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Glycosyltransferases in Cellular Function: Carbohydrate Biosynthesis and Diseases by UDP-*N*-acetylglucosaminyltransferases-III and -V

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Oligosaccharides of glycoproteins and glycolipids alter a wide variety of structures in mammalian cells. These various alterations of the carbohydrate structures are known to associate with development, differentiation and malignant transformation. This suggests that cell surface oligosaccharides play a specific role in cell-cell interaction. Carbohydrate structures are mainly determined by genes encoding glycosyltransferases and glycosidases.

Neoplastic transformation of cells is associated with changes in complex type *N*-linked oligosaccharides on surface proteins and lipids. Alterations of oligosaccaride chains in malignant cells include increased branching on the trimannosyl core, increased polylactosaminoglycan chain formation, and increased sialylation. The formation of the branches is governed by the activities of a set of *N*-acetylglucosaminyltransferase(GnT)s. GnT-III catalyzes the addition of *N*-acetylglucosamine through a β1-4 linkage (bisecting *N*-acetylglucosamine) to the β-linked mannose of the trimannosyl core structure of *N*-linked oligosaccharides of glycoproteins, in contrast, GnT-V catalyzes the transfer of GlcNAc from UDP-GlcNAc to an α-D-mannoside. The β-1,6 branched oligosaccharides may contribute directly to the malignant or metastatic properties of tumor cells since blocking the glycosylation pathway prior to the formation of the β-1,6 linked antenna leads to a loss of mestastatic potential and inhibits organ-organ colonization.

The changes of GnT-III level seem to play a key role in the alteration of *N*-glycan structures. It is known that *GnT-III* expression is tissue-specific, being prominent in rat kidney, and it was dramatically increased in proportion with the enzyme activity in metastatic lesion or during hepatocarcinogenesis in LEC (Long-Evans with a cinnamon-like coat color) rat. Analysis of several putative promoter elements in the human *GnT-III* 5'-flanking region, upstrem from that corresponding to the cDNA, revealed a similarity to that of the well characterized murine 1,4-galactosyltransferase. No TATA-like sequence is present within this region. However, eleven *IRE* consensus sequences, seven potential AP-2-binding sites, two SP1 consensus sequences (GC boxes), two *GRE* sequences similar to the half-palindromic glucocorticoid-responsive

element and two possible sequences similar to the cAMP responsive regulatory element were present. This may be of significance in relation to the role of cAMP in the developmental control of *GnT-III* and the impact of diabetes on *GnT-III* expression. No regions of close similarity to other well characterized *cis*-acting elements were found. Considering that the expression of *GnT-III* mRNA and enzyme activity are dramatically increased during hepatocarcinogenesis, it is notable that consensus recognition sequences for hepatocyte-specific transcription factor (HNF)-1 and HNF-4 are not found in the 5'-flanking region of *GnT-III* gene. However, though induction of GnT-III activity by glucocorticoids, insulin and cAMP has not been specified, the existence of sequences required for the response to them indicates that they may have a direct effect on *GnT-III* expression in stage-specific steps or a tissue-specific manner.

To examine the biological function of GnT-III in hepatocytes, we have used the transgenic mouse system to target the expression of human GnT-III to the liver of the mouse. The GnT-III transgene was designed to be expressed in the transgenic animal directed by the mouse albumin enhancer'promoter and SV40 polyA addition signal. Total 5 transgenic founder mice were generated by pronyclear microinjection and in 4 cases transformation of the transgenes was confirmed in the G1 generation. Northern blot analysis of total RNAs from various organs of the transgenic mice revealed that the expression of the human GnT-III was specific to the liver. Upon Western analysis the human GnT-III protein was detected only in the liver of transgenic mice, Moreover, these mice showed a gross abnormality in the abdomen, i.e., an abnormal inflation and had a swollen oval-like morphology, with many lipid droplets. We demonstrated with these results that aberrant glycosylation leads to the generation of fatty liver and aberdon. Thus, these results indicate a novel mechanism for the pathogenesis of fatty liver. On the other hand, we have established 6 hybridomas which produce anti-GnT-III monoclonal antibodies, and applied these for the simple diagnosis of liver diseases by sandwich-ELISA method using the monoclonals. The GnT-III monoclonals was also applied positively for diagnosis of human hepatitis, cirrhosis and hepatocarcinoma. Finally, the relationships between expressions of matrix metalloproteinase-9 and GnT-III during hepatooncogenesis will be discussed.