101 Characterization of Collagen Phenotype in Cultured Hepatic Stellate Cells

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Stellate cells also called Ito cells, the principal cells residing in the "space of Disse" in the liver is now known to play an important role in the development of liver fibrosis. Cultures of stellate cells thus appear to provide a good model to study modulation of hepatic collagen synthesis in fibrotic liver. The stellate cells were isolated from normal male albino rats according to the procedure discussed by Friedman & Roll, 1987, with minor modifications. The liver was dispersed by perfusion with pronase and collagenase and the resulting non parenchymal cell suspension was filtered through cotton gauze and cells were then separated through a discontinuous gradient of 35, 50, 100%, Percoll. Pure stellate cells were recovered from the interface between Percoll and medium. The cells were washed with PBS and plated in DMEM supplemented with 10% fetal calf serum at a density of 2 x 10^6 cells per 10^2cms and incubated at 37 °C, in a 5% CO_2 air humidified atmosphere. Subcultures were obtained by trypsinization. Stellate cells were identified by their positive immunofluorescent staining for Desmin. Collagen was determined in culture media and cell layers after radiolabelling with [^{3}H] proline, subject to pepsinization and reduction and separates the different collagen α chains by interrupted electrophoresis. The major ECM Collagen produced was Type I Collagen and accounted for ~75% of total Type I and Type III collagens. Matrix related changes in collagen production were investigated. The results showed ECM could modulate matrix production, suggesting that changes in hepatic subendothelial matrix may underlie stimulation of stellate matrix production and progression in liver fibrosis

102 Platelet-derived growth factor modulates adhesion of smooth muscle cells to fibronectin.

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Convergence of intracellular pathways activated by growth factors and integrin-mediated cell adhesion is required for normal cell growth. We investigated the effect of platelet-derived growth factor (PDGF-BB) on adhesion of smooth muscle cells (SMC) to fibronectin.

Adhesion of SMC to fibronectin was inhibited by pre-treatment with PDGF-BB. This inhibition decreased (from 80 % to 50 %) with increasing concentrations of fibronectin (from 0.5 to 5 µg/ml).

Moreover, when activation of extracellular signal-regulated kinase (ERK) was inhibited by PD 98059, a remaining inhibition of cell adhesion (50 %) was still observed and was independent of fibronectin concentration. Effect of PDGF-BB on inhibition of cell adhesion with RGD peptide was investigated: in the presence of 0.2 mM RGD peptide 45 % of PDGF-BB-treated cells versus 80 % of untreated cells adhere to fibronectin, suggesting a decrease in integrin avidity. This effect of PDGF-BB was abolished by liposomal transfection with MAP kinase antisens oligonucleotides, suggesting that ERK might participate to the decrease in integrin avidity.

PDGF-BB also inhibited focal adhesion assembly and stress fiber formation. Moreover, pre-treatment of cells with PDGF-BB diminished the adhesion-induced tyrosine phosphorylation of paxillin but did not alter activity of Src family tyrosine kinase. Since, tyrosine phosphorylation of paxillin was dependent of Src kinases, these results suggested that inhibition of integrin signaling by PDGF-BB occurred downstream of Src.

In conclusion, PDGF-BB inhibited SMC adhesion by two mechanisms: 1) decreasing integrin avidity, 2) inhibiting signal transduction induced by integrins.

103 FAP-1 is a pro-apoptotic protein tyrosine phosphatase inhibiting the IGF-1 survival pathway in human breast cancer cells.

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We previously showed that antiestrogens (ICI 182,780 or OH-Tam) prevented EGF and IGF-1 proliferative activities in estrogen receptor positive human breast cancer cells (MCF-7, T47D). This growth inhibition was correlated with the induction of FAP-1 (Fas-Associated Phosphatase-1) expression. The use of stable transfectants expressing FAP-1 antisense RNA allowed us to implicate this PTP in the inhibitory action of OH-Tam on IGF-1 pathway. While IGF-1 is promoting survival of breast cancer cells, we showed that OH-Tam was drastically increasing apoptosis in MCF-7 cells. This negative action of OH-Tam was accompanied by a large decrease in PI3-kinase and Akt activities which are no longer abolished in FAP-1 antisense transfectants. In our ongoing studies, we are analyzing the mechanism of the pro-apoptotic activity of FAP-1 in these cells. To achieve this purpose, we are developing a "substrate-trapping" approach by fusing to N-terminal tags (HA, GFP, GST) the complete FAP-1 cDNA or its catalytic domain containing the Asp-Ala mutation within the WPD domain. This mutation has been shown to stabilize the interaction between PTPs and their substrates. Using such mutant, we are attempting to characterize FAP-1 targets along the IGF-1 pathway by co-immunoprecipitation and pull-down experiments. FAP-1 possesses a 4.1 band domain and several protein-protein interacting domains (PDZ), we therefore aim to define the regions which are necessary and sufficient to target FAP-1 to its substrates by the use of such fused proteins. Altogether, this study should precise the role of FAP-1 as a pro-apoptotic molecule which might be helpful in identifying new ways to control breast cancer cell proliferation. (Supported by INSERM, ARC n°5405, Ligue Nationale contre le Cancer – Comités de l'Aude et du Gard).

104 The EGF Signaling Cascade is Involved in Rerouting of Soluble and Membrane Proteins in Endocrine Cells.

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Endocrine cells have the ability to store selected endogenous proteins within the secretory pathway, and can be stimulated to secrete at a higher rate by treatment with secretagogues such as $BaCl_2$ and the phorbol ester, PMA. However, the involvement of mitogen-activated signal transduction events in the regulated secretion of pro-hormones and neuropeptides has not been fully elucidated. In this study, we sought to determine whether epidermal growth factor (EGF) affected the regulated secretory pathway by examining the localization and secretion of soluble and membrane bound proteins from mouse pituitary corticotrope cells (AtT-20). Following EGF treatment, $BaCl_2$-stimulated secretion of endogenous prohormone convertase 1 (PC1), POMC-derived peptides and peptidylglycine-α-amidating monooxygenase was decreased. Immunofluorescent staining also revealed rerouting of PC1 from the tips of cellular processes to the *trans*-Golgi network region, and concentration of adaptor protein 1 to the TGN. In addition, increased FITC-phalloidin staining of filamentous actin at the cell surface was observed, indicating changes in the actin cytoskeleton. These studies suggest a role for the EGF-induced protein kinase phosphorylation cascade in the trafficking of proteins via the regulated secretory pathway of endocrine cells. Supported by National Institutes of Drug Abuse Grant, DA-00266.