CHAPTER IV

DISCUSSION

As a number of reports have shown that AFP is related to hepatomas of experimental animals as well as those of human being, except the rate of liver regeneration is faster than in man. AFP has been found in the sera of the patients with hepatocellular carcinoma, it is also detected in the early stage of experimental hepatocarcinogenesis (62-66).

Histologically, the degenerated hepatocyte appearing in the initial stage, is followed by nodular or adenomatous proliferation of the hepatic cells in the following stage, and the production of hepatocellular carcinoma occurres in some of the areas of the nodules in a later stage. When hepatocytes suffer severe and prolong damage, some of them show necrosis, the remainder will become hyperfunctional cells to compensate for the decline of the total function (67).

Regeneration of the liver, caused by hepatotoxic carcinogens is accompanied by a sharp outbrust of AFP production. Comparison of the dynamics of AFP synthesis during the acute phase of chemical hepatocarcinogenesis with the population dynamics of different cell types in the liver, has suggested that AFP is synthesized by cells which are transitional from oval cells to small hepatocytes (68).

The early AFP is believed to be produced by oval cells. The cell composing the nodular hyperplasia or the area of hyperplasia,

which replaced the major part of the liver in the next stage has been considered the most probable forerunner of the carcinoma. AFP in the late phase is produced by the carcinoma.

By electron microscopical observations, the oval cells reveal pale cytoplasm and scanty organelles resembling the cholangiole cells. The small hepatocytes have round nuclei and more organelles, but are still fairly immature compared with the original mature hepatocytes (63).

It may be considered that the protein structure of mammalian sera, including human, undergoes typical evolution in ontogenesis. One of the most characteristic features of such an evolution is the presence of an embryo-specific-α-globulin in the fetal sera. It is the main component of the fetal serum in the earliest stages of ontogenesis. Furthermore AFP can be detected in normal human serum by sensitive RIA method but in a small amount. The presence of AFP in normal human serum indicates that the repression of AFP synthesis at birth is not complete at the adult stage. The presence of AFP in malignant disease is usually thought to result from depression of a fetal gene that is normally suppress at the adult stage (69). As the fact that, the site of AFP production is the liver cells in the fetus, the tumours arising from hepatocytes may synthesize the protein (9,17,25,62,70,71,72)

In the present study, the relationship between serum AFP-positive cases and histological grading could not be ascertained absolutely in each individual case without exception. The peak incidence of AFP-positive cases was highest in poorly differentiated grade III hepatocellular carcinoma (71.4 %), followed by moderately differentiated

grade II hepatocellular carcinoma (50 %). This finding agreed with that of Sakurai, M., and Miyaji, T. (73), which indicated that relatively poorly differentiated hepatocellular carcinoma might mainly produced this protein.

In cases of metastatic carcinoma of the liver serum AFP turned out to be negative in all 11 cases. This finding was in accordance with the result of the other observation (25).

According to the above observation, negative serum-AFP did not rule out the presence of a suspected liver carcinoma.

For the direct immunofluorescence antibody technique, in this experiment, AFP was detected in 14 of 25 cases (56 %), with histologically confirmed liver carcinoma. Of these 14 cases were hepatocellular carcinoma and 11 were metastatic carcinoma of the liver. Based on these observations, the occurrence of AFP fluorescence did not seem to be correlated with the degree of differentiation of the tumours. As shown in Table 21, six patients had grade II moderately hepatocellular carcinoma, seven cases with grade III poorly differentiated hepatocellular carcinoma, and undifferentiated hepatocellular carcinoma grade IV in 1, with marked intensity of AFP. For grade II and grade III hepatocellular carcinoma showed moderately intensity than those of marked, faint or negative fluorescence.

No information was available regarding the differentiating histological grades of tumour cells containing AFP in comparison with those not containing AFP.

The distribution of tumour cell containing AFP varied with the case and the specimen. Intense fluorescence in the cytoplasm of hepa-

toma cells, appeared either homogeneous or somewhat granular. It was present in single cells or in a clump of cells. The overall average of tumour cells containing AFP was 30 % or less of the total. In some cases the brightness of granular cytoplasm and of cytoplasmic membrane were difficult to distinguish. The brighter cells were found usually near the capillaries and blood vessels. The reason for an intense bright fluorescence, a faint or negative in tumour cells was unclear.

It was interesting that, the cases of marked intense fluorescence, the brightness could be seen in all three areas. Diffuse, finely granular fluorescence of the cytoplasm of tumour cells appeared to be more common in grade II hepatocellular carcinoma. The positive fluorescence in the cytoplasmic membrane was demonstrated in both grade II and grade III hepatocellular carcinoma. However, it must be memtioned that 2 cases (18.2 %) of metastatic carcinoma of the liver moderate fluorescence were observed. The main portion in which AFP was stained was the fibroblasts. The distribution of the fibroblasts containing AFP was 10 % of the total. Onoé et al, (67) stated that fibroblast showed in the light microscope, might be an oval cells examined in electron microscope. Recently Hamashima, Y. (74) reported that positively fluorescent cells were detected in 4 of 71 cases (5.6 %) of metastatic carcinoma of the liver.

There was no correlation between the serum AFP and the occurrence of fluorescence in the tumour cells. The sera turned positive-AFP in 9 cases of 14 cases with hepatocellular carcinoma by counterimmunoelectrophoresis. Eight of nine patients having AFP in their sera had specific fluorescence in the tumour cells. Two cases of each grade II and grade

III hepatocellular carcinoma in whom sera AFP could not be detected, a specific AFP was detected in the cytoplasm of tumour cells by this method. This observation suggested that the immunofluorescent technique was more sensitive for the detection of AFP than counterimmunoelectrophoresis. The reason for these differences was not clear at the moment.

According to Houstek et al (75) higher concentrations of AFP was detected in hepatoma tissues. However, body fluids such as blood and ascites also had the highest AFP level. This suggested that tumour cells rapidly secreted AFP into the serum after synthesizing it. For this reason, it was difficult to come to a clear correlation between the levels of serum AFP and the occurrence of AFP in the tumour cells.

The amount of serum AFP decreased after surgical removal of the tumours (76). In this experiment, most of the cases were surgical biopsy, 21 cases, and 4 cases were needle biopsy. The percentage (64.3 %) of serum AFP-positive from hepatocellular carcinoma observed in this study was lower than that of Pongpipat et al 73.1 % (43). This agreed with the statement mentioned earlier (76).

It has been suggested that soon after birth there is "repression" of the genes responsible for coding for AFP synthesis during fetal life (1). The reappearance of AFP in the serum of patient with liver carcinoma may be due to derepression of these genes by malignant liver cells (30).

As is well illustrated in this study of AFP localization in the cytoplasm of the tumour cells confirmed the synthesis of AFP in the cells. The presence of AFP was rare in the absence of a hepatoma. The individual hepatomas had a different amounts of AFP.