Diminished Hepatic Binding Protein for Desialylated Glycoproteins during Chemical Hepatocarcinogenesis

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ABSTRACT

The lectin hepatic binding protein has a specific binding capacity for desialylated serum glycoproteins and is limited to hepatocyte membranes. This binding capacity was reduced by approximately 60% in the neoplastic nodules which resulted from exposure of rat livers to N-2-acetylamino-fluorene. The binding capacity of the primary hepatocellular carcinomas which resulted from this regimen was reduced by 95%. The loss of binding capacity was found to be proportional to the decreased concentration of immunologically detectable lectin in the altered tissues.

INTRODUCTION

There is an active and continuing interest in the possibility that cell surface membrane alterations play an obligate role in the conversion of normal cells to malignant phenotype. A large number of reports have described differences in composition, structure, and function between normal and malignant cells. In addition, a variety of alterations of cell membranes are evident during chemically induced carcinogenic sequences.

The mammalian HBP is a membrane constituent which is responsible for the receptor-mediated endocytosis of a number of desialylated glycoproteins, thus initiating their catabolism (1). Purified HBP isolated from rabbit and rat liver membranes as an aqueous soluble glycoprotein (7, 12, 18) forms specific complexes with oligosaccharide moieties which are constituents of cell surfaces or with soluble glycoproteins that terminate in galactosyl or N-acetylgalactosaminyl residues (7, 16).

It appeared, therefore, that examination of the content and function of this specific hepatic receptor throughout the sequential alterations induced in the rat by AAF might offer additional insight into the process of hepatocarcinogenesis. Utilizing antibody to HBP, we have correlated the specific binding activity in AAF-induced neoplastic nodules and PHC with the content of HBP.

MATERIALS AND METHODS

Orosomucoid was isolated from pooled human serum by the procedure of Whitehead and Sammons (20). ASOR was prepared by enzymatic hydrolysis with neuraminidase from Clostridium perfringens (Sigma type IX, Sigma Chemical Co., St. Louis, Mo.). Iodination of ASOR was performed with carrier-free Na125I (Amersham/Searle Corp., Arlington Heights, Ill.) by a chloramine-T method (6), and the 125I-labeled protein was isolated by Sephadex G-25 chromatography. Protein concentrations were estimated by the technique of Lowry et al. (9). HBP was solubilized from an acetone powder of rat liver using Triton X-100 and purified from the rabbit protein by affinity chromatography as described previously (7).

RESULTS

The level of receptor binding capacity (325 pmol/g liver) demonstrated in control rats was in good agreement with that reported by Pricer and Ashwell (13) (385 pmol/g) using a somewhat different assay system (Table 1). The level of ASOR-binding capacity of neoplastic nodules at the termination of carcinogen exposure was approximately 37% that of normal. Of additional interest was the finding that the reduced degree of activity which was evident at the cessation of the carcinogenic feeding regimen remained constant for as long as 3 months afterward. The level in the 4 PHC’s which were studied was approximately 5% that of normal.

It has been previously demonstrated that the binding activity of HBP has several identifying characteristics (7, 13). This activity required the presence of calcium ions and a pH above...
6.0 and was readily abolished by neuraminidase treatment. In order to determine if the HBP activity in nodules and PHC was identical to that of normal livers, the standard assay conditions were modified (Table 2). The results indicated that in all instances the characteristics of the residual activity in altered tissue were identical to those of controls.

Tissue samples washed with EDTA to remove any prebound or soluble asialoglycoproteins present in the homogenates showed no increase in ASOR-binding capacity (Table 2). Further, an HBP antibody titration was performed to determine if the residual activity was related to a loss of functional activity or reflected a decreased concentration of HBP characteristic of normal hepatocytes. As demonstrated in Chart 1, the residual HBP activity was inhibited by the same amount of anti-HBP as was required for the same number of binding units in normal liver.

**DISCUSSION**

A number of enzymes and/or their functional cofactors in membranes have been reported to undergo alteration during hepatocarcinogenesis (5, 8). In particular, components of the mixed oxidase system are reduced during regimens similar or identical to the one described herein (2, 11, 12). HBP is also a membrane constituent and is limited exclusively to the hepatocyte. Although the uptake of certain desialylated glycoproteins by HBP has not yet been demonstrated to exert control over any hepatocyte function, as a nonezymatic component it might represent a prototypic example of altered receptor and transport functions during carcinogenic evolution.

Since the presence of asialoglycoprotein inhibitors, which have been reported in the sera of patients with hepatocellular carcinoma (10), was eliminated as a potential cause of reduced ASOR binding, the diminished binding capacity in nodules and PHC is evidently the result of a reduction in characteristic HBP content. Equal amounts of anti-HBP are required to inhibit equal numbers of binding units whether present in low tissue concentrations as in PHC or in concentrations many times this amount as in neoplastic nodules or normal liver. If carcinogen treatment had resulted in loss of HBP activity without loss of immunologically detectable HBP, disproportionately more anti-HBP would have been required for inhibition of activity in the altered tissue samples (18). This indicates either that an antigenically modified inactive lectin is present or that a reduction of synthesis of HBP occurs during exposure to the carcinogen which is almost complete in PHC. The synthesis of membranes with decreased content of functional components appears to be quite characteristic of the process of hepatocarcinogenesis, and functional significance has been inferred from this process (2, 5, 8). For example, a loss of the enzymes of the microsomal oxidase system is associated with decreased carcinogen activation and might be responsible for a decreased toxicity in these altered cells (4).

Thus, the possibility remains that the diminution of HBP which was demonstrated may represent a decreased synthesis in all cells of a nodule or a defect as great as that detected in PHC limited to a subpopulation of cells. If the latter pertains, then this loss might be considered as a specific biochemical marker for premalignant cells. A suitable histochemical technique might then be applied to nodules and nonnodular foci as well for the identification of the premalignant population.

**REFERENCES**

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