Mapping the interaction between uPAR and high molecular weight kininogen

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The urokinase plasminogen activator receptor (uPAR) is a GPI-linked receptor in membrane rafts on HUVEC that shuttles between proteins, modulating their behavior. In order to describe the regulatory functions of uPAR, its interaction with high molecular weight kininogen (HK) was mapped. Using purified HK, kallikrein-cleaved 56 kDa HK (56HKa) and 46 kDa HK (46HKa), low molecular weight kininogen (LK) and the isolated 56 kDa light chain of HK (LC), we mapped where uPAR binds to HK and where HK binds to soluble, recombinant uPAR (suPAR). The regions on HK that bind to uPAR were determined first. Biotin-HK binding to HUVEC is inhibited by HK, 56HKa or 46HKa with an IC_{50} of 110, 110 or 55 nM, respectively. Biotin-HK binding to suPAR also is inhibited by HK, 56HKa, or 46HKa with an IC_{50} of 60, 100, or 9 nM, respectively. The region on the light chain of HK and 46HKa that binds best to suPAR is H477-G496. Biotin-HK or -46HKa also binds by its heavy chain to suPAR at 10-fold lower affinity. The heavy chain HK/46HKa binding region to suPAR is C333-K345. Biotinylated peptides of these suPAR binding regions on HK specifically bind to suPAR. The HK binding site(s) on uPAR was determined next. Mab 3139 that blocks HK binding to HUVEC and suPAR immunoblots epitopes on uPAR's domains 2 and 3. Purified domain 1 (D1) of uPAR blocks biotin-HK and -46HKa binding to suPAR 18-35%. Purified isolated domains 2 & 3 (D2D3) block biotin-HK or -46HKa binding 55-60%. Combined D1 and D2D3 completely inhibit biotin-HK or -46HKa binding to suPAR. Mutagenizing the uPA binding region on the aminoterminal portion of domain 2 of suPAR does not interfere with HK binding. Fine mapping of the HK binding site on uPAR was performed by using sequential and overlapping 20 amino acid peptides prepared from uPAR. Two regions on uPAR contribute to HK binding. One region on the carboxyterminal end of D2 (L166-T195) blocks HK binding to HUVEC or suPAR and, when biotinylated, directly binds to HK. A second region on the aminoterminal portion of D3 (Q215-N255) blocks HK binding to HUVEC or suPAR and, when biotinylated, directly binds to HK. Both suPAR and recombinant cytokeratin 1 block biotin-HK or -46HKa binding to suPAR with equal affinity (IC_{50} = 1 µM). These investigations indicate that HK has the ability to intimately interact with uPAR. This activity influences vitronectin's interactions with

uPAR and uPAR's role in kallikrein formation, angiogenesis, and thrombosis prevention.

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