Purification and characterization of Perlecan derived from Human Synovium

Arivalagan Muthusamy, Charles M. Davis, III, and Ronald R. Gomes, Jr.
Department of Orthopedics and Rehabilitation, The Pennsylvania State University College of Medicine, Hershey, PA-17033, USA.

Rheumatoid arthritis (RA) is a chronic systemic disease marked by neovascularization and associated synoviocyte hyperplasia. This process produces a pannus of a highly vascularized tissue that invades and erodes articular cartilage and leads to joint destruction. Heparin-binding growth factors and perlecan (Pln), a multifunctional heparan sulfate proteoglycan, contributes to the neovascularization. We hypothesized that the glycosaminoglycan chains of human Pln isolated from rheumatoid synovium are differentially sulfated and may differentially modulate the bio-availability of heparin-binding growth factors relative to Pln derived from non-diseased synovium. To test our hypothesis, Pln was localized and purified from disease-free/"normal" synovial tissues, biochemically characterized, and then assessed for their growth factor binding abilities. Localization of Pln in the synovium by immunohistochemistry using anti-perlecan antibodies revealed that Pln was highly abundant in the epithelial surface and vascular bed region of the synovium similar to the staining pattern of vascular endothelial growth factor 165 ($VEGF_{165}$). Initially Pln was isolated by cesium chloride density gradient centrifugation, then enriched and purified by anion exchange and gel filtration chromatography. Perlecan was monitored during the purification process by protein and uronic acid assays, and by dot blot analysis using anti-perlecan antibodies. Perlecan was eluted on anion-exchange column with equilibration buffer containing 0.4 M NaCl suggests that they are either moderately glycosylated or not highly sulfated. Western blot analysis using anti-perlecan domain I and domain IV specific monoclonal antibodies confirms the presence of Pln protein core. Carbohydrate analysis demonstrated the presence of both chondroitin sulfate (CS) and heparan sulfate (HS) chains on Pln. Disaccharide analysis of human Pln revealed that their HS chains contain the higher amount of *0*-sulfate (95%) and lesser amount of 6-*0*-sulfate (1.5%) and 2-*0*-sulfate (1%). Solid phase binding assays indicate that Pln binds FGF-2 and $VEGF_{165}$ relative to recombinant PlnDI and human Pln derived from osteoarthritic articular cartilage tested. Removal of chondroitin sulfate chains enhances binding of Pln to growth factors, whereas removal of heparan sulfate chains abolished binding, suggesting that growth factor binding to Pln is heparan sulfate dependant. Future studies will functionally characterize synovial Pln ability to modulate capillary tube-like formation of synovial microvascular endothelial cells with or without various heparin binding growth factors.
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