S13.3

Expression of Tumor-Associated Ganglioside and its Expression Mechanism in IL-3 Independent NSF-60 Cells Introduced with IL-3 Gene

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Murine IL-3 gene was introduced into IL-3-dependent mouse myelogenous leukemia cell line, NSF-60. Several IL-3-independent clones were established and shown to acquire capabilities of autonomous growth and tumorigenicity. Using these cells, we analyzed ganglioside metabolism associated with the acquisition of autonomous growth. Main constituents of cell surface gangliosides in the parental cells were *GM1b* and *GD1a*. By contrast, the IL-3-transfected cells expressed ***GD1a*** in addition to *GM1b* and *GD1a*. With the progression of malignant transformation stage, GD1a was newly synthesized through so-called "a"-pathway from GM3. This shift of metabolic flow, i.e. from asialo-pathway alone to asialo-pathway plus "a"-pathway, was a consequence of up-regulation of GM3 synthase activity, the upstream key enzyme of this metabolic pathway. In addition, inhibition of IL-3 gene expression using IL-3 anti-sense DNA resulted in immediate decrease in the level of GDIa. These data strongly suggested that the ganglioside metabolism is tightly coupled with the progression of malignant transformation stage and signaling pathways of IL-3.

S13.4

Biosynthesis of Glycosphingolipids (GSLs) is Reduced in the Absence of a Vimentin Intermediate Filament (IF) Network

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Our previous observations (1, 2) on the colocalization of GSLs and IFs led us to analyze the role of IFs in the biosynthesis and intracellular transport of GSLs. Cells with and without a vimentin IF network (IF+ and IF−) were cloned from the human adrenal carcinoma cell line SW13. IF+ cells synthesized LacCer, Gb3Cer and GM3 3-, 10- and 2-fold more rapidly, respectively, than IF− cells. The increased rates of synthesis were not accounted for by the levels of synthetase activity in IF+ and IF− cells. There was less difference in the biosynthesis rates for complex neutral GSLs and gangliosides. Cell content of GSLs, especially LacCer and Gb3Cer, was greater in IF+ cells, but metabolic turnover was not significantly different. To determine whether the presence of an IF network is responsible for the observed differences in biosynthesis, mouse vimentin cDNA was transfected into IF− clones. Transfectants demonstrated a two-fold or greater increase in the rate of neutral GSL and ganglioside biosynthesis. The decreased biosynthesis of GSLs in IF− cells may be due to decreased transport of ceramide from the endoplasmic reticulum to the Golgi, and/or to altered ultrastructural organization of the Golgi.

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S13.5

Leukemia Inhibitory Factor, Interferon γ and Dexamethasone Control *N*-Glycosylation of α1-Protease Inhibitor in Human Hepatoma Cells

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Hepatocytes respond to inflammatory stimuli by changing the synthesis and *N*-glycosylation of acute phase proteins (APP). Until now interleukin (IL) 6, transforming growth factor β(TGFβ), tumor necrosis factor α and IL-1 have been found to control *N*-glycosylation pattern of APP. Cytokines either increased (type I) or decreased (type II) the ratio of bi- relative to more branched complex type *N*-glycans on APP. Here we describe the effect of leukemia inhibitory factor (LIF), interferon γ (INFγ) and dexamethasone (dex) on production of α1-protease inhibitor (PI) and α1-antichymotrypsin (ACT) and on glycosylation of PI in the human hepatoma cell line Hep G2. Cytokines and dex were used separately and in various combinations including also IL-6 and TGFβ . Production of antiproteases was quantitated by immunoelectrophoresis of the proteins accumulated in the culture medium. Glycosylation pattern of PI was assessed by crossed immunoaffinity electrophoresis (CIAE) with Concanavalin A (Con A) as a ligand. The production of ACT and PI was increased by LIF, decreased by INFγ and unaffected by dex. LIF and INFγ each like IL-6, decreased PI-Con A reactivity while dex like TGFβ enhanced PI-Con A reactivity. Combination of dex with LIF yielded additive effects while combination of dex with either INFγ, IL-6 or TGFβ acted synergistically on PI-Con A reactivity. Combinations of multiple cytokines and dex produced additive, inhibitory or synergistic effects. The type of glycosylation profile of PI secreted by Hep G2 cells depended on the composition and amounts of interacting cytokines and dex.

S13.6

Corticosteroid Inhibition of the Synthesis and Release of Ferret Tracheal Glycoconjugates

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Corticosteroid has been shown to inhibit secretion of glycoconjugates from human respiratory epithelia (1). We have demonstrated that organ culture of ferret trachea continues to secrete mucins and other high molecular weight glycoconjugates (2). The objective of this study was to elucidate the mechanism of the corticosteroid inhibitory action on tracheal "mucin" secretion. High molecular weight glycoconjugates (HMG) including mucin, and proteoglycans synthesized *in vitro* by ferret tracheal strips were labeled with ^{3}H-glucosamine in the presence and absence of dexamethasone (dex). Inhibition of the secretion of total HMG and mucin (bovine testicular hyaluronidase resistant HMG) occurred with onset at 6–8 and 12–14 h after dex treatment, respectively.