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Heparan sulfate and cell division

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Abstract

Heparan sulfate is a component of vertebrate and invertebrate tissues which appears during the cytodifferentiation stage of embryonic development. Its structure varies according to the tissue and species of origin and is modified during neoplastic transformation. Several lines of experimental evidence suggest that heparan sulfate plays a role in cellular recognition, cellular adhesion and growth control. Heparan sulfate can participate in the process of cell division in two distinct ways, either as a positive or negative modulator of cellular proliferation, or as a response to a mitogenic stimulus.

Key words
- Heparan sulfate
- Glycosaminoglycan
- Proteoglycan
- Cell division
- Cell cycle
- Growth factors

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Introduction

Heparan sulfate was discovered 50 years ago by Jorpes and Gardell (1). Nevertheless, its biological functions are still under investigation. The heparan sulfate proteoglycans, that correspond to the glycosaminoglycan chains covalently linked to a core protein, are present in all tissues from every species that show tissue organization, and has a highly variable structure (2,3) which may account for at least one of their presumable biological roles, i.e., cell-cell recognition (4,5). Also, heparan sulfates have been implicated in other biological processes such as interaction with extracellular matrix components (laminin, fibronectin, collagen) (6), participation in focal adhesion formation (7), and cell growth control (8,9). This review focuses on the possible roles of heparan sulfates in cell division.

Heparan sulfate and the control of cell division

Heparan sulfate can participate in the process of cell division in two different ways, either acting as a positive or negative modulator, or as a response to a mitogenic stimulus.

Heparan sulfate as cell growth modulator

Heparan sulfate can either be stimulatory of cell growth, e.g., when it is a co-receptor for growth factors, or inhibitory, depending on the experimental model.

The action of heparan sulfate as a positive modulator of cell proliferation is due to its capacity to bind and act as a co-receptor for growth factors, such as the fibroblast growth factor (FGF) (Figure 1). Growth factors are polypeptide molecules that elicit several responses in target cells. These responses include proliferation, differentiation, survival and apoptosis of the cells. FGF, for instance, binds to the extracellular domain of its receptor, which is a transmembrane molecule with protein-tyrosine kinase activity in the cytoplasmic region. After growth factor binding, the dimerization of the receptor occurs, followed by autophosphorylation of the intracellular domain of the receptor. This process will initiate a signal transduc-

tion cascade which will generate signals that reach the nucleus activating the expression of specific genes, leading to the cellular response.

It has been extensively reported that proteoglycans, due to their highly negative charge, bind to several growth factors and this binding is thought to have an important regulatory role (10,11). It has also been demonstrated that heparin binds to FGF and is necessary for its action. As heparin is present exclusively inside the cells, the heparan sulfate proteoglycan would be the "candidate" molecule to play this role *in vivo*. The results suggested that both heparin and heparan sulfate proteoglycan may protect FGF from inactivation and proteolysis (12,13) and function as a reservoir for the growth factor which would be released according to the proliferation needs of the cells (14). Other data, however, show that heparan sulfate proteoglycan mediates the binding of FGF to its high affinity receptor, which bears tyrosine kinase activity (15,16).

The potentiation of the mitogenic activity of aFGF (FGF-1) on 3T3 fibroblasts by highly sulfated oligosaccharides obtained from heparin and heparan sulfate has been demonstrated (17). One curious finding was that, although heparan sulfates extracted from several mammalian tissues potentiated the mitogenic activity of aFGF, in general, they were less efficient than heparin, on a mass basis. On the other hand, heparan sulfate prepared from 3T3-conditioned culture medium proved to be more efficient than heparin both on a mass and molar basis. These results suggest that there is a structural specificity for this interaction.

Members of the FGF family mediate, at least in part, the outgrowth of the mesoderm of the developing limb bud in response to the apical ectodermal ridge. It has been shown that syndecan-3, a heparan sulfate proteoglycan, plays an essential role in this process, mediating the interaction of the FGFs produced by the apical ectodermal ridge with the underlying mesoderm of the limb bud (18).

All these results implicate heparan sulfate as a positive modulator of mitogenic stimuli. However, there are several other results indicating that heparan sulfate, as well as heparin, can function as an inhibitor of cell proliferation. The action of heparan sulfate inhibiting cell proliferation is corre-

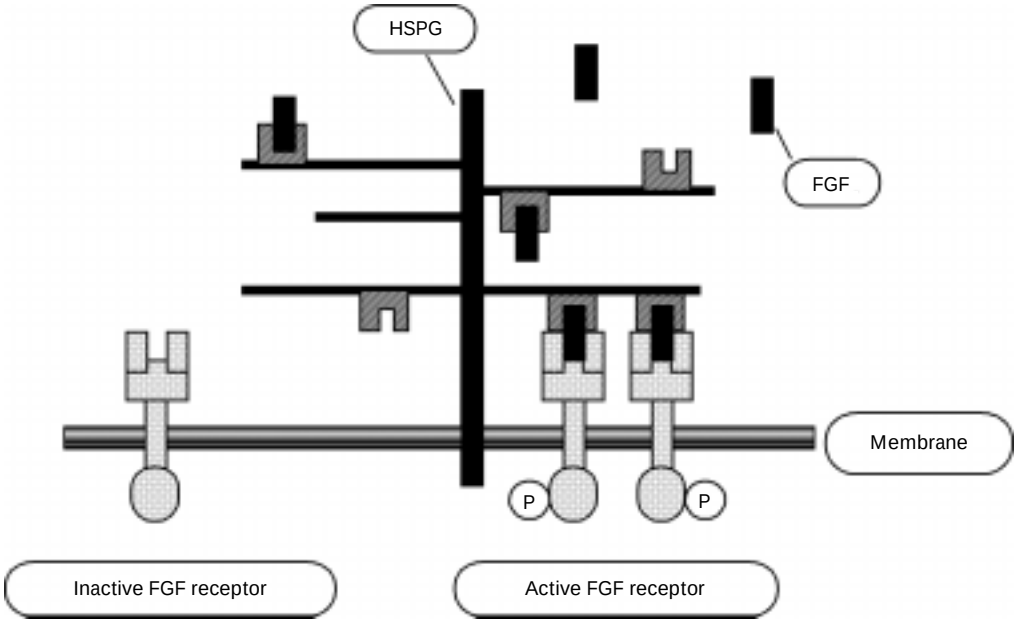

Figure 1 - Ativaction of fibroblast growth factor (FGF) receptor by interaction with the heparan sulfate proteoglycan (HSPG)-FGF complex (modified from Ref. 11).

lated with the same action exerted by heparin in a variety of experimental models. Stimulation of quiescent rat mesangial cells with serum induces the expression of *c-fos* mRNA. Addition of heparin and heparan sulfate prepared from rat mesangial cell layers and conditioned medium inhibits both cell proliferation and expression of *c-fos* mRNA (19).

Cell surface heparan sulfate proteoglycan prepared from log and confluent monolayers of a rat hepatoma cell line was added to a hepatoma cell culture and the proliferation of the cultures was analyzed (20). The results showed that heparan sulfate proteoglycan synthesized by confluent hepatocytes prevents cell division due to a block in the G_1 phase of the cell cycle prior to the G_1/S transition. On the other hand, heparan sulfate proteoglycan synthesized by exponentially growing cultures stimulated cell proliferation.

Transfection of NIH 3T3 cells with the mouse syndecan-1 cDNA induced high expression of this heparan sulfate proteoglycan on the cell surface and increased its shedding into the culture medium (21). Another result of transfection was the inhibition of bFGF (FGF-2)-induced cell proliferation, suggesting that the expression of heparan sulfate proteoglycan might provide a mechanism to restrict the action of FGF.

When heparan sulfate proteoglycans extracted from rabbit aorta were applied periadventitially in the rabbit carotid artery injury model provoked by a balloon catheter, they played an inhibitory role in neointimal formation (22). *In vitro,* the heparan sulfate proteoglycans maintained smooth muscle cells in a quiescent state.

These results, together with others, illustrate the statement we made at the beginning of this section, that heparan sulfate proteoglycans might play at least two distinct and apparently opposite biological roles as modulators of cell growth. As has been extensively reported, they might act as positive effectors of cell proliferation, functioning as co-receptors for growth factors, such as those from the FGF family. On the other hand, heparan sulfate proteoglycans seem to modulate cell growth negatively, just like heparin. Actually, heparin also plays both roles in cell growth control, and heparan sulfate would then be the biological molecule involved in these processes. It is not clear if these opposite actions are a consequence of the distinct experimental models used or if, in reality, they are different biological roles played by heparan sulfate proteoglycans due to the characteristic structural variability of these molecules.

Heparan sulfate synthesis and secretion is a response of cells to mitogenic stimuli

Changes in heparan sulfate synthesis and secretion during the different phases of the cell cycle have been described in a few experimental models and questions still remain to be answered. A significant decrease of heparan sulfate is observed in regenerating rat liver after partial hepatectomy. The peak of mitotic activity coincided with the smallest amounts of heparan sulfate (23). Also, quiescent cells exhibit an increase in DNA synthesis after treatment with a crude extract of *Flavobacterium heparinum,* which contains enzymes that degrade heparan sulfate (24).

In the early 70's, Kraemer and Tobey (25) described a premitotic loss of cell surface heparan sulfate in CHO cell culture, with indications that this loss was not merely part of a general premitotic secretion of cell surface components but, rather, a specific loss. Furthermore, data from studies using the murine melanoma cell line B16-F10 showed that the turnover of heparan sulfate and chondroitin sulfate is decreased in the S phase when compared to the turnover during the G_1 and G_2 + M phases (26).

Another experimental model shows that bovine corneal endothelial cells in culture

respond to bFGF (FGF-2) by increasing the amount of heparan sulfate proteoglycan synthesized after stimulation by the growth factor (27).

We have demonstrated that fetal calf serum specifically stimulates the synthesis of heparan sulfate proteoglycan by an endothelial cell line derived from rabbit aorta (28). The effect of serum stimulation on heparan sulfate synthesis is related to the phase of the cell cycle. The effect was not observed for chondroitin sulfate. The synthesis and secretion of heparan sulfate proteoglycan by these cells is down-regulated during the S phase in relation to the G_1 and G_2 + M phases of the cell cycle, showing a physiologic response of the cells (Figure 2) (29). The phorbol ester, phorbol 12-myristate 13-acetate (PMA), which activates protein kinase C, promotes a 10-fold increase in the amount of heparan sulfate proteoglycan secreted into the medium. Curiously, the secretion curve along the cell cycle presents the same pattern as observed for fetal calf serum alone (Figure 2).

We have also reported that PMA causes a cell cycle block at the G_1/S phase transition. This observation, together with the increase in heparan sulfate proteoglycan synthesis, suggests a coordinated cell response elicited by PMA that was not realized before, which links a cell cycle checkpoint with the metabolism of cell surface proteoglycans.

Heparan sulfate synthesis is mediated by the activation of protein kinase C

Protein kinase C has a crucial role in signal transduction for a variety of biologically active substances, such as growth factors and hormones, whose activities balance cellular proliferation and differentiation (30). On the other hand, PMA was first known for its tumor-promoting activity (31), and only later on recognized as a strong and specific activator of protein kinase C (32), mimicking the natural modulator of this enzyme, i.e., diacylglycerol (33).

As stated above, PMA specifically stimulates the synthesis of heparan sulfate proteoglycan by endothelial cells in culture, an effect that is likely to be mediated by protein kinase C activation. Staurosporine and n-butanol, two kinase inhibitors (34,35), abolish the PMA effect whereas activation of the cAMP/protein kinase A pathway by both forskolin and 8-bromo-cAMP is not effective in triggering stimulation of heparan sulfate proteoglycan synthesis.

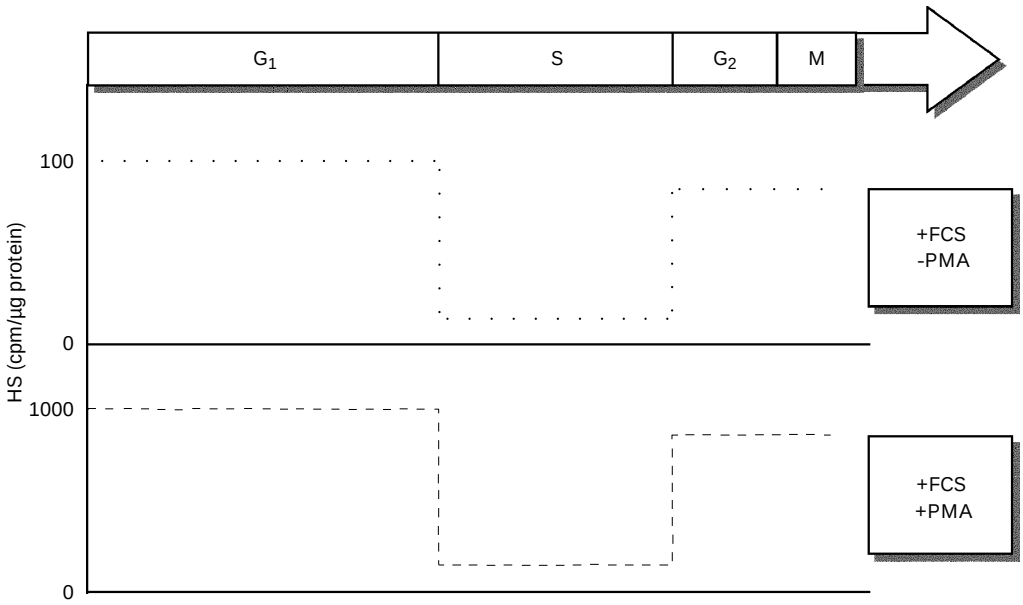

Figure 2 - Effect of fetal calf serum (FCS) and phorbol 12-myristate 13-acetate (PMA) on the synthesis of heparan sulfate (HS) secreted into the culture medium in different phases of the cell cycle.

In spite of the largely accepted view that protein kinase C is important in signal transduction mechanisms, it is not known how the isoforms of this enzyme operate. Specifically, it is not clear how protein kinase C is located in the regulation circuitry that controls the cell cycle. Therefore, the dual effect of PMA on this cell line is being considered in connection with the pathways that regulate the gene transcription presumably relevant to cell cycle control. Curiously, the mapping of the syndecan (heparan sulfate proteoglycan) genes has shown a linkage with members of the *myc* gene family (36). Also, *in vitro* experiments have demonstrated that protein kinase C is able to phosphorylate two of the four tyrosine residues in the cytoplasmic domain of syndecan (37). We are presently investigating the links between cell surface proteoglycan metabolism and intracellular regulatory circuitry that maintain the homeostasis of proliferation and differentiation.

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