Endothelial barrier protective effects of activated protein C (APC) require the endothelial protein C receptor (EPCR), protease activated receptor 1 (PAR1), and PAR3. In contrast, PAR1 and PAR3 activation by thrombin results in barrier disruption. Non-canonical PAR1 and PAR3 activation by APC versus canonical activation by thrombin provide an explanation for the functional selectivity of these proteases. APC induces non-canonical PAR3 activation at Arg41 and synthetic peptides representing the tethered-ligand sequence of PAR3 after non-canonical cleavage (P3R) induce barrier protective effects in vitro and vascular integrity in vivo. However, signaling mechanisms employed by PAR3 remain undefined. To obtain better insights into the relation between coagulation proteases with endothelial barrier protective effects and canonical/non-canonical PAR1 activation, we investigated the role of PAR3 in FXa-mediated endothelial barrier integrity.

The PAR3 proteolysis analysis was extended to factor Xa (FXa). Similar to APC, FXa-mediates endothelial barrier protective effects and PAR proteolysis analysis was extended to factor Xa in FXa-vivo. However, signaling mechanisms employed by PAR3 in FXa-protective effects in vitro and vascular integrity in vivo. 2 However, signaling mechanisms employed by PAR3 remain undefined. To obtain better insights into the relation between coagulation proteases with endothelial barrier protective effects and canonical/non-canonical PAR1 activation, we investigated the role of PAR3 in FXa-mediated endothelial barrier integrity.

1. PAR1 and PAR3 cleavage by FXa is dependent on EPCR

2. Canonical and non-canonical cleavage of PAR1 and PAR3 by FXa on cells

3. FXa-mediated changes in endothelial barrier integrity

4. Modulation of PAR1-mediated signaling by PAR3

5. Non-canonical PAR3 activation induces Tie2 activation

6. Stabilization of tight junctions by non-canonical activated PAR3-mediated Tie2

Results (cont.)

Phosphorylation of ERK1/2 and Akt by P3R (residues 42-54) and P3K (residues 39-54) was determined in the absence and presence of TRAP-peptides TFLRN or SFLRN. (A) Time-dependent ERK1/2 phosphorylation by P3R (50 µM) and P3K (50 µM). (B, C) ERK1/2 phosphorylation at 15 minutes induced by (B) TFLRN (25 µM) or (C) SFLRN (25 µM) after pretreatment (1 hr) with P3R (50 µM) or P3K (50 µM). (D) Phosphorylation of Akt by P3R (50 µM) and P3K (50 µM) over time. (E, F) Akt phosphorylation at 15 minutes induced by (E) TFLRN (25 µM) or (F) SFLRN (25 µM) after pretreatment (3 hr) with P3R (50 µM) or P3K (50 µM).

Activation of Tie2 was determined by analysis of Tie2 phosphorylation at Ser1119/Tie2. (A) Time-dependent Tie2 phosphorylation by FXa (25 nM), APC (50 nM) and thrombin (10 µM). (B) FXa (25 nM) induced phosphorylation of Tie2 at 90 min after pretreatment with antibodies against Tie2 (anti-hTie2; 25 µg/mL), PAR1 (WEDE15/ATAP2; 25 µg/mL), PAR3 (Moab19b; 25 µg/mL), EPCR (rcr-252; 25 µg/mL), or an inhibitor against PAR1 (Vorapaxar; 1 µM). (C) Induction of Tie2 phosphorylation at 15 and 90 min by the tethered-ligand peptides of PAR1, PAR2 and PAR3: TRAP (SFLLRN or SFLLRN). (D) Phosphorylation of Akt by P3R (50 µM) induced ZO-1 upregulation after 3 hours. (E) Effect of blocking Tie2 (anti-hTie2; 25 µg/mL) and PAR3 (Moab19b; 25 µg/mL) antibodies on FXa-induced (25 nM, 3 hr) ZO-1 upregulation. (F) Immunofluorescent staining for ZO-1 and DAPI in EA.hy926 cells incubated for 3 h in the absence or presence of FXa (50 nM) or P3R (50 µM).

Summary & Conclusions

Here we identified a novel pathway for Tie2 activation by non-canonical PAR3 activation that promoted tight-junction formation and endothelial barrier protective effects. In contrast, canonical activation of PAR3 enhanced PAR1-mediated barrier disruptive effects by thrombin.

References & acknowledgement

