P098

G-protein Coupled Estrogen Receptor Stimulates Capillary Formation by Human Umbilical Vein Endothelial Cells via ALK1-SMAD1/5/8 Pathway Activation

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Objectives:

Endothelial dysfunction and damage are associated with vasooclussive disorders. Moreover, estradiol (E2) induces vascular repair by promoting endothelial growth and capillary formation. The molecular mechanisms via which E2 mediates its vasoprotective effects remain unclear. Based on our previous findings that the capillary stimulating effects of E2 are mimicked by its non-permeable analog, BSA-tagged E2, we hypothesize that the stimulatory effects are potentially mediated via the newly discovered membrane bound G-protein Coupled Estrogen Receptor (GPER). Hence, in the present study, we assessed whether E2 stimulates the capillary formation in Human Umbilical Vein Endothelial Cells (HUVECs) via GPER by using GPER specific agonist G1, which mimicks E2 effects. Finally, we explored the role of ALK1-SMAD1/5/8 and PI3K-Akt, both prominent angiogenic pathways, in mediating the effects of E2.

Methods:

The role of GPER in E2-regulated capillary formation was assessed using 2D-matrigel based capillary assay. Cells were treated with the GPER specific agonist (G1), GPER specific antagonist (G15), G-protein pathway inhibitor Pertussis Toxin (PTX), ALK1 antagonizing antibody (ALK1Fc), PI3K-inhibitor LY292400 (LY), GPER and SMAD1 siRNA. Western Blotting was used to evaluate changes in ALK1 – and ID-1 expression, in SMAD1/5/8 and Akt phosphorylation and the efficiency of GPER and SMAD1 silencing siRNA transfection.
Experiments were conducted three times. *p<0.05 relative to control, §p<0.05 relative to G1 treated cells.

Results:

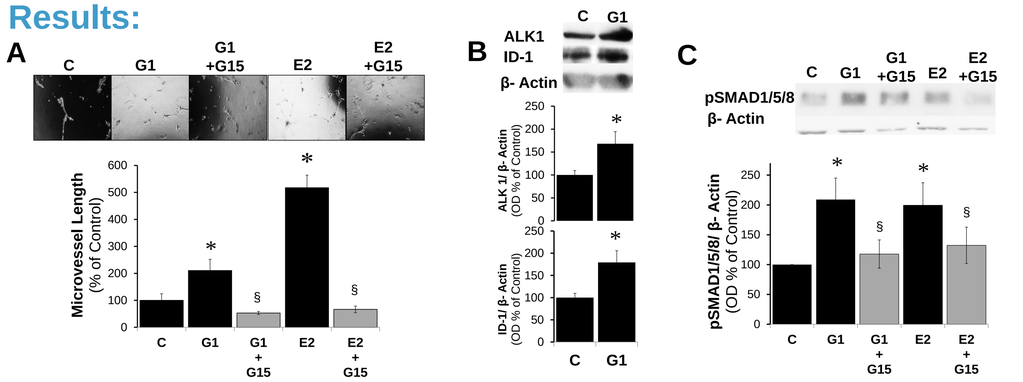


Figure 1: A: Treatment with GPER specific agonist G1 (10nM) and E2 (10nM) induced an increase in capillary formation and these effects were abrogated by GPER specific antagonist G15 (100nM). **B:** Treatment with G1 for 24h induced ALK1– and ID-1 expression. **C:** Treatment with G1 and E2 induced SMAD1/5/8 phosphorylation within 45min and this effect was blocked significantly by G15.

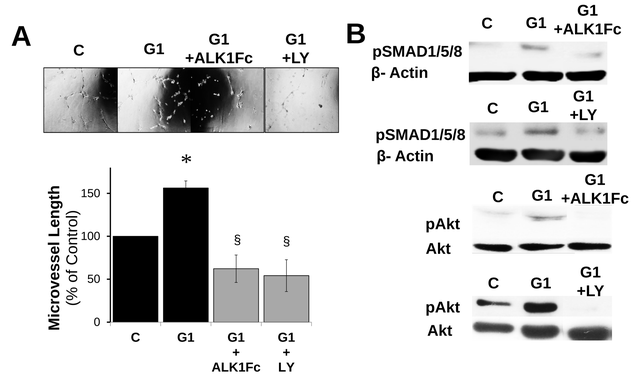


Figure 2: A: Treatment with ALK1 antagonizing antibody ALK1Fc (100ng/ml) and PI3K inhibitor LY294002 (5µM) decreased G1 (10nM) induced capillary formation. **B:** G1 induced pSMAD1/5/8 and pAkt were blocked by PI3K and ALK1 inhibitors.

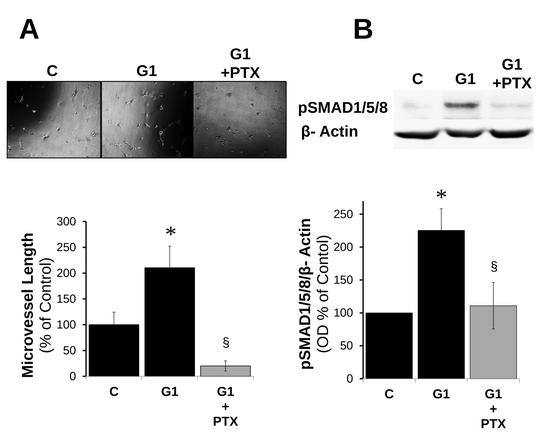


Figure 3: A: Treatment with G-protein pathway inhibitor PTX (0,1ng/ml) decreased G1 (10nM) induced capillary formation. **B:** G1 induced pSMAD1/5/8 was blocked by PTX.

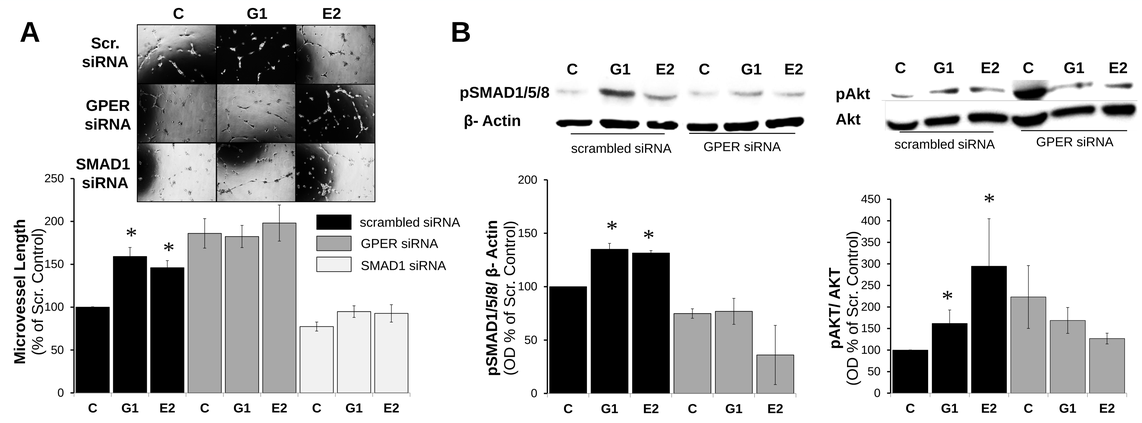
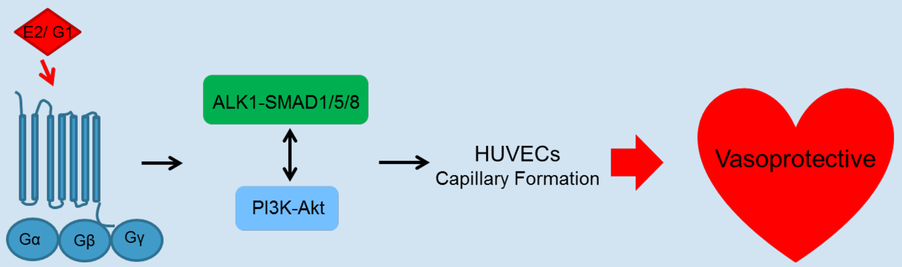


Figure 4: A: siRNA silencing of GPER and SMAD1 in HUVECs abrogated the stimulatory effetcs of G1 (10nM) and E2 (10nM) on capillary formation. Whereas in cells, treated with scrambled siRNA, G1 and E2 induced capillary formation. **B:** Similar to capillary formation GPER siRNA, but not scrambled siRNA, abrogated the stimulatory effects of G1 and E2 on SMAD1/5/8 and Akt phosphorylation.

Outcomes:

We provide the first evidence that GPER plays an active role in mediating the effects of E2 on capillary formation. Importantly we demonstrate that GPER activates two major angiogenic pathways i.e. ALK-1 → SMAD1/5/8 and PI3K → Akt. Furthermore our results suggest a cross-talk/ interplay between these pathways inducing capillary formation. Our findings provide evidence for a potential role of membrane bound GPER in vascular remodeling and improving endothelial recovery and function. Use of GPER specific agonists may be of therapeutic significance in preventing endothelial damage by promoting endothelial cell activity and inducing endothelial recovery and capillary formation following injury.



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Disclosures: None