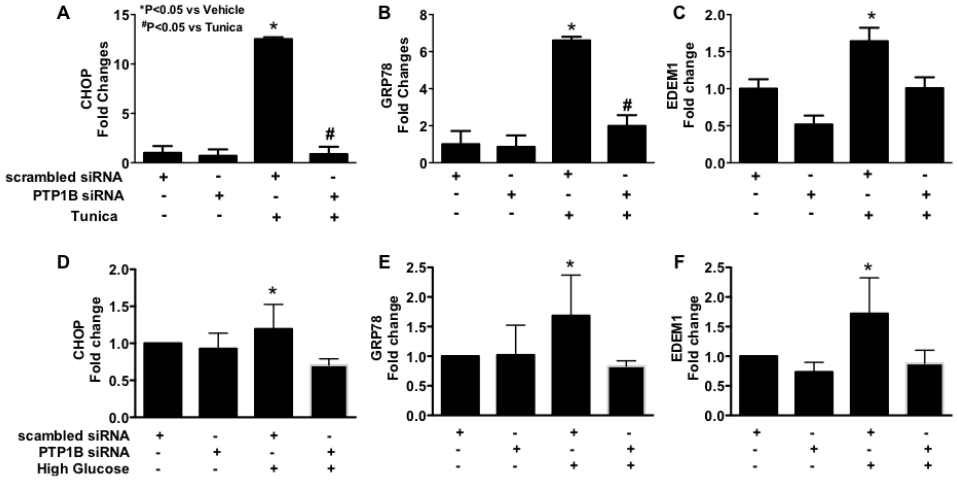

Figure 3: **Deletion of PTP1B in primary HAECs reduces ER stress reponse**. Reduction of PTP1B expression via gene silencing protects primary human aortic endothelial cells from tunicamycin (Tunica, A, B, C, D) and high glucose (25mM, 5 days, E, F, G)- induced ER stress. Data are mean±sem, *p<0.05 *vs. vehicle,* #p<0.05 *vs. Tunica*. n=6 per group.

Results: While analyzing the endothelial function of wild-type and PTP1B KO mice submitted to type 1 diabetes via streptozotocin injection, we reported that ER stress blockade in WT mice and PTP1B deletion respectively restored and prevented diabetes-induced endothelial dysfunction (figure not shown). While investigating the underlying mechanisms we reported that ER stress-mediated reduction in endothelium-dependent relaxation involves apoptosis. Indeed, inhibitors of JNK and P38, two effectors of the apoptosis pathway, prevented ER stress-induced endothelial dysfunction (Fig.4). We also showed that reduction in PTP1B expression in endothelial cells via siRNA prevented ER stress-induced endothelial cell apoptosis (Fig.5).

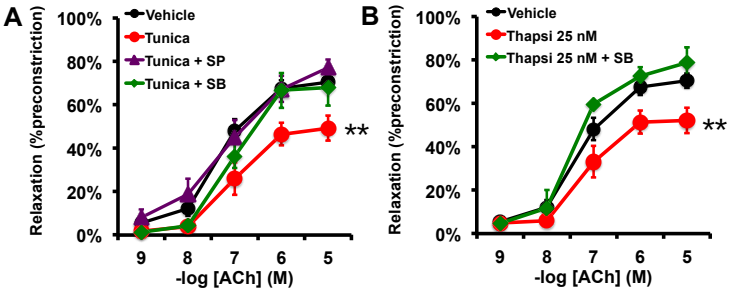

Figure 4: **Inhibition of the pro-apoptotic signaling pathway prevents ER stress-induced endothelial dysfunction.** Endothelium-dependent relaxation measured in aortic rings from wild-type mice incubated or not with the ER stress inducer Tunica or thapsigargin (Thapsi), in the presence or absence of the p38 (SB) or JNK (SP) inhibitor. Data are mean±sem, **p<0.01 *vs.* vehicle. n=6 per group.

In our search for the molecular mechanisms whereby PTP1B protects endothelial cells from apoptosis, we focused our attention on AMPKα, which is a major regulator of cell survival under stress conditions and a protective mechanism against atherosclerosis-associated ER stress. As PTP1B deletion increases AMPKα activity in metabolic tissues, we investigated whether PTP1B deletion increases AMPKα expression in the vasculature providing a protective mechanism against apoptosis. We reported that decreases in PTP1B expression in the aorta

of TNFα KO mice is associated with an increased AMPKα activity (ratio pAMPKα^{TH172}/AMPKα, (Fig.6), while a reduction in AMPKα and pAMPKα^{TH172} accompanies increases in PTP1B expression (Fig.7)**.**

Based on all these data we develop the working hypothesis (Fig. 8) according to which diabetes mediated endothelial dysfunction involves TNFα-mediated increase in PTP1B expression which leads to ER stress and endothelial cell apoptosis.

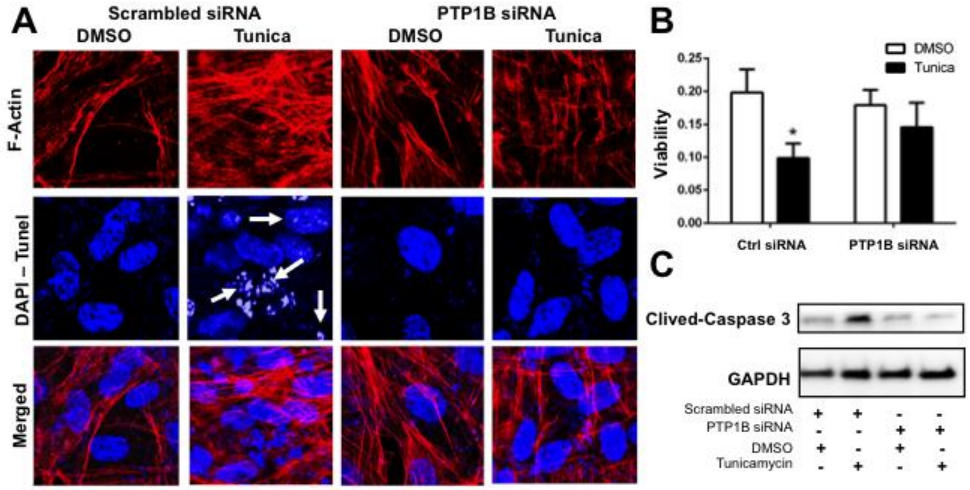

Figure 5: **Reduction in PTP1B expression via gene silencing prevents ER stress-induced endothelial cell apoptosis**. (A) immuno-histochemistry staining for F-actin, nuclei (DAPI) and DNA strand breaks (tunel), of HAECs expressing PTP1B or nor, in the presence or absence of tunica, (B) MTT assay conducted in HAECs expressing or not PTP1B and exposed to tunica, and (C) quantification of cleaved caspase 3 expression by western-blot. Data are mean±sem. n=6 per group, *p<0.05. Experiment conducted 1 time.

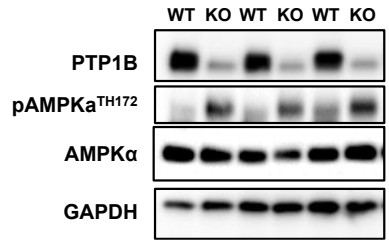

Figure 6: **Genetic deletion of TNFα reduces vascular PTP1B expression**. Western-blot quantification of PTP1B and AMPKα expression in aortic rings from wild-type and TNFα KO animals.

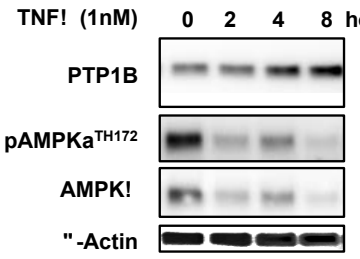

Figure 7: **TNFα increases endothelial PTP1B**. Western-blot quantification of PTP1B and AMPKα expression in HAECs treated with TNFα for 2 to 8 hours.

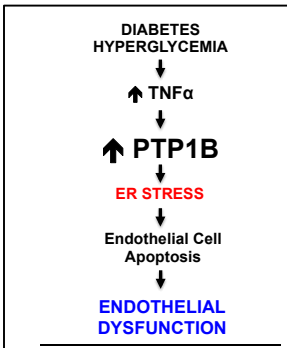

Figure 8: **Working Hypothesis**

2. Publications:

No publication resulted from this project yet. However, thanks to the data gathered, a R01 application was submitted in June 2015 that received a score of 31% after a first submission.