ULTRASTRUCTURAL OBSERVATION OF α-FETO-
PROTEIN PRODUCING CELLS IN HUMAN
HEPATOCELLULAR CARCINOMA USING
IMMUNOPEROXIDASE METHODS
—COMPARISON WITH FETAL LIVER—

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Summary

The precise sites of α-fetoprotein (AFP) synthesis and ultrastructural features and differences of AFP-producing cells were observed in periodate-lysine-paraformaldehyde fixed, frozen liver tissues from four human hepatocellular carcinoma (HCC) patients and three human fetuses using the direct (horseradish peroxidase-labeled Fab' fraction of anti-human AFP) immunoperoxidase method. We demonstrated that AFP was located in the membrane and cisternae of rough endoplasmic reticulum, membrane-bound ribosomes, perinuclear space and Golgi apparatus. The location and intensity of immunoreaction products of AFP in hepatoma cells varied from cell to cell and case to case, while these features tended to be regular in fetal hepatocytes. We did not observe ultrastructural differences between AFP-producing and non-producing cells adjacent to each other. These observations indicate that AFP production does not occur in morphologically distinct cell populations of hepatoma tissue and that hepatoma tissue is functionally much more heterogeneous than fetal liver.

Key Words: α-fetoprotein, Fetal liver, Hepatocellular carcinoma, Immunoelectron microscopy, Peroxidase-labeled antibody method.

Introduction

The α-fetoprotein (AFP), an onco-fetal protein, has been widely studied for more than twenty years since it was discovered by Abelev in 1963¹). It is well known that AFP synthesis occurs under both physiological condition during embryonic and fetal life and certain pathological conditions, including hepatocellular carcinoma (HCC)²,³ and teratocarcinoma⁴). At present, this protein is recognized as the best tumor marker for the diagnosis of HCC.

Numerous immunohistochemical studies
have revealed the cellular and subcellular localization of AFP in human and rat fetal liver\textsuperscript{5,6} as well as rat hepatoma induced by chemical carcinogens\textsuperscript{7,8}. However, there have been few immunocytochemical studies of AFP in human HCC\textsuperscript{9}, and the precise subcellular localization of AFP remains to be clarified.

In this paper, we describe ultrastructurally the precise localization of AFP, morphological features and differences in the localization of AFP in human HCC and human fetal liver.

**Materials and Methods**

Liver tissues surgically removed from 12 patients with HCC, described in the Table 1, and livers from three human fetuses, 10, 16 and 17 weeks old, were studied. The serum level of AFP in the 12 cases of HCC measured by radioimmunoassay (SPAC α-Feto kit, Daichii Radioisotope Labs., Ltd., Tokyo) ranged from 12.0 ng/ml to 109,300 ng/ml. HBsAg was shown to be positive in 4 HCC cases (nos. 5, 7, 10, 12) by reversed passive hemagglutination methods.

Surgically resected tissues were cut into small pieces approximately in size $1 \times 1 \times 5$ mm within 4 hours after resection and fixed in a periodate-lysine-paraformaldehyde (PLP) fixative\textsuperscript{10} for immunohistochemical study and in a 10% neutral formalin or Bouine's solution for routine light microscopy. After fixation, the tissues were washed in 0.05 M sodium phosphate buffer (pH 7.2) with 8.5% sucrose for 4 hours, in the same buffer containing 15% sucrose overnight, and finally in buffer containing 20% sucrose and 10% glycerin for 2 hours. Subsequently the tissues were embedded in Ames OCT compound.

Immunohistochemical study of AFP was performed by the direct immunoperoxidase method\textsuperscript{11}. The specific antiserum against human AFP was raised in rabbits in our laboratory. Purified human AFP was obtained from fetal liver homogenate by the method of Nishi and Hirai\textsuperscript{12}. After immunization of rabbits, antiseraum was absorbed by normal human immunoglobulins. The specificity of the antiseraum was checked by the Ouchterlony test. The Fab' fragment purified from the IgG fraction of antiserum was conjugated with horse-radish peroxidase (HRP) (Sigma Chemical Co., St. Louis, MO, type VI) by the method of Nakane et al.\textsuperscript{13}. Frozen sections (6 μm) were made with a cryostat, air-dried and washed in PBS three times for 5 min. For light microscopy, the tissue sections were incubated with HRP-Fab' for 15 min and diaminobenzidine (DAB)-peroxide for 10 min. The sections were dehydrated in graded ethanol and mounted. For immunoelectron microscopy, the sections were incubated with HRP-Fab' for 4 hours, postfixed with 2% glutaraldehyde and reacted with DAB for 30 min and DAB-peroxide for 2 min. After fixation with 1% osmium tetroxide for 30 min, they were dehydrated in graded ethanol and embedded in Epon-araldite. Ultrathin sections were observed without counterstaining under a JEM-100S electron microscope.

The following controls were used: 1) HRP-rabbit Fab' of anti-AFP absorbed with purified AFP and 2) HRP-Fab' of normal rabbit serum instead of specific HRP-rabbit Fab' of anti-AFP.

**Results**

(1) Histology

According to Edmondson and Steiner's grading\textsuperscript{14}, the 12 HCC were classified as: Grade I, 2 cases, Grade II, 4 cases, and Grade III, 6 cases (Table 1).

(2) HCC

a) Light microscopy

In 12 cases with HCC, AFP was detected in the liver of 5 patients who showed elevation of serum AFP (475–109300 ng/ml) and were classified into Edmondson's grade II or III. How-