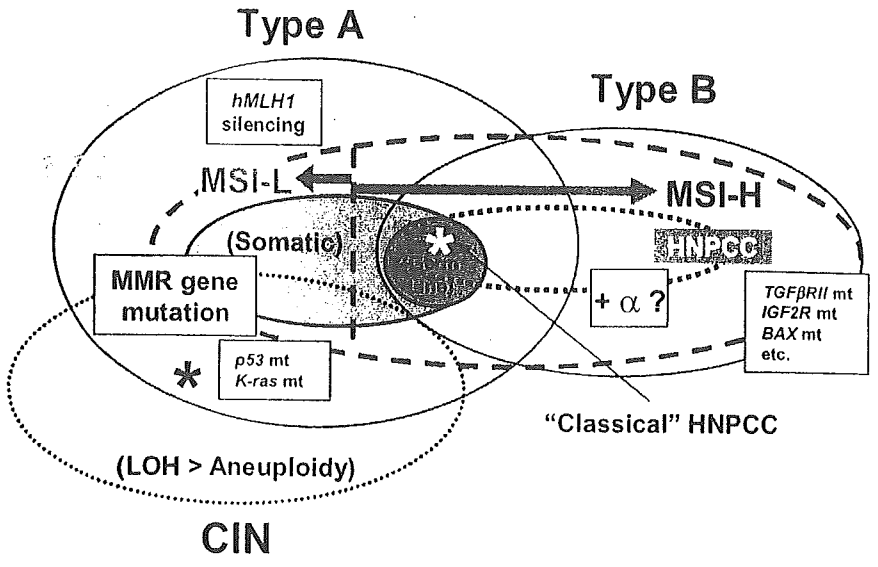

Fig. 2. Complex relationships between known molecular backgrounds in human colorectal cancer. Possible relationships between the categories of microsatellite instability are schematized. Typical genetic alterations are also shown in *rectangles*. The *black* and *white asterisks* indicate the chromosomal instability (CIN) and the classical microsatellite mutator phenotype (MMP) pathways. *MSI*, microsatellite instability; *dMMR*, defective mismatch repair; *CIN*, chromosomal instability; *LOH*, loss of heterozygosity; *mt*, mutation

stable microsatellites also appear to exist. They represent a biologically distinct phenotype with a significantly higher metastatic potential at an early clinical stage. This complexity in the relationship between the CIN pathway and the MMP pathway may derive from an oversimplification of both phenotypes. CIN may be comprised of numerical and structural instability in chromosomes. The former is characterized by aneuploidy, the latter by diverse chromosome aberrations including LOH. Different molecular abnormalities may underlie the two CIN phenotypes. Detailed characterization of the CIN and MMP pathways is required for a better understanding of tumorigenesis in the colorectum and, possibly, other organs.

Gastric Cancer

The first report of MSI in gastric cancer appeared in the literature in 1993. The initially reported frequencies of MSI in sporadic cases of gastric cancer vary from 25% to 50%.[70–73] After the Bethesda panel has been recommended, MSI-H and MSI-L have been reported in a range of 5%–50% and 5%–80%,[74–79] respectively. Using high-resolution fluorescent microsatellite analysis (HRFMA),[80] the frequencies for MSI-H and MSI-L were determined as 11% and 13%, respectively (unpublished data and Ref. 18). In contrast to colorectal cancer, sporadic or germ line mutations in MMR genes have not been elaborately explored in MSI-positive gastric tumors. Instead, as in colorectal cancer, *hMLH1* silencing has been recently suggested to be a cause of MSI in sporadic gastric cancer.[81] From the data accumulated in the literature, it appears difficult to discuss the relationship between MSI phenotypes and mutations in major MMR genes in gastric cancer.

In gastric cancer, the MSI$^+$ phenotype has been reported to correlate with several clinicopathological features,[82–84] i.e., older age, lower clinical stages, antral location, "intestinal" histological subtype, and better patient outcome. However, in the literature, some reports differ in conclusion.[85,86] Among the clinical aspects of MSI$^+$ gastric tumors, more attention has been directed toward multiple cancers and familial predisposition. Several reports have pointed out that MSI is more common in patients with multiple gastric cancers, either synchronous or asynchronous, than in those with its solitary forms.[70,78,87,88] Intriguingly, Miyoshi et al. reported that MSI-L correlated significantly with synchronous and asynchronous multiplicity in gastric cancer.[78] It is known that familial gastric cancer comprises approximately 1% of sporadic cases of gastric cancer. The entity of familial gastric cancer, however, remains unclear. Gastric cancer has been histologically classified into two subtypes, i.e., "intestinal" and "diffuse" types, and familial gastric cancer appears to be composed of several different entities depending on these histological subtypes. In the diffuse type, hereditary diffuse gastric cancer (HDGC) has been difined.[89] In HDGC pedigrees, mutations in the *E-Cadherin* gene have been reported.[90] However, *E-Cadherin* mutation is not found in all of HDGC individuals, and appears to account for only a limited part of this type of familial cancer.[89] Intestinal type familial gastric cancer has been defined based on the Amsterdam Criteria II for HNPCC.[10] Intriguingly, gastric cancer is a possible phenotype of HNPCC, and indeed occurs in 10% of HNPCC individuals.[91] In these HNPCC-related gastric tumors, intestinal type predominates. However, in designating familial gastric cancer, cancer-prone syndromes causing gastric cancer as a phenotypes, i.e., HNPCC, Li–Fraumeni syndrome, familial adenomatous polyposis (FAP), and Peutz–

Jeghers syndrome, are excluded. Nevertheless, both intestinal and diffuse type tumors are found in patients with familial gastric cancer who fulfill the criteria. Thus, familial gastric cancer may comprise, at least, three categories: HDGC, the intestinal type familial gastric cancer, and familial gastric cancer related to other cancer-prone syndromes such as HNPCC (intestinal type). In gastric cancer, a number of reports point out a correlation between MSI in proband gastric tumors and a family history of gastric cancer.[72–74,92] Reported frequencies of MSI in familial gastric cancer are largely high, varying from 25% to 70%.[93–95] Intriguingly, Akiyama et al. reported that in MSI$^+$ tumors in familial gastric cancer patients no mutations were found in the two major MMR genes, *hMSH2* and *hMSH1*.[93] This finding may suggest a possibility that molecular abnormalities other than defective MMR cause MSI and underlie familial predisposition to gastric cancer.

Esophageal Cancer

MSI in esophageal cancer has been controversial. Some groups reported that its frequency is relatively high,[71,96–99] while others found it to be low.[100–102] This discrepancy appears to derive from the high frequency of LOH in this cancer. As pointed out in the above-mentioned NCI workshop, some forms of LOH are indistinguishable from MSI. Using high-resolution fluorescent microsatellite analysis (HRFMA), Araki et al. have obtained similar figures of less than 2%–4% frequency in two geographically independent panels of patients with squamous cell carcinoma in the esophagus.[103] LOH was uniformly high in both panels. These findings are compatible with the report of Uchida et al., in which mutation or aberrant expression were not found in the major MMR genes in squamous cell carcinoma cell lines.[104] On the other hand, in squamous cell carcinoma in the head and neck, MSI has been reported to be frequently observed.[105–107] Wang et al. reported a higher incidence of MSI in younger patients,[106] and Ha et al. found that MSI is more common MSI in chronologically advanced tumors compared with precancerous dysplastic lesions.[107] Intriguingly, however, inactivation of the major MMR genes was not found in the former report.

As discussed above, in colorectal carcinogenesis, two mutually exclusive pathways, i.e., CIN and MMP, have been proposed. In the CIN pathway, mutations in various oncogenes or tumor suppressor genes such as *APC*, *K-ras*, and *p53* are associated. Considering that defective MMR is rare in squamous cell carcinoma in the esophagus, CIN may comprise a major pathway of tumorigenesis in the esophagus. This is compatible with the findings that LOH is frequent in esophageal cancer, and that mutation and LOH in the *p53* gene are also frequently observed.[108] The sources of mutations in oncogenes and tumor suppressor genes are still unknown. Defective MMR greatly contributes to an increased mutation rate in cells. However, this molecular abnormality has been excluded. Molecular mechanisms causing mutations in tumorigenic genes, as well as ones of CIN, warrant particular attention.

What Problems Remain?

A New Marker Problem — Mono- Versus Dinucleotide Microsatellites

Careful attention has recently been paid to the methodological aspects of MSI assays and their effects on the results of analyses, and, consequently, sensitivity and quantitativity of analyses have been greatly improved. Fragment analysis using fluorescent PCR primers and an automated sequencer is now widely employed. However, selection of targets for analysis has become controversial again. Jass et al. pointed out that instability in dinucleotide microsatellites is not identical to mononucleotide MSI, and that use of dinucleotide markers for detecting MSI-H is problematic.[40] Mononucleotide markers, such as BAT25 or BAT26, exhibit typical "jump"-like band shifts in MSI-H tumors. On the other hand, instability in dinucleotide microsatellites is observed mainly in MSI-L. The "working reference panel" recommended by NCI includes two dinucleotide markers, which appear to be adopted because of their sensitivity to MSI-L. In fact, molecular mechanisms causing repeat instability are not single. In addition to polymerase slippage and defective mismatch repair (MMR), erroneous proofreading in polymerase complexes and misalignment in the processes of double-stand break repair by gene conversion are also possible. These mechanisms may work differently on mono-, di-, and trinucleotide repeats. Clarifying the relationships between these molecular mechanisms and each category of repeat instability is of urgent necessity.

Frequency or Qualitative Change?

Tomlinson and colleagues[14] have concluded in their recent report that MSI-L occurs in most colorectal tumors, and that the difference between MSI-L and the microsatellite-stable phenotype is not qualitative but quantitative. In this report, no difference in clinicopathological features and molecular backgrounds has been found between MSI-L and microsatellite-stable tumors. Conversely, other studies have shown significant correlations between MSI-L and mutation in *K-ras* or *p53*,[12,15,16] which implies that MSI-L tumors form a unique entity. Although MSI-H tumors appear

to form specific clinicopathological and molecular entities, there seems to be a limit in discussing MSI merely from the frequency of changes in a given set of markers. As mentioned above, it may be also informative to note the qualitative aspects of MSI, since the three above-mentioned molecular mechanisms should work differently on repeat sequences with different unit lengths, and may lead to different modes of length changes in repeat sequences. Qualitative differences in dinucleotide MSI have been reported by Thibodeau et al.[2] and other investigators.[9,18]

MSI and MMR Gene Mutation — Sporadic Versus Hereditary Settings

In HNPCC individuals, deleterious mutations in major MMR genes are often found. On the other hand, in sporadic MSI-H colorectal tumors the generally accepted frequencies for mutations in the major MMR genes, *hMSH2* and *hMLH1*, are beneath 30%, and missense mutations with unknown pathogenic significance predominate. Instead, a role of epigenetic silencing of hMLH1 is regarded as more important. According to several reports, colorectal tumors that do not express hMLH1 comprise approximately 70% of all MSI$^+$ tumors.[47–51] However, it is not always easy to designate a loss of expression using immunohistochemistry, considering the quality of fixed tissue specimens, the reactivity of antibodies used, and other technical variables. In fact, methylation of the proximal region of *hMLH1* promoter and its extent correlate well with loss of hMLH1 expression.[109–111] However, the results in immunohistochemistry are not always parallel with those in methylation analyses.[112] These observations may suggest a possibility that MSI-H tumors arise via two mutually exclusive pathways. Indeed, Jass and colleagues have proposed that MSI-H tumors in these two settings form clinicopathologically different entities,[40,50,54] which may imply that additional and previously unrecognized molecular abnormalities may underlie the differential tumorigenesis. Nevertheless, MSI-H tumors exhibit a specific and uniform mode of instability, i.e., Type B MSI, either in the hereditary or the sporadic setting. The relationship between defective MMR and this form of MSI may be more complex than has been suspected.

Defective Mismatch Repair as a Source of Mutation

It remains controversial whether a state with an elevated mutation rate plays an important role in tumorigenesis. Loeb and colleagues[113,114] propose a state with an elevated mutation rate in tumorigenesis, and this state is now referred to as "mutator phenotype." On the other hand, Bodmer and colleagues[115,116] pointed out that tumor cells which harbor mutations in tumor suppressor genes or oncogenes can be selected merely by phenotypical advantage, without an elevated mutation rate. Spontaneous mutation rate on the genome is invariably controlled. Previous studies using *E. coli* mutators suggest that there are several cellular systems, the failure of which will lead to a significant increase in the mutation rate. MMR is also categorized in these systems, and in its mutator the spontaneous mutation rate is 100-fold higher than wild-type cells. Nevertheless, point mutations, particularly base substitutions, in acknowledged oncogenes or tumor suppressor genes are not found in MSI-H tumors that are regarded as MMR-deficient. Instead, only insertion/deletion mutations are observed in mononucleotide runs within the reading frame in several genes of a different variety, such as *TGFβRII*, *IGF2R*, *BAX*, *Caspase 5*, etc. It is widely accepted that CIN predominates in tumors which are not categorized as MSI-H, and that point mutations in oncogenes or tumor suppressor genes are frequent in the CIN pathway. Some recent reports have pointed out a connection between MSI-L and mutations in *p53* or *K-ras* genes.[12,15,16] Mutator phenotype may also underlie tumorigenesis in some MSI-L tumors.

Where do the mutations found in tumorigenic genes come from? In MSI$^+$ tumors, defective MMR is the most likely candidate for the source of mutations, However, there is a paradox, as discussed above. In tumors with stable microsatellites, such as squamous cell carcinoma in the esophagus,[103] non-small cell lung cancer,[117] and breast cancer,[118] the source of mutation is unknown. Based upon previous studies using *E. coli* mutators, it is known that disruption of other cellular components, particularly DNA repair enzymes counteracting oxidative DNA damage and replication polymerases, also leads to a marked increase in the spontaneous mutation rate. Abnormalities of these cellular functions in cancer are of particular interest. It appears essential to test these possibilities in various cancers for a better understanding of the mutator phenotype underlying tumorigenesis.

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Suppressed MKP-1 Is an Independent Predictor of Outcome in Patients with Hepatocellular Carcinoma

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Key Words
Hepatocellular carcinoma · Mitogen-activated protein kinases · MKP-1 · Tumor marker

Abstract
Objective: An increase in the activity of mitogen-activated protein kinases (MAPKs) has been correlated with a more malignant phenotype in several tumor models in vivo. This study was designed to clarify the expression of MKP-1 in surgically resected hepatocellular carcinoma (HCC). ***Methods:*** We reviewed the cases of 77 patients who had undergone initial liver resection for HCC without preoperative treatment. Immunohistochemical analysis of MKP-1 was performed on paraffin-embedded tissues. The correlation between MKP-1 expression and clinical outcome was investigated. ***Results:*** Tumor cells were immunohistochemically stained for MKP-1 expression, and the same levels as in normal hepatocytes were detected in 66 (85%) of 77 HCC patients, being decreased in 11 (15%) HCCs. Decreased MKP-1 expression significantly correlated with serum α-fetoprotein levels and tumor size ($p < 0.05$). The disease-free survival rates in MKP-1-negative and -positive patients were 0 and 31.0% at 5 years, respectively ($p < 0.01$). The survival rates after a surgical resection in MKP-1-negative and -positive patients were 18.2 and 65.5% at 5 years, respectively ($p < 0.01$). ***Conclusions:*** The MKP-1 expression in HCC was an independent prognostic factor for outcome in HCC patients. In the future, it will be useful to explore whether the phosphatase expression might account for the response to HCC treatments targeting at MAPK activation.

Introduction

Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine kinases with three main components: extracellular signal-regulated kinase (ERK), c-jun NH2-terminal kinase (JNK) and p38 [1, 2]. MAPK dephosphorylation is carried out by some phosphatases, similar to the dual specificity of MAPK phosphatases (MKPs), which simultaneously dephosphorylate both serine/threonine and tyrosine residues [3, 4]. The first member of this family which was characterized was MKP-1. It is controlled by an early response gene, which is transiently induced by mitogens and stress signals such as serum, cytokines, UV radiation, heat shock and hypoxia [5–9].

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The MAPK pathway has been shown to be very relevant in human carcinogenesis [10, 11]. The reported expression patterns of MKP-1 in carcinogenesis vary depending on the organ studied. Prostate, colon and bladder tumors have high MKP-1 expression in the early phases of carcinogenesis, but expression levels decrease at advanced stage [12, 13]. Besides, in prostate tumors, a correlation between an increased activation of ERK, JNK or both and MKP-1 overexpression has been described previously [14]. In other cancers, e.g. breast carcinomas, a high expression of MKP-1 in poorly differentiated or late disease stages has been shown [12].

In hepatocellular carcinoma (HCC) cell lines, increased apoptosis, inhibition of tumorigenicity and cell cycle control were induced by ERK or MEK inhibitors [15]. In HCC, expression of MAPK and Stat3 was raised compared to non-cancerous tissues [16]. ERK activation correlated positively with tumor size [17].

However, to date MKP-1 expression has not been studied in human HCC. Therefore, the aim of this study was to characterize the expression and localization of MKP-1. This study was performed to clarify the expression of MKP-1 in surgically resected HCC. In addition, we also analyzed the potential significance of these markers in the prognosis of HCC.

Patients and Methods

Patients

Seventy-seven patients, 58 men and 19 women, who underwent a first curative liver resection for treatment of HCC, were retrieved from the files of the Second Department of Surgery (Kyushu University Hospital in Japan) from January 1991 to February 2004. In all patients, HCC was confirmed histologically, based mainly on the examination of sections stained with hematoxylin and eosin. Patients ranged in age from 42 to 83 years, with an average age of 61.9 years.

Clinicopathological variables were defined according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer of the Liver Cancer Study Group of Japan [18]. Tumors were histologically diagnosed as well-differentiated HCC (n = 7), moderately differentiated HCC (n = 32) or poorly differentiated HCC (n = 38). Fibrous capsule formation was seen in 52 patients (81%), portal venous invasion was observed in 26 patients (41%), hepatic venous invasion was found in 6 patients (9%), and intrahepatic metastases were noted in 15 patients (23%). After surgical resection, HCC specimens from each patient were fixed in 10% buffered formalin, embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically.

Histologic Examination and Immunohistochemistry

The resected specimens were cut along the largest diameter and fixed with formalin. The entire cut surface was sectioned from the slice of the largest diameter and embedded in paraffin. The microscopic sections were stained with hematoxylin and eosin. We assessed tumor diameter, vascular invasion of cancer cells and histological grade.

Immunohistochemical studies were performed on adjacent deparaffinized sections using the peroxidase-labelled streptavidin-biotin technique using a Histofine SAB-PO kit (Nichirei, Tokyo, Japan) [19]. Sections (5 μm) were deparaffinized with xylene and dehydrated in ethanol. Then, endogenous peroxide activity was blocked by methanol containing 0.3% hydrogen peroxide for 30 min. After exposure to 10% non-immunized goat serum in PBS for 10 min, sections were treated at 4°C overnight with primary rabbit antibodies to MKP-1 (Santa Cruz Biotechnology, Santa Cruz, Calif., USA). The sections were then incubated with biotinylated anti-rabbit immunoglobulin serum for 30 min, followed by incubation with peroxidase-labelled streptavidin for 20 min at room temperature. Reaction products were visualized by diaminobenzidine tetrahydrochloride as chromogen. Finally, the sections were counterstained with hematoxylin. The normal hepatocyte was considered positive regarding the cutoff used for this molecular marker [20]. In normal hepatocytes, MKP-1 protein expression was detectable in all cases. The MKP-1 expression was recorded as the percentage of cancer cells showing cytoplasmic staining. The specimens of cancer cells examined which showed more than 30% MKP-1-positive cells were considered to have a positive response to MKP-1.

Statistical Analysis

All data were expressed as medians. We analyzed the association of each of the variables using Mann-Whitney's U test. The survival curves were generalized using the Kaplan-Meier method, and then compared using the log-rank test. Multivariate survival analysis was calculated according to Cox's proportional hazards model in a forward stepwise manner. A p value less than 0.05 was considered to be statistically significant [21].

Results

Expression of MKP-1 Protein in Clinical Samples

Immunohistochemical staining for MKP-1 expression was in all normal hepatocytes positive (fig. 1). In tumor tissues of 66 cases (85.7%), staining did not differ compared to normal hepatocytes, but 11 cases (14.3%) exhibited decreased cytoplasmic MKP-1 expression (fig. 1). Macrophages and endothelial cells were also stained. Generally, immunostaining was found in the cytoplasm.

Relationships between Immunohistochemical Results and Clinicopathological Factors

The patients with HCC were divided into MKP-1-negative (n = 11) and MKP-1-positive (n = 66) groups. Table 1 shows a comparison of the clinicopathological factors between MKP-1-positive and MKP-1-negative

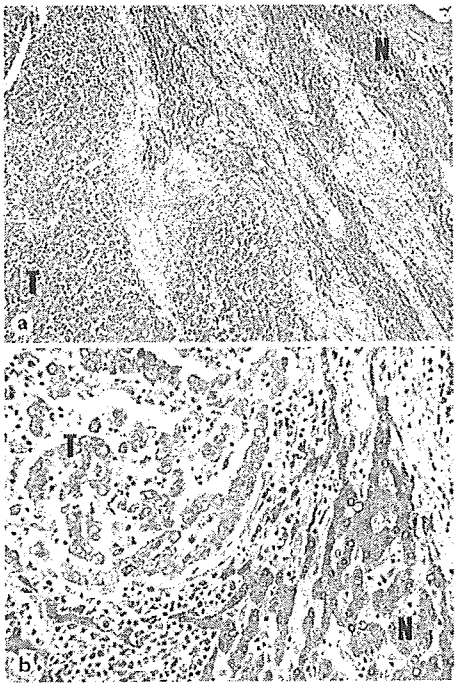

Fig. 1. Expression of MKP-1 in HCC. Normal hepatocytes (N) express MKP-1 in the cytoplasm and decreased expression in carcinoma cells. T = Tumor. **a** ×100. **b** ×400. Immunostaining (streptavidin-biotin-peroxidase staining).

Table 1. Relationship between MKP-1 expression and clinicopathological factors

Factors	MKP-1 positive (n = 66)	MKP-1 negative (n = 11)	p value
Gender, males/females	52/14	6/5	NS
Age, years[a]	62	63	NS
HBs-Ag positive, %	13 (20)	4 (36)	NS
HCV-Ab positive, %	46 (70)	6 (54)	NS
Albumin, g/dl[a]	3.8	3.8	NS
Total bilirubin, mg/dl[a]	0.8	0.7	NS
HPT, %[a]	70.0	70.4	NS
ICG-R15, %[a]	15.4	14.0	NS
AST, U/l[a]	52	48	NS
ALT, U/l[a]	61	56	NS
Plt, $\times 10^4/\mu l$[a]	12.0	12.3	NS
Child score, A/B/C	54/10/2	10/1/0	NS
AFP, ng/ml[a]	29	288	<0.05
PIVKA-2, mIU/l[a]	79	199	NS
Tumor size, cm[a]	3.5	5.6	<0.05
Intrahepatic metastases positive, %	23 (35)	4 (36)	NS
Portal vein invasion, %	28 (42)	6 (54)	NS
TNM stage	10/31/17/8	1/6/2/2	NS
Grade, well/moderately/poorly differentiated	7/27/32	0/5/6	NS

[a] Median.

NS = Nonsignificant; HPT = hepaplastin test; ICG -R15 = indocyanine green retention rate at 15 min; Plt = platelet; PIVKA-2 = protein induced by vitamin K absence or antagonist II.

tumors. MKP-1 expression correlated significantly with serum level of α-fetoprotein (AFP) and tumor size ($p < 0.05$) in univariate analysis. No association between phosphatase expression and clinicopathological variables, e.g. liver function, serum albumin, AST, ALT or ICG-R15 (indocyanine green retention rate at 15 min) and tumor factors, PIVKA-2 (protein induced by vitamin K absence or anagonist II), intrahepatic metastasis rates and portal vein invasion, or immunohistochemical patterns was observed.

Prognostic Value of MKP-1 in HCC

We also performed survival analysis. Kaplan-Meier plots of all patients with clinical follow-up whose tumors had either stained positively or negatively for MKP-1 were carried out. The survival analysis revealed a better outcome of patients with positive staining ($p < 0.01$; fig. 2). The disease-free survival rates after a surgical resection in patients with MKP-1-negative and -positive tumors were 31.8 and 77.6% at 1 year, and 0 and 31.0% at 5 years, respectively ($p < 0.01$). The survival rates after a surgical resection in MKP-1-negative and -positive patients was 45.5 and 91.9% at 1 year, and 18.2 and 65.5% at 5 years, respectively ($p < 0.01$ by log-rank test).

A multivariate Cox regression analysis revealed that negative MKP-1 expression ($p = 0.0037$) as well as TNM stage and serum AFP level were independent prognosticators in HCCs (table 2). The hazard ratio of death for negative staining was 3.357.

Discussion

HCC is the third most common malignant tumor in Japan, prognosis is relatively poor, and progression of disease is fast [22, 23]. We have described the suppression of MKP-1 in HCC patients and its relationship with clin-

ical outcome. High cytoplasmic expression of MKP-1 was also detected in normal hepatocytes, and an association between decreased cytoplasmic MKP-1 expression levels and decreased survival was found in HCC patients (fig. 2). Clinicopathologically, decreased MKP-1 expression in HCC was one of the independent prognosticators for the survival of a patient after hepatectomy. This finding partially parallels the expression patterns found in several other human cancers [12].

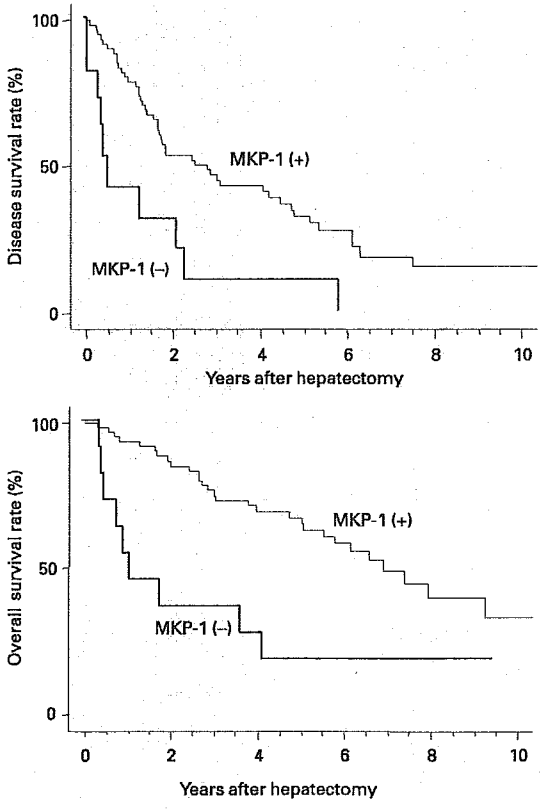

Fig. 2. Survival rates with respect to MKP-1 expression in HCC patients. $p < 0.01$ for MKP-1-positive (n = 66) vs. MKP-1-negative (n = 11) patients.

While MKP-1 was overexpressed in the early phases of prostate, colon and bladder carcinogenesis in clinical specimens, there is a steady decrease in expression of MKP-1 at late stages of disease and in metastases [12], and decreased expression was correlated with a poor prognosis [24]. The expression of MKP-1 decreases from normal epithelium to early-stage cancers and essentially disappears in advanced cancer [13, 14, 24].

The downregulation of MKP-1 occurs predominantly as the tumor progresses from localized to advanced disease. This stage in disease progression corresponds with the important clinical transition from an essentially curable HCC to incurable HCC. However, the mechanism by which MKP-1 expression decreases remains to be determined [14].

In addition, MAPK activation has previously been studied in liver tumor cells in vitro [25–27], but MKP-1 activation was not analyzed in detail. Our present results in HCC were similar to other research data published on tumors showing sustained ERK overexpression in a large part of the tumor [17], and the possibility for the activation of MAPK. In a rat model of hepatocarcinogenesis, mRNA levels of MKP-1 were increased in primary hepatomas but decreased in rat ascites hepatomas, malignant phenotypes of hepatomas, compared with a normal liver [28]. This study also suggests that the decreased expression of MKP-1 may correlate to a worse phenotype of HCC.

In this study, decreased MKP-1 expression correlated with higher AFP levels. Serum AFP is often elevated in HCC. A high AFP level is correlated with late stage, early recurrence and poor prognosis in hepatocellular carcinoma [29, 30]. Previously, we have also reported that high serum AFP was an independent prognosticator after curative resection for HCC [31].

Table 2. Significant variables determined by Cox's proportional hazard model

Factors	Coefficient	SE	Coefficient/SE	p value	Hazard ratio
MKP-1	1.211	0.417	2.905	0.0037	3.357
AFP	0.760	0.368	2.066	0.0388	0.468
Stage	1.395	0.706	1.976	0.0482	0.248

MKP-1 = Positive or negative in tumor tissue of HCC; AFP = serum AFP level; Stage = TNM stage I, II, III or IV.

In an in vitro model, using the AFP-producing and AFP-nonproducing clones of the McA-RH 7777 rat hepatoma cell line, Khamzina and Borgeat [32] revealed that the AFP-producing phenotype is clearly associated with enhanced tyrosine phosphorylation. The tyrosine phosphorylation of PI3K was observed in AFP-producing clones, whereas the same proteins were not phosphorylated in AFP-nonproducing clones. The growth rate in cells of AFP-producing clones was higher than that measured in cells of AFP-nonproducing clones, and inhibition of PI3K blocked the stimulated DNA synthesis only in cells of AFP-producing clones. Therefore, we hypothesized that decreased MKP-1 and high AFP levels were both associated with phosho-PI3K, but the mechanism resulting in decreased MKP-1 and increased AFP levels remains to be elucidated. This can only be confirmed by future studies.

The greater tumor size is one of the risk factors for recurrence after HCC resection [33–35]. With regard to tumors, the HIF-1α expression was significantly associated with larger tumor size [36]. With respect to HepG2, suppression of MKP-1 enhances HIF-1 transactivation [37]. Consequently, we investigated whether HIF-1α protein expression correlated with MKP-1 downregulation using an immunohistochemical technique. However, no relationship was seen between MKP-1 and HIF-1α expression (data not shown). Unfortunately, because of insufficient staining using the TUNEL method, we were unable to investigate the relationship between MKP-1 expression and the apoptosis ratio in HCC tissues. However, in this study, decreased expression of MKP-1 correlated with a larger tumor size. Low-oxygen regions (hypoxia) and necrosis are common features of solid tumors [38–40]. Hypoxia can induce resistance to apoptosis through the MAPK signaling pathway [41]. The mechanisms by which MKP-1 alters cell growth are less clear but may involve altered expression levels of proteins in those processes through attenuation of MAPK signaling.

In summary, we have described MKP-1 suppression in HCC patients and its relationship with clinical outcome. It will be useful in the future to explore whether the phosphatase expression might account for the response of HCC treatments targeting MAPK activation [42]. The correlation between downregulation of MKP-1 and an increased risk of HCC recurrence after hepatectomy, independent of other pathological or clinical parameters, support the potential use of MKP-1 as a prognostic marker in HCC, a possibility that needs to be validated in a larger clinical study.

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Surgical outcome of patients with hepatocellular carcinoma originating in the caudate lobe

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Abstract

Background: Hepatocellular carcinoma (HCC) originating in the caudate lobe is rare, and the treatment for this type of carcinoma is difficult because of its unique anatomic location.

Methods: This retrospective study assessed the surgical outcome of patients with caudate lobe HCC. There were 20 cases of HCC originating in the caudate lobe among 435 patients with primary HCC who underwent hepatic resection in our department from 1990 to 2002. The caudate tumors were located in the Spiegel lobe in 3 patients, the paracaval portion in 15 patients, and the caudate process in 2 patients. Surgical procedures consisted of limited resection of the caudate lobe in 6 patients and extended caudate lobectomy in 14 patients. Recurrence was recognized in 12 patients, including 8 patients with multiple intrahepatic recurrences, 1 with peritoneal dissemination, and 1 with lymph node metastasis.

Results: There was no significant difference in postoperative survival rate between patients who underwent limited resection of the caudate lobe and those who underwent extended caudate lobectomy. Compared with 415 patients with HCC originating in other locations, the 20 patients with caudate lobe HCC showed significantly more intraoperative blood loss ($P < .05$), longer operation time ($P < .0001$), and more postoperative complications ($P < .005$). Intrahepatic recurrence was more frequent in the caudate lobe HCC compared with HCC originating in other locations (40% vs 17.6%; $P < .05$). There was a significantly poor survival rate in the postoperative patients with caudate HCC (25.9% vs 54.1% for five-year survival; $P = .01$). Intrahepatic multiple recurrences were frequently recognized in the patients with caudate lobe HCC, indicating no significance for extended caudate lobectomy.

Conclusions: Because of the relatively poor prognosis in patients with caudate lobe HCC, adjuvant therapy combined with surgical operation should be considered.

Keywords: Caudate lobe; Hepatocellular carcinoma; Prognosis; Recurrence

Hepatocellular carcinoma (HCC) arising from the caudate lobe has been reported to be relatively rare [1]. Anatomically, the caudate lobe can be divided into 3 parts: the left Spiegel lobe, the right caudate process, and the paracaval portion. Because intrahepatic and extrahepatic metastases are seen earlier than metastases from HCC in other parts of the liver [2], we previously reported the difficulties experienced in the surgical treatment of caudate lobe HCC [3] as well as innovative treatment in the use of isolated caudate lobectomy [4]. Resection of the caudate lobe is associated with considerable technical difficulty and is challenging for hepatic surgeons [5–8]. However, the surgical outcome of the patients with caudate lobe HCC has not been well established [2,9,10]. We retrospectively reviewed 20 patients who underwent caudate lobectomy to evaluate surgical competence and results of different treatments.

Patients and Methods

From April 1990 to December 2002, hepatic resection for primary HCC originating in the caudate lobe was performed in 20 patients in the Department of Surgery and Science at Kyushu University Hospital. Seventeen patients

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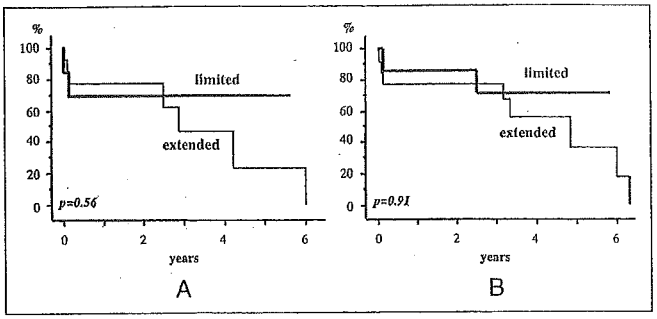

Fig. 1. Cumulative survival curves in patients who underwent limited resection for caudate lobe HCC (thick line) and those who underwent extended caudate lobectomy (thin line). (**A**) Disease-free survival rate. The statistical difference is $P = .56$ (log-rank test). (**B**) Survival rate. The statistical difference is $P = .91$ (log-rank test).

were male, and 3 were female; mean age was 62.8 ± 7.1 years (range 46 to 73). Preoperative evaluation included hepatitis B surface antigens, hepatitis C viral antibodies, conventional liver function test (including Child-Pugh grading), aspartate amino transferase, alanine amino transferase, gamma-globulin, indocyanine green R15, hepaplastin, platelet count, and imaging studies. Assays for hepatitis B surface antigens were positive in 3 patients (15%), and those for hepatitis C viral antibodies were positive in 14 patients (70%). All of the patients had bilirubin <1.5 mg/dL and ICG15 <30%. Fourteen patients were Child-Pugh A status, 5 were B status, and 1 was C status. Tumors were detected before surgery by computed axial tomography scanning, ultrasonography, and angiography. Additionally, intraoperative ultrasonography was used to stage patients and detect additional tumors. No metastasis to lymph nodes or other organs was recognized at surgery. The caudate tumors were located in the Spiegel lobe in 3, the paracaval portion in 15, and the caudate process in 2 patients. According to International Union Against Cancer (UICC)-TNM staging [11], 1 patient was stage I, 10 patients were stage II, 8 patients were stage III, and 1 patient was stage IV. In addition, 415 patients who underwent hepatic resection in our department for primary HCC originating in other locations during the same period were analyzed to enable comparison with the 20 patients who had caudate lobe HCC. Assays for hepatitis B surface antigens for these patients were positive in 84 (20.2%) and for hepatitis C viral antibodies were positive in 291 (70.1%). Sixty-four patients were UICC-TNM stage I, 198 patients were stage II, 113 patients were stage III, and 40 patients were stage IV. There was no significant difference in patient background between the caudate lobe HCC group and the non–caudate lobe HCC group (data not shown).

Postoperative follow-up of patients undergoing hepatic resection included contrast-enhanced helical CAT scanning of the liver within 14 days and then every 3 months thereafter. CAT results were analyzed for local control and development of new intrahepatic and extrahepatic diseases. Tumor markers were followed-up every month. Cumulative disease-free survival and patient survival rates were determined using the Kaplan-Meier method.

Results

Twenty patients with primary HCC originating in the caudate lobe underwent hepatic resection. The surgical margin was tumor negative (>5 mm) in all of the 20 cases. Surgical procedures were classified as limited resection within the caudate lobe for 6 patients and extended resection over the caudate lobe for 14 patients. There was no statistical difference in tumor staging between the 2 groups ($P = .38$): The limited resection group included 4 patients in UICC-TNM stage II and 1 patient in stage III, whereas the extended resection group included 1 patient in stage I, 5 patients in stage II, 7 patients in stage III, and 1 patient in stage IV. Limited resection of the caudate lobe included total caudate lobectomy in 1 patient, sole Spiegel lobectomy in 3 patients, resection of the sole paracaval portion in 1 patient, and resection of the sole caudate process in 1 patient. Extended caudate lobectomy included caudate lobectomy with extended posterior segmentectomy in 3 patients, Spiegel lobectomy with extended posterior segmentectomy in 1 patient, paracaval portion resection with right lobectomy in 2 patients, paracaval portion resection with extended posterior segmentectomy in 1 patient, paracaval portion resection with posterior segmentectomy in 2 patients, paracaval portion resection with anterior segmentectomy in 1 patient, and paracaval portion resection with subsegmentectomy in 4 patients.

Twelve patients experienced postoperative complications that consisted of uncontrollable ascites and pleural effusion in 6 patients, bile leakage in 3 patients, jaundice in 1 patient, intraperitoneal abscess in 1 patient, and acute dilatation of the stomach in 1 patient. Recurrence was noted in 12 patients. Ten patients were identified as having intrahepatic recurrence, 1 patient as having peritoneal dissemi-

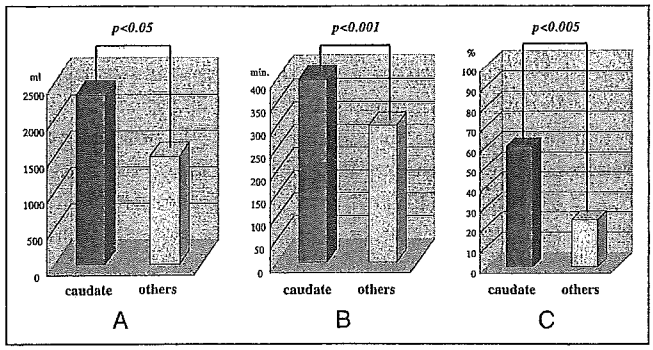

Fig. 2. Outcome of patients with caudate lobe HCC (n = 20) compared with those having HCC in other locations (n = 415). (**A**) Intraoperative blood loss. The statistical difference is $P < .05$ (Student t test). (**B**) Operation time. The statistical difference is $P < .001$ (Student t test). (**C**) Ratio of postoperative complications. The statistical difference is $P < .005$ (Fisher's Exact test).

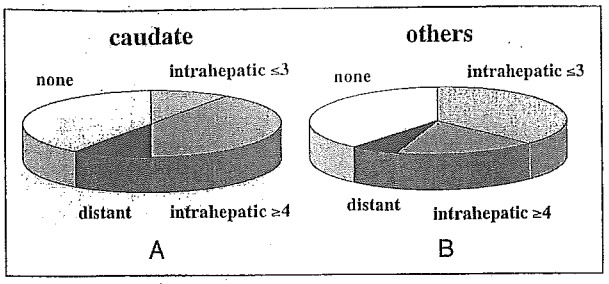

Fig. 3. Recurrence pattern of the patients with caudate lobe HCC (**A**) and those with HCC in other locations (**B**). The divisions indicate intrahepatic recurrence with <3 tumors (light gray), intrahepatic recurrence with >4 tumors (dark gray), distant metastasis (black), and no recurrence (white). In the caudate group, 2 of 20 patients with intrahepatic recurrence had <3 tumors (10%); 8 patients with intrahepatic recurrence had >4 tumors (40%); 2 patients had distant metastasis (10%); and 8 patients had no recurrence (40%). In the other-locations group, 156 of 415 patients with intrahepatic recurrence had <3 tumors (37.6%); 73 patients with intrahepatic recurrence had >4 tumors (17.6%); 26 patients had distant metastasis (6.3%); and 160 patients had no recurrence (38.6%).

nation, and 1 patient as having mph node metastasis. The postoperative disease-free survival rate as well as the patient survival rate was analyzed, which enabled comparison between limited caudate lobectomy (n = 6 patients) and extended caudate lobectomy (n = 14 patients). There was no significant difference in survival rates between the 2 groups (Fig. 1).

Outcome of the 20 patients with caudate lobe HCC was compared with that of the 415 patients with primary HCC originating in other locations. The caudate lobe group showed significantly more intraoperative blood loss (caudate = 2332.5 ± 1695.5 mL vs others = 1493.3 ± 1500.2 mL; $P < .05$), longer operation time (399.5 ± 123.5 vs 298.8 ± 103.6 minutes; $P < .0001$), and more postoperative complications (60% vs 26.5%; $P < .005$) as shown in Figure 2. The surgical margin was tumor negative (>5 mm) in all patients. The evidence of either portal or hepatic vein invasion was detected in 6 patients (30%) in the caudate lobe group and 156 patients (37.6%) in the other-locations group (no significant difference). The recurrence pattern of the intrahepatic tumors differed between the groups: Multiple recurrence >4 tumors occurred in 8 patients in the caudate group (40% vs 17.6% [73 of 415 patients]), and only 2 patients had <3 tumors in the caudate group (10% vs 37.6% [156 of 415 patients]; $P < .5$; Fig. 3). It is worth noting that there was a significantly poor survival rate in the postoperative patients with caudate lobe HCC (25.9% for 5-year survival) compared with patients having HCC in other locations (54.1% for 5-year survival; $P = .01$; Fig. 4), despite no significant difference in disease-free survival rates (Fig. 4). There was significant relationship between poor survival rate and vascular invasion in the patients with HCC in other locations ($P = .04$) but not in the patients with caudate lobe HCC ($P = .46$) as shown in Figure 5. Stratification according to UICC-TNM staging revealed a significantly poor survival rate in the patients with caudate lobe HCC in stage II (21.4% for 5-year survival) compared with patients having HCC in other locations (57.4% for 5-year survival; $P = .03$; Fig. 6) despite no significant difference in stage III patients ($P = .34$; Fig. 6).

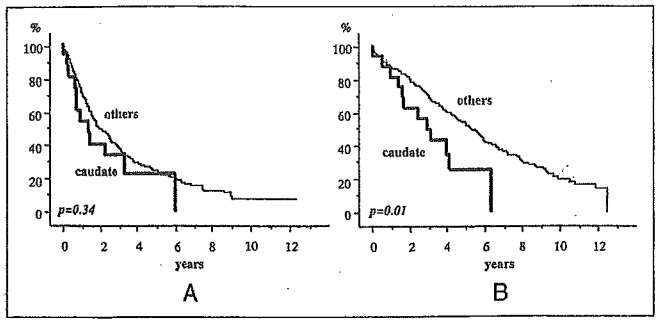

Fig. 4. Cumulative survival curves in patients with caudate lobe HCC (thick line) and those with HCC in other locations (**A**) Disease-free survival rate. The statistical difference is $P = .34$ (log-rank test). (**B**) Survival rate. The statistical difference is $P = .01$ (log-rank test).

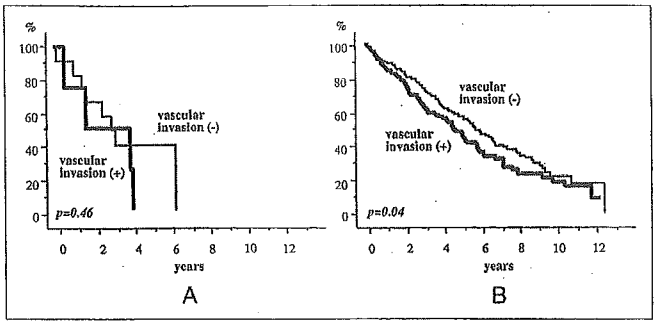

Fig. 5. Cumulative survival curves in patients undergoing resection for HCC with (thick line) or without (thin line) vascular invasion. (**A**) Actuarial survival rate of patients with caudate lobe HCC. The statistical difference is $P = .46$ (log-rank test). (**B**) Actuarial survival rate of patients with HCC in other locations. The statistical difference is $P = .04$ (log-rank test).

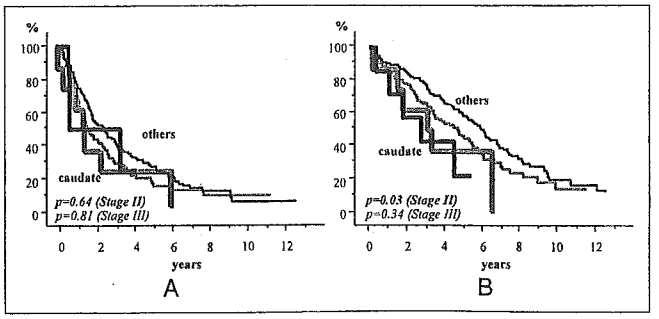

Fig. 6. Cumulative survival curves in patients with caudate lobe HCC (thick line) compared with those having HCC in other locations (thin line) in UICC-TNM stage II (black) or stage III (gray). (**A**) Disease-free survival rate. The statistical difference is not significant in either stage II ($P = .64$; log-rank test) or stage III ($P = .81$; log-rank test). (**B**) Survival rate. The statistical difference is significant in stage II ($P = .03$; log-rank test) but not in stage III ($P = .34$; log-rank test) patients.

Comments

The caudate lobe is generally divided into 3 regions–the left Spiegel lobe, the right process portion, and the paracaval portion–according to Kumon's classification [1]. The Spiegel lobe is located below the lesser omentum and to the left of the intrahepatic inferior vena cava (IVC). The paracaval portion is in front of the intrahepatic IVC, just to the right of the Spiegel lobe, and is surrounded by the right and middle hepatic veins. The caudate process is a tongue-like projection between the IVC and the adjacent portal vein, just to the right of the paracaval portion. Kumon studied the anatomy of the paracaval portion and concluded that this portion is the liver parenchyma ventral to the hepatic IVC, between the Spiegel lobe and the right lobe, adjacent to the middle hepatic vein ventrally [1]. Couinaud also confirmed the existence of a paracaval portion in the caudate lobe and classified this portion separately as segment IX [12]. Couinaud postulated that the paracaval portion, or the S9, is not defined by its supplying portal vein branch but by its "dorsal location" in the liver [13]. The caudate lobe has 5 surfaces: the dorsal, the left and hilar-free surfaces, and the right and ventral border planes. The paracaval portion corresponds to the dorsally located parenchyma in front of the IVC. All three parts are supplied by primary branches originating from the left and right portal veins as well as the hilar bifurcation area [14,15]. It is noteworthy that the hilar bifurcation branch not only supplies the paracaval portion, it also extends its territory to the left Spiegel lobe (29%) and the right caudate process (21%) [15]. This complicated blood flow of vessels might mediate metastasis of HCC cells to the entire liver [2,3].

We studied 20 patients with HCC originating in the caudate lobe who underwent hepatic resection. Recurrence was noted in 12 patients, 10 of whom had intrahepatic recurrence and 2 had extrahepatic recurrence (peritoneal dissemination and lymph node metastasis). Of note, 8 of 10 patients with intrahepatic recurrence had >4 tumors. Such frequency of extrahepatic recurrence possibly indicates low significance of extended hepatectomy exceeding the caudate lobe. We previously proposed that for cirrhotic patients with primary HCC, the mortality and survival rates in patients undergoing "limited" hepatic resection were superior to those in patients undergoing standard major hepatic resection [16]. Indeed, there was no significant difference in postoperative survival rates between patients who underwent limited caudate lobectomy and those who underwent extended caudate lobectomy (Fig. 1).

Recent advances in surgical techniques, such as isolated caudate lobectomy as reported by us and others [4,17–19], might improve the outcome of patients with caudate lobe HCC. Our data certainly demonstrated that there was no significant difference in disease-free survival rates between patients with caudate lobe HCC and those with HCC in other locations (Fig. 4). In contrast, there were significantly poor prognoses concerning patient survival. As shown in Figure 4, a 25.9% 5-year survival rate was noted for patients with caudate lobe HCC, but 5-year survival for patients with HCC in the other locations was 54.1% ($P = .01$). The poor survival rate was noted especially in patients with caudate lobe HCC in stage II (Fig. 6). The discrepancy between patient survival rates and disease-free survival rates might be associated with the frequency of multiple or extrahepatic recurrence after the caudate lobectomy (Fig. 3).

Although several methods for treatment for HCC—such as percutaneous ethanol injection, microwave coagulation, radiofrequency ablation, and transarterial embolization—have been developed they may have been thought to be technically difficult and unsafe because of the HCC's unique anatomic location [1,13]. These conventional methods are not effective for the treatment of caudate lobe tumors because there are often multiple feeding arteries, and the tumor is adjacent to the IVC and lesser omentum [14,15]. In our study, the frequency of vascular invasion was not different between patients with caudate lobe HCC and those with non–caudate lobe HCC. Interestingly, vascular invasion affected the prognosis of the non–caudate lobe HCC group but not that of the caudate lobe HCC group (Fig. 5). Multiple or extrahepatic recurrence is so common that all patients with caudate HCC are particularly well suited for enrollment in prospective randomized clinical trials investigating the utility of adjuvant therapy.

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Genetic mutual relationship between PTEN and p53 in gastric cancer

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Abstract

Both PTEN (encoding phosphate and tensin homologue) and p53 are known as cancer suppressor genes, and they are assumed that their gene mutations and loss of heterozygosity (LOH) occur frequently in various types of carcinoma. In the present study, we investigated both the p53 mutation and LOH of PTEN in 113 gastric cancer patients. We observed the LOH of PTEN in 11.1% of the patients with normal p53s and 46.2% of the patients with p53 gene mutations. The result that LOH of PTEN was frequently observed in the cases with p53 gene mutations and other data in this study suggested that both PTEN and p53 have complimentary roles in gastric carcinoma development.

Keywords: PTEN; p53; Gastric cancer; Loss of heterozygosity

1. Introduction

The encoding phosphate and tensin homologue (PTEN) gene, a tumor suppressor candidate, is located on chromosome 10q23 and has an extensive homology with the cytoskeletal proteins auxilin and tensin [1,2]. PTEN mutations have been frequently observed in various neoplasms, including glioblastoma, melanoma, prostate cancer, and breast cancer [1–7]. In glioblastoma, melanoma, and prostate cancer, PTEN mutations and allelic deletions are observed during the late stages, while in thyroid and endometrial cancers, PTEN mutation alterations are found during the early stages and include endometrial hyperplasia and benign thyroid tumors [3–6,8,9].

PTEN encodes an enzyme with phosphatase activity towards the acidic protein substrates and the lipid second messenger, phosphatidylinositol-3,4,5-triphosphate (PIP3) [10]. The phosphatase activity of PTEN is crucial in controlling the phosphatidylinositol-3

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(PI-3) kinase signal transduction pathway and in the activation of the protein kinase B (PKB/Akt) proto-oncogene. This indicates that PTEN exerts its tumor-suppressor function by negatively regulating the anitapoptotic PI3-kinase/AKT signaling pathway [11,12].

The p53 tumor suppressor gene is the most commonly mutated gene in various kinds of cancers [13]. A short-lived and non-abundant protein in normal cells, it plays a major role in regulating the response of mammalian cells to stresses and damage. This is partly done through the transcriptional activation of genes involved in cell cycle control, DNA repair, senescence, angiogenesis, and apoptosis [14,15]. Research [16] has discovered that when these functions are disrupted, a cell moves toward cancer development. The p53 mutation and allelic loss were found in various kinds of cancers and were considered to be tumor suppressor genes since the mouse without the p53 gene develops tumors with high frequency [16].

The absence of functional PTEN leads to the loss of p53 activity and the inability of cancer cells to respond to a DNA damaging agent in an apoptotic manner [15]. It has been shown that the human PTEN promoter contains a p53 binding site and that p53 induces a transcription of the PTEN gene and elevates the cellular level of the PTEN protein [17]. Genetic interactions between PTEN and p53 in radiation-induced lymphoma development were recently reported. The double p53+/− PTEN+/− mice were equivalent to p53 null mice in their radiation sensitivities for tumor development [18]. These results suggest that PTEN and p53 are tumor suppressors that mutually form a network.

Although gastric cancer is one of the leading causes of cancer-related deaths in Japan [19], the pathogenesis and progression of it are not yet clearly understood. Previous studies have shown that approximately 10–50% of gastric adenocarcinomas exhibit a loss of 10q or 17q in which PTEN and p53 are, respectively, located [20–25]. Although mutational alteration in gastric cancer is rarely observed in PTEN, the alteration of p53 is frequently observed [21], [26,27]. To determine whether or not the mutational inactivation of p53 and the allelic loss of PTEN are implicated in gastric tumorigenesis, we examined the LOH of PTEN and p53 mutations in 113 gastric cancer patients.

2. Patients and methods

2.1. Tissue samples

Pairs of primary gastric carcinoma tissue and corresponding normal mucosa were obtained from 113 patients who underwent surgery in the Department of Surgery II at Kyushu University Hospital from the years 1996–2000. Informed consents were obtained from all of the patients beforehand. In all of the cases, the histopathological type of tumor was adenocarcinoma. Cancer tissues and well-separated normal gastric mucosa obtained by gastrectomy were immediately snap-frozen and kept in liquid nitrogen. Genomic DNA was prepared by proteinase K digestion and phenol/chloroform extraction, which was followed by ethanol precipitation.

2.2. DNA sequencing

The base sequence was determined from Exon 4 to Exon 8 of p53 using a PCR direct sequence. The PCR product of p53 was purified with a microcon-100 microconcentrator (Amicon, Inc., Beverly, MA, USA). The direct sequencing of PCR products was performed using the ABI Prism Big dye terminator cycle sequencing ready reaction kit (Perkin-Elmer Corp.). The cycle sequence product was electrophoresed and analyzed on the Applied Biosystems model 311 genetic analyzer (Perkin-Elmer Corp.).

2.3. LOH analysis

LOH was analyzed using a DNA sequencer with microsatellite markers. The oligonucleotide primers for D10S796 and D17S1765 were synthesized and purified with HPLC. Any case with a peak value of one gene locus diminishing more than 30% in the carcinoma was judged as having LOH. The PCR reactions and running conditions with Perkin-Elmer Genetic Analyzer 310 (Norwalk, CT, USA) were previously described [20,21].

2.4. Statistical method

The statistical significance was determined using the Student's *t*-test, the Chi-square test with Yates's correlation factor. The difference was considered