tumor cell implantation. Fresh human ovarian tumors grown on mesothelial monolayers maintained on extracellular matrix (ECM) had an absolute preference for adhesion to ECM. These adhesion events are partially mediated via galaptin, a β-galactoside binding lectin. Cell surface receptor molecules for galaptin have been identified as lysosomal associated membrane proteins (lamps), the major cell glycoproteins carrying the poly-*N*-acetyllactosamine chains. Carbohydrate analogs, 2-acetamido-1,4,6-tri-*O*-acetyl-2,3-dideoxy-3-fluor-α-D-glucopyranose and 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluor-α-D-glucopyranose were synthesized to act as oligosaccharide chain modifiers and terminators. These sugar analogs specifically inhibited incorporation of labeled oligosaccharide precursors in a time and a concentration dependent manner. Changes in cell surface carbohydrate structures caused by drug treatment were confirmed by lectin binding studies, indirect immunostaining and Western blot analysis. HOCC treatment with both sugar analogs caused a significant (40 – 50%) inhibition of HOCC adhesion to polymerized galaptin confirming (i) a modification in carbohydrate structures on cell surface and (ii) the importance of lectin mediated adhesion of human ovarian carcinoma cells.

Supported by NCI grants CA 42898 and 13038.

S.5 BIOLOGICAL FUNCTIONS OF PROTEOGLYCANS

S5.1
Regulation of Growth Factor Activities by Proteoglycans

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S5.2
FGF-Binding Sequences in Heparan Sulphate: Structural Characterisation and Regulation of FGF Activity

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Heparan sulphate proteoglycans (HSPGs) have a role as modulators of the activity of basic FGF (Yayon *et al.*, *Cell* (1991) **64**, 841; Rapraeger *et al.*, *Science* (1991) **252**, 1705). The HS chains may induce a conformational change in bFGF which is a prerequisite for binding to the signal transducing receptor. Using affinity chromatography we have investigated the structural properties of fibroblast HS required for it to bind to bFGF. HS chains bound strongly to bFGF, with the major peak eluting at 1.25 M NaCl. Specific enzymic and chemical scission of fibroblast HS, together with chemical *N*-de-sulphation, revealed that *N*-sulphate groups and iduronate-2-sulphates [IdoA(2S)] were essential for the interaction. bFGF-affinity chromatography of sulphated oligosaccharides released from HS by heparitinase scission led to the identification of an oligosaccharide component (oligo-H) with a similar affinity for bFGF as the parent molecule (Turnbull *et al.*, *JBC* (1992) **267**, 10337). Structural analysis indicated that the sequence of oligo-H was:

ΔGlcAβ1,4 $GlcNSO_3$ α1,4 [IdoA(2S) α1,4 $GlcNSO_3$]$_5$ α1,4 IdoA α1,4GlcNAc

Sulphated oligosaccharides of similar size but with a lower affinity for bFGF (eluting at 1 M and 0.75 M NaCl) contained less IdoA(2S) but correspondingly more $GlcNSO_3$(6S) and GlcNAc(6S). Probable sequences for these oligosaccharides have been established. Our data indicates a primary role for contiguous sequences of IdoA(2S)α1→4$GlcNSO_3$ in mediating high affinity binding between HS and bFGF.

Our recent studies focus on the relationship between oligosaccharide affinity and activation of aFGF and bFGF, using a HS-dependent assay of FGF-stimulated mitogenesis. 3T3 fibroblasts grown in the presence of chlorate (which suppresses polysaccharide sulphation) do not respond to aFGF or bFGF. Responsiveness is restored by addition of HS or heparin (as little as 1-10 ng/ml) to the culture medium, thus allowing testing of the ability of exogenous HS oligosaccharides to activate FGFs. Oligosaccharides with a range of structures and affinities for FGFs are being studied in this assay. Preliminary results suggest that oligosaccharides six or more disaccharides in length are active, indicating that large oligosaccharides of particular structure excised from HS are capable of full biological activation of FGFs.

S5.3
The Effects of Brefeldin A on the Biosynthesis of Aggrecan and Hyaluronic Acid in Rat Chondrosarcoma Cells

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Brefeldin A (BFA) is a fungal metabolite that causes the Golgi complex to reversibly disassemble. In this study, Swarm rat chondrosarcoma cells were used to study the effects of BFA on the synthesis of the large cartilage proteoglycan, aggrecan. Aggrecan synthesis provides an ideal model for intracelluar processing, since it encompasses pre-Golgi, Golgi and post-Golgi events; including protein biosynthesis, sorting and targeting; *N*-linked oligosaccharide, *O*-linked oligosaccharide and glycosaminoglycan chain initiation, biosynthesis and processing; secretory vesicle packaging; exocytosis and integration into an extracellular matrix. For comparison, the effect of BFA on hyaluronic acid (HA) synthesis was also studied. The size of aggrecan (~2×10^6 daltons) and HA (~10×10^6 daltons) as well as their preferential labeling with select radiolabeled precursors allow easy isolation and discrimination of these molecules. Results indicate that HA synthesis occurs at normal levels in the presence of BFA whereas chondroitin sulfate (CS) synthesis on aggrecan is rapidly inhibited (<15 min.) by more than 95%. These results are consistent with HA synthesis occurring at the plasma membrane and independent of the Golgi complex. β-Xylosides, exogenous acceptors for CS synthesis, are unable