

[O-S-089] SCUPA OR FXII STIMULATE ERK1/2 OR AKT THROUGH UPAR AND BETA 1 INTEGRINS

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Introduction: Studies have shown that high molecular weight kininogen (HK), single chain urokinase (ScuPA), and factor XII (XII) compete each's binding to the urokinase plasminogen activator receptor (uPAR). New studies show outside-in signaling or its regulation through uPAR by these proteins.

Methods: Using antibodies to phospho and total ERK1/2 or Akt, immunoblot investigations were performed. Further, the influence of ScuPA or FXII on HUVEC proliferation and growth was ascertained.

Results: ScuPA (4-200 nM) or XII (3-200 nM) in the presence of 0.05 mM Zn²⁺ stimulates ERK1/2 (MAPK 42 and 44) and Akt (Ser473) phosphorylation in endothelial cells (HUVEC). ScuPA or XII phosphorylation of ERK1/2 is blocked by PD98059, but partially decreased by wortmannin or LY294002. Akt phosphorylation (Ser473) by ScuPA or XII is blocked by wortmannin or LY294002, but not by PD98059. The region on uPAR that mediates this signaling is the same 22 amino acid sequence on uPAR domain 2 (D2) (peptides LRG20, PGS20) that binds ScuPA, XII, HK, and vitronectin. Cleaved HK (HKa) or peptide (HKH20) from the HK domain 5(D5) cell binding region block HUVEC ERK1/2 phosphorylation. Activated forms of ScuPA or XII were not required for signaling and cholesterol depletion had no influence. Antibody 6S6 to beta-1-integrin blocks ScuPA- or XII-induced phosphorylation of ERK1/2 or Akt. Investigations also show that ScuPA or XII induces HUVEC proliferation and growth and these activities are blocked by the same inhibitors, peptides and antibodies that block ERK1/2 or Akt phosphorylation.

Conclusions: These studies indicate that uPAR mediates ScuPA and XII outside-in signaling and activated forms of kininogen regulate these cellular activities connected to apoptosis and angiogenesis.

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