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THE UROKINASE-SENSITIVE REGION OF THE UROKINASE RECEPTOR IS RESPONSIBLE FOR ITS POTENT CHEMOTACTIC ACTIVITY

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Urokinase (uPA)-induced chemotaxis is physiologically important since uPA-/- mice are impaired in inflammatory cell recruitment (Gyetko et al., 1997). uPA must interact with its receptor (uPAR/CD87) which then activates intracellular src tyrosine kinases through an unidentified trans-membrane adaptor. Here we locate and functionally characterize a potent uPAR epitope that mimics the effects of the uPA/uPAR interaction. It lies in the region linking domains 1 and 2, the only protease-sensitive region of uPAR, efficiently cleaved by uPA at physiological concentrations. Synthetic peptides carrying this epitope promote chemotaxis and activate p56/p59hck tyrosine kinase. Both chemotaxis and kinase activation are pertussis-toxin (PT) sensitive, involving a Gi/o protein in the pathway.

Rat aortic smooth muscle cells show a prouPA-induced chemotaxis with all of the above properties. These cells allow an accurate determination of the cell shape changes consequent to chemotactic stimulation. Pro-uPA stimulation specifically causes reversible, PT-sensitive cytoskeleton and focal adhesion sites rearrangements.

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IDENTIFICATION OF A NOVEL MEMBRANE PROTEIN THAT INTERACTS SPECIFICALLY WITH THE uPAR-PROUROKINASE COMPLEX.

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A high molecular weight protein on human U937 cells interacts specifically with the uPAR-prourokinase complex in a reaction that can be fixed covalently by tissue transglutaminase (Behrendt, N. et al. (1993) FEBS Lett. **336**, 394-396). We have now isolated approx. 1 µg of this membrane protein and subjected it to various analyses based on mass spectrometry. Tryptic digestion was found to yield a peptide map distinct from all known proteins in the data base of derived composite peptide masses. Partial sequence information was obtained by nano-electrospray tandem mass spectrometry of tryptic peptides. This analysis confirmed that the protein is distinct from any known human sequence, but it was found to be closely related to a recently cloned murine cDNA hypothesized to code for an integral membrane protein, the function of which is unknown. Based on this information we have isolated a human cDNA clone that spans approx. 90 % of the complete sequence, as judged by comparison with the murine cDNA. Our preliminary studies suggest that this protein serves to bind various extracellular proteins in events that may play a role in adhesion, as well as in presenting substrate proteins for degradation mediated by the PA system. This type of function might have important implications for the role of this proteolytic system in invasive processes.