HEPARAN SULFATES: OLD FELLOWS IN THE CELL CYCLE SCENARIO.`

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The syndecans, heparan sulfate proteoglycans (HSPG) are molecules associated with the cell surface and extracellular matrix (ECM), and consist of a protein core to which HS chains are covalently attached. The functions of cell surface heparan sulfate proteoglycan have been centered on the role of heparan sulfate chains, located at the outer face of the cell surface, in the binding of a wide array of ligands, including extracellular matrix (ECM) proteins and soluble growth factors. The core proteins of the syndecan family transmembrane proteoglycans have also been implicated in cell signaling through the interaction with integrins and tyrosine kinase receptors. HSPG are present on the cell surface of mammalian cells in culture and in most vertebrate and invertebrate tissues. They are composed of alternating units of glucosamine and uronic acid (glucuronic or iduronic) and the hexosamine is either N-acetylated or N-sulfated and/or 6-sulfated, and the uronic acid can be 2-O sulfated. The fine structure of heparan sulfates will be presented as well as structural requirements for interaction with growth factors. The data suggest that the mitogenic activity is primarily potentiated by interacting with highly sulfated regions of heparan sulfates chains. Also, it can be concluded that the glucuronidic domain in heparan sulfates hampers the active oligosaccharide domain to interact with the protein. Phorbol esters with different activities on PKC (PMA, PD, PDD, PDBu activators and 4α-PDD, P, PM biologically inactive) were used to study the role of the kinase on the expression of HSPG and cell cycle. The most remarkable aspect of those results, was the correlation between stimulation of HSPG synthesis and a block of G_1-S phase traversing, both triggered by the active phorbol esters. Staurosporine and RO potent kinase inhibitors abolished the effect of all the phorbol esters that activate PKC. On the other hand activation of cAMP/PKA pathway by, both, forskolin and/or 8-BrcAMP, is not effective to trigger stimulation of HSPG synthesis. Downregulation of PKC renders endothelial cells resistant to a second PMA treatment. The data suggest a relationship between PKC activation and endothelial cell cycle blockage. Furthermore, the combination of xylosides with PMA produced some cumulative effect. PMA stimulates the synthesis of heparan sulfate mainly at G_1 phase whereas the highest enhancement of synthesis produced by the xylosides is in the S phase of the endothelial cell cycle. These results led us to investigate the involvement of the ras-raf-MAPK pathway. EJ-*ras*-transfected endothelial cells (EJ-*ras* EC) display morphological changes, much higher expression of the *ras* oncogene and deregulation of the cell cycle, becoming more densely populated and serum-independent. In addition, EJ-*ras* EC display increased levels of the syndecan-4 mRNA. Besides the increase in the core protein, there is an increase in the glycosylation of the syndecan-4 protein, a proteoglycan that bears heparan sulfate chains. This increase is observed for the heparan sulfate proteoglycan (HSPG) synthesized by the cells and secreted to the culture medium. This enhancement in heparan sulfate synthesis was observed through the metabolic labeling of the cells, immunoprecipitation of syndecan-4 and heparitinases treatment. Furthermore, transfected cells do not exhibit decreased synthesis of heparan sulfate during G_1-S phase transition, as observed for the parental cell line. Also, heparan sulfate synthesis is not stimulated by PMA as in parental endothelial cells. Significant structural changes of heparan sulfate such as decreased O-sulfation were observed in the EJ-*ras*-transfected cells. A decrease in the mRNA levels of epimerase, 2-O-sulfotransferase and N-sulfotransferase, enzymes involved in the biosynthetic pathway of heparan sulfate was also observed. We also observed a decrease in the expression of the G1 regulatory proteins, cyclins D and E. The combined results suggest that the EJ-*ras* oncogene, through the signal transduction cascade, alter the cell cycle and the expression of proteoglycans in endothelial cells. The *Ras* family of small proteins which bound to GTP plays a central role in regulating integrin activation. Recent evidences suggest that the extracellular signal-regulated kinase (ERK) pathway contributes to cell migration and adhesion in a manner independent of its ability to promote gene transcription or cell proliferation. Thus the effect of the EJ-*ras* oncogene transfection on the ability of the cells to adhere and migrate to different substrata (fibronectin, laminin, collagen I and collagen IV) was also investigated. The results suggest that EJ-*ras* oncogene, through the signal transduction cascade, alters adhesion and migration in endothelial cells.

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