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IMMUNOCYTOCHEMICAL INVESTIGATION OF α-FETOPROTEIN-POSITIVE CELLS IN HEPATOCARCINOCENESIS AND HEPATOMAS INDUCED BY 3'-ME-DAB

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Summary

AFP-positive cells during 3'-Me-DAB hepatocarcinogenesis and in hepatomas induced by 3'-Me-DAB were observed by the light microscope immunoperoxidase method, and the location of AFP-immunoreaction products in hepatocytes was determined by the electron microscope immunoperoxidase method. Simultaneously serum concentrations of AFP were measured by radioimmunoassay.

Serum concentrations of AFP during hepatocarcinogenesis showed a biphasic pattern, and proliferation of cholangiolar cells in the periportal areas was associated with the first peak of serum AFP in the early stage of hepatocarcinogenesis. AFP-containing cells in the late stage were distributed in clusters in the peripheral areas of hyperplastic nodules. The clusters were composed of two different types of AFP-positive cells, one atypical in shape and structure and the other preserving the structure of hepatic cords. In neoplasms, a heterogeneous AFP immunoreaction was recognized in hepatoma cells. In non-neoplastic nodules, cholangiofibrosis surrounded by AFP-positive cholangiolar cells developed.

Ultrastructural immunoperoxidase studies revealed immunoreaction products of AFP on the membrane of r-ER, outer nuclear membrane, s-ER and Golgi complex in hepatocytes of hyperplastic nodules as well as of hepatocellular carcinoma cells.

It is suggested that cholangiolar cells detected in hepatocarcinogenesis may form proliferating bile ductules, and the appearance of a cluster in association with AFP in hyperplastic nodules supports the hypothesis that some hyperplastic nodules with clusters of AFP-positive cells are important cell populations which may develop into hepatocellular carcinoma.

Key Words: α-fetoprotein-positive cells, hepatocarcinogenesis, hyperplastic liver nodules, hepatomas, immunocytochemistry.

Introduction

It has long been known that experimentally induced hepatocellular carcinoma is preceded by the sequential appearance of focal and nodular changes in liver tissues1-3). It is important to investigate the origin and properties of these to study the essential events in malignant transformation. The properties of some putative preneoplastic cell population have been studied extensively with the use of several
pheno
typic markers of neoplasms, 5). In 1963, Abelev et al. first reported the appearance of AFP in the serum of mice with transplantable mouse hepatoma, and it has been shown that AFP is synthesized not only by immature hepatocytes during normal ontogeny but also by hepatocellular carcinoma cells. Since AFP was recognized as one of the oncofetal proteins, many reports have been published on its nature, biological significance and value in the diagnosis of hepatocellular carcinoma.

Hirai et al. reported the appearance of AFP in rats serum during azo dye hepatocarcinogenesis, and many investigators have demonstrated AFP-positive cells in the early stage of hepatocarcinogenesis by immunofluorescent technique. It is important, however, to clarify the response of the original liver cells to injury by several carcinogens and their acquisition of a property that can evolve into hepatocellular carcinoma. New cell populations in the liver are often called hyperplastic nodules in the late stage of hepatocarcinogenesis. The aims of the present study are to immunocytochemically clarify the AFP-positive cells not only in the early and late stage of hepatocarcinogenesis but also in the stage of development of hepatocellular carcinoma; to demonstrate the immunoreaction products of AFP in hepatocytes of hyperplastic liver nodules by electron microscopic immunocytochemistry; to identify under light and electron microscopy the morphological features of AFP-positive cells; and to throw light on the question of which cell populations in the liver might be associated with evolution into hepatocellular carcinoma.

Materials and Methods

Male Donryu rats weighing about 180 g were fed normal diets for two weeks and then a solid diet containing 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) for 20 weeks. The control group was fed the normal diet for 22 weeks.

Two, 4, 6, 8, 12, 14, 16, and 20 weeks after the start of 3'-Me-DAB administration, 5 rats were sacrificed, the liver was examined histologically and blood was collected by puncture of the inferior vena cava for the determination of serum AFP levels.

For immunocytochemical studies of AFP, rats were perfused with cold phosphate buffered saline (PBS: 0.1 M, pH 7.4), followed by perfusion with periodate-lysine-4% paraformaldehyde (PLP) through the portal vein under sodium pentobarbital anesthesia, and the liver was post-fixed in PLP for an additional 6 hours at 4°C and washed in cold PBS containing 10% sucrose for 48 hours.

For light microscopic immunocytochemical studies, the immunoperoxidase method was used. Frozen sections of the liver were sliced into sections 10–20 μm thick and washed in PBS containing 0.3% Triton X-100 for 48 hours at 4°C. They were incubated in rabbit anti-rat AFP solution for 2 days at 4°C and treated with swine peroxidase conjugated anti-rabbit IgG solution for 2 hours at 4°C and finally incubated in 3,3'-diaminobenzidine (DAB) solution for 10 minutes at 20°C. Serial sections were stained with hematoxylin-eosin (HE) to demonstrate morphological changes during the experiment.

For the ultrastructural immunocytochemical method, vibratome sections (20 μm thick) were made in PBS containing 0.05% Triton X-100 for 48 hours at 4°C. They were then incubated in rabbit anti-rat AFP for 4 days at 4°C, followed by treatment with swine peroxidase-conjugated anti-rabbit IgG solution for 12 hours and washed in PBS. Tissues were fixed with 1% glutaraldehyde at 20°C for 10 minutes, washed in PBS for 30 minutes at 20°C and incubated in DAB solution for 10 minutes at 20°C. They were fixed with 2% OsO₄ for 1 hour at 20°C,