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Protein Tyrosine Phosphatase (PTP) 1B Inhibition Improves Endoplasmic Reticulum Stress-Induced Apoptosis and Impaired Angiogenic Response in Endothelial Cells

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Background

- Endothelial cells are the inner most layer of blood vessels.
- Continuously produce Nitric oxide (NO) and other vasoactive molecules, to promote vascular homeostasis.

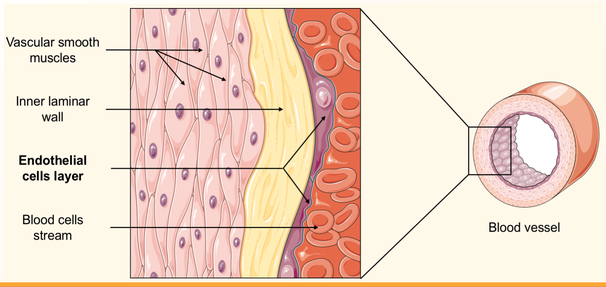
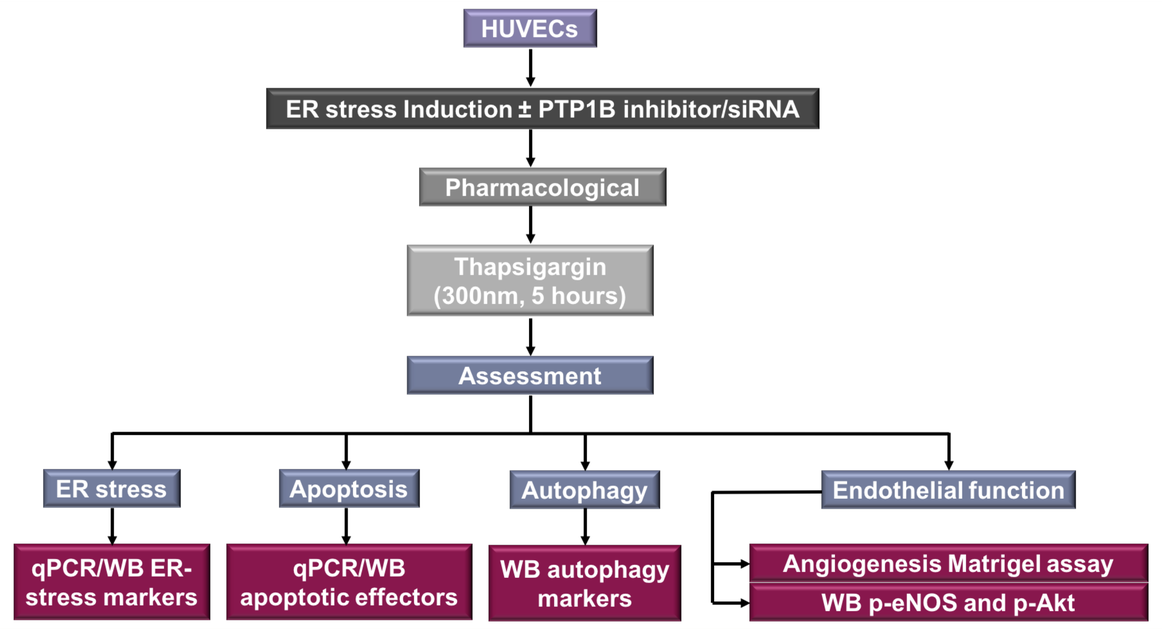


Figure 1. The vascular wall. showing the endothelial cell layer being the inner most layer directly in contact with the circulation.

- Impairment in these cells function is known as endothelial dysfunction.
- It has been shown that PTP1B expression plays a key role in the pathogenesis of endothelial dysfunction.
- ER stress activation has been linked to several diseases including diabetes and cardiovascular disorders that are both marked by endothelial dysfunction.
- There is a complex interplay between PTP1B and ER stress, However, the role of this crosstalk in endothelial cell death and dysfunction has never been investigated.

Methods



Study Objectives

1. Investigate the impact of PTP1B inhibition on ER stress-mediated endothelial dysfunction and impaired angiogenesis.
2. Determine the effects of PTP1B inhibition on ER stress-induced apoptosis and activation of apoptotic sub-pathways in endothelial cells.

Results

1. The impact of PTP1B inhibition on ER stress in endothelial cells

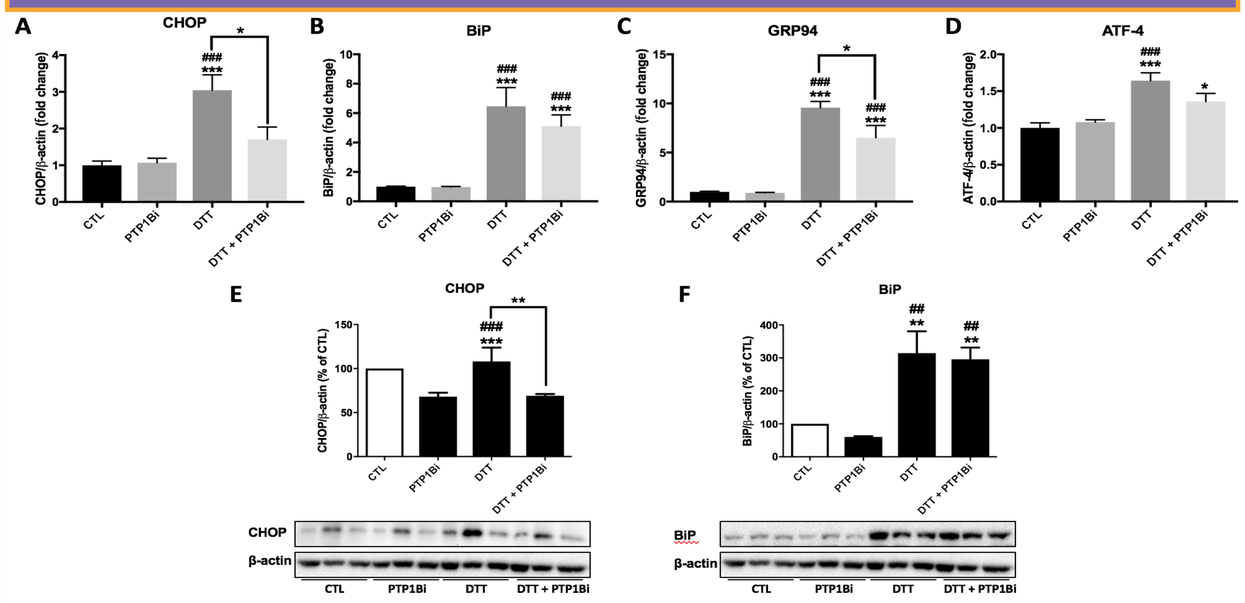


Figure 2. qPCR analysis to assess mRNA expression of CHOP (A), BiP (B), GRP94 (C) and ATF-4 (D) and western blot analysis to assess protein expression levels of CHOP (E) and BiP (F) in HUVECs exposed to thapsigargin [TG; 300 nM, 5 hours] in the presence or absence of PTP1B inhibitor [BML, 20 μM, added 1-hour prior to treatment]

2. Impact of PTP1B inhibition on eNOS and Akt activation HUVECs subjected to pharmacological ER stress

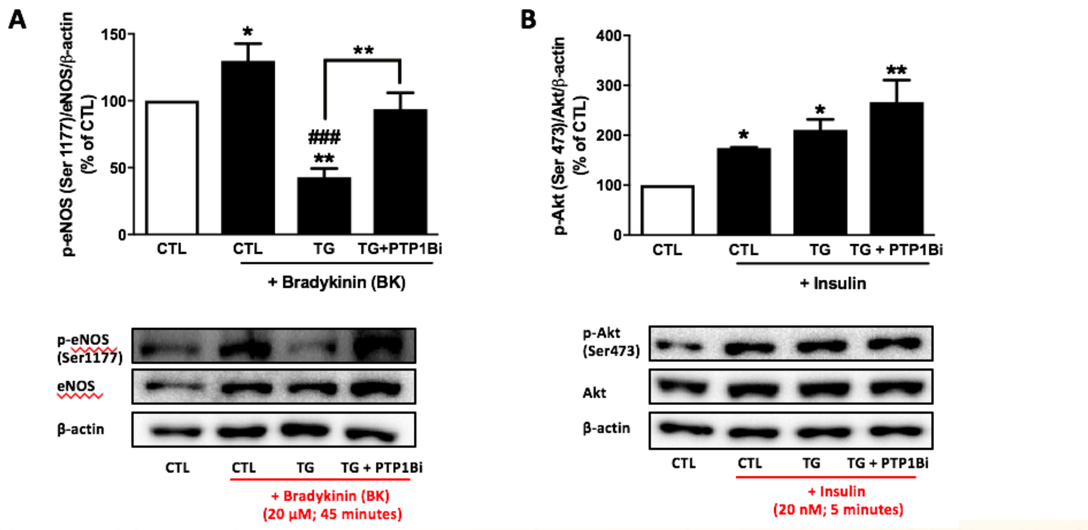


Figure 3. Western blot analysis to assess protein expression levels p-eNOS (Ser1177) (A) and p-Akt (Ser473) (B) in HUVECs treated with TG [300 nM for 5 hours] in the presence or absence of PTP1B inhibitor [BML, 20 μM, added 1-hour prior to treatment], followed by the incubation of cells with either bradykinin (BK) [20 μM, 45 minutes] (A) or insulin [20 nM, 5 minutes] (B)

3. Impact of PTP1B inhibition on angiogenic capacity of HUVECs subjected to pharmacological ER stress

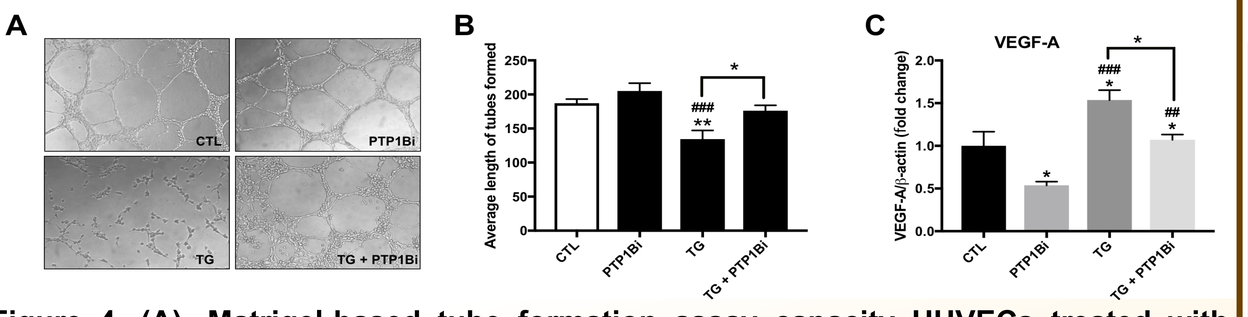


Figure 4. (A), Matrigel-based tube formation assay capacity HUVECs treated with thapsigargin [TG; 300 nM, 5 hours] in the presence or absence of PTP1B inhibitor [BML, 20 μM, added 1-hour prior to treatment] and grown tri-dimensional Matrigel matrix. Images are representative of three independent experiments. (B), Bars represent pooled data of quantification of angiogenic capacity, expressed as the average of length of tubes formed that were counted in five random fields for each well using WimTube software (n=3 per group). (D), relative mRNA expression of VEGF-A normalized against housekeeping gene β-actin

4. Impact of PTP1B inhibition and knockdown on cell survival of HUVECs subjected to pharmacological ER stress

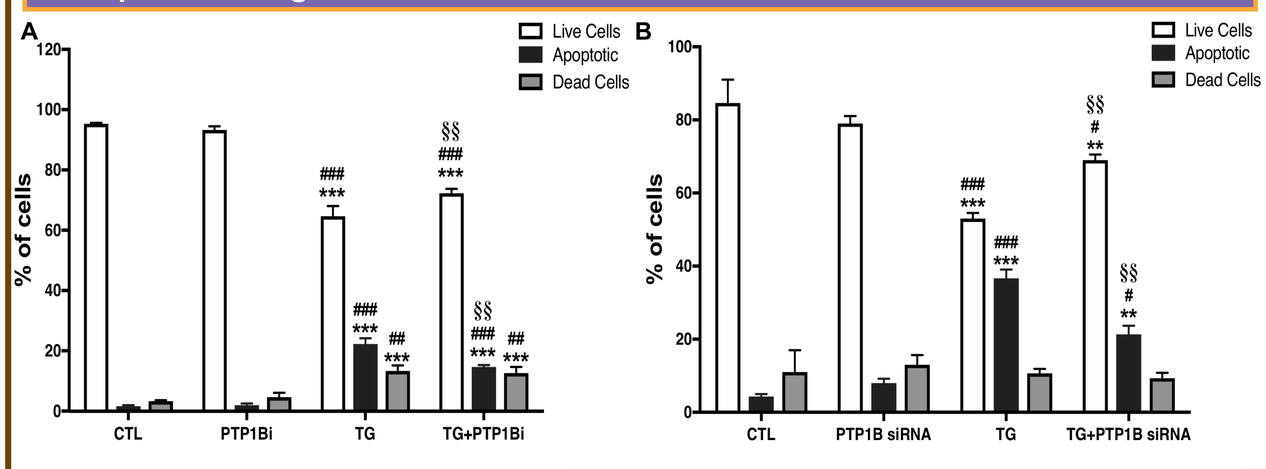


Figure 5. Tali image-based cytometer analysis of apoptosis in HUVECs treated with TG [300 nM, 5 hours] (A) in the presence or absence of PTP1B inhibitor (PTP1Bi) [BML, 20 μM, added 1-hour prior to treatment] and (B) in the presence or absence of PTP1B siRNA duplexes.

5. Impact of PTP1B inhibition on apoptotic signals in HUVECs subjected to pharmacological ER stress

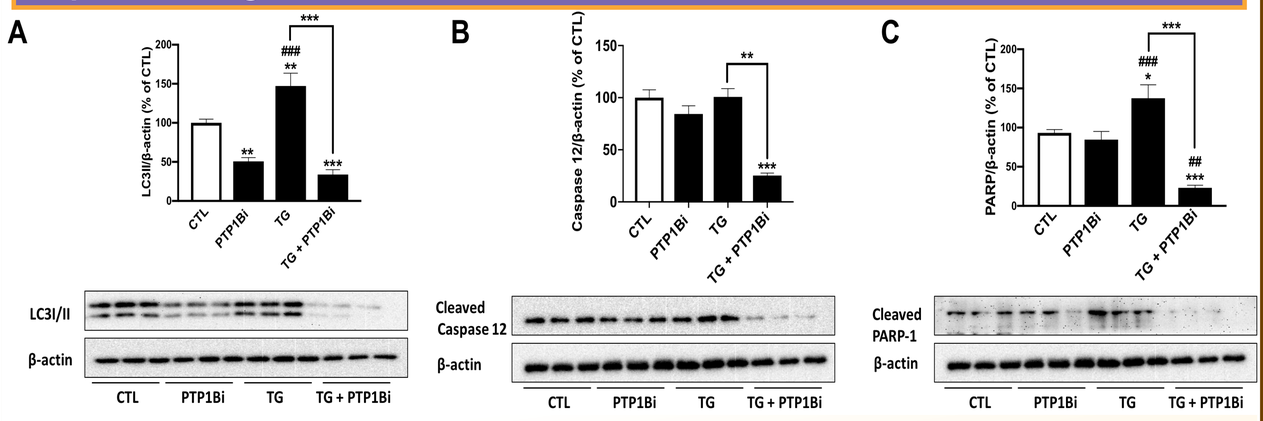


Figure 6. Western blot analysis to assess protein expression levels of LC3I/II (A), PARP-1 (B), Caspase 12 (C), Caspase 9 in HUVECs treated with thapsigargin [TG; 300 nM, 5 hours] in the presence or absence of PTP1B inhibitor [BML, 20 μM, added 1-hour prior to treatment]

Conclusion

- ✓ Our study to highlight the role of crosstalk between PTP1B and ER stress in endothelial cell dysfunction and shed light on central role of ER stress-mediated apoptosis in this process.
- ✓ Inhibiting PTP1B protected endothelial cells against ER stress-mediated apoptosis and impairment of endothelial function.
- ✓ Our work emphasized the critical implication of PTP1B in ER stress-mediated autophagy.

Future directions

- Investigate the impact of PTP1B inhibition on VEGF-A signaling as a reflector of angiogenesis.
- Determine the relative contribution of insulin resistance in PTP1B-mediated endothelial dysfunction.
- Identify the molecular targets of PTP1B in endothelial cells, especially those related to insulin response and NO pathway, using immunoprecipitation technique and PTP1B gene silencing combined with phosphor-proteomics.

Acknowledgements

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