Collagen metabolism in dimethylnitrosamine induced hepatic fibrosis in rats

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HEPARIN AND HEPARAN SULFATE WITH AFFINITY FOR THE NEUROTROPHIC FACTOR PEDF. E. Alberdi and S.P. Becerra. National Eye Institute, NIH, Bethesda, MD USA

Glycosaminoglycans and proteoglycans are structural and functional modulators of extracellular matrices. Pigment epithelium-derived factor (PEDF) is an extracellular neurotrophic factor and a serpin protein (50-kDa) of the retina. We have shown that PEDF has affinity for immobilized heparin, chondroitin sulfates-A, -B and -C and heparan sulfate proteoglycan. Conditioned media of retinoblastoma cell cultures enhance the binding of PEDF to these cells by >4-fold. In addition, PEDF forms a complex larger than 100-kDa with soluble component(s) of the media. To identify glycosaminoglycans secreted by retinal cells with affinity for PEDF, we subjected the media conditioned by retinoblastomaY-79 cells to PEDF-affinity column chromatography. Components that bound to the column were eluted with 3 M NaCl. The eluted sample was treated with subtilisin to digest the protein components. The presence of glycosaminoglycans was analyzed by spectrophotometric measurement of the unsaturated disaccharide products from chondroitinase ABC, heparinase and heparitinase digestion reactions. Retinoblastoma cultures ($5x10^6$ cells/ml) incubated at 37°C for 16 h secreted heparin and heparan sulfate at 14 µg/ml and 13 µg/ml, respectively, and no detectable amounts of chondroitin sulfates. About 17% of heparin and 4.5% heparan sulfate bound to an excess of immobilized PEDF. In addition to binding to PEDF, heparin and heparan sulfate might be cofactors of the PEDF neurotrophic activity.

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ADENOSINE NUCLEOTIDES CAN MODULATE INTEGRIN αIIbβ3 RECOGNITION OF FIBRINOGEN(FG), FIBRIN AND NON-FG LIGANDS, J.M. Wysocki, B. Dhutia, J. Styrsky and D. B. Taylor, Benedictine University, Lisle, IL, USA.

ATP inhibits platelet aggregation apparently by modulating FG receptor [integrin αIIbβ3] function. The goal(s) of these studies are to determine the effect(s) of ATP and related nucleotides on clot retraction and integrin αIIbβ3 recognition of immobilized FG, fibronectin(FN) and von Willebrand factor (vWf). In clot retraction assays, platelet rich plasma(PRP) ± nucleotides was clotted by the addition of thrombin, and retraction was followed with time by measuring clot length. In platelet adhesion assays, PRP, or activated washed platelets ± nucleotides were transferred to substrate coated wells and platelet adhesion was quantitated colorimetrically. ATP and adenosine analogs were tested at various concentrations for their effects on clot retraction(n=6) and platelet adhesion(n=3) to immobilized FG, FN and vWf. 8-azido ATP(8AA) or ATP potently inhibited the rate of clot retraction in a dose dependent manner, compared to adenosine or ADP. ATP or 8AA, compared to adenosine, inhibited platelet adhesion to FG. In assays with nonFg ligands, ATP decreased platelet attachment, while ADP increased platelet adhesion to FN or vWf. Our results demonstrate that ATP inhibits the rate of clot retraction and the adhesion of platelets to immobilized FG, FN and vWf. This work was supported by HHMI Grant #71191-528601.

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MOLECULAR BASIS FOR HIGHLY SPECIFIC CELL ADHESION, E.C.K. Lin and J.W. Smith, The Burnham Inst., La Jolla, CA, USA.

Integrins are the predominant cell surface receptors for the extracellular matrix. Integrins are directly involved in many tissue remodeling events, including those associated with the progression of osteoporosis and cancer. It is still not evident how integrins direct adhesion and migration in a highly specific manner. Here, this question is adressed by constructing molecular chimeras between the integrin β3 and β5 subunits. The chimeras were examined for their ligand binding phenotype. We have identified a 39 amino acid region of the β3 subunit (5% of the total protein) which confers ligand binding specificity. This small domain is predicted to be a flexible loop, and is highly divergent among all integrin β subunits. These findings provide the first molecular basis for the mechanism by which integrins distinguish between the complex array of proteins found in extracellular matrices.

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COLLAGEN METABOLISM IN DIMETHYLNITROSAMINE INDUCED HEPATIC FIBROSIS IN RATS

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Collagen plays the key role in the pathogenesis of hepatic fibrosis. The rate of biosynthesis and metabolic degradation of liver collagen were investigated in Dimethylnitrosamine (DMN) induced liver fibrosis in adult male albino rats, after a single intraperitoneal injection of ^3H proline (1110 Kbq/100 g body weight). The total and ^3H hydroxyproline were determined after extraction and fractionation of liver collagen into neutral, salt soluble, acid soluble and pepsin solubilized fractions. A significant increase was noted in the rate of biosynthesis of liver collagen in all DMN treated animals with a maximum on the 21st day. About 4 fold raise was recorded in the amount of total liver collagen . The urinary excretion of total and labelled hydroxy proline,studied to assess the metabolic degradation of collagen, was also increased significantly with a maximum excretion on the 7th day. The results suggest that liver collagen undergoes alterations in both synthesis and degradation during DMN induced hepatic fibrosis. The molecular characteristics of purified pepsin solubilized collagen from control and DMN induced fibrotic rat liver also were studied. A significant increase in the aldehyde content, decreased α/β ratio and an increased rate of fibril formation were observed in DMN induced fibrotic liver collagen. The deposition of Type III collagen was more pronounced than Type I collagen in early fibrosis. It is concluded that the regulation of collagen metabolism is almost maintained in the early stages of fibrosis, but it is impaired in the later stages resulting in the accumulation of collagen in the liver.

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CONNECTIVE TISSUE PROTEIN SYNTHESIS IN VARIOUS SKIN DISEASES Haile F. Yancy and Agnes Day* Department of Biology and Department of Microbiology* Howard University, Washington D.C. 20059

Many skin conditions have been described that are directly attributable to abnormal connective tissue protein synthesis. The goal of this study was to identify the connective tissue proteins which demonstrate altered transcription. The total RNA from fibroblasts of four pathologic skin cell lines; Gardner's syndrome(GS), maligant melanoma(MM), Xeroderma pigmentosum (XP), Pseudoxanthoma elasticum(XPE) and Ehlers-Danlos syndrome(EDS) was extracted, spotted onto nitrocellulose membrane strips and singly hybridized with ^{32}P cDNA probes encoding for the connective tissue proteins (CTPs) fibronectin, osteonectin, decorin and type I collagen. Autoradiograms of the slot blots were quantitated and compared to normal fibroblast CTP expression by densitometric measurements. *In situ* hybridization showed reduced levels of type I collagen mRNA in (GDS) and(MM), reduced expression of osteonectin in (EDS) and (XPE) reduced amounts of decorin in (XPE), (XP) and (MM), and reduced expression of fibronectin in all cell lines. Our data indicate that these cell lines have decreased levels of one or more CTPs which may play a major role in the development of these diseases. Supported by NIH/NCI grant RO3CA68991 and NIH/NIGMS grant SO6GM08016.

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THE DIFFERENT PATTERNS OF ACTIN CYTOSKELETON ORGANISATION IN CELLS SPREADED ON LAMININ AND FIBRONECTIN, I.V. Voronkina, A.F. Are and G.P. Pinaev, Institute of Cytology, RAS, Sankt-Petersburg, Russia.

The regulation of cell functional activity is carried out by effect of different ligands, such as extracellular matrix proteins on its surface receptors. The interactions are accompanied by formation of contacts of cytoplasmatic receptors domains with cytoskeleton. According to one of assumptions, the cytoskeleton participates in signal transduction from cell surface to nucleus and different forms of cytoskeleton space organization arising at formation of ligand-receptor complexes can provide the specifity of provided ligands. In this work the influence of extracellular proteins - laminin and fibronectin on space organisation of cytoskeleton of three types of cells which attach and spread on such substrates is considered. Morphology and actin cytoskeleton of cells spreaded on laminin were significantly different from those of cells spreaded on fibronectin. The cells which are used for this work are: human epidermoid carcinoma A-431 cells, rat embryonal fibroblasts and coelomocytes of Arenicolas marina. The organization of actin cytoskeleton was analysed with the rodamin-phalloidin staining.

This substrate proteins interact with cells with binding sites of different types. Here we show that binding to cells through the RGD site as it is on fibronectin can differ the cytoskeleton arrangement from binding by another mechanism such on the laminin.