Abstract

Previous studies established that uterine epithelial cells and cell lines express cell surface heparin/heparan sulfate (HP/HS)-binding proteins (Wilson, O., Jacobs, A. L., Stewart, S., and Carson, D. D. (1990) J. Cell. Physiol. 143, 60-67; Raboudi, N., Julian, J., Rohde, L. H., and Carson, D. D. (1992) J. Biol. Chem. 267, 11930-11939). The accompanying paper (Liu, S., Smith, S. E., Julian, J., Rohde, L. H., Karin, N. J., and Carson, D. D. (1996) J. Biol. Chem. 271, 11817-11823) describes the cloning of a full-length cDNA corresponding to a candidate cell surface HP/HS interacting protein, HIP, expressed by a variety of human epithelia. A synthetic peptide was synthesized corresponding to an amino acid sequence predicted from the cDNA sequence and used to prepare a rabbit polyclonal antibody. This antibody reacted with a protein with an apparent Mr of 24,000 by SDS-polyacrylamide gel electrophoresis that was highly enriched in the 100,000 x g particulate fraction of RL95 cells. This molecular weight is similar to that of the protein expressed by 3T3 cells transfected with HIP cDNA. HIP was solubilized from this particulate fraction with NaCl concentrations > or = 0.8 M demonstrating a peripheral association consistent with the lack of a membrane spanning domain in the predicted cDNA sequence. HIP was not released by heparinase digestion suggesting that the association is not via membrane-bound HS proteoglycans. NaCl-solubilized HIP bound to heparin-agarose in physiological saline and eluted with NaCl concentrations of 0.75 M and above. Furthermore, incubation of 125I-HP with transblots of the NaCl-solubilized HIP preparations separated by two-dimensional gel electrophoresis demonstrated direct binding of HP to HIP. Indirect immunofluorescence studies demonstrated that HIP is expressed on the surfaces of intact RL95 cells. Binding of HIP antibodies to RL95 cell surfaces at 4 degrees C was saturable and blocked by preincubation with the peptide antigen. Single cell suspensions of RL95 cells formed large aggregates when incubated with antibodies directed against HIP but not irrelevant antibodies. Finally, indirect immunofluorescence studies demonstrate that HIP is expressed in both lumenal and glandular epithelium of normal human endometrium throughout the menstrual cycle. In addition, HIP expression increases in the predecidual cells of post-ovulatory day 13-15 stroma. Collectively, these data indicate that HIP is a membrane-associated HP-binding protein expressed on the surface of normal human uterine epithelia and uterine epithelial cell lines.