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Heparan sulfate and control of cell division: adhesion and proliferation of mutant CHO-745 cells lacking xylosyl transferase

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Abstract

We have examined the role of cell surface glycosaminoglycans in cell division: adhesion and proliferation of Chinese hamster ovary (CHO) cells. We used both wild-type (CHO-K1) cells and a mutant (CHO-745) which is deficient in the synthesis of proteoglycans due to lack of activity of xylosyl transferase. Using different amounts of wild-type and mutant cells, little adhesion was observed in the presence of laminin and type I collagen. However, when fibronectin or vitronectin was used as substrate, there was an enhancement in the adhesion of wild-type and mutant cells. Only CHO-K1 cells showed a time-dependent adhesion on type IV collagen. These results suggest that the two cell lines present different adhesive profiles. Several lines of experimental evidence suggest that heparan sulfate proteoglycans play a role in cell adhesion as positive modulators of cell proliferation and as key participants in the process of cell division. Proliferation and cell cycle assays clearly demonstrate that a decrease in the amount of glycosaminoglycans does not inhibit the proliferation of mutant CHO-745 cells when compared to the wild type CHO-K1, in agreement with the findings that both CHO-K1 and CHO-745 cells take 8 h to enter the S phase.

Key words
- CHO cells
- Extracellular matrix proteins
- Proteoglycans
- Glycosaminoglycans
- Cell adhesion
- Cell proliferation

Introduction

Heparan sulfate proteoglycans are ubiquitously present on the cell surface and in the extracellular matrix (ECM). The major cell surface proteoglycan families are syndecans and glypicans. The expression of heparan sulfate proteoglycans is cell type specific (1), and different cells can express specific core protein with heparan sulfate (HS) chains of distinct disaccharide sequences and binding properties (1). Studies with different tissues and cell lines during the last decade have revealed the putative roles of heparan sulfate proteoglycans in cell adhesion, modulation of growth factor activities, and ECM organization. All of these roles are mediated by interactions with extracellular and cytoplasmic ligands (2).

HS chains contain the same disaccharide

units albeit in different proportions among organisms as diverse as insects, mollusks, and mammals. The conservation of HS binding by extracellular proteins suggests that their association with HS is ancient and functionally relevant (3).

HS has been implicated in a variety of biological processes including the regulation of glomerular basement membrane permeability, the assembly of basement membranes, the regulation of nuclear metabolism (4), blood coagulation (5), viral and bacterial infection (6), and tumor cell malignancy (4). Several disease conditions such as cardiovascular diseases (4) and diabetes (7) are associated with changes in the expression of proteoglycans as well as with structural and functional alterations of their glycosaminoglycan chains. HS chains are known to interact with a variety of proteins such as heparin-binding growth factors, ECM, selectins, protease inhibitors and lipoprotein lipase (4) and are thereby implicated in various cell processes. HS binds to and regulates the activity of growth factors, cytokines, superoxide dismutase and antithrombin, which contribute to aberrant cell proliferation, migration and matrix production (4). They are clearly involved in adhesion, morphological regulation during development, remodeling and changes in cell shape (8-10).

Effect of heparan sulfate on cell adhesion

Cell adhesion plays a key role in the maintenance of tissue organization in multicellular organisms. Cell adhesion requires HS-ligand binding and direct or indirect interactions of core protein with cytoskeletal and signaling molecules. The ECM ligands bind to cell surface heparan sulfate proteoglycans and cause actin filament reorganization (4). The interaction of HS chains with extracellular ligands may help the formation of proteoglycan clusters, which appears to be critical for focal adhesion formation (4). Focal adhesions in many cell types grown on various substrates contain syndecan-4 (11). HS binding to cell adhesion molecules and syndecans localized at adherens junctions (4) show that cell surface heparan sulfate proteoglycans mediate physiological cell-cell interactions. HS binds the immunoglobulin superfamily of adhesion molecules PECAM-1 and N-CAM (4). Cell surface-associated heparan sulfate proteoglycans, predominantly perlecan, are involved in the process of binding and endocytosis of thrombospondin-1 by vascular endothelial cells (12).

Cell attachment is a reaction that depends on time of incubation as well as on the concentrations of cell surface receptors and ligands. Since cell surface proteoglycans have been proposed to participate in cell adhesion processes, we have examined the attachment of wild-type (CHO-K1) and mutant (CHO-745) cells that are unable to synthesize proteoglycans to wells coated with fibronectin (FN) at different concentrations and times of exposure (Figure 1). The different strains behaved almost identically, i.e., wild-type and mutant cells attached to a fixed amount of FN in a time-dependent reaction that was completed in ≅40 min for wild-type and ≅60 min for mutant cells (Figure 1A). Both cells also attached similarly to limiting amounts of FN adsorbed in the wells (Figure 1B). Wells coated with <4 µg FN allowed only submaximal numbers of cells to attach. We also demonstrated that both cell lines show poor adhesion to laminin (LN) and type I collagen. This was observed with a limited number of cells. On the other hand, there is a change in cell adhesion in the presence of FN and vitronectin (VN) when the mutant cells are plated at higher concentrations. The results of our study suggest that although negligible amounts of glycosaminoglycans are present in mutant CHO-745 cells, their attachment to some ECM components (FN and VN) is not affected (Figure 2). When type IV collagen was the substrate, CHO-K1 demonstrated a time-dependent cell adhe-

sion. Furthermore, little adhesion observed in CHO-745 cells was not time-dependent (Figure 2).

Heparan sulfate proliferation and cell cycle

Several studies have suggested that heparan sulfate proteoglycans are involved in the control of cell growth (8-10,13). Cell proliferation and differentiation are regulated by a broad range of growth factors and cytokines, many of which bind heparin or HS with a relatively high affinity (14), regulating cell proliferation and differentiation. These include the family of fibroblast growth factors (FGF), hepatocyte growth factor, platelet-derived growth factor, vascular endothelial growth factor (VEGF), γ-interferon, and various chemokines (13,15). These molecules bind to high-affinity cell surface receptors, but in many cases, efficient signal transduction also requires HS, since in mutant cell lines lacking heparan sulfate proteoglycans or in cells treated with chlorate or heparitinase, cellular responses are dramati-

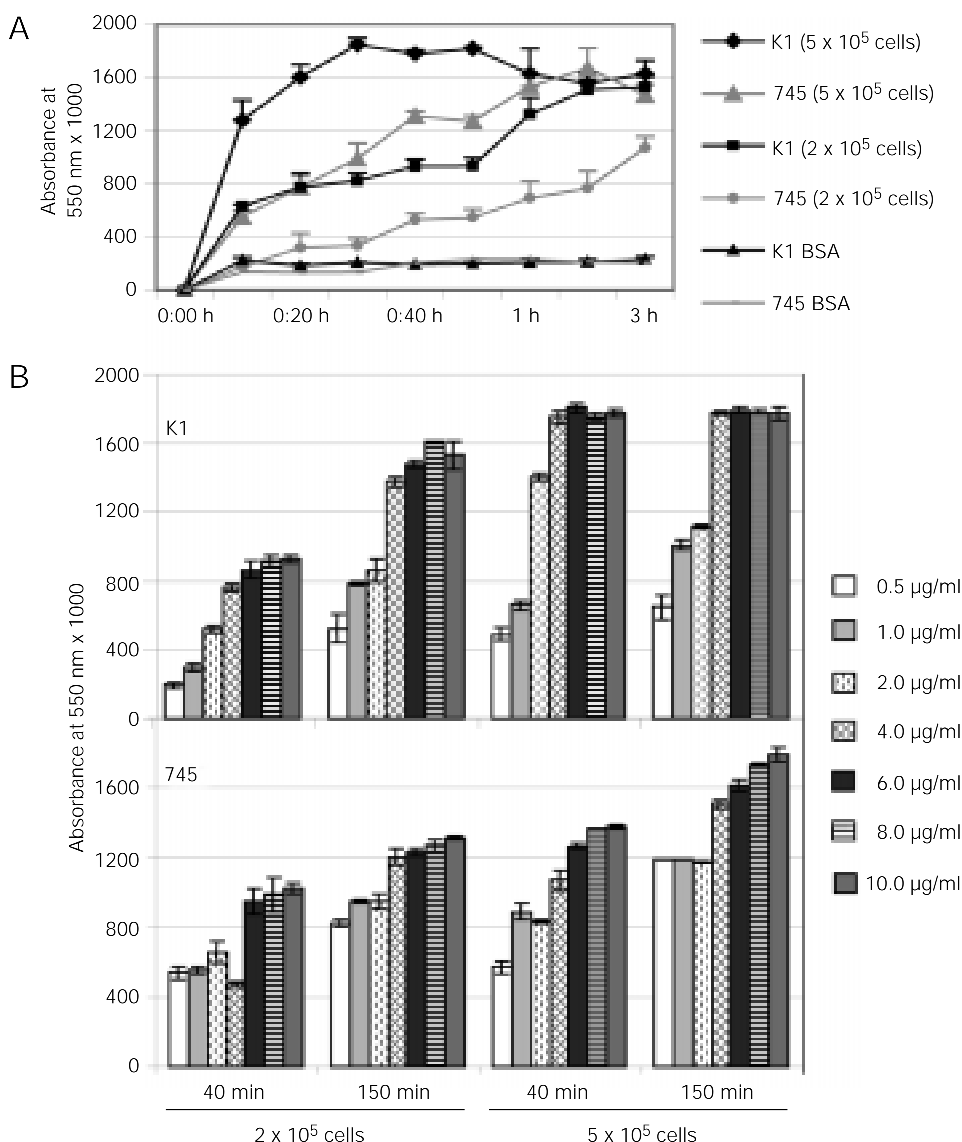

Figure 1. Attachment of wild-type (CHO-K1) and mutant (CHO-745) cells to fibronectin substrates as a function of fibronectin concentration and time. Cell attachment was quantified after staining the cells with 0.8% crystal violet in 20% ethanol, washing with PBS, pH 7.4, eluting the dye with 0.1 M sodium citrate and ethanol (1:1), pH 4.2, and measuring absorbance at 550 nm. Data are reported as mean ± SD of four determinations. A, Cells (5 x 10^5 or 2 x 10^5 in F-12 medium) were seeded onto 24-well plates coated with 10.0 µg/ml fibronectin and incubated for different periods of time as indicated. Nonadhesive substrates were prepared by coating the wells with 1% BSA. B, Cells (2 x 10^5 or 5 x 10^5 in 0.5 ml F-12 medium) were seeded onto 24-well plates coated with 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 µg/ml well fibronectin and incubated for 40 and 150 min.

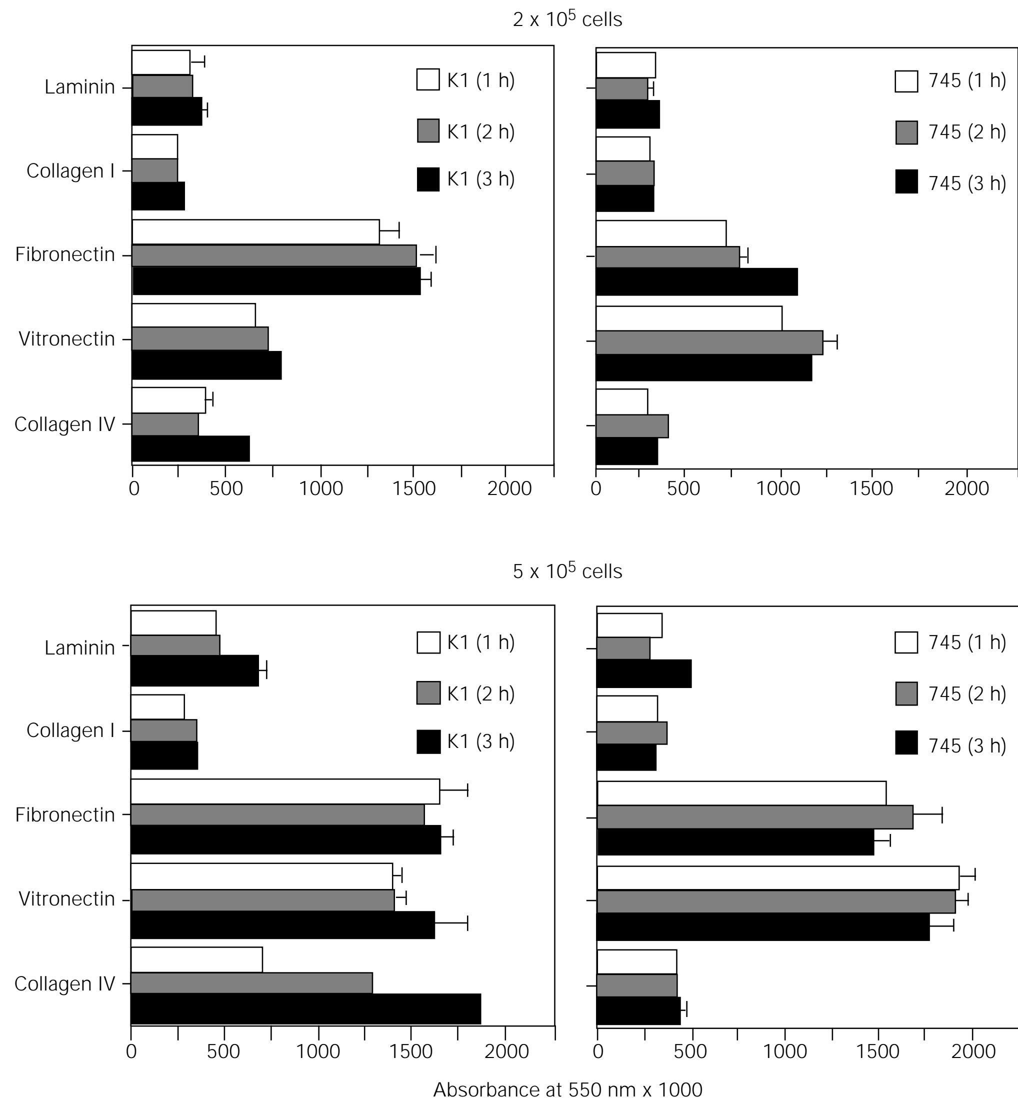

Figure 2. Adhesion of of wild-type (CHO-K1) and mutant (CHO-745) cells to different matrix proteins. Attachment of CHO-K1 and CHO-745 cells to substrates containing laminin, collagen I, fibronectin, vitronectin or collagen IV. Cells (2 x 10^5 or 5 x 10^5 in 0.5 ml F-12 medium) were seeded onto 24-well plates coated with 10 µg/ml of the different matrix proteins and incubated at 37ºC for the indicated periods of time. Nonadhesive substrates were prepared by coating the wells with 1% BSA previously heat-treated at 56ºC for 60 min. Cell attachment was quantified after staining the cells with 0.8% crystal violet in 20% ethanol, washing with PBS, pH 7.4, eluting the dye with 0.1 M sodium citrate and ethanol (1:1), pH 4.2, and measuring absorbance at 550 nm. The comparison of attachment was possible because both cell types show similar absorbance (0.8% crystal violet) for the same number of cells (1 x 10^5 to 1 x 10^6).

cally decreased (16). Syndecans-1, -2 and -4 as well as glypican-1 possess HS which carry out this function (17). The action of HS as a positive modulator of cell proliferation is due to its capacity to bind and act as co-receptor for growth factors, such as FGF (14). VEGF, FGF, heparin-binding epidermal growth factor, interleukin-3, and γ-interferon form strong complexes with heparan sulfate proteoglycans (18). The crystal structures of FGF-2 and heparin oligosaccharides show specific interactions between Asp^{28}, Arg^{121}, Lys^{126}, and Gln^{135} of the growth factor and the iduronic acid 2-O-sulfate and glucosamine N-sulfate residues as well as other carboxyl groups present in the heparin oligosaccharides (19).

HS can participate in the process of cell division in two different ways, either as a modulator or in response to a mitogenic stimulus (14). A significant decrease in HS is observed in regenerating rat liver after partial hepatectomy. The peak of mitotic activity coincided with the smallest amounts of HS (13). It was also shown that increased HS synthesis mediated by protein kinase C inhibits cell proliferation in endothelial cells in culture. On the other hand, a decrease of

surface HS was observed at the G1 phase of the cell cycle (20). Corroborating the previous observations for endothelial cells (20), the present results clearly demonstrate that a diminished amount of glycosaminoglycans does not inhibit the proliferation of mutant CHO-745 cells, which is similar to that of the wild-type CHO-K1 (data not shown). Both cell types enter and progress through the S phase after about 8 h. It is interesting to note that the wild-type CHO-K1 cell has a slightly higher cell proliferation than the mutant CHO-745 cell type when we analyze the number of cells (data not shown). This could possibly explain why wild-type CHO-K1 incorporates more thymidine (data not shown).

Cell adhesion is a process dependent on intact proteoglycan molecules since it requires both HS-ligand binding and direct or indirect interactions of the protein core with cytoskeletal and/or signaling molecules (2). The use of the HS-deficient mutant CHO-745 and the wild-type CHO-K1 demonstrated that heparan sulfate proteoglycans were not involved in cell adhesion to FN, VN, LN and type I collagen or in cell cycle and cell proliferation. Finally, our results strongly suggest that adhesion of the cells to type IV collagen is dependent on heparan sulfate proteoglycans since the mutant cell line lacks this property.

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