gery are shown in **Table 3**. On the basis of previous reports (4), 60pg/ml was chosen as the cutoff. Fungi were isolated in cultures from the specimens in only 4 out of 16 patients. Twelve patients were given antifungal agents (FLCZ to 6, MCFG to 6) when the serum levels of ß-D glucan were above high values regardless of the isolation of fungi. Four deceased patients showed high serum levels of ß-D glucan at relatively late POD (23, 25, 12, and 39, respectively) and they were all complicated with bacterial infections.

DISCUSSION

In a clinical setting, distinguishing between fungal colonization and infection is often difficult because non-sterile sites such as the pharynx or nasal cavity frequently harbor fungi without evidence of inflammation or invasion (5). Generally, in order not to delay therapy, we begin administration of antifungal agents if fungus is isolated in cultures from specimens normally considered sterile, as immunosuppressive conditions easily lead to fungal colonization overgrowth and infection. Historically, although *C. albicans* has accounted for more than 80% of isolated *Candida* species in liver transplant recipients, the incidence of FLCZ-resistant *non-albicans Candida* increased after the late 1990s partially due to widespread use of FLCZ (6). In our assessed period, the emergent rate of *C. albicans* was only 37.5% and we chose MCFG for treatment after 2004.

We assessed some known factors that are associated with an increased risk of developing fungal infection (**Table 2**). In the present study, multivariate analysis indicated that only fungal carriage is associated with fungal isolation after surgery. One reason for the dissociation from previous reports probably originated from our sample extraction; namely, we assessed fungus-identified patients as including both colonization and infection.

Early identification and treatment should be quite essential but sensitivity of surveillance culture is not sufficient (8). Obayashi *et al.* have reported that the sensitivity of blood culture for the detection of fungal infection is only 8.3% (4). In addition, identification of fungi can take days to weeks (9). Measurement of ß-D Glucan, which is derived from fungal cell walls, has emerged as a rapid adjunct diagnostic strategy for invasive fungal infection, especially in Japan. Recently, this test was included as one of the microbiological criteria for probable invasive fungal disease in the Consensus Revised Definitions Draft VI produced by the joint committee of the European Organization for Research and Treatment of Cancer and the United States Mycology Study Group (10). Forty-three (71.7%) patients showed serum levels of ß-D glucan above 20pg/ml, which is the manufacturer's recommended cutoff. Autopsy study showed the sensitivity and specificity of the assay to be 85.4% and 95.2%, respectively, at a cutoff value of 60pg/ml, with the same commercial kit as ours (4). Based on this report, the patients who showed serum levels of ß-D glucan at greater than 60pg/ml were chosen in this study, and we then evaluated their characteristics (**Table 3**). Serum ß-D glucan can show a false-positive by the influence of blood product (11), dialysis membrane (12), and use of cotton gauze during surgery (13), and intra- or immediately after operation, many recipients can be affected by these products. Additionally, one of the major sites of eliminating ß-D glucan is Kupffer cells in the liver (14), whose phagocytic function is impaired immediately after liver transplantation (15). Less ß-D glucan may be eliminated from the blood stream, and it is likely to show a false-positive at early time points after surgery. Recipients showing positive serum levels of ß-D glucan soon after surgery with no other symptoms or findings, may not need to be given antifungal agents under a strict follow-up. If recipients are suspected of having fungal infection with high serum levels of ß-D glucan or any other findings, however, not only control of fungal infection but also bacterial infection is quite important.

Deceased donors are generally in the ICU, on mechanical ventilation, and receiving antibiotics and/or corticosteroids, and these clinical circumstances may allow fungi to colonize in their oropharyngeal and respiratory tract. In contrast, donors are basically healthy in LDLT and there should be fewer chances to have fungal colonization. In addition, most LDLTs are carried out electively and it is possible to make a full investigation of infections. Recently, Kawagishi, *et al.* have reported that 8.3% of recipients of LDLT suffer from definitive or probable invasive fungal infection in a single institute (3). In order to compare characteristics of fungal infection between LDLT and DDLT, further investigations are needed in the future.

In conclusion, all fungi that were isolated after LDLT in our institute were *Candida species*. Preoperative fungal carriage was associated with fungal isolation after LDLT. If fungal infection is suspected, antifungal therapy should be carried out concomitantly with antibacterial management.

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BRIEF ARTICLE

mPGES-1 expression in non-cancerous liver tissue impacts on postoperative recurrence of HCC

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Abstract

AIM: To investigate whether microsomal prostaglandin E synthase-1 (mPGES-1) expression in hepatocellular carcinoma (HCC) and in non-cancerous liver affects HCC prognosis after hepatectomy.

METHODS: The relationship between patient clinical profiles, tumor factors, surgical determinants, and mPGES-1 expression and the recurrence-free survival rate were examined in 64 patients who underwent curative hepatectomy between March 2003 and December 2006.

RESULTS: The scores for mPGES-1 expression were higher in well differentiated and moderately differentiated HCC tissues than in poorly differentiated HCC tissues (well differentiated, 5.1 ± 2.7; moderately differentiated, 5.1 ± 1.7; poorly differentiated, 3.0 ± 1.8). In non-cancerous liver tissues, the mPGES-1 levels were higher in injured liver tissues than in normal tissues. Cirrhotic livers had higher mPGES-1 levels than livers with chronic hepatitis (normal livers, 3.3 ± 0.7; chronic hepatitic livers, 5.4 ± 1.9; cirrhotic livers, 6.4 ± 1.6). A univariate analysis revealed that the recurrence-free survival rate was significantly lower in patients with vascular invasion, a higher mPGES-1 level in non-cancerous liver tissue, a larger tumor diameter (≥ 5 cm), and a lower serum albumin level (≤ 3.7 g/dL). The mPGES-1 expression in HCC tissues did not correlate well with postoperative recurrence. A multivariate analysis demonstrated that the presence of vascular invasion and higher mPGES-1 levels were statistically significant independent predictors for early postoperative recurrence of HCC.

CONCLUSION: Increased mPGES-1 expression in non-cancerous liver tissues is closely associated with the early recurrence of HCC after curative resection.

Key words: Curative resection; Hepatocellular carcinoma; Microsomal prostaglandin E synthase-1; Non-cancerous liver tissue; Recurrence-free survival

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a common cause of cancer death worldwide[1,2]. Hepatectomy is one of the best treatment modalities for HCC. Recent advances in surgical techniques and perioperative management have led to improved survival after curative resection. However, the rates of postoperative recurrence remain high (60%-80%)[3], and such recurrences can originate from intrahepatic metastases of the primary HCC and from the multicentric occurrence of new tumors[4]. With regard to the latter, many studies have reported a significant association between HCC development and underlying liver disease[5,6]. Therefore, HCC tumor factors as well as the underlying hepatic status should be carefully examined to predict tumor recurrence after curative resection and to choose optimal treatments.

A variety of malignant tumors in many visceral sites have appeared after chronic inflammation[7]. Clinical and biochemical evidence suggests that prostaglandin E_2 (PGE_2) produced at inflammation sites and its receptors play an important role in the development of malignant tumors, including HCC and other cancers[8,9]. The biosynthesis of PGE_2 from arachidonic acid requires two enzymatic activities that include cyclooxygenase (COX) and prostaglandin E synthase (PGES), which is the terminal enzyme for PGE_2 biosynthesis. Three PGES isoforms have been identified, including microsomal PGES-1 (mPGES-1), mPGES-2, and cytosolic PGES[10,11]. In particular, mPGES-1, an enzyme induced by pro-inflammatory stimuli, has received much attention[8,11]. Previous studies have indicated that mPGES-1 overexpression was associated with various types of cancer, including HCC[12,13]. Therefore, mPGES-1 may play an important role in HCC recurrence in the remnant liver tissue after curative resection for HCC.

The aim of the present study was to clarify whether mPGES-1 expression in HCC and non-cancerous liver tissues affects the clinical course of HCC patients undergoing curative resection.

MATERIALS AND METHODS

Patients and follow-up

Sixty-four consecutive patients (42 males and 22 females) underwent curative liver resection for HCC at the Division of Surgery, National Hospital Organization, Nagasaki Medical Center, between March 2003 and December 2006. In all cases, the diagnosis of HCC was confirmed by pathological examination of the resected specimens.

The inclusion criteria for the study were as follows: (1) the absence of extrahepatic metastasis; (2) curative resection defined as histological evidence of the complete removal of HCC tumors; and (3) no additional therapies or multi-modality treatment for HCC until the development of recurrence. Written informed consent was obtained from all the patients. They were regularly followed up at our outpatient clinic and were prospectively monitored for disease recurrence by serum levels of α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), and ultrasonography or computed tomography every 3 mo. Suspected intra-hepatic recurrence was confirmed by hepatic angiography, and if necessary, by percutaneous needle biopsy. The follow-up period was at least 12 mo or until death in patients who died within 12 mo of their operation. The study was conducted in accordance with the Helsinki Declaration and the guidelines issued by the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Ethics Committee at National Hospital Organization, Nagasaki Medical Center.

Tissue samples

HCC tissues and non-cancerous liver tissues from the opposite liver lobe in which HCC developed were obtained. The tissues were frozen in liquid nitrogen and stored at -80℃ until use. For immunohistochemical analysis, the tissues were formalin-fixed and paraffin-embedded.

Histologically "normal" livers (free of hepatitis B or C viral infections and without any significant pathological abnormalities) were obtained from 7 patients with liver metastases from colorectal cancer.

Immunohistochemistry

For immunohistochemical analysis of the mPGES-1 protein, formalin-fixed and paraffin-embedded tissue blocks were cut into 4 μm-thick sections. The sections were deparaffinized in xylene and subsequently rehydrated in sequential ethanol (100%-70%). After washing 3 times with 10 mmol/L phosphate-buffered saline (PBS) (pH 7.4), antigen retrieval was performed by first heating in a microwave at 95℃ for 20 min, then by washing twice in PBS for 10 min. The sections were treated with peroxidase-blocking solution (DAKO Japan, Kyoto, Japan) for 5 min, and incubated with the primary antibody for 60 min at room temperature. The primary antibody used was a 1:100 dilution of a mPGES-1 polyclonal antibody (Cayman Chemical, Ann Arbor, MI, USA). A standardized two-step method with ENVISION plus (DAKO) was used for detection. The reaction products were visualized using diaminobenzidine as a chromogen (DAKO), and counterstained with Mayer's hematoxylin (DAKO). The specificity of the antibody was checked by the adsorption with corresponding blocking peptides (Cayman Chemical) using a 1:1 ratio of primary antibody to blocking peptide.

Scoring criteria for mPGES-1 expression

Two blinded investigators (MI and KN) evaluated the immunostained sections. To assess the mPGES-1 protein staining results, the cytoplasmic immunoreactive intensity was scored as previously described[14]. In summary, the staining intensity for mPGES-1 was scored in each specimen on a scale of 0-3, with 0 = negative staining, 1 = weakly positive staining, 2 = moderately positive staining, and 3 = strongly positive staining (Figure 1). The staining intensity was evaluated for the maximum intensity among

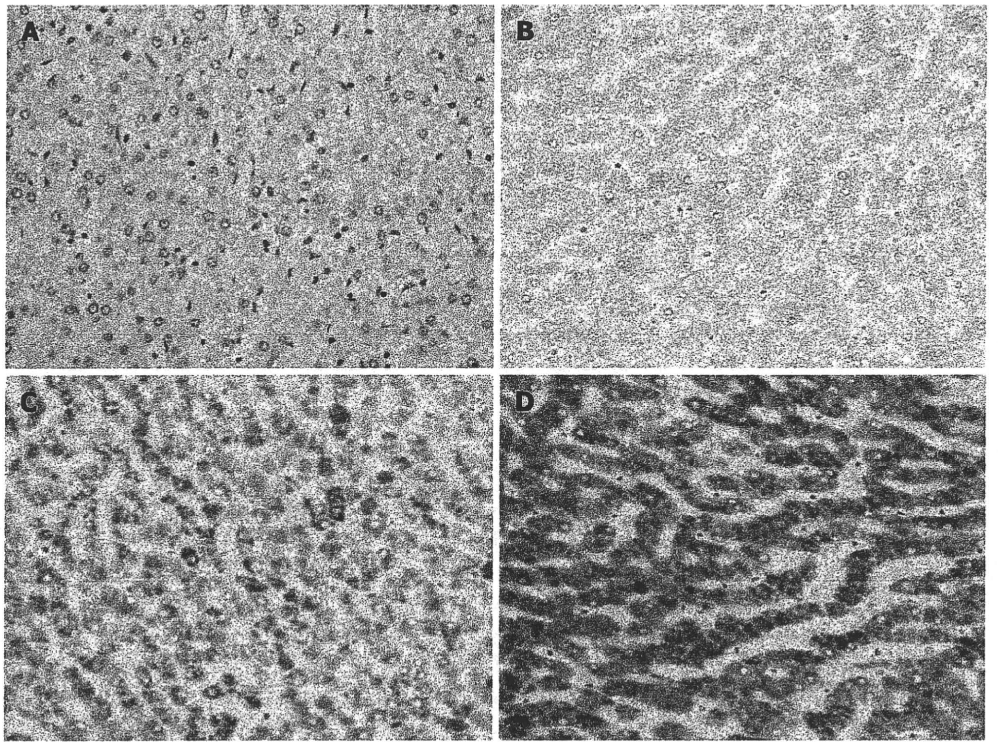

Figure 1 Grading of immunohistochemical staining for microsomal prostaglandin E synthase-1 protein in representative liver tissue (original magnification, × 200). A: No immunoreactivity for microsomal prostaglandin E synthase-1 (mPGES-1) (grade 0); B: Weakly positive for mPGES-1 (grade 1); C: Moderately positive for mPGES-1 (grade 2); D: Strongly positive for mPGES-1 (grade 3).

positive cells ("maximum intensity of staining", I) and the intensity level observed in the largest number of positive cells ("most extensive intensity level", II). The extent to which positive cells were observed in each specimen ("extent of distribution of positive cells", III) was estimated and scored on a scale of 0-4, with 0 = negative, 1 = positive in 1%-25% of cells, 2 = positive in 26%-50% of cells, 3 = positive in 51%-75% of cells, and 4 = positive in 76%-100% of cells. Each section was evaluated for the sum of these three parameters (I + II + III). Immunoreactivity for mPGES-1 protein was compared statistically using the average of the sum in each histological category. The patients in the present study were divided into two groups, including the higher expression (the sum of the categorical score, 6 to 10) and the lower expression groups (the sum of the categorical score; less than 6).

Western blotting analysis

We performed a Western blotting analysis on representative samples of HCC and non-cancerous liver tissues. The tissues were homogenized on ice in RIPA buffer [PBS, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (SDS)] containing 100 ng/mL phenylmethylsulphonyl fluoride, 4 mg/mL aprotinin, 2 mg/mL leupeptin, 1 mg/mL pepstatin, 10 mg/mL antipain, 10 mg/mL soybean trypsin inhibitor, and 2 mmol/L ethylenediaminetetraacetic acid. The homogenates were clarified by centrifugation. Protein concentrations were measured using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). After boiling for 5 min in the presence of 2-mercaptoethanol, samples containing 50 mg of tissue lysates were separated on 12.5% SDS-polyacrylamide gels and then transferred onto equilibrated Hybond PVDF membranes (Amersham International, Buckinghamshire, UK). After skim milk blocking, the membranes were then incubated with the mPGES-1 polyclonal antibody (at a dilution of 1:500). Bound antibodies were detected with horseradish peroxidase-labeled rabbit anti-goat IgG (Southern Biotechnology Associates, Birmingham, AL, USA) using an enhanced chemiluminescence detection system (ECL kit; Amersham International, Buckinghamshire, UK).

Analysis of the risk factors for HCC recurrence after curative resection

The following clinicopathological factors were evaluated for their association with HCC recurrence: age, gender, presence of hepatitis B surface antigen (HBsAg) or anti-hepatitis C virus antibody (anti-HCV Ab), platelet count, preoperative blood chemistry (serum levels of total bilirubin, alanine aminotransferase and albumin), presence of liver cirrhosis, and mPGES-1 expression. The evaluated operative factors included the intraoperative blood loss and the hepatectomy method. The tumor factors were the greatest tumor diameter, the number of tumor nodules, the presence of vascular invasion, the presence of capsular formation, the histological grade, and the serum levels of AFP and DCP. The hepatectomy method was classified as anatomical or non-anatomical resection ac-

Table 1 Microsomal prostaglandin E synthase-1 expression in hepatocellular carcinoma and non-cancerous liver tissue *n* (%)

	No. of cases	Patients with higher scores	Patients with lower scores	Scores (mean ± SD)	*P*
Hepatocellular carcinoma tissues					
Well differentiated	18	7/18 (38.9)	11/18 (61.1)	5.1 ± 2.7	-
Moderately differentiated	40	14/40 (35.0)	26/40 (65.0)	5.1 ± 1.7	0.959[a]
Poorly differentiated	6	0	6/6 (100)	3.0 ± 1.8	0.009[a]
Non-cancerous liver tissues					
Normal	2	1/2 (50.0)	1/2 (50.0)	3.3 ± 0.7	-
Chronic hepatitis	31	19/31 (61.3)	12/31 (38.7)	5.4 ± 1.9	0.006[b]
Cirrhosis	31	15/31 (48.4)	16/31 (51.6)	6.4 ± 1.6	0.002[b], 0.039[c]
Normal livers from colorectal cancer	7	0	7/7 (100)	3.5 ± 0.5	-

[a]*vs* well differentiated hepatocellular carcinomas; [b]*vs* normal livers; [c]*vs* chronic hepatitis.

cording to the methods described by Makuuchi *et al*[15] and Takayama *et al*[16]. The anatomic resection consisted of the systematic removal of the hepatic segment which is confined by the tumor-bearing portal tributaries. In the non-anatomic resection, the liver was divided along a line so as to secure a surgical margin of at least 5 mm, if possible.

Statistical analysis

Statistical analyses were performed using either Student's *t*-test or the Mann-Whitney *U* test to compare variables between the groups. A recurrence-free survival curve was plotted using the Kaplan-Meier method. A statistical comparison of the recurrence-free survival was performed using the log-rank test. A multivariate analysis by the Cox proportional hazard model was used to identify the independent risk factors for tumor recurrence. A P value < 0.05 was considered statistically significant. Statistical analyses were performed using the StatView for Windows software program (version 5.0, SAS Institute Inc., Cary, NC, USA).

RESULTS

Characteristics of the patients

There were 42 male (65.6%) and 22 female (34.4%) patients. The mean age was 64 years (range, 38-86 years). Twenty-one patients were positive for HBsAg, 32 were positive for anti-HCV Ab, and 11 were negative for both. Thirty-one patients had a cirrhotic liver, while 33 did not. The maximum tumor size was 12 cm, and 57 patients (89.1%) had a solitary tumor. More than 90% of the patients enrolled in the study had a Child-Pugh classification of A for liver function. Fifty percent of the patients had a tumor size > 3 cm. In the pathological differentiation, HCC was well differentiated in 18 patients, moderately differentiated in 40, and poorly differentiated in 6. The median observation period was 49 mo (range, 3-74 mo).

Immunohistochemical analysis of mPGES-1 protein

The expression of mPGES-1 protein in the HCC and non-cancerous liver tissues was examined immunohistochemically. Various degrees of staining for mPGES-1 protein were observed. The scores for mPGES-1 expression in the HCC and non-cancerous liver tissues are summarized in Table 1. The marked expression of mPGES-1 was demonstrated in well differentiated as well as in moderately differentiated HCC tissues (scores; 5.1 ± 2.7 and 5.1 ± 1.7, respectively). Conversely, mPGES-1 expression was significantly weaker in poorly differentiated HCC tissues (score; 3.0 ± 1.8, $P < 0.05$). Seven of 18 cases (38.9%) with well differentiated HCC and 14 of 40 cases (35.0%) with moderately differentiated HCC had high expression scores, whereas none of the patients with poorly differentiated HCC had high expression scores.

The mPGES-1 levels increased significantly with fibrotic stage of the liver tissues (scores; normal liver 3.3 ± 0.7, chronic hepatitic livers 5.4 ± 1.9, cirrhotic livers 6.4 ± 1.6). High expression scores were observed in 1 of 2 normal livers (50%), 19 of 31 chronic hepatitic livers (61.3%), and 15 of 31 cirrhotic livers (48.4%). There was no significant correlation between tumor differentiation and non-cancerous liver tissue in the expression of mPGES-1 (data not shown). Additionally, mPGES-1 expression in normal livers obtained from 7 patients with liver metastasis was lower than that in damaged livers ($P < 0.05$).

Western blotting analysis of mPGES-1

To confirm the specificity of the mPGES-1 antibody and the presence of mPGES-1 protein in the specimen, Western blotting analysis was performed on representative samples of HCC and non-cancerous liver tissues. Both tissue types yielded a single band with a molecular weight of 16 kDa, indicating the presence of mPGES-1 protein (Figure 2).

Correlation between the levels of mPGES-1 expression and recurrence-free survival time

We evaluated the correlation between the levels of mPGES-1 expression in HCC and non-cancerous liver tissues and recurrence-free survival time. No statistically significant difference was observed in the recurrence-free survival time between the higher and lower expression groups in HCC tissues (Figure 3A). In contrast, a statistically significant difference in the recurrence-free survival time was observed between the higher and lower expression groups in non-cancerous liver tissues ($P = 0.006$, Figure 3B).

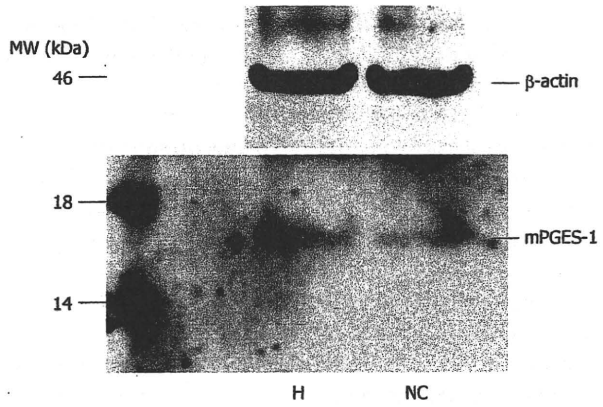

Figure 2 Western blotting analysis for microsomal prostaglandin E synthase-1. A band of 16 kDa in molecular weight, thus indicating the presence of microsomal prostaglandin E synthase-1 (mPGES-1) protein, is identified in both hepatocellular carcinoma tumors (H) and non-cancerous liver (NC) tissues. MW: Molecular weight.

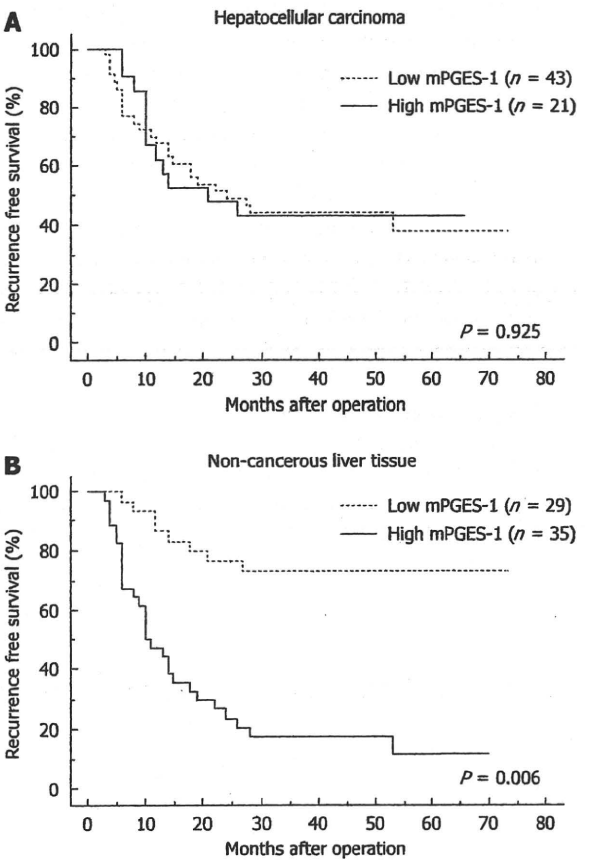

Figure 3 Recurrence-free survival time based on microsomal prostaglandin E synthase-1 expression in hepatocellular carcinoma tissues (A) and non-cancerous liver tissues (B). The recurrence-free survival time is significantly shorter in patients with an increased expression of microsomal prostaglandin E synthase-1 (mPGES-1) in non-cancerous liver tissues.

Correlation between various clinicopathological parameters and recurrence-free survival time

Various clinicopathological parameters were evaluated for their association with HCC recurrence (Table 2). A univariate analysis revealed that recurrence-free survival time was

Table 2 Univariate analysis of clinicopathological features related to postoperative recurrence

	No. of patients	Postoperative recurrence		*P*
		Yes	No	
Age (yr)				
≥ 60	45	23	22	0.733
< 60	19	9	10	
Gender				
Male	43	20	23	0.481
Female	21	12	9	
Hepatitis B surface antigen				
Positive	21	13	8	0.147
Negative	43	19	24	
Hepatitis C virus antibody				
Positive	32	12	20	0.151
Negative	32	20	12	
Total bilirubin (mg/dL)				
≥ 1.0	29	7	22	0.987
< 1.0	35	13	22	
Alanine aminotransferase (IU/L)				
≥ 50	26	11	15	0.635
< 50	38	21	17	
Albumin (g/dL)				
≥ 3.7	49	24	25	0.022
< 3.7	15	13	2	
Platelets (10^4/μL)				
≥ 10	46	21	25	0.465
< 10	18	11	7	
Liver cirrhosis				
Present	31	16	15	0.772
Absent	33	16	17	
T mPGES-1				
High	21	12	9	0.925
Low	43	25	18	
NC mPGES-1				
High	35	23	11	0.006
Low	29	9	21	
Hepatectomy				
Anatomic	31	21	10	0.149
Non-anatomic	33	16	17	
Operative blood loss (mL)				
≥ 500	15	12	3	0.091
< 500	49	25	24	
α-fetoprotein (ng/mL)				
≥ 100	15	10	5	0.347
< 100	49	27	22	
DCP (mAU/mL)				
≥ 400	22	15	7	0.081
< 400	42	19	23	
Tumor diameter (cm)				
≥ 5	16	12	3	0.018
< 5	48	20	29	
Tumor number				
Multiple	7	3	4	0.789
Solitary	57	29	28	
Histological grade				
Well	18	8	10	0.495
Moderate	40	21	19	
Poor	6	3	3	
Capsular formation				
Present	49	25	24	0.320
Absent	15	7	8	
Vascular invasion				
Present	16	11	5	< 0.001
Absent	48	9	39	

DCP: Des-γ-carboxy prothrombin; mPGES-1: Microsomal prostaglandin E synthase-1; T mPGES-1: mPGES-1 expression in hepatocellular carcinoma tumor tissue; NC mPGES-1: mPGES-1 expression in non-cancerous liver tissue.

Table 3 Multivariate analysis of the risk factors for postoperative recurrence

Variables	Hazard ratio	95% CI	*P*
Vascular invasion (present)	4.116	1.813-9.344	< 0.001
NC mPGES-1 expression (high)	4.074	1.760-9.428	0.001
Tumor diameter (≥ 5 cm)	2.060	0.860-4.935	0.105
Albumin (< 3.7 g/dL)	1.745	0.589-3.165	0.315

NC mPGES-1: Microsomal prostaglandin E synthase-1 expression in non-cancerous liver.

shorter in cases with vascular invasion, higher mPGES-1 levels in non-cancerous liver tissue, a larger tumor diameter (≥ 5 cm), and lower levels of serum albumin (< 37 g/L). The operative factors were not significantly correlated with recurrence-free survival time. A multivariate analysis demonstrated that the presence of vascular invasion and higher mPGES-1 levels in the non-cancerous liver tissue were significant independent predictors for the early recurrence of HCC after curative hepatectomy (Table 3).

DISCUSSION

The present study demonstrated that the rate of HCC recurrence was high after curative resection. This finding was consistent with those described in other recent reports[1-6]. Tumor recurrence is caused by metastatic lesions, residual microscopic lesions that remain after curative resection, or multicentric occurrence in the setting of hepatitis or cirrhosis[17,18]. The prevention of tumor recurrence is key to the improvement of prognosis for HCC patients after a hepatectomy[19]. In the present study, a multivariate analysis indicated that the two independent predictors for HCC recurrence after curative resection were the presence of vascular invasion and increased mPGES-1 expression in the non-cancerous liver tissue.

Vascular invasion is a well-known risk factor for a poor prognosis after curative resection. The presence of vascular invasion is considered one of the strongest predictors of intrahepatic metastasis caused by the spread of cancer cells *via* the portal venous system[17-19]. Although several reports have demonstrated that postoperative adjuvant therapy prevented postoperative HCC recurrence[20,21], its efficacy has yet to be determined. Other therapeutic modalities for treating postoperative recurrence are urgently needed.

The most interesting finding in the present study was that increased mPGES-1 expression in the non-cancerous liver tissue was an independent predictor for early HCC recurrence after curative resection. Increased mPGES-1 levels induce PGE_2 synthesis, which may create a suitable environment for occult intrahepatic metastases to survive and spread after hepatectomy. This hypothesis is supported by several studies showing that PGE_2 was implicated in migration, secretion of various types of matrix metallo-proteinases, and cell adhesion in HCC cells[22-24]. Additionally, increased PGE_2 levels in the non-cancerous liver tissue leads to prolonged acceleration of necroinflammation and regeneration in the remnant liver[25]. The inflamed liver may also provide a good environment for occult intrahepatic metastases to grow in response to different growth factors[26]. In the present study, active hepatitic and/or cirrhotic livers had increased mPGES-1 expression compared to normal livers. The repeated cycles of necroinflammation, degeneration, and regeneration increase hepatocyte turnover, which facilitates spontaneous mutation and may hinder DNA repair[26]. The release of reactive oxygen species including superoxide and H_2O_2 in this situation may also contribute to uncontrolled cell growth, apoptosis, and senescence[27]. Another possible mechanism is that mPGES-1 itself may act as a landscaping tumor promoter. mPGES-1 lies downstream of the PGE_2-biosynthetic pathway of COX-2. Recent studies reported that mPGES-1 was expressed in several cancers and was linked to carcinogenesis[12,28]. mPGES-1 derived from the stromal component may promote tumor growth by producing bioactive PGE_2, which acts angiogenetically or immunosuppressively, and affects carcinoma cells in a paracrine fashion[12,28]. Therefore, the increased expression of mPGES-1 in the non-cancerous liver tissue may create conditions suitable for HCC recurrence from metastasis or multicentric occurrence. However, the precise mechanisms remain to be elucidated.

The mPGES-1 expression in HCC tissues did not correlate well with postoperative recurrence. This finding suggested that the mPGES-1 in HCC tissues *per se* did not determine the malignant potential of HCC tissues, although overexpression of mPGES-1 was associated with various types of cancer[12,13].

The data indicate that COX-2 inhibitors are chemopreventive for several kinds of cancers[29], however, there have been no reports on HCC patients. Although the COX-2 inhibitors have a reduced gastrointestinal toxicity in comparison to traditional non-steroidal anti-inflammatory drugs, some adverse effects have been reported[30]. From this standpoint, more selective inhibition of the prostanoid pathway to PGE_2 is thus highly desirable. mPGES-1 is the terminal enzyme for PGE_2 biosynthesis, and thus it is considered the most selective agent for that pathway. Although there have been several reports concerning the selective inhibitors of mPGES-1[31,32], further studies are still needed in clinical settings.

In conclusion, increased mPGES-1 expression in non-cancerous liver tissue is closely associated with the early recurrence of HCC after curative resection. The present study also indicates that an inhibitor of mPGES-1 may be a new therapeutic option to improve the survival rate of HCC patients after curative resection.

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COMMENTS

Background

Microsomal prostaglandin E synthase-1 (mPGES-1) is the terminal enzyme in the formation of prostaglandin E_2 from prostaglandin H_2. Data indicate that increased expression of mPGES-1 is associated with various types of cancers. However, the impact of mPGES-1 expression on the clinical course of hepatocellular carcinoma (HCC) has not yet been elucidated.

Research frontiers

In HCC, tumor recurrence is caused by metastatic lesions, residual microscopic lesions that remain even after curative resection, and multicentric occurrence in the setting of hepatitis or cirrhosis. The research was performed to clarify the risk factors for the recurrence of HCC after curative resection in Nagasaki Medical Center.

Innovations and breakthroughs

The present study demonstrates that various degrees of mPGES-1 expression occur in HCC and non-cancerous liver tissues. This is the first report to demonstrate that increased expression of mPGES-1 in non-cancerous liver tissue is an independent predictor for HCC recurrence after curative resection.

Applications

mPGES-1 expression in non-cancerous liver tissue could be a useful biomarker for screening high risk groups of patients with HCC after curative resection. In the near future, a selective mPGES-1 inhibitor may prevent postoperative recurrence of HCC and improve the prognosis of HCC patients.

Terminology

mPGES-1 is a protein belonging to the membrane-associated proteins involved in eicosanoid and glutathione metabolism super family. mPGES-1 is induced by pro-inflammatory stimuli, down-regulated by anti-inflammatory glucocorticoids, and functionally coupled with cyclooxygenase-2. Thus, mPGES-1 plays a central role in the biosynthesis of prostaglandin E_2.

Peer review

The paper reported that increase in mPGES-1 in non-cancerous liver was an independent prognostic factor in patients received surgical therapy to HCC. Although the results might be of importance, several questions are addressed, and several points to be improved are suggested.

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Predictor for Histological Microvascular Invasion of Hepatocellular Carcinoma: A Lesson from 229 Consecutive Cases of Curative Liver Resection

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Abstract
Background Microscopic vascular invasion is an important risk factor for recurrent hepatocellular carcinoma (HCC), even after curative liver resection or orthotopic liver transplantation. To predict microscopic portal venous invasion, the following two questions were examined retrospectively: Is it possible to detect microvascular invasion preoperatively? What are the characteristics of a group of early HCC recurrences even with no microvascular invasion?
Methods Study 1 included 229 patients with HCC who underwent curative liver resection between 1991 and 2008; 127 had HCC without microscopic portal venous invasion, and 52 had HCC with microscopic portal venous invasion (MPVI). These two distinct groups were analyzed with regard to various clinicopathologic factors. Subsequently, we specifically investigated if HCCs <5 cm with vascular invasion ($n = 32$) have some characteristics that would allow detection of latent microvascular invasion. Study 2 included 127 HCC patients without MVPI; 42 had a recurrence within 2 years, and 85 patients were recurrence-free for at least 2 years. These two distinct groups were analyzed with regard to various clinicopathologic factors.
Results HCC diameter of >5 cm, the macroscopic appearance of HCC, and high levels of preoperative des-γ-carboxyprothrombin are significant prognostic factors in identifying microvascular invasion of HCC. The strongest predictor of early recurrence (within 2 years) was the serum α-fetoprotein level in patients without clear microvascular invasion.
Conclusions Tumor size, macroscopic appearance, and high tumor marker levels are important elements in identifying the group of patients with a low HCC recurrence rate after curative liver resection.

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Introduction

Microvascular invasion is a strong prognostic factor for hepatocellular carcinoma (HCC), even after curative liver resection. Moreover, after orthotopic liver transplantation (OLT), which is the ultimate removal of a malignant tumor, microvascular invasion remains a significant prognostic factor as HCC becomes systemic through invasion of peripheral portal or hepatic veins and subsequent spread [1–3]. Therefore, vascular invasion has always been included as an indication for OLT, including in the Milan criteria [4]. Macrovascular invasion to the first and second ramifications of the portal vein can be diagnosed by computed tomography (CT) or other imaging techniques; however, microvascular invasion to minute peripheral areas, such as the third branch of the portal vein, is difficult to detect with current imaging modalities. Therefore, to predict the outcome of liver transplantation or curative liver resection more accurately, it is necessary to identify factors that indicate or predict the microvascular invasion of HCC.

We performed 229 curative liver resections for HCC during a period of 17 years. Using the data from these cases, we carried out a retrospective analysis to identify factors that can predict the recurrence of HCC based on the histological findings of HCC in the resected liver.

Patients and methods

Between 1991 and 2008, a total of 229 curative liver resections were performed in the Department of Surgery of Nagasaki University Hospital, Nagasaki, Japan. Of 229 HCCs, 50 had vascular invasion up to the second branch of the portal vein. The remaining 179 patients were analyzed in the study. The average age of the patients was 65 years (range 20–85 years). There were 143 men and 36 women, and the median follow-up period was 45.5 months.

The macroscopic appearance of HCC was classified into four types: type 1, single nodular type; type 2, single nodular type with extranodular growth; type 3, contiguous multinodular type formed by a cluster of small, contiguous nodules; type 4, infiltrative type [5]. As tumor markers, serum levels of α-fetoprotein (AFP) and des-γ-carboxy-prothrombin (DCP) were measured. We conducted two distinct retrospective studies.

Study 1

To identify factors for detecting microscopic portal venous invasion preoperatively, we examined 179 of our 229 patients (excluding those with vascular invasion to the first and/or second branches of the portal vein). Of these 179 patients, 127 had no histologically proven microscopic portal venous invasion, hepatic venous invasion, or intrahepatic metastasis. The remaining 52 had microscopic vascular invasion regardless of the presence or absence of intrahepatic metastasis of HCC. Subsequently, after we learned that more microvascular invasion occurred in large tumors (≥5 cm), we were interested in whether HCCs <5 cm with microvascular invasion have some characteristics that would allow detection of latent microvascularity. When we limited our examination to patients with a single HCC lesion of <5 cm in diameter, we had 102 patients without and 31 patients with microvascular invasion.

Study 2

The second study was performed to identify the group of patients who suffered early recurrence—defined as recurrence within 2 years—even though they showed no microscopic portal venous invasion. Of the 127 patients without proven microvascular invasion, 42 suffered early recurrence and 85 experienced recurrence after 2 years. These two distinct groups were analyzed with regard to various clinicopathologic factors. For this study, necroinflammatory activity (grade) and the degree of fibrosis (stage) as determined by Knodell et al. were calculated by routine histologic examination [6].

Statistical analysis

All analyses were conducted with Stat-View. Univariate analysis was performed using the Pearson chi-squared test for categorical factors and the Mann–Whitney test for numerical values. Multivariate analysis was conducted with a logistic regression model. Odds ratios (ORs) and the corresponding 95% confidence interval (CI) were computed to assess the strength of association. Any P values of <0.05 were considered statistically significant.

Results

Study 1

Univariate analysis revealed that the size of the HCC, the number of HCC lesions, the macroscopic appearance of HCC, and tumor markers (AFP, DCP) had a significant predictive value (Table 1). Multivariate logistic regression analysis revealed that the size of the HCC and its macroscopic appearance were significant independent risk factors for microvascular invasion by HCC (Table 2).

When we limited our examination to patients with a single HCC lesion of < 5 cm diameter, we had 102 patients without and 31 patients with microvascular invasion. Significant predictive factors for microvascular invasion were the macroscopic appearance of the HCC and a high DCP level (Table 3). With respect to the macroscopic appearance of the HCC, types 2 and 3 were significantly predictive of microvascular invasion of HCC.

Study 2

Only AFP had a significant predictive value for identifying patients likely to experience recurrence within 2 years even if there was no histologic evidence of microvascular invasion (Table 4). Moreover, a positive rate of hepatitis C antibody in the early recurrence group was higher than in the group with recurrence after 2 years. Grading (necroinflammatory response) and staging (fibrosis) were not statistically different between the two groups.

Discussion

In the present study, a tumor diameter of ≥5 cm, its macroscopic appearance, and the DCP level were significant predictive factors for microvascular invasion, which cannot be detected by current imaging techniques; this is consistent with the findings of a previous report by Shirabe et al. [7] The macroscopic appearance of type 2 or type 3 HCC, which can be evaluated in imaging studies, also

Table 1 Association of microvascular invasion of HCC: all HCCs

Parameter	Microvascular invasion		
	Positive ($n = 52$)	Negative ($n = 127$)	P
Age (years), median and range	64 (20–85)	65 (44–81)	NS
Sex (M:F)	41:11	102:25	NS
HBsAg-positive	20 (38.5%)	36 (28.3%)	NS
HCV Ab-positive	20 (38.5%)	63 (50.8%)	NS
Liver damage (A:B)	48:4	114:13	NS
HCC size (cm), median (range)	5.2 (0.5–17)	3 (0.8–11.5)	<0.001
No. of HCCs, median and range	1 (1–5)	1 (1–6)	<0.01
Macroscopic appearance of HCC (types 1/2/3/4)	8/17/19/1 (7 unclassified)	65/26/20/2 (14 unclassified)	<0.001
Tumor markers (median and range)			
AFP	95 (1.6–454,300)	12.7 (1.2–13,840)	<0.001
DCP	845 (6–76,600)	24 (0–69,150)	<0.05

HCC hepatocellular carcinoma, *HBsAg* hepatitis B virus surface antigen, *HCV Ab* hepatitis C virus antibody, *AFP* α-fetoprotein, *DCP* des-γ-carboxyprothrombin

Table 2 Logistic regression of factors associated with microvascular invasion of HCC: all HCCs

Parameter	Coefficient	Odds ratio (95% CI)
Size of HCC	0.517	1.678* (1.275–2.208)
No. of HCC	−0.42	0.657 (0.181–2.384)
Macroscopic appearance of HCC		
Type 1		Reference
Type 2	2.569	13.047** (1.514–112.439)
Type 3	3.229	25.253* (3.289–193.913)
Type 4	4.098	60.205** (2.574–1408.257)

CI confidence interval

* $P < 0.01$

** $P < 0.05$

predicts microvascular invasion. Therefore, in cases of type 2 or 3 HCC, early recurrence can be carefully monitored even after OLT.

With respect to tumor markers, the DCP level was an important factor in estimating the malignant potential of HCC without microscopic vascular invasion even after curative liver resection. Furthermore, even when the HCC is limited to a single lesion <5 cm in diameter (as described in the Milan criteria), an elevated DCP level implies a poor prognosis after curative resection. For these small, single HCCs, the DCP level was found to be a better predictor of vascular invasion than the macrovascular appearance of the HCC.

According to the results of the present study 2, AFP predicted the early recurrence of HCC even without proven microvascular invasion in the resected specimen. As documentation of microvascular invasion may be difficult because of the width of the slice in the tumor, the possibility of microvascular invasion in patients with early recurrence cannot be ruled out. Our findings indicate that even when an HCC that meets the Milan criteria (a single lesion <5 cm diameter) is removed by curative liver

Table 3 Association of microvascular invasion of HCC: single HCCs <5 cm diameter

Parameter	Microvascular invasion		
	Positive ($n = 31$)	Negative ($n = 102$)	P
Age (years), median and range	61 (37–85)	65 (44–81)	NS
Sex (M:F)	2:4	81:21	NS
HBsAg-positive	12 (38.7%)	28 (27.4%)	<0.05
HCV Ab-positive	12 (38.7%)	52 (50.9%)	NS
Child-Pugh (A:B)	30:1	94:8	NS
Macroscopic appearance (types 1/2/3/4)	6/11/10/1 (3 data sets missing)	55/23/13/1 (10 data sets missing)	<0.001
Tumor markers (median and range)			
AFP	97.9 (1.6–454,300)	13 (1.2–81)	NS
DCP	1307 (1.8–76,600)	71 (0–8520)	<0.001

Table 4 Factors for early recurrence within 2 years without proven microvascular invasion

Parameter	Early recurrence		*P*
	Yes ($n = 42$)	No ($n = 85$)	
Age, median (range)	65 (45–77)	66 (44–81)	NS
Sex (M:F)	35:7	67:18	NS
HBsAs-positive	12 (28.6%)	27 (31.7%)	NS
HCV Ab-positive	23 (54.8%)	39 (45.9%)	<0.05
Child Pugh (A:B)	36:6	80:5	NS
HCC size (cm), median and range	3 (1–11.5)	3 (0.8–11.4)	NS
No. of HCCs, median and range	1 (1–6)	1 (1–2)	NS
Macroscopic appearance (types 1/2/3/4)	21/10/5/1 (5 unclassified)	45/14/16/2 (8 unclassified)	NS
Tumor markers (median and range)			
AFP	41.4 (2–1714)	8.3 (1.2–13,840)	<0.05
DCP	61 (0–69,150)	24 (6–3,999)	NS
HAI			
Grading	5 (0–13)	5 (0–13)	NS
Staging	3 (0–4)	2 (0–4)	NS

HAI hepatitis activity index

resection and OLT, patients should be carefully monitored for early recurrence when the AFP level is elevated. Usually, after curative liver resection, recurrence within 2 years appears mostly as an intrahepatic metastasis through vascular invasion, whereas recurrences occurring 2 years after R0 are regarded as multicentric HCCs, which are a different clone from the first resected HCC. In other words, it is not usual that recurrence occurs after 2 years through microvascular invasion.

When considering expansion of the indication criteria for OLT for HCC, the prediction of vascular invasion should be a key point because a previous report showed its importance even after total eradication of the diseased liver [8]. The present study found that the macroscopic appearance of the HCC and tumor markers are important as predictors of microvascular invasion and that DCP in particular can be used to detect latent microvascular invasion of HCC even in patients with a single lesion of <5 cm diameter. Furthermore, the AFP level can be used to predict early recurrence after curative removal of HCC, which implies latent microvascular invasion because early recurrence is generally thought to indicate intrahepatic metastasis of a primary HCC through the portal vein [9]. In contrast, recurrence after 2 years is usually regarded as a second occurrence of HCC in the diseased liver (multicentric occurrence) [10]. Because the diseased liver is removed during OLT, intrahepatic metastasis through microvascular invasion is more important than the multicentric occurrence of HCC after the procedure.

Recently, using thin-sliced explant liver we showed that preoperatively undetectable HCC does not have a prognostic impact on outcome or recurrence of HCC after liver transplantation [11]. The characteristics of undetectable HCCs included a minute (median size 6 mm), well-differentiated appearance (80%), with indistinct margins (85.3%) and without vascular invasion (94%). There was no recurrence in any patients at the time of follow-up (median follow-up period was 30.1 months). In fact, tumor markers in almost all patients were within normal limits. Together with these results, it was found that in small HCCs with low tumor marker levels there was an absence of microvascular invasion of the HCC.

As a subgroup analysis, we investigated the group of patients with HCCs <5 cm in diameter to determine predictors for microscopic vascular invasion. As it has already been widely reported that HCCs ≥5 cm in diameter have a greater chance of spreading through microvascular invasion, the prediction of microvascular invasion is not important for those patients. Even when the patient meets other criteria for liver transplantation, if the HCC is ≥5 cm OLT is contraindicated because of the high risk of recurrence. In the studies described herein, therefore, we tried to find potential microscopic vascular invasion using criteria other than the size of the HCC.

In conclusion, tumor size, the macroscopic appearance of the HCC, and the DCP level are important factors that can be used to identify the group of patients with a low probability of recurrence of HCC after curative liver resection. The AFP level can also be used as a predictor of latent microscopic vascular invasion and early recurrence.

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Significance of PET/CT in Determining Actual TNM Staging for Patients With Various Lung Cancers

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We investigated the difference in TNM stage of lung cancer provided by PET/CT (combining positron emission tomography and computed tomography) as compared with TNM stage obtained with conventional imaging studies (CI) with contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) with iron contrast media. Sixty-seven cases of lung cancer were included in this study. Overall, the rate of correction of TNM staging was 70.1% after PET/CT. The correction rate for each factor was 32.8% in T, 37.3% in N, and 37.3% in M. High rates of correction were observed in small cell lung cancer (SCLC), with 75% (6/8 cases) obtained by PET/CT. When SCLCs were divided into limited disease (n = 6) involving 1 hemithorax, including mediastinal and contralateral hilar lymph nodes, and others (extensive disease, n = 2), the correction rate was as high as 80% for limited disease. In conclusion, PET/CT can provide actual TNM staging and recognition for oncologists in staging, which would not mislead to selection of inadequate subsequent treatment.

Key words: PET/CT – TNM stage – Lung cancer

With the development of fluoro-deoxy-glucose (FDG) in the late 1900s, positron emission tomography (PET) emerged as a new technology for cancer diagnosis.[1] However, it was difficult to learn the precise anatomic locations of lesions because images obtained by PET were as vague as those obtained by scintigraphic images.

After the appearance of PET combined with computed tomography (CT) devices (hereinafter abbreviated as "combined PET/CT") in 2000, CT images of cancer lesions and the sites thereof were clearly enhanced by FDG, thereby further increasing the precision of images of the range and metastasis of a lesion, resulting in the provision of very

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convincing images for the patient.[2–4] Meanwhile, remarkable developments in cancer treatment have been reported in succession in each field of chemotherapy, radiotherapy, and surgical treatment. The methods of treating cancer have drastically changed, bringing enormous benefits to patients with cancer.

The question of which of these effective treatment methods should be selected is dependent upon TNM staging of cancers.[5] To make an error in staging means to make an error in selecting a treatment method. For instance, a patient with latent distant metastasis might be indicated not for surgical resection but for chemotherapy. We therefore investigated the significance of the combined use of PET/CT for the staging of cancer by examining the rates of correction of TNM staging obtained through conventional modalities such as CT and magnetic resonance imaging (MRI) (conventional imaging; hereinafter abbreviated as CI) as compared with "staging" done with PET/CT.

Patients and Methods

Sixty-seven cases of lung cancer between April 2005 and April 2006 were included in this study. The male-to-female ratio was 45:22, with a mean age of 66 years for males and 68.2 years for females. The clinical diagnosis was small cell carcinoma in 8 cases, squamous cell carcinoma in 28, large cell carcinoma in 1, and adenocarcinoma in 30. Small cell lung cancer (SCLC) was usually divided into 2 groups—limited disease (LD) and extensive disease (ED)—because of differences in biological behavior. LD involves 1 hemithorax, including mediastinal and contralateral hilar lymph nodes. Ipsilateral but not contralateral supraclavicular lymph nodes may also be involved. Usually patients with LD are considered for curative-intent combined chemotherapy and radiotherapy. All subjects refrained from exercise on the day before the study and fasted for 6 hours before the study began. FDG was administered at 4 MBq/kg with an automated syringe. Diabetic patients with fasting plasma glucose of 150 mg/dL and above were excluded from the study. With the use of PET/CT (General Electronics [GE] Discovery ST, Tokyo, Japan), initial images were taken at 45 minutes after administration (after rest), and later images were taken after another 45 minutes of rest. The images (CT, PET, and PET/CT images) were read at the workstation, and the standardized uptake value (SUV) was measured on the PET images. CI was performed with CT (Activion 16, Toshiba, Toshigi, Japan) and MRI (Excelart Vantage 1.5T, Toshiba). Informed consent was obtained from all participating patients.

Evaluation criteria for lung cancer

Cases that met the following 3 criteria were included in the study for the evaluation of cancer: (1) The reference SUV for evaluating cancer varied depending on the type of organ. However, the SUV was set to approximately 3 or 4 and above.[6] In addition, the SUV was measured at the lesion site where the highest level of accumulation was observed. (2) Images were taken in the early phase and at a later phase, and cases in which a significant increase was observed in later-phase images were included in the study.[7] (3) Cases were confirmed as cancer in the histologic findings. For classification of stage, the TNM Classification of Malignant Tumors was used.[8]

Imaging study

(1) Preoperatively, staging with CI was performed in advance in 67 cases; after restaging was performed with PET/CT, we examined whether this resulted in a correction in "stage" (upstage, downstage, or no change). (2) The number of corrections for each factor was examined to determine which of the T, N, and M factors were involved in the change in TNM stage of lung cancer.[8] (3) Involvement of both histology and correction in stage was examined for lung cancer, which requires different treatment methods depending on the histology.

Results

Rate of correction in TNM stage

The correction rate of TNM staging was 70.1% in whole patients with lung cancer, while which of the T, N, and M factors were involved in the correction was observed (Fig. 1). When stratified by histology of lung cancers, the correction rate was 75% in SCLC, 71.4% in squamous cell carcinoma, and 63.3% in adenocarcinoma. Table 1 describes which of the TNM factors was involved in correction by PET/CT. Overall, the correction rate of each factor was 32.8% in T, 37.3% in N, and 37.3% in M, and various factors were equally corrected by PET/CT. When stratified with histology, combined factors were involved in the corrections (Table 2); in many cases, multiple factors were corrected by PET/CT.

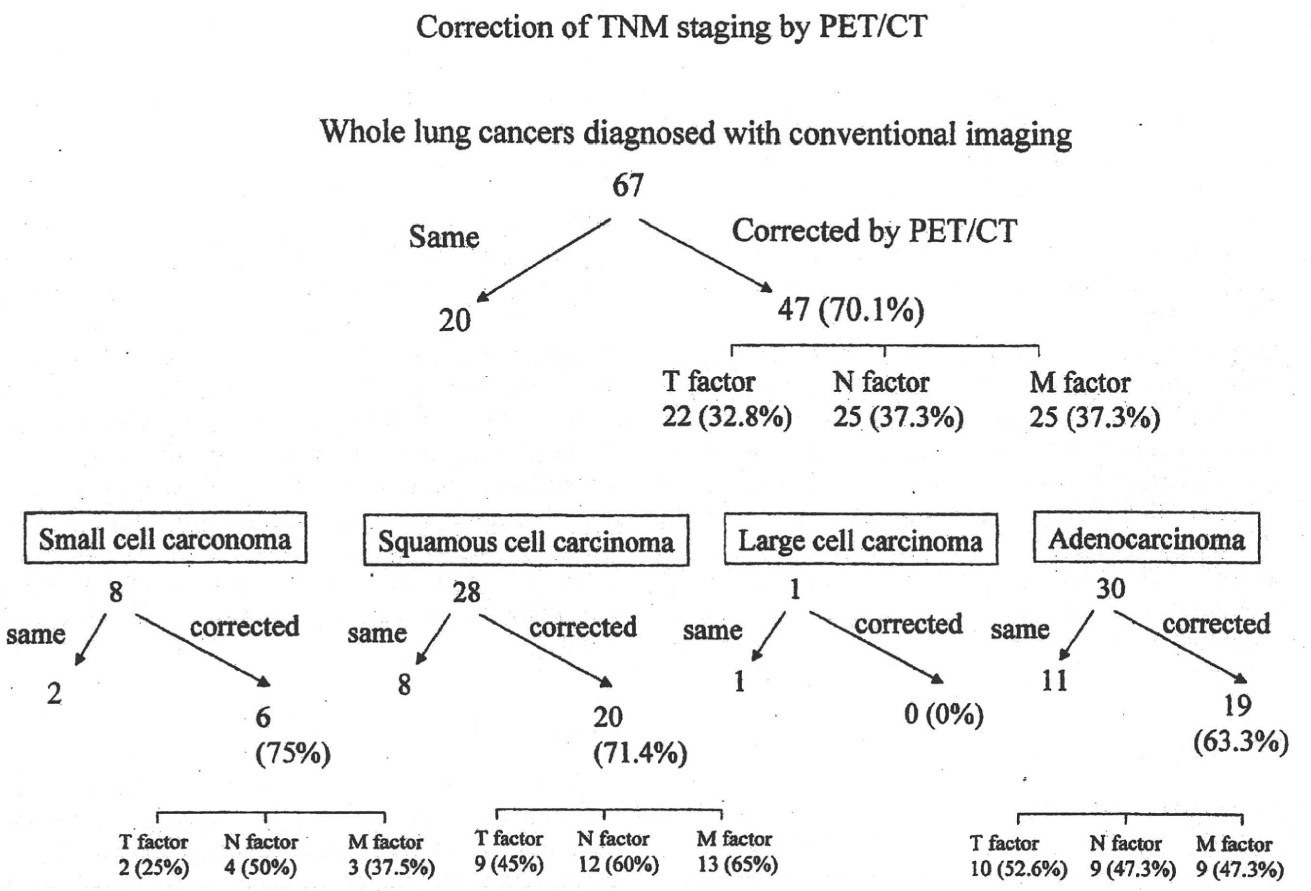

Fig. 1 Correction of TNM staging by PET/CT.

Value of PET/CT in differentiating the histology of lung cancer

Higher rates of correction were observed in SCLC (6/8, 75%) than in squamous cell carcinoma (20/28, 71.4%) and adenocarcinoma (19/30, 63.3%). However, for the pathologies of all types of lung cancer, nearly the same number of each TNM factor was involved in the correction (Fig. 1).

Differences in SUV were examined in the 67 cases of lung cancer (under the condition that the tumor size in each case was nearly the same) based on the mean accumulation value for each pathology in Fig. 2. As to differences in FDG accumulation, this was in the order of large cell carcinoma, squamous cell carcinoma, SCLC, adenocarcinoma, and alveolar cell carcinoma.

When SCLCs were divided into LD (n = 6) involving 1 hemithorax, including mediastinal and contralateral hilar lymph nodes, and others (ED, n = 2), the correction rate was as high as 80% in LD (Table 2). Three LD patients were found to be ED after the correction was made (50%, 3/6).

Table 1 T, N, M factors corrected by PET/CT

	T factor	N factor	M factor
Total number of patients corrected by PET/CT	22 (32.8%)	25 (37.3%)	25 (37.3%)
Up	15 (22.4%)	24 (35.8%)	25 (37.3%)
Down	7 (10.4%)	1 (1.5%)	0 (0%)
Total lung cancers	67	67	67

Case reports

Figure 3 illustrates a case of double lung cancer in which preoperative staging with PET/CT alone enabled the estimation of pathology and thereby contributed to the selection of a treatment method. The case was a 73-year-old male. A mass was found in both lower lung fields with the use of CT. The right lesion was diagnosed as adenocarcinoma after bronchoscopic cytology was performed. However, because of the difficulty of performing cytology on the left mass, histologic confirmation was not

Table 2 T, N, M factors corrected by PET/CT in each cancer

	3 factor (T, M, N)	2 factor (T, M)	2 factor (T, N)	2 factor (M, N)	T factor, only	N factor, only	M factor, only	Not corrected
Small cell carcinoma (n = 8)								
Total	1	0	1	0	0	2	2	2
Up	1	0	1	0	0	1	2	0
Down	0	0	0	0	0	1	0	0
Limited disease (n = 6)	1	0	1	0	0	1	2	2
Extended disease (n = 2)	0	0	0	0	0	1 (down)	0	0
Squamous cell carcinoma (n = 28)								
Total	2	1	4	4	2	2	6	7
Up	2	1	4	4	2	2	6	0
Down	0	0	0	0	0	0	0	0
Adenocarcinoma (n = 30)								
Total	1	1	3	3	6	2	4	10
Up	1	1	3	3	3	2	4	0
Down	0	0	0	0	3	0	0	0

obtained. PET/CT staging showed that the right and left tumors in this case were nearly the same size, but a low SUV of 3.4 was observed in FDG accumulation of the lesion in the right lobe; it was therefore diagnosed as adenocarcinoma, which coincided with the results of the cytology. The lesion in the left lobe was estimated to be squamous cell carcinoma because FDG accumulation showed a

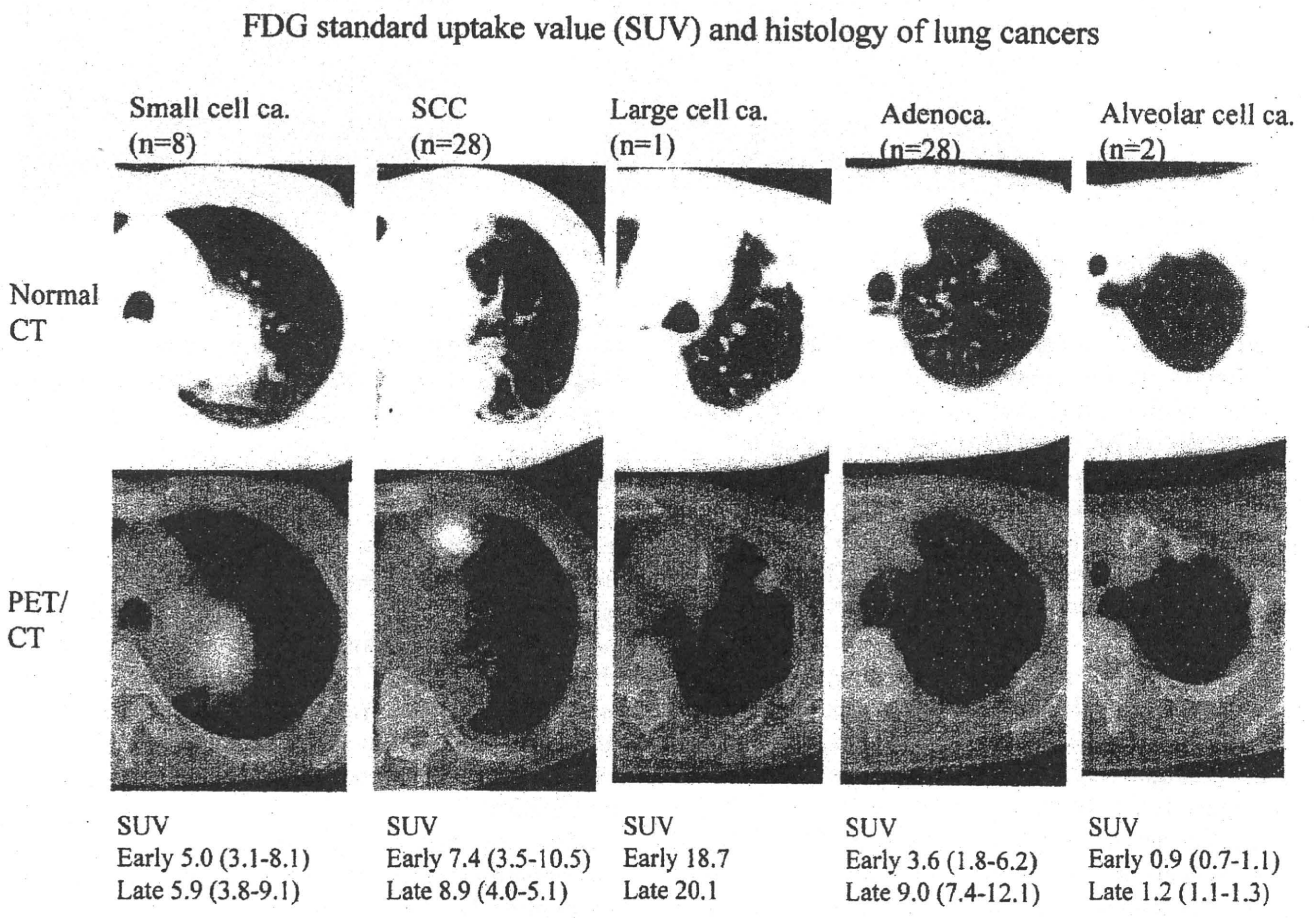

Fig. 2 FDG SUV and histology of lung cancers.

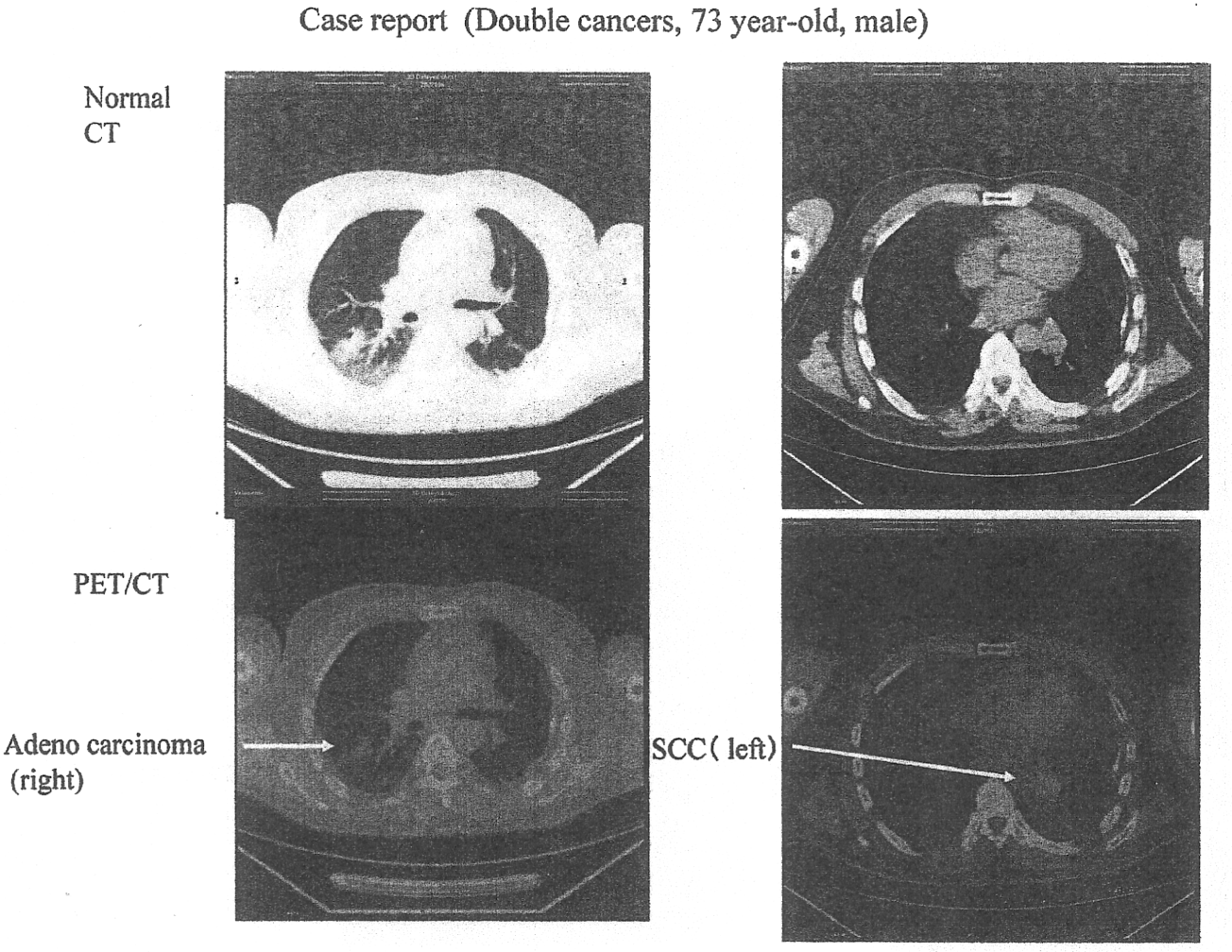

Fig. 3 Case report 1 (double cancers, 73-year-old male).

high SUV of 8. Consequently, as the first choice of treatment, a resection of the right lower lobe, including the tumor and a dissection of the lymph node were selected for the right tumor, and left lobe preservation as well as anticancer drug and radiotherapy was selected for the left tumor. As can be seen in this case, it is possible to estimate the pathology preoperatively by using PET/CT, thereby allowing preoperative selection of a treatment method.

Figure 4 shows the case of a 56-year-old male with SCLC. In this case, distant metastasis (M) was discovered using PET/CT, and the stage of the case was changed from LD to ED. The patient was preoperatively diagnosed as stage IIIB of T2N3M0 with the use of CI, but it was revealed by PET/CT that the cancer had metastasized to the pelvic bone, and the stage was then changed to stage III of T2N3M1. Accordingly, the treatment method based on radiotherapy in combination with anticancer drug therapy was changed to a palliative treatment in which an anticancer drug was primarily used.

Discussion

In the present study, we discussed the rate of correction in TNM stage using combined PET/CT for lung cancer. As is shown in Table 1, the rate of correction in stage of lung cancer was as high as 70% when combined PET/CT was used. Metastasis is missed with a high probability when the staging is based only on CI without the use of PET/CT. Such oversights can be improved by using PET and CT in combination.

Conventionally, the pathology of a lung cancer was presumed only on images. If PET/CT is used independently, the amount of cellular uptake of FDG (i.e., SUV accumulation) should vary depend-