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# IMMUNOHISTOCHEMICAL STUDY ON HEPATIC TUMORS -KM01 STAINS COMPARED WITH AFP, CEA, CA19-9 AND RAS P21-

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## INDEXING WORDS

KM01; AFP; hepatic tumors; PAP methods

## SYNOPSIS

Pathological diagnosis of hepatic tumors is sometimes difficult when performed with only routine examinations such as Hematoxylin and Eosin(H.E.) stain. The diagnostic usefulness of KM01 was compared to that of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), CA19-9 and ras p21 in this immunohistochemical study.

AFP was positive in about half of the cases of hepatocellular carcinoma and hepatoblastoma, and AFP-positive cells were frequently found at the periphery of acini in both diseases. Absorbed CEA stain was mostly negative in hepatocellular carcinoma, but was positive in the cells of mixed hepatocellular and cholangiocellular carcinoma (MHCC) and metastatic liver cancer, especially in their cytoplasm. CA19-9 immunostaining was completely negative, and was only 3% positive in hepatocellular carcinoma.

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KM01 stain was positive in about half of the cases of hepatocellular carcinoma, hepatoblastoma and MHCC. It was positive in proliferated bile ducts around the capsule in the former two diseases but positive in the tumor cell of both parts of the cytoplasm in the latter.

The histological positivity of ras p21 was high in all tumor cells of these three types of tumors. Negative absorbed CEA and KM01 in pseudoglandular hepatocellular carcinoma differentiated from MHCC and metastatic liver cancer. However these tumor markers were occasionally positive and nonspecific in cancer-like lesions, implying no advantage for differential diagnosis between hepatocellular carcinoma and apparent cancer-like lesions. The above results demonstrate that AFP, CEA and KM01 are effective for differentiating hepatocellular carcinoma among various hepatic tumors.

## INTRODUCTION

Hepatic tumors could be more easily detected by recently developed diagnostic methods such as ultrasonography, computed tomography and angiography. However, preoperative differential diagnosis by use of tiny specimen obtained by needle biopsy can be sometimes more difficult in some cases, therefore those are left uncertain until the final diagnosis. The effective combination of AFP and/or other markers for immunohistochemical stains of hepatic tumors has been reported by Thung et al.[24] Others have investigated further several markers.[5, 18, 19] However, these conventional markers such as AFP and CEA were incomplete for the immunohistochemical differential diagnosis of various types of hepatic tumors.[2, 3, 11, 13, 18, 19, 20] The purpose of this study is to examine KM01[17] as a new marker, in comparison to those conventional markers, and finally to use it for differential diagnosis of various types of hepatic tumors.

## MATERIALS AND METHODS

We examined pathologically 92 cases of hepatic tumors from surgery and autopsy in Kobe University Hospital from 1980 to 1987. They were composed of hepatocellular carcinoma (n=71),

mixed hepatocellular and cholangiocellular carcinoma (MHCC) (n=2), metastatic liver cancer from the colon (n=5), hepatoblastoma (n=10) and cancer-like lesions (n=4). Four cancer-like lesions consisted of a liver cell adenoma, a focal nodular hyperplasia (FNH) and two cases of adenomatous hyperplasia. All these hepatic tumors were fixed in 10% buffered formalin solution and processed according to the routine pathologic procedure, then embedded in paraffin and cut at 4-micron sections. HE stain was carried out in all cases. Immunohistochemical stains were performed with peroxidase-antiperoxidase (PAP) methods[14,22,23] using AFP (UNIPATH), CA19-9 and KM01 (Green Cross) as a monoclonal antibody tumor-marker; CEA, absorbed and unabsorbed (DAKO) as polyclonal antibody tumor-markers; and ras p21 (Oncor) as a polyclonal antibody. The mouse monoclonal antibody of KM01, against an established human colon cancer cell line, COLO-201, has been developed with the routine hybridoma technique[8] in the First Department of Surgery, Kobe University School of Medicine and Central Research Laboratory, The Green Cross Corporation. It is immunologically cross-reactive against CA19-9 though some differences exist.[17]

The intensity of staining was estimated in four degrees: strongly positive (++), positive(+), weakly positive( ± ) and negative(-), compared to the control. Positive areas were estimated, and staining patterns such as cytoplasmic, apical and bile canalicular were distinguished morphologically.

## RESULTS

The positive ratios (positive/positive-plus-negative areas) of monoclonal AFP antibody in hepatocellular carcinoma and hepatoblastoma were 45 and 63% respectively. Positive fine granules of AFP were often found at the periphery in trabecular and cytoplasmic patterns of their neoplasm (Fig. 1). Though hepatoblastoma was stained more strongly and massively, the staining patterns were not different from hepatocellular carcinoma. Most of the metastatic liver cancer and cancer-like lesions were negative for AFP (Table 1).

Unabsorbed CEA had high positive ratio in hepatocellular

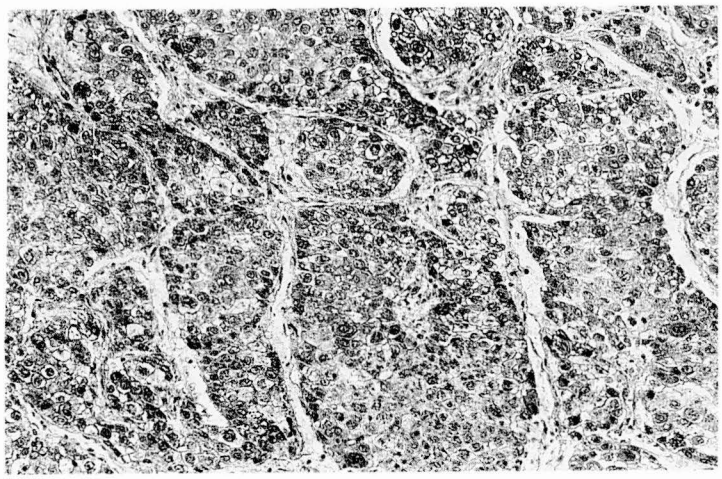
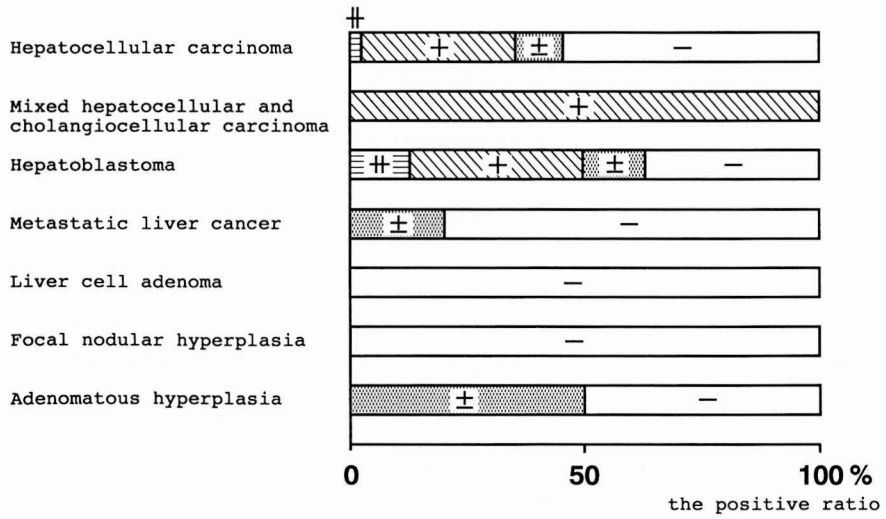


Fig. 1 Hepatocellular carcinoma showing cytoplasmic staining(+) for AFP at the periphery in the trabecular type (x66, original magnification)

Table 1 The positive ratio of AFP

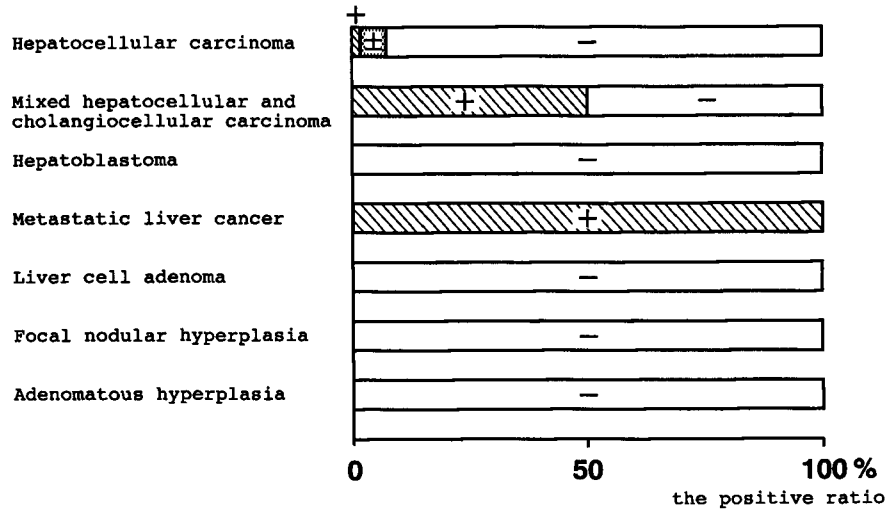


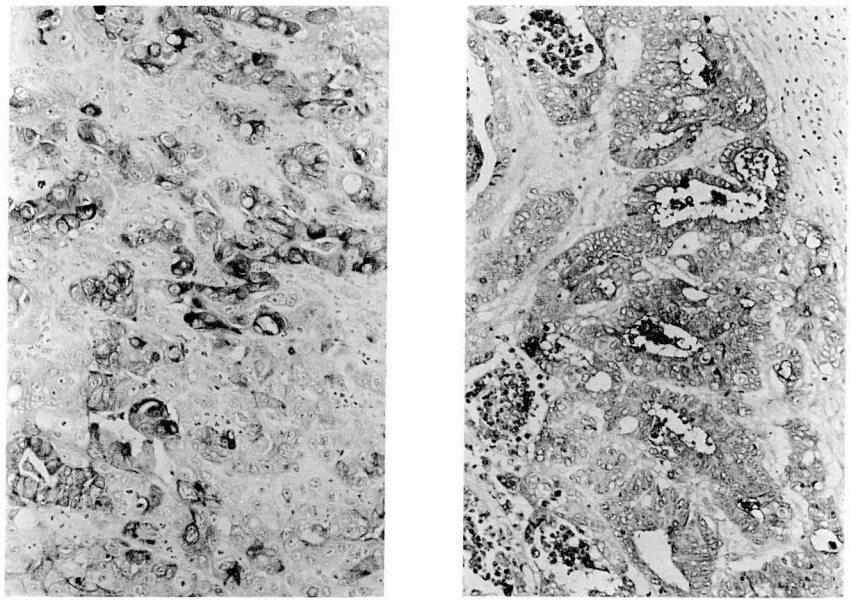
carcinoma, hepatoblastoma and metastatic liver cancer. Both cytoplasmic and canalicular sites of these tumors were stained positively. Absorbed CEA stained weakly in only 7% of hepatocellular carcinoma. It was nagative both in hepatoblastoma and cancer-like lesions. The positive ratio was 100% in metastatic liver cancer and 50% in MHCC. Then, absorbed CEA was thought to be chiefly specific for metastatic liver cancer and MHCC (Table 2). Its staining pattern was glandular cytoplasmic (Fig. 2).

CA19-9 was negative except for 3% of hepatocellular carcinoma and 50% of MHCC (Table 3). The CA19-9 was negative for most of the cells in hepatocellular carcinoma including the bile ducts proliferated in the tumor capsule (Table 3)(Fig. 3).

The positive ratios of KM01 for hepatocellular carcinoma, MHCC, hepatoblastoma and metastatic liver cancer were 54, 50, 57 and 60% respectively. However, cancer-like lesions except adenomatous hyperplasia were negative (Table 4).

Table 2 The positive ratio of absorbed CEA

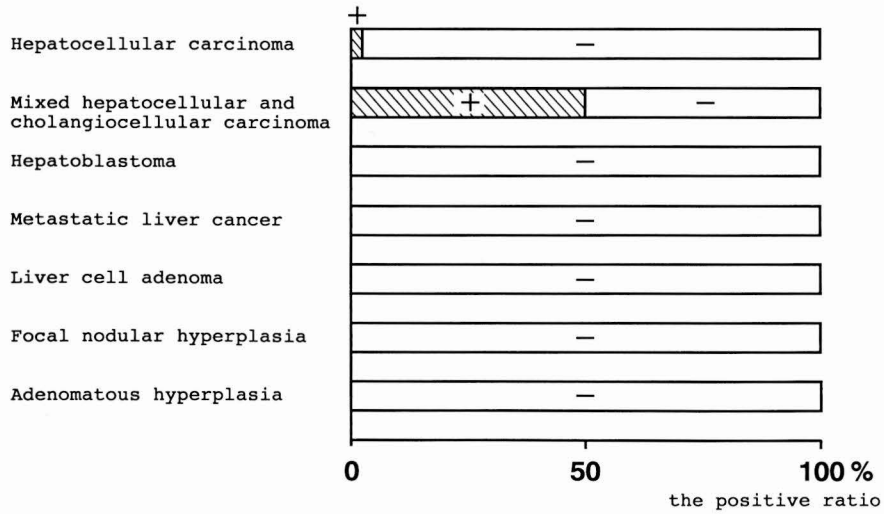




left right

Fig. 2 Mixed hepatocellular and cholangiocellular carcinoma(left) and metastatic liver cancer(right) showing positive staining(+) for absorbed CEA of the cytoplasmic pattern in the glandular formation (x66, original magnification)

Table 3 The positive ratio of CA19-9



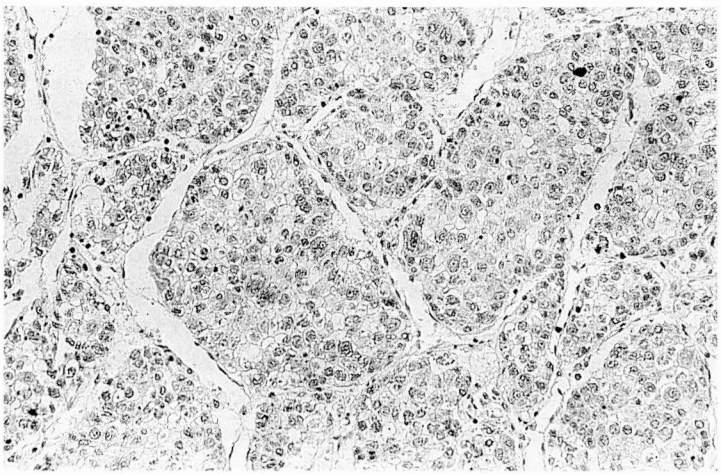
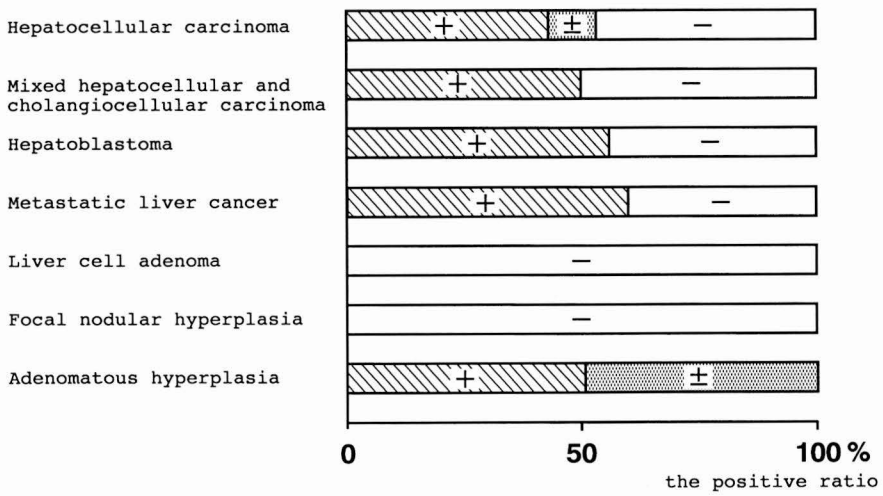


Fig. 3 Hepatocellular carcinoma showing negative finding for CA19-9 (x66, original magnification)

Table 4 The positive ratio of KMO1



The staining pattern of KM01 in hepatocellular carcinoma was histologically specific. Tumor cells were negatively stained but the cytoplasm of bile ducts proliferated in the capsule around the cancer lesion had intense positivity (Fig. 4).

Compared to proliferated bile ducts in cirrhotic areas of the liver with hepatocellular carcinoma, proliferated bile ducts around hepatocellular carcinoma showed a higher positive ratio, and a greater intensity. The staining pattern of hepatoblastoma was almost the same as that of hepatocellular carcinoma. The cytoplasm of tumor cells in MHCC showed diffuse positivity (Fig. 5). The positive portion of metastatic liver cancer was apical. Hepatocellular carcinoma in MHCC showed a partly adenocarcinomatous pattern and KM01 positivity.

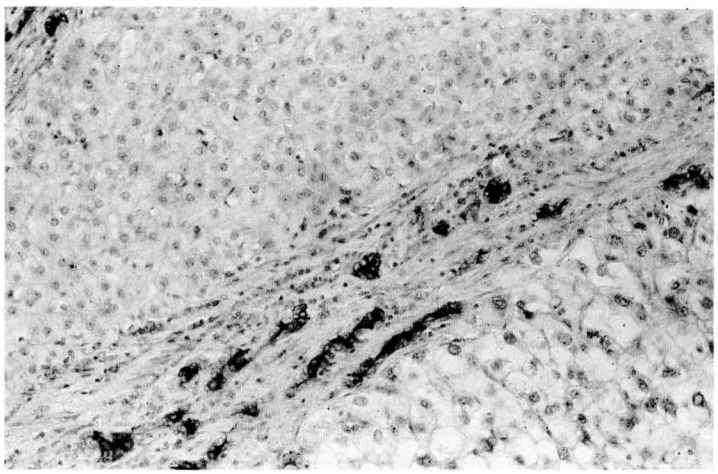


Fig. 4 Hepatocellular carcinoma showing strongly positive(++) finding for KM01 in the cytoplasms of bile ducts proliferated in the capsule surrounding the cancer (x66, original magnification)

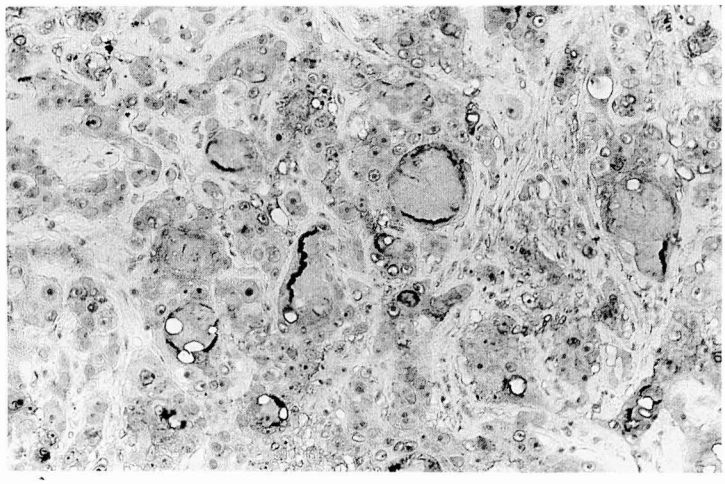
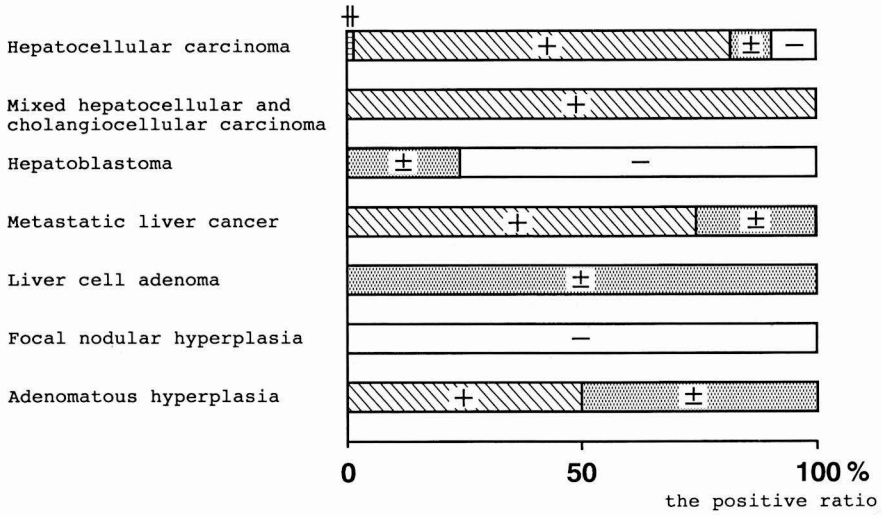


Fig. 5 Mixed hepatocelluar and cholangiocellular carcinoma showing positive(+) finding for KM01 in the cytoplasms of both carcinoma(x66, original magnification)

On the other hand, ras p21 of a oncogene protein showed a high positive ratio of 90% in hepatocellular carcinoma. It showed also the positive reactivity in liver cell adenoma and adenomatous hyperplasia. Thus no ras p21 specificity was found histologically in these tumors (Table 5). The cytoplasms of hepatocellular carcinoma stained weakly positive as fine granules for ras p21 (Fig. 6), but it showed very low positive ratio in hepatoblastoma.

These results mentioned above are summarized as follows: AFP, KM01 and ras p21 tend to show high positive stain in well differentiated hepatocellular carcinoma.

Table 5 The positive ratio of ras p21



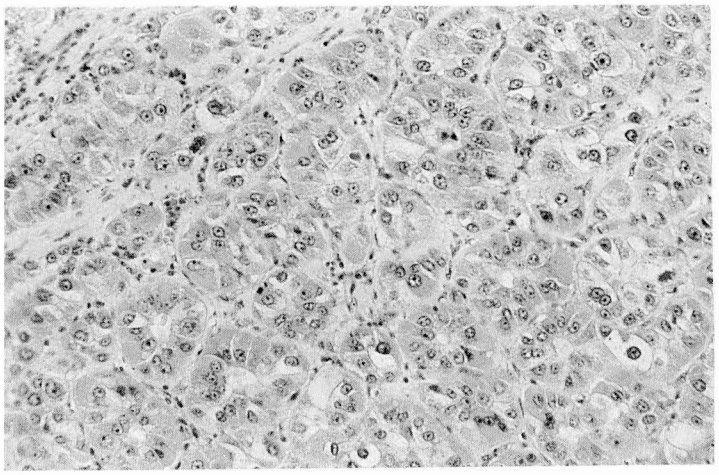



Fig. 6 Hepatocellar carcinoma showing weakly positive( ± ) staining for ras p21 in the cytoplasms as fine granules (x66, original magnification)

## DISCUSSION

AFP is known as the most useful serum marker for hepatic tumors.[1,3,6,10] CEA and other new markers are also applied for clinical and pathological diagnosis.[11,12,15,19,20,24] In immunohistochemical studies using these markers , however, the positive ratios and/or their distribution patterns are not always similar.

It is obvious that staining procedure and thickness of histological sections are more important in immunohistochemical examination with PAP methods. The markers are false negative when the specimen is badly fixed or contains necrotic areas, embolisms, thrombi, or negatively charged red blood cells. To obtain correct information from these stains, we must avoid these troubles.

Hepatocellular carcinoma is histologically classified by WHO[9] as trabecular, pseudoglandular, compact and scirrhous types. Especially in the pseudoglandular type, differential diagnosis is difficult from cholangiocellular carcinoma, MHCC and metastatic liver cancer. Immunohistochemical examinations including the new monoclonal antibody, KM01, may be worthwhile to evaluate these markers on the diagnosis of hepatic tumors. AFP positive ratios was 45%, 100%, 63%, 0%, and 0% in hepatocellular carcinoma, MHCC, hepatoblastoma, metastatic liver cancer and cancer-like lesions, respectively, while metastatic liver cancer and cancer-like lesions were negative. Forty five % of sensitivity in hepatocellular carcinoma may reveal that negative AFP staining does not exclude the existence of hepatocellular carcinoma and is insufficient to differentiate malignant primary hepatic tumors. These immunohistochemical results against AFP agree with previous report[13,24] and indicate the necessity of a combination with other markers. Absorbed CEA, which has a high positivity for adenocarcinoma, is a useful marker for metastatic liver cancer. High positive ratios by unabsorbed CEA in hepatocellular carcinoma, hepatoblastoma and metastatic cancer arise from positive staining and cytoplasmic sites in the tumor cells of these tumors and in bile canalicular around tumors. According to the reports by Nap et al.,[16] a positive bile canalicular reaction suggests nonspecific

reaction, involving one of its nonspecific cross-reacting antigens such as biliary glycoprotein I (BGPI).[5,16] CEA staining has little merit in differential diagnosis of hepatocellular carcinoma. As CA19-9 was negative for most hepatocellular carcinoma, including bile duct proliferation in and around the tumor capsule, it cannot be used as a decided effective criterion for the diagnosis of hepatocellular carcinoma but is of interest histochemically in comparison to the results of KM01, which has a very close immunoreactivity to CA19-9.

KM01 antibody is made in novel mouse injected by monoclonal antibody which was successfully reactive against an established human colon carcinoma cell line, COLO-201.[21] The antigenic determinant region of KM01, detected by this monoclonal antibody, is compatible to three components. The main site is sialylated lacto-N-fucopentaose II . The other two are monosialogangliosides with molecular weights slightly larger than lacto-N-fucopentaose II . As pointed out, KM01 antibody has similar reactions to CA19-9, whereas its essential nature is different immunologically and chemically.

KM01 antibody can be applied for immunohistochemical examination especially on the diagnosis of hepatic tumors. Though the positive ratio of KM01 as the serum tumor marker was about 50% as compared to hepatocellular carcinoma,[17] our study showed also its nagativity in the tumor cells and positivity in proliferated bile ducts in and around the boundary capsule of the hepatocellular carcinoma. This suggests that serum KM01 is not secreted by the tumor cells themselves, but increases from proliferated bile ducts in the capsule of hepatocellular carcinoma. According to the positivity by KM01 in the proliferated bile duct, hepatocellular carcinoma may be differentiated from liver cirrhosis nodules, which are weakly positive for KM01. As in MHCC, KM01 is also positive not only in cholangiocellular carcinoma but also in hepatocellular carcinoma.

Therefore, it is concluded that some cells of hepatocellular carcinoma in MHCC have a possibility of adenocarcinomatous nature different from that of the usual hepatocellular carcinoma.

Then the hepatomatous carcinogenesis may closely correlate

to the development of hepatocytic series cells. As CA19-9 immunologically cross-reactive against KM01 and almost negative findings, there are likely to be some differences in the distribution in hepatocellular carcinoma between KM01 and CA19-9. Based on these results, it is expected that KM01 can be applied to AFP-negative and capsule-formation cases of hepatocellular carcinoma and it is useful for the differential clinical diagnosis.

Recently, cancer-like lesions have been increasing and been discussed in their etiology and differentiation from hepatocellular carcinoma. We expected at first that ras p21 would show higher positivity as an oncogene protein in hepatocellular carcinoma than benign tumors, whereas ras p21 showed positive reactivity in liver cell adenoma and adenomatous hyperplasia as well as in the hepatocellular carcinoma, MHCC and metastatic liver cancer.[4,7] Ras p21 might be useful for differential diagnosis between hepatocellular carcinoma and hepatoblastoma, because the positive ratio of the latter was very low.

Based on our immunohistochemical studies on hepatic tumors, each tumor marker could possibly be applied for differential diagnosis of hepatocellular carcinoma in the following combinations. AFP staining should be performed first. If the case was negative for AFP, KM01 should be applied to detect hepatocellular carcinoma. Ras p21 stain may be useful for differential diagnosis between hepatocellular carcinoma and hepatoblastoma.

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