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THE EXPRESSION OF α-FETOPROTEIN AND ALBUMIN GENES IN RAT LIVER DURING CHEMICAL CARCINOGENESIS

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Summary. The effect of the hepatocarcinogen 3'-methyl-4-dimethylaminoazobenzene on α-fetoprotein (AFP) and albumin gene expression in rat liver was studied. Serum concentrations of AFP and albumin were measured. Amounts of AFP mRNA and albumin mRNA in rat livers were determined by hybridization of total cytoplasmic RNAs to their cDNAs. Dramatic increases in serum AFP concentrations coincided with increases in AFP biosynthesis and amount of AFP mRNA in livers of carcinogen-treated rats. In contrast, no or little change in albumin mRNA concentration was found in livers of rats treated with 3'-methyl-4-dimethylaminoazobenzene. Concomitantly, there was little change in liver albumin biosynthesis or serum albumin concentrations during hepatocarcinogenesis.

Cells exhibit a number of marked morphologic and metabolic changes during their neoplastic transformation. Accumulated evidence indicates that changes in biological, biochemical and morphologic properties associated with neoplastic cells were also accompanied by alteration of gene expression (1-7). Albumin is the major plasma protein synthesized by the adult liver. Albumin synthesis is retained in hepatomas but at reduced levels as compared to the normal rat liver (8-12). In contrast, α-fetoprotein (AFP), which is synthesized in large amounts in embryonic liver and yolk sac but not in adult liver,

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was induced dramatically in hepatomas (13,14). The synthesis of AFP and albumin in the liver has been shown to change reciprocally as a function of neonatal development. Tamaoki et al (15) demonstrated that neonatal growth was accompanied by a decrease in AFP mRNA activity and a marked increase in albumin mRNA activity.

Both AFP and albumin are actively synthesized in large quantities in liver and hepatoma. Based on the striking homology in amino acid sequences and protein structure, it has been suggested that AFP and albumin may share a common ancestral gene (16-18). Consequently, we examined the expression of AFP and albumin genes in neoplastic and preneoplastic livers of rats treated with the hepatocarcinogen, 3'-methyl-4-dimethylaminoazobenzene.

MATERIALS AND METHODS

Animals and Treatments

Hepatomas and preneoplastic livers were produced by feeding Fischer rats (125 gm initial weight) with rat chow (Purina) containing 10% corn oil (Mazola) and 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'-MDAB) which was dissolved in the corn oil (19). At each experimental point, a group of 5 rats were sacrificed. Sera were collected for determination of AFP and albumin levels. Livers were immediately removed from rats and placed in cold buffered saline (0.15 M NaCl - 0.15 M sodium citrate).

The Measurements of Serum AFP and Albumin Concentrations

The concentration of AFP in the serum was measured by a double antibody radioimmunoassay described previously (20). The serum albumin concentration was determined by the immunodiffusion method of Mancini et al (21).

The Determination of AFP and Albumin Synthesis in Liver Slices

One gram of rat liver or neoplastic liver was cut into 1 mm thick slices and incubated at 37°C in water bath under 95% O_2/5% CO_2 for 5 min in 13 ml of Hank's balanced salt solution (Grand Island Biological Co.) containing 6 mM sodium bicarbonate, 7 mM Hepes buffer (pH 7.4), and tenfold concentrated plasma amino acids (25) minus methionine as described by Tse et al (22) with minor modifications. After preincubation, [^{35}S]methionine (sp. act. 1228.3 Ci/mmol) was added to the reaction mixture at a final specific radioactivity of 100 μCi/ml and incubated under the same conditions for an additional 10 min. Tissue slices were then rinsed thoroughly and homogenized in 15 ml of phosphate-buffered saline (0.01 M sodium phosphate, pH 7.4, containing 0.15 M NaCl). The quantities of [^{35}S]AFP and [^{35}S]albumin in cell lysates were determined by immunoprecipitation of in vivo translational products with anti-AFP or anti-albumin as described (23). The pelleted immunoprecipitate was washed and its radioactivity was measured.

Extraction of Liver Cytoplasmic RNA

Livers were homogenized in 5 volumes of 0.25 M sucrose, 5 mM magnesium chloride, 25 mM NaCl, and centrifuged at 10,000 x g for 10 min. The super-

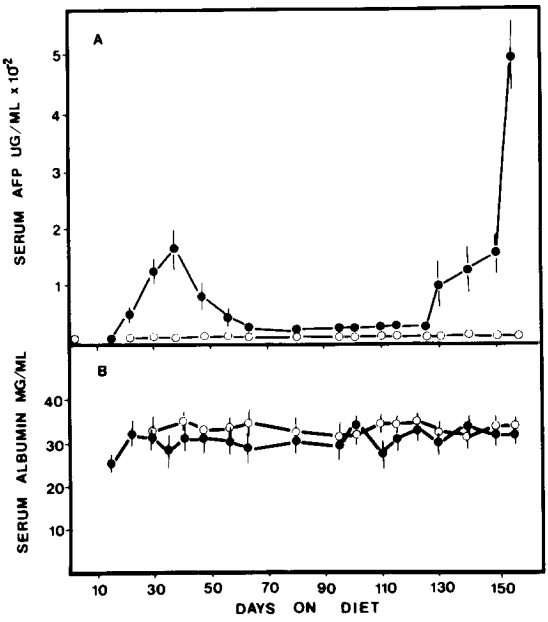

Figure 1. The concentration of AFP (A) and albumin (B) in sera of rats fed with diet containing (●) or without (○) 3'-MDAB.

natant was then brought to 25 mM sodium acetate, pH 5.0, 10 mM EDTA, 0.5% SDS, and the solution was extracted once with an equal volume of buffered-saturated phenol and 2-3 times with phenol/chloroform/isoamyl alcohol (49:49:2). The RNA in the aqueous phase was precipitated with ethanol overnight at -20°C.

Preparation of cDNA and Molecular Hybridization

AFP mRNA purification and cDNA preparation have been described previously (24). [^{32}P]Labeled cDNA to albumin mRNA was prepared essentially as described (25). RNA excess hybridizations were performed in a final volume of 20 µl of 0.5 M NaCl, 25 mM Hepes, pH 7.4, 2 mM EDTA, 0.1% SDS, containing 2000 cpm of cDNA and a known amount of RNA. Following heat denaturation at 100°C for 1 min, incubations were carried out at 68°C. Hybrids were determined by measuring the S_1 nuclease-resistant radioactivity (24).

RESULTS AND DISCUSSION

Chemically-induced hepatoma and preneoplastic livers were produced by feeding rats with a diet containing the hepatocarcinogen, 3'-MDAB. At various times the levels of serum albumin and AFP were determined. As shown in Figure 1, AFP was present in adult rat serum at levels of 40-60 ng/ml at the beginning of the experiment. The concentration of AFP started to increase in the sera of rats shortly after the initiation of feeding with the diet containing 3'-MDAB. It reached a peak at approximately 40 days and then decreased drarratically after this period. After 164 days of treatment, the serum level

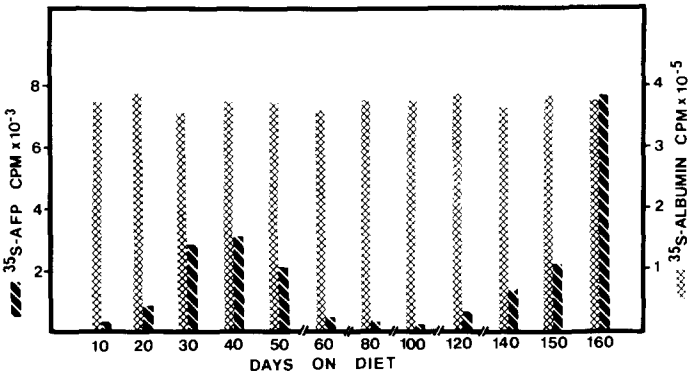

Figure 2. Incorporation of L-[^{35}S]methionine into AFP (▨) and albumin (▩) in liver slices of rats fed with diet containing 3'-MDAB.

of AFP increased markedly concomitant with the appearance of palpable liver tumors. The final permanent elevation of AFP is associated with the appearance of malignant cells (26). Similar patterns of serum AFP concentrations during hepatocarcinogenesis with 3'-MDAB have been reported by Chiu et al (19) and Kelleher et al (27). The serum level of albumin in the rats undergoing chemical hepatocarcinogenesis is also shown in Figure 1. Interestingly, there is little, if any, change in the serum albumin concentration in the rats treated with chemical carcinogen.

In order to determine whether the effect of the chemical carcinogen on concentrations of AFP and albumin resulted from protein synthesis in liver cells, intracellular AFP and albumin biosynthesis was studied. As shown in Figure 2, the synthesis of AFP in livers of rats treated with 3'-MDAB was found to increase in parallel with AFP serum concentrations. However, the biosynthesis of albumin in carcinogen-treated rat livers did not show any changes during the course of hepatocarcinogenesis.

The quantities of AFP and albumin mRNA were also determined by nucleic acid hybridization (Figure 3). We conclude that the serum concentrations of AFP and albumin are a consequence of the content of translatable AFP mRNA and albumin mRNA in carcinogen-treated neoplastic liver. The increase in AFP mRNA at approximately 18 days coincided with the increase in serum AFP concentration and reached a peak after 30-40 days of exposure to carcinogen. Subsequently, the concentration of AFP mRNA dropped to a normal level. Liver AFP

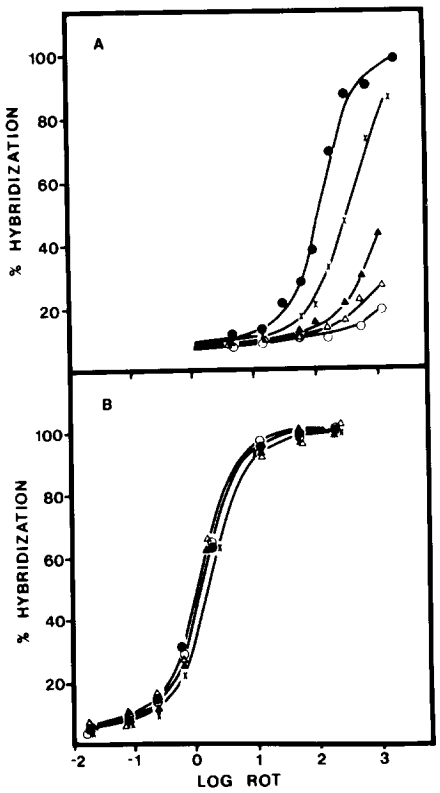

Figure 3. Hybridization assays of AFP (A) and albumin (B) mRNA concentrations in total liver cytoplasmic RNA. Cytoplasmic RNA from the liver of rats treated with diet containing 3'-MDAB for 18 days (▲-▲), 34 days (x-x), 96 days (Δ-Δ), and 164 days (●-●). Cytoplasmic RNA isolated from the livers of rats fed with control diet for 8 days as described in the text (o-o). RNA in control livers showed identical curves at all time points.

mRNA content increased markedly again in rats fed the diet containing 3'-MDAB for 164 days. In contrast to AFP, the content of albumin mRNA in livers of rats exposed to carcinogen was identical to that of normal rat livers.

These data indicate that early in hepatocarcinogenesis the production of albumin may be unaltered from normal. They are in contrast to reports (8-12) that the genetic regulation of albumin synthesis is altered in established hepatomas. Certain transplantable hepatomas were found to retain their capacity to produce serum albumin but at much reduced levels as compared to normal rat liver. Tse *et al* (22) reported that there was approximately a fourfold reduction in the level of albumin synthesis in hepatoma 7777 as compared to the normal rat liver.

Recently Liao et al (16) have studied the expression of AFP and albumin during normal development. They found that the levels of AFP and albumin do not show a direct reciprocal relationship during the fetal-neonatal period. Chiu et al (28) and Belanger et al (29) also have demonstrated that there is no direct reciprocal relationship between AFP and albumin gene expression. Both groups have shown that dexamethasone suppresses AFP gene expression in newborn rat liver. However, there is no significant effect of dexamethasone on albumin gene expression.

In conclusion, even though the two proteins share similar biological, physical, and chemical properties, a striking homology in amino acid sequences and protein structure, and a possible common ancestral gene, the expression of albumin and AFP genes appear to be under separate control. This communication demonstrates quantitative and qualitative differences between gene expression in normal and neoplastic liver, consistent with the concept of alterations in the control of gene expression upon neoplastic transformation.

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