Fig. 1 Insulin signaling and downstream intracellular events. Insulin action begins with binding to its specific receptor with tyrosine kinase activity. Signaling by the activated insulin receptor then promotes the phosphorylation of IRS-1 (insulin receptor substrate-1) and transmission of the insulin signal via two major phosphorylation cascades: PI3K (phosphoinositide 3-kinase) and MAPK (mitogen-activated protein kinase) cascades. *P* phosphorylation, *mSos* mammalian Son of sevenless

already been rendered hypersensitive by over-expression of insulin signal components.

Indirect action of insulin on growth promotion

Previous studies have described that insulin and insulin-like growth factor I (IGF-1) act as growth factors, leading to cell proliferation and the inhibition of apoptosis [26]. It has been clarified that hyperinsulinemia can promote the synthesis and biological activity of IGF-1 [27]. The liver is the source of over 80% of circulating IGF-1 and the principal stimulus for IGF-1 synthesis in the liver is provided by growth hormone (GH) signaling. Insulin can up-regulate human hepatic GH receptors [28]. Thus, hyperinsulinemia would produce and release a large amount of IGF-1 from the liver. In patients with type II diabetes, hyperinsulinemia accompanied by an up-regulation of hepatic GH-receptor, enhances IGF-1 production.

IGF-1 signaling via the IGF-1 receptor (IGF-1R) has effects on cell proliferation and survival, which is obviously stronger than those of insulin [29]. IGF-1 can act as a potent growth factor for cancer cells both in vivo [30] and in vitro [31], and in vivo over-expression of IGF-1 can promote tumor formation [32]. Conversely, its down-regulation can inhibit tumorigenesis [33]. Thus, the role of IGF system and IGF-1R signaling has been emphasized in tumorigenesis. Epidemiologic evidence has described elevated circulating IGF-1 levels in the development of a variety of cancers, including colorectal, prostate and breast cancers, and HCC [34–36].

In addition, hyperinsulinemia can enhance IGF-1 activity by means of modulating the availability of IGF binding
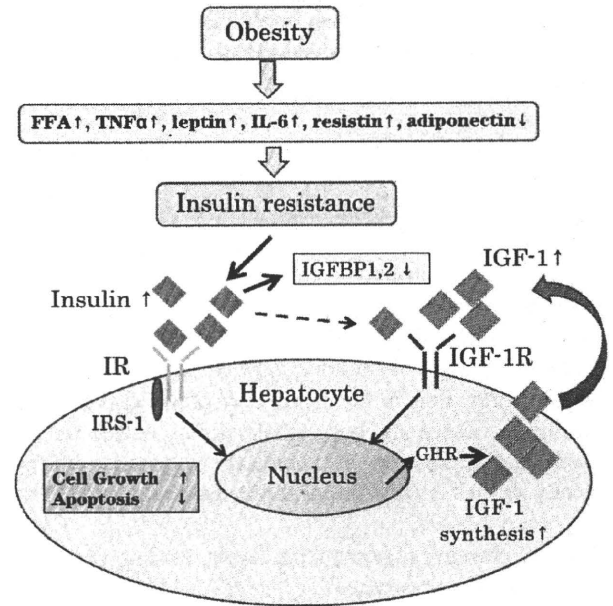
Fig. 2 Effect of obesity on cell growth and survival through insulin and IGF-1 signaling. Obesity is associated with increased release of FFA, multiple pro-inflammatory cytokines including TNFα, leptin, IL-6, resistin, reduced release of adiponectin (an anti-inflammatory polypeptide from adipose tissue), which gives rise to insulin resistance and compensatory hyperinsulinemia. Hyperinsulinemia, in turn, promotes the synthesis and biological activity of insulin-like growth factor I (IGF-1) through GH signaling, and reduces IGFBP1 and IGFBP2 levels, leading to an increase in bio-available IGF-1. Since considerable homology has been identified between insulin receptor and IGF-1 receptor, insulin can bind to IGF-1 receptor and enhance IGF-1 signaling. *IR* insulin receptor, *IGF-1* insulin-like growth factor 1, *IGF-1R* IGF-1 receptor, *GHR* growth hormone receptor, *IRS-1* insulin receptor substrate 1

proteins (IGFBPs). Over 80% of IGF-1 in the circulation is bound to IGFBP3, while the remainder of IGF-1 is bound to at least five IGFBPs. The actions of IGFBPs vary among their subtypes. Because IGFBP3, having anti-tumorigenic property, is up-regulated by GH signaling, hyperinsulinemia is often associated with higher levels of IGFBP3. Conversely, IGFBP1 and IGFBP2, which play an important role in regulating IGF-1 bioactivity, are suppressed by insulin. Hyperinsulinemia, thus reduces liver synthesis and blood levels of IGFBP1 and IGFBP2, leading to an increase in bio-available IGF-1. In accordance with this, an inverse relationship has been reported between cancer risk and blood levels of IGFBP1 and IGFBP2 [37].

Since considerable homology has been identified between insulin receptor and IGF-1R, insulin can bind to IGF-1R and enhance IGF-1 signaling. Provided that signaling through IGF-1R is more closely linked to growth promotion than through insulin receptor, enhancement of IGF-1R-mediated signaling by insulin would contribute greatly to cell proliferation and survival (Fig. 2).

Obesity and inflammation may promote hepatocarcinogenesis independently of their effects on insulin resistance

As described above, insulin resistance and its complementary hyperinsulinemia can promote hepatocarcinogenesis, and such an effect can interact with other growth-promoting signals.

Conversely, insulin resistance and hyperinsulinemia may be a consequence of other conditions that can themselves promote tumorigenesis by other pathways. In this context, two key conditions have been clarified: obesity and inflammation in the liver, both of which can induce insulin resistance and both of which may induce the initiation and promotion of hepatocarcinogenesis, independently of their effects on insulin resistance.

Obesity changes adipocytokine levels, leading to cell proliferation and survival

In obesity, adipose tissues increase the release of a variety of adipocytokines, including TNFα and IL-6, both of which have pro-oncogenic effects, leading to insulin resistance. Obesity is also associated with leptin resistance and hyperleptinemia. Leptin has been shown to have pro-oncogenic effects and enhance proliferation and angiogenesis [38]. In addition, obesity is associated with reduced levels of anti-inflammatory pro-apoptotic adiponectin. Adiponectin has an anti-proliferation effect through the activation of AMP-activated protein kinase (AMPK), and is inversely associated with insulin resistance. Taking these findings together, changes in adipocytokine levels associated with obesity would lead to cell proliferation and survival, independent of the effect on insulin resistance.

Hepatic steatosis induced by insulin resistance generates reactive oxygen species, accompanied by cytokine production

Obesity is often associated with hepatic steatosis through insulin resistance and hyperinsulinemia as increased levels of insulin and glucose enhance fatty acid and triglyceride (TG) synthesis. In addition, insulin resistance hampers the inhibitory action of insulin on hormone-dependent lipase, thus increasing TG hydrolysis and FFA release from the adipose tissue. Increased plasma FFA levels are associated with a higher hepatic uptake of FFA. An increase in hepatic uptake and synthesis of fatty acid (FA) is, in turn, compensated by a faster removal of fatty acids, which will take place via increased mitochondrial β-oxidation of FA. In accordance with increased levels of TNFα released from adipose tissue, increased β oxidation will enhance reactive oxygen species (ROS) production in the liver.

Consequently, ROS overproduction generates chemically reactive lipid peroxidation products such as 4-hydroxy-nonenal (4-HNE), and also increases the expression of cytokines in the liver, such as TNFα, transforming growth factor beta (TGFβ) and IL-8. Among them, TGF-β, IL-8 and 4-HNE are chemo-attractants for neutrophil, which may account, in part for neutrophil infiltration and inflammation in the liver [39].

### Inflammation in the liver generates ROS and RNS

Inflammation in the liver has been thought to contribute to the initiation and progression of HCCs. In this regard, oxidative stress that occurs through overproduction of ROS or reactive nitrogen species (RNS) is recognized as playing an important role in the initiation and promotion of carcinogenesis events [40, 41]. Oxidative stress generated not only by obesity but also HBV infection, HCV infection and alcohol, has emerged as a key player in the pathogenesis of chronic liver diseases and pre-cancerous lesions. Polymorphonuclear neutrophils (PMNs) infiltrated into the liver, produce and release a vast amount of oxidants [42], and the whole spectrum of oxidants generated by PMNs is due to the actions of four different enzymes. Among these enzymes, NADPH oxidase is the one by which oxidant generation is initiated. In addition, PMNs also produce RNS, which is generated by inducible nitric oxide synthase (iNOS). The four types of oxidants, including O_2^- and H_2O_2, constantly interact with each other, causing the formation of a myriad of oxidants among which the hydroxyl radical (OH·) is the most DNA-reactive compound [43]. Furthermore, non-parenchymal cells including Kupffer cells and macrophages, which can release pro-inflammatory cytokines, are another source to induce ROS in the liver [43].

Oxidative stress generates intracellular responses leading to carcinogenesis

Oxidative stress can react with a wide range of intracellular molecules, and cause cytostatic/cytotoxic damage to cellular DNA, protein and lipids [44] (Fig. 3).

Nuclear DNA damage

As described above, the hydroxyl radical (OH·) in particular has been shown to generate a number of oxidized DNA lesions. Recent attention has focused on the formation of 8-hydroxy deoxyguanosine (8-OHdG) in the DNA. This 8-OHdG lesion results in a site-specific mutagenesis that is widely found in mutated oncogenes and tumor suppressor genes [45]. Further support for the involvement
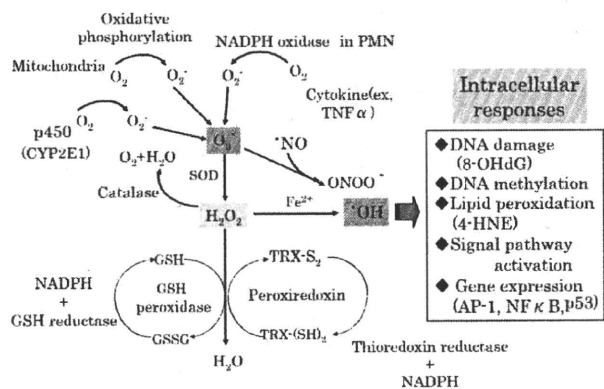
Fig. 3 Oxidative stress generates a variety of intracellular responses. Reactive oxygen species (ROS) encompass a variety of partially reduced metabolites of oxygen possessing higher reactivities than molecular oxygen, and are generated endogenously as a consequence of normal cell functions or derived from external sources. A number of anti-oxidant defense systems have evolved to combat the accumulation of ROS. These include enzymatic and non-enzymatic molecules. Oxidative stress can occur through overproduction of ROS or reactive nitrogen species (RNS), and interacts with a wide range of intracellular molecules. *CYP2E1* cytochrome p450 2E1, *SOD* super-oxide dismutase, *GSH* reduced glutathione, *GSSG* oxidized glutathione

Fig. 4 Interaction of insulin resistance induced by obesity and hepatocarcinogenesis. Insulin resistance can promote growth by the action that insulin may have, and also amplify growth by other growth factors, particularly IGF-1. Conversely, insulin resistance and hyperinsulinemia may be a consequence of other conditions, including obesity and inflammation in the liver, which can themselves promote tumorigenesis, mainly through cytokine production and/or generation of oxidative stress. Because insulin itself does not induce somatic mutations, intracellular responses to oxidative stress induced by inflammation and/or obesity, are indispensable for hepatocarcinogenesis
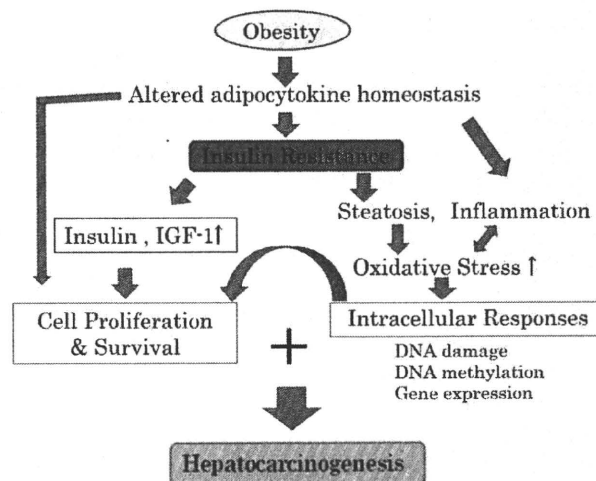
of 8-OHdG in carcinogenesis comes from studies showing that 8-OHdG produces dose-related increases in cellular transformation, which can be prevented by anti-oxidants [46].

DNA methylation

DNA methylation is an important regulator of gene expression with decreased methylation being associated with increased gene expression. In this context, many cancer cells have been shown to exhibit global hypomethylation of DNA compared with control cells. In particular, hypomethylation of tumor-promoting genes has been proposed as a possible mechanism for cancer development. On the contrary, hypermethylation of genes may inhibit transcription. Tumor suppressor genes are methylated, resulting in their inactivation [47]. ROS can modify DNA methylation and, in particular, oxidative DNA damage elicited by ROS can result in decreased DNA methylation. For instance, the formation of 8-OHdG in DNA can lead to hypomethylation. 8-OHdG formation can also interfere with the normal function of DNA methyltransferase and alter DNA methylation [48].

Signal pathway

MAPKs are divided into three subfamilies based on structural differences: the extra-cellular signal-regulated

kinases (ERK), the c-Jun N-terminal kinases (JNK) and the p38kinases (p38MAPK). The latter two are categorized as stress-activated protein kinases (SAPKs). The ERK pathway is mostly linked to the regulation of cell proliferation, while the SAPK pathways (JNK and p38MAPK) are more strongly linked to stress.

The SAPK pathways are noted for their activation by a wide range of stresses. For oxidative stress-induced activation of these pathways, change in the cellular redox state appears to be a key factor. Under normal condition, the redox regulatory protein thioredoxin (Trx) has been shown to bind and inhibit apoptosis signal-regulating kinase (ASK1), which is involved in both JNK and p38MAPK activation [49]; however, oxidative stress causes dissociation of the Trx-ASK1 complex, leading to activation of JNK and p38MAPK. As is the case with Trx, under nonstressed conditions, glutathione S-transferase (GST) binds to JNK and inhibits its activity, but this interaction is disrupted by oxidative stress [50].

Thus, oxidative stress may act at multiple levels in the SAPK pathways to modulate their activities. The influence of JNK activation on cell survival following oxidative stress is complex and controversial. Many studies have shown that JNK activation is correlated with cell death or apoptosis. The role of p38MAPK is also controversial; previous studies have yielded evidence for pro-apoptotic [51] as well as anti-apoptotic [52].

Gene expression

The most significant effects of oxidative stress on gene expression have been observed in the expression of transcriptional factors including activating protein-1 (AP-1) and nuclear factor-kappa B (NF-κB) [53]. Activation of these transcriptional factors is involved in both cell proliferation and apoptosis. The cellular redox state appears to influence the selective activation of these transcriptional factors and, therefore, may explain the observation that either cell death or cell proliferation might result from exposure to oxidative stress.

One of the target genes of AP-1 is cyclinD1, which supports the fact that AP-1 promotes entry into the cell division cycle [54]. AP-1 proteins also participate in oncogenic transformation through interaction with activated oncogenes [55].

On the other hand, the NF-κB family of transcriptional factors is composed of homodimers or heterodimers of Rel proteins [56]. Almost every step of the NF-κB signaling cascade consists of redox-sensitive proteins whose activities are modulated by oxidative stress [57]. Activation of NF-κB has been considered to be linked to carcinogenesis, because NF-κB regulates several genes involved in cell transformation, proliferation, angiogenesis and cell survival [58]. In this context, a large number of NF-κB target genes have anti-apoptotic functions. These include TNFα, TNF receptor-associated factor 1 (TRAF1), TRAFs, and cellular inhibitors of apoptosis proteins (CIAPs) [59]. NF-κB is also involved in regulating the expression of Bcl-Xl, an anti-apoptotic member of the Bcl-2 family.

Activation of p53 by oxidative stress can result in either growth arrest or apoptosis. Oxidative stress contributes to p53 activation through SAPK cascade. Downstream targets of p53 activation include p21/Waf1, GADD45, and 14-3-3, which are important in mediating G2/M arrest [60], while genes linked to apoptosis include Bax, a pro-apoptotic Bcl-2 family member, and Fas. P53 activation can also interfere with survival signals to render cells permissive to apoptosis [61].

Although the above events may be derived by different mechanisms, a common theme is the involvement of ROS in the development of HCCs. In particular, unrepaired damage to DNA may result in mutation, provided that cell replication ensues prior to repair of the modified bases. In addition to oxidative nuclear DNA damage, the formation of mitochondrial DNA damage and the mutation and alteration of mitochondrial genomic function have been proposed as contributing much to the process of carcinogenesis.

At least three distinct stages of the carcinogenesis process, including initiation, promotion and progression, have been identified. Apart from the role of oxidative stress in the induction of mutation, it is apparent that ROS and cellular redox state mediate cell signaling pathways that are involved in cell growth and survival, leading to promotion and progression of HCCs.

Conclusion

Insulin resistance and its complementary hyperinsulinemia can promote growth by insulin action, and also amplify growth by other growth factors, particularly IGF-1. Conversely, insulin resistance and hyperinsulinemia may be a consequence of other conditions, including obesity and inflammation in the liver, that can themselves promote tumorigenesis, mainly through cytokine production and/or generation of oxidative stress. Because insulin itself does not induce somatic mutations, intracellular responses to oxidative stress induced by inflammation and/or obesity, are indispensable for hepatocarcinogenesis. Thus, the above components need to work together to increase the cancer risk beyond that of the individual component alone (Fig. 4).

Metabolic syndrome has been considered as the association between obesity, insulin resistance and the risk of a variety of chronic diseases, including cancer. Because the trend for increasing obesity, which began in the West, is now spreading worldwide, insulin resistance is certain to be put forth as a central factor for hepatocarcinogenesis in the foreseeable future, not only in developed countries but also in developing countries.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Alpha-fetoprotein above normal levels as a risk factor for the development of hepatocellular carcinoma in patients infected with hepatitis C virus

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Abstract

Background Noninvasive risk factors are required for predicting the development of hepatocellular carcinoma (HCC) not only in patients with cirrhosis but also in those with chronic hepatitis who are infected with hepatitis C virus (HCV).

Methods A total of 707 patients with chronic HCV infection without other risks were evaluated for the predictive value of noninvasive risk factors for HCC, including age, sex, viral load, genotype, fibrosis stage, aspartate and alanine aminotransferase levels, bilirubin, albumin, platelet count, and alpha-fetoprotein (AFP) at entry to the study, as well as interferon (IFN) therapy they received.

Results The ten-year cumulative incidence rates of HCC for patients with fibrosis stages F0/F1, F2, F3, and F4 were 2.5, 12.8, 19.3, and 55.9%, respectively. Multivariate analysis identified age ≥57 years [hazard ratio (HR) 2.026, $P = 0.004$], fibrosis stage F4 (HR 3.957, $P < 0.001$), and AFP 6–20 ng/mL (HR 1.942, $P = 0.030$) and ≥20 ng/mL (HR 3.884, $P < 0.001$), as well as the response to IFN [relative risk (RR) 0.099, $P < 0.001$], as independent risk factors for the development of HCC. The ten-year cumulative incidence rates of HCC in the patients with AFP levels of <6, 6–20, and ≥20 ng/mL at entry were 6.0, 24.6, and 47.3%, respectively.

Conclusions Not only high (>20 ng/mL), but also even slightly elevated (6–20 ng/mL) AFP levels, could serve as a risk factor for HCC to complement the fibrosis stage. In contrast, AFP levels <6 ng/mL indicate a low risk of HCC development in patients infected with HCV, irrespective of the fibrosis stage.

Keywords Alpha-fetoprotein · Hepatitis C virus · Hepatocellular carcinoma

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Introduction

Worldwide, an estimated 170 million people are persistently infected with hepatitis C virus (HCV) [1, 2], and they are at high risk of developing hepatocellular carcinoma (HCC) [1, 3–5]. Several factors have been identified that increase the risk of HCC, including, age, male gender, and alcohol intake, as well as cirrhosis and the duration of infection [3, 5]. Of these factors, the stage of liver fibrosis parallels the risk for HCV-associated HCC. The annual incidence of HCC in patients with HCV-related cirrhosis ranges from 1 to 7% [6, 7]. Although liver biopsy is the gold standard for the assessment of hepatic fibrosis [8, 9], it is too invasive a procedure to be acceptable as a routine test [10, 11]. In place of liver biopsy, the platelet count is used to estimate the degree of fibrosis [12–14], and low platelet counts have been shown to be a risk factor for the development of HCC in cirrhotic patients [13, 15, 16]. In this study, we tried to identify noninvasive markers for predicting the development of HCC in a large cohort of

patients with chronic HCV infection during a long obser-. vation period.

Patients and methods

Study design

Between January 1992 and December 2003, 832 patients were identified who were positive for both anti-HCV, by a second or third-generation enzyme-linked immunosorbent assay (ELISA), and for HCV RNA by polymerase chain reaction (PCR). These patients underwent liver biopsy guided by ultrasonography (US) at the National Nagasaki Medical Center. Of the 832 patients, 125 (15.0%) were excluded according to the following criteria: (1) positive for hepatitis B surface antigen (HBsAg) ($n = 12$); (2) heavy habitual drinking defined as an average daily consumption of >100 g ethanol ($n = 26$); (3) presence of autoimmune hepatitis (AIH), primary biliary cirrhosis, or idiopathic portal hypertension ($n = 8$); (4) positive anti-nuclear antibody (defined as a titer of >320×) without a diagnosis of AIH ($n = 8$); or (5) a short follow-up period (<180 days) ($n = 71$). The remaining 707 patients were analyzed retrospectively for the incidence of HCC. Their medical histories had been recorded, with the results of routine tests for blood cell counts, liver biochemical parameters, and markers for HCV infection at the time of US-guided liver biopsy at regular intervals. Complete blood cell counts and biochemical tests were performed, using automated procedures, at the clinical pathology laboratories of the National Nagasaki Medical Center. Iinformed consent was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a-priori approval by the institution's human research committee.

Staging of hepatic fibrosis

Liver biopsy was taken by fine-needle aspiration (18G or 16G sonopsy) guided by US. Liver tissue specimens were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. They were evaluated for the stage of hepatic fibrosis by a pathologist according to the criteria of Desmet et al. [17].

HCV RNA, HCV core antigen, and HCV genotypes

HCV RNA was determined by reverse transcriptase (RT)-PCR using a commercial kit (Amplicor HCV; Roche Diagnostic Systems, Basel, Switzerland). HCV core antigen was determined using the lumispot EIKEN HCV antigen assay (Eiken Chemicals, Tokyo, Japan). HCV core antigen levels were classified as low or high with the cutoff at 1,000 fmol/L [18, 19]. Genotypes of HCV were determined by RT-PCR with genotype-specific primers (HCV RNA core genotype; Roche Diagnostics, Tokyo, Japan) [20, 21].

Interferon therapy

During the observation period, 373 of the 707 (52.8%) patients received interferon (IFN) monotherapy, pegylated (PEG)-IFN monotherapy, combination therapy with IFN and ribavirin, or PEG-IFN and ribavirin. Sustained virological response (SVR) was defined as the absence of detectable HCV RNA by the end of treatment that persisted for longer than 6 months thereafter, while failure in meeting these criteria was judged as non-SVR. There was no relapse of viremia after 6 months among SVR patients.

Diagnosis of hepatocellular carcinoma

Patients were followed up with hematological and biochemical tests at intervals of 1–12 months. Liver imaging was performed by US at 6- to 12-month intervals in most patients at fibrosis stages F0–F2, while computed tomography (CT), magnetic resonance imaging (MRI), or US was performed at 3- to 6-month intervals in patients at fibrosis stages F3 and F4. HCC was diagnosed by typical vascular patterns on CT, MRI, or angiography, or by fine-needle biopsy of space-occupying lesions detected in the liver.

Statistical analysis

Continuous variables [platelet counts, albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alpha-fetoprotein (AFP), HCV core antigen] were dichotomized with respect to the median value or clinically meaningful values in a multivariate analysis. To estimate the cumulative risk of developing HCC, the Kaplan–Meier method and the log-rank test were used. Cox proportional hazards regression analysis was performed to evaluate risk factors for HCC. Analysis was performed by Bonferroni's correction and data analysis was performed with SPSS ver. 11.0 (SPSS, Chicago, IL, USA).

Results

Characteristics at enrollment

Table 1 lists the characteristics of the 707 patients at enrollment. The median age was 57.0 years; 120 (17.0%)

Table 1 Demographic, clinical, and virological characteristics of 707 patients persistently infected with hepatitis C virus (HCV)

Age (years)	57.0 (19–79)
Male	351 (49.6%)
Observation period (years)	8.2 ± 4.4[a]
Interferon therapy	373 (52.8%)
Habitual alcohol intake	135 (19.1%)
Fibrosis stage	
F0/F1	273 (38.6%)
F2	193 (27.3%)
F3	121 (17.1%)
F4	120 (17.0%)
Platelet count ($\times 10^3/mm^3$)	156 (30–391)
Albumin (g/dL)	4.2 (2.7–5.3)
Total bilirubin (mg/dL)	0.7 (0.1–2.5)
Aspartate aminotransferase (AST; IU/L)	53 (11–422)
Alanine aminotransferase (ALT; IU/L)	82 (1–1,057)
Alpha-fetoprotein (AFP; ng/mL)	6 (1–510)
HCV core antigen	
≥1,000 fmol/L	539 (76.2%)
HCV genotype	
1b	510 (72.1%)
2a/2b	195 (27.6%)
Unknown	2 (0.3%)

Values are medians with ranges in parentheses, or means with SD in parentheses

[a] Mean ± SD

patients were diagnosed histologically with liver cirrhosis (fibrosis stage: F4) and the remaining 587 had chronic hepatitis (fibrosis stage F0, F1, F2, or F3). The median value of AFP was 6 ng/mL. The average follow-up period was 8.2 years. The patients were classified into three categories by the level of AFP; 350 patients (49.5%) had AFP levels of <6 ng/mL, 254 (35.9%) had levels between 6 and 20 ng/mL, and the remaining 103 (14.6%) had levels of ≥20 ng/mL.

IFN therapy and IFN response

Of the 120 patients with cirrhosis (fibrosis stage F4), 46 (38.3%) received IFN while the remaining 74 (61.7%) did not. The proportions of IFN-treated patients showing an SVR were 40.8% (56/137) in patients with F1; 37.6% (44/117) in those with F2; 32.8% (24/73) in those with F3; and 32.6% (15/46) in those with F4.

Risk factors for HCC

Cox regression analysis was performed on several variables, including age, sex, alcohol consumption, IFN therapy during the observation period, and biochemical as well as virological parameters. The following factors were identified as showing an increased risk for HCC by the univariate analysis: age; IFN therapy; fibrosis stage; platelet count; albumin; AST, ALT, and AFP levels; and HCV genotype (Table 2). Multivariate analysis was performed on these factors (Table 3), and the following were identified as independent risk factors: fibrosis stage (F4), AFP (6–20 and ≥20 ng/mL), age (≥57 years), and IFN therapy (SVR).

Development of HCC

During the follow-up period, HCC developed in 110 (15.6%) patients. Of the 110 patients with HCC, 58 (52.7%) were diagnosed with the disease by histological examination of biopsy-obtained or resected liver specimens. Of these 58 patients, 24 (41.3%) had hypovascular HCC.

Among the patients with HCC, only eight (7.2%) had AFP <6 ng/mL at the time of diagnosis of HCC. Figure 1 shows Kaplan–Meier estimates of the cumulative risk of HCC with respect to fibrosis stage at entry. The 10-year cumulative incidence rates of HCC for stages F0/F1, F2, F3, and F4 were 2.5, 12.8, 19.3, and 55.9%, respectively.

There were significant differences in cumulative incidence rates among the three groups of patients with different AFP levels. The 10-year cumulative risk of HCC was 6.0% in the 350 patients with AFP <6 ng/mL at the study entry, 24.6% in the 254 patients with AFP 6–20 ng/mL, and 47.3% in the 103 patients with AFP ≥20 ng/mL ($P < 0.001$) (Fig. 2). Of the 350 patients with AFP <6 ng/mL, 21 eventually developed HCC during the observation period. Fourteen of these 21 patients were ≥57 years old and 10 had fibrosis stage F3 or F4. In remarkable contrast, HCC ultimately developed in 84.5% of the patients with AFP ≥20 ng/mL.

The 10-year cumulative incidence rates of HCC were 3.1% in patients with SVR to IFN, 14.6% in patients with non-SVR, and 29.5% in the patients without IFN therapy (Fig. 3). Of the 139 patients with SVR, three (2.2%) eventually developed HCC during the observation period. These three patients had advanced fibrosis stages at the study entry (1 with F3 and 2 with F4). Figure 4 shows the cumulative incidence of HCC in the patients with different AFP levels, stratified by the fibrosis stage. In the patients with fibrosis stage F4, there were significant differences in HCC incidence between those with AFP levels of <6 and those with levels of ≥20 ng/mL.

Figure 5 shows the proportions of patients with different AFP levels stratified by the fibrosis stage. The proportion of patients with AFP <6 ng/mL decreased with the advance of fibrosis stage, and conversely, the proportion of patients with AFP ≥20 ng/mL increased with the advance of fibrosis stage. There was a strong correlation between AFP levels and the fibrosis stage.

Table 2 Factors increasing the risk for hepatocellular carcinoma (HCC), determined by univariate analysis

Features	Hazard ratio	P value
Age		
<57 years	1	
≥57 years	3.889	<0.001
Sex		
Female	1	
Male	1.146	0.475
Alcohol intake		
None	1	
Habitual	1.012	0.962
Interferon therapy		
None	1	
Non-SVR	0.523	0.002
SVR	0.063	<0.001
Fibrosis stage		
F0/F1	1	
F2	1.863	0.096
F3	3.985	<0.001
F4	13.045	<0.001
Platelet count		
≥150 × 10^3/mm^3	1	
<150 × 10^3/mm^3	4.644	<0.001
Albumin		
≥4.2 g/dL	1	
<4.2 g/dL	2.952	<0.001
Total bilirubin		
<0.7 mg/dL	1	
≥0.7 mg/dL	1.438	0.065
AST		
<53 IU/L	1	
≥53 IU/L	2.501	<0.001
ALT		
<82 IU/L	1	
≥82 IU/L	1.514	0.035
AFP		
<6 ng/mL	1	
6–20 ng/mL	4.628	<0.001
≥20 ng/mL	10.335	<0.001
HCV core antigen		
<1,000 fmol/L	1	
≥1,000 fmol/L	1.112	0.645
HCV genotype		
2a/2b	1	
1b	1.730	0.027

SVR sustained virological response

Table 3 Factors increasing the risk for HCC, determined by multivariate analysis

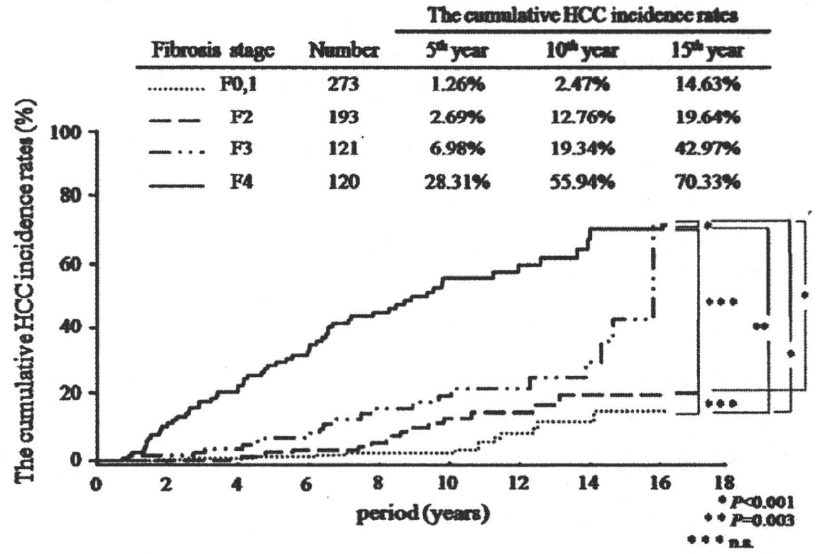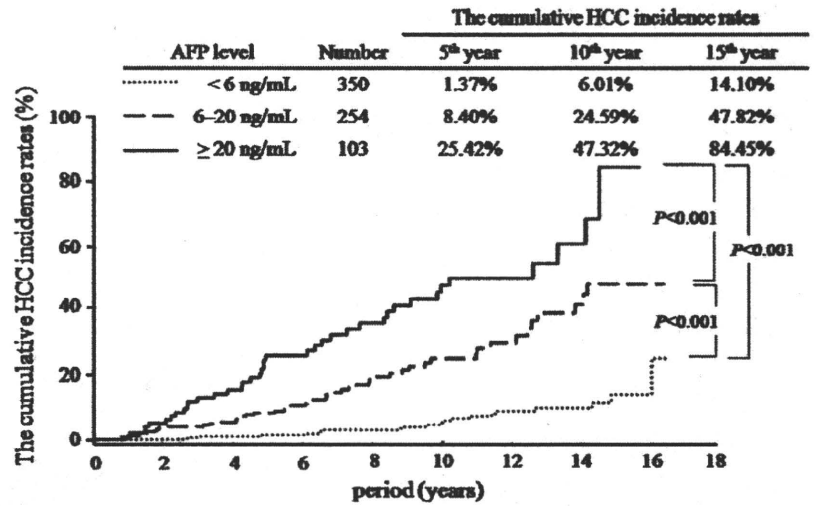| Features	Hazard ratio (95% CI)	P value
Fibrosis stage		
F0/F1	1	
F2	1.030 (0.471–2.253)	0.942
F3	1.682 (0.632–3.713)	0.198
F4	3.957 (1.861–8.411)	<0.001
AFP		
<6 ng/mL	1	
6–20 ng/mL	1.942 (1.066–3.538)	0.030
≥20 ng/mL	3.884 (2.014–7.433)	<0.001
Age		
<57 years	1	
≥57 years	2.026 (1.261–3.255)	0.004
Interferon therapy		
None	1	
Non-SVR	0.704 (0.453–1.094)	0.119
SVR	0.099 (0.029–0.334)	<0.001

CI confidence interval

Discussion

In the present study, four variables were identified as risk factors for HCC in patients with chronic HCV infection: fibrosis stage, AFP level, age, and IFN therapy. Previous reviews have analyzed risk factors for the development of HCC [3, 22–25]. Yoshida et al. [6] have reported that the annual incidence increases with the stage of liver fibrosis, from 0.5% in patients with stage F0 or F1 to 7.9% in patients with stage F4 (cirrhosis). In our study, the cumulative incidence of HCC increased along with the advance of fibrosis stage. AFP is used as a serological marker of HCC, and is employed in combination with US for screening HCC [3]. Several reports have shown an elevated AFP level as a risk factor for the development of HCC among patients infected with HCV [16, 25–32]. Most of the studied patients had cirrhosis that was not definitely diagnosed by clinical symptoms and ultrasonographic findings. There have been few studies on patients with chronic hepatitis C, in addition to those with cirrhosis [27]. Thus, it has been unclear whether elevated AFP levels are a risk factor for the development of HCC in patients infected with HCV. Against this background, we were prompted to analyze the utility of AFP as a risk factor for the development of HCC in patients who had been histologically diagnosed by US-guided liver biopsy. In the present study,
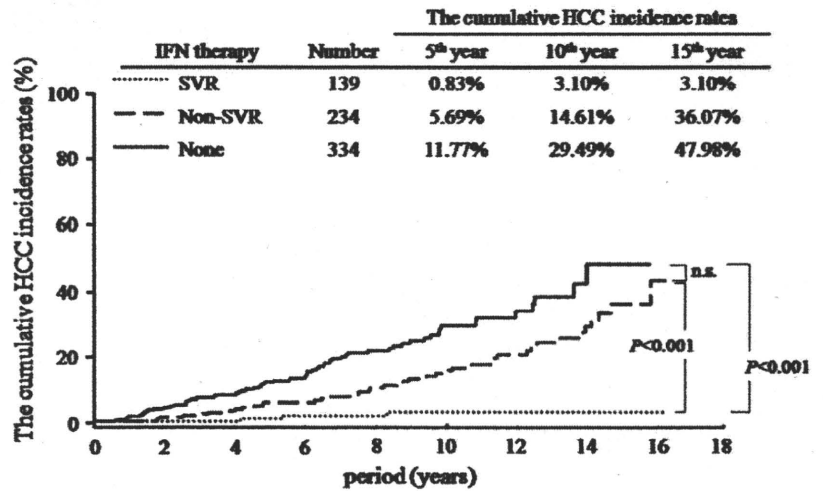
Fig. 1 Cumulative incidence of hepatocellular carcinoma (*HCC*) according to the fibrosis stage

Fibrosis stage	Number	The cumulative HCC incidence rates		
		5th year	10th year	15th year
F0,1	273	1.26%	2.47%	14.63%
F2	193	2.69%	12.76%	19.64%
F3	121	6.98%	19.34%	42.97%
F4	120	28.31%	55.94%	70.33%

* *P* <0.001
** *P* =0.003
*** n.s.

Fig. 2 Cumulative incidence of HCC according to alpha-fetoprotein (*AFP*) levels

AFP level	Number	The cumulative HCC incidence rates		
		5th year	10th year	15th year
<6 ng/mL	350	1.37%	6.01%	14.10%
6–20 ng/mL	254	8.40%	24.59%	47.82%
≥20 ng/mL	103	25.42%	47.32%	84.45%

P <0.001
P <0.001
P <0.001

Fig. 3 Cumulative incidence of HCC according to interferon (*IFN*) therapy. *SVR* Sustained virological response

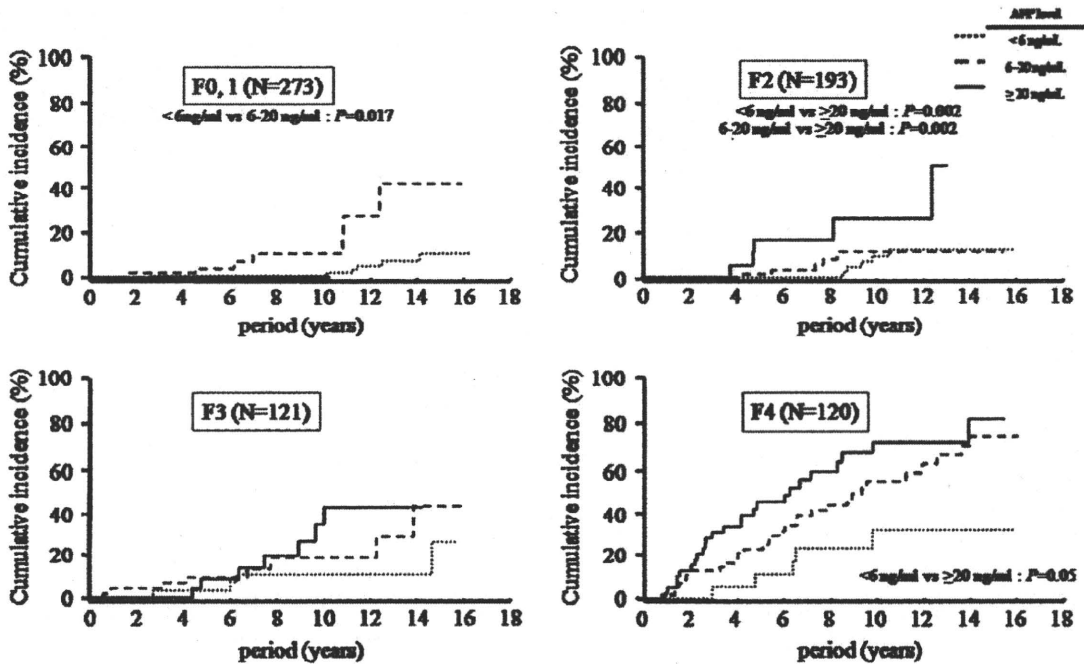| IFN therapy	Number	The cumulative HCC incidence rates		
		5th year	10th year	15th year
SVR	139	0.83%	3.10%	3.10%
Non-SVR	234	5.69%	14.61%	36.07%
None	334	11.77%	29.49%	47.98%

n.s.
P <0.001
P <0.001

Fig. 4 Cumulative incidence of HCC according to AFP levels, stratified by the fibrosis stage

Fig. 5 Proportions of patients with three different AFP levels (<6 ng/mL, 6–20 ng/mL, and ≥20 ng/mL) at different fibrosis stages
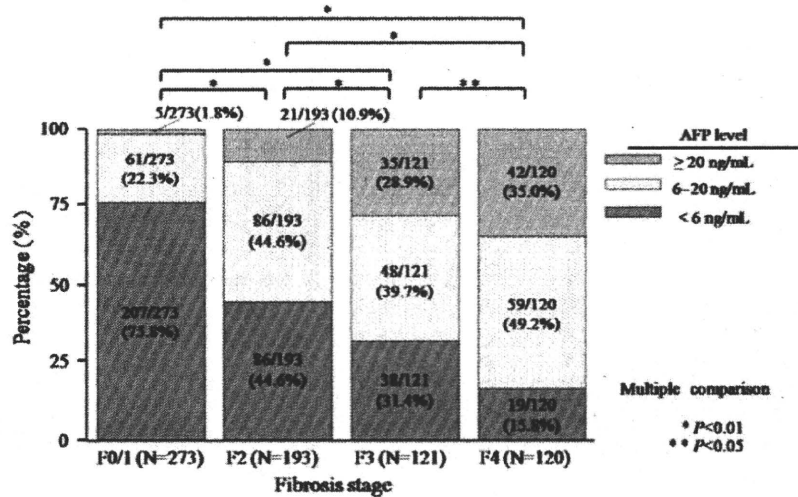
among patients infected with HCV, including not only those with cirrhosis but also those with chronic hepatitis, we found AFP levels to be a dependable risk factor for HCC, in addition to the fibrosis stage. Of particular note, not only the patients with high AFP levels (≥20 ng/mL) but also those with even slightly elevated AFP levels (between 6 and 20 ng/mL) had increased risks for the development of HCC. In the patients in this study, the median AFP level was 6 ng/mL. It deviated slightly from serum levels of AFP in healthy adults that have been reported to range from 0.1 to 5.8 ng/mL [33]. Hence, we performed analyses by setting various AFP cutoff levels for

evaluating their performance as risk factors. However, there were no significant differences in the analysis with the use of AFP cutoff levels exceeding 7 ng/mL. On the basis of these observations, an AFP cutoff level of 6 ng/mL was adopted in this study. In previous reports, AFP levels were associated with advanced fibrosis stage in patients infected with HCV in the absence of HCC [34–38]. In the present study, AFP levels were elevated in parallel with advanced fibrosis stages and correlated well with the fibrosis stage. As the patients with even slightly elevated AFP levels, between 6 and 20 ng/mL, had moderately advanced liver fibrosis stages, these AFP levels could

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indicate an elevated risk for HCC in patients with chronic HCV infection.

Hu et al. [36] found that an AFP level of 15.0 mg/mL could detect severe fibrosis with a sensitivity of 22.8% and specificity of 94.5%. Moreover, they reported, during observation for 6 months of patients with chronic hepatitis C, that AFP levels stayed within the normal range (<10 ng/mL) in 60%, were persistently elevated in 24%, and fluctuated in the remaining 15%. By multivariate analysis, they identified AST, INR, and fibrosis as risk factors for AFP levels of >10 ng/mL. In view of the correlation between AFP levels and fibrosis stages, the AFP level at the time of liver biopsy was taken into account in the analysis in the present study; ALT levels are reported to be persistently elevated in the majority (60%) of patients with chronic hepatitis C.

Liver biopsy is the gold standard for assessing hepatic fibrosis [8, 9]. However, the needle liver biopsy has a sampling error and is too invasive as a routine procedure [10, 11]. Therefore, AFP levels may be used as a noninvasive and predictive marker in place of the fibrosis stage. The platelet count is known to reflect the severity of chronic hepatitis C [12, 13], and is used to estimate the degree of fibrosis without resort to liver biopsy [12–14]. Previous reports have shown low platelet counts to represent a risk factor for HCC in cirrhotic patients [13, 15, 16]. Matsumura et al. [13] reported that age and serum platelet count were significant risk factors for the development of HCC, and as such, they were a major clinico-laboratory means of evaluating the fibrosis stage. In the present study, however, the platelet count was not an independent risk factor for HCC development. When Cox regression analysis was performed on variables other than the fibrosis stage, platelet count and serum albumin levels were identified as independent risk factors for the development of HCC (data not shown).

IFN has been used to treat patients with HCV infection. Failure to achieve an SVR to IFN-based therapies, and preexisting advanced hepatic fibrosis and/or cirrhosis, are major predictors of HCC [6, 23, 25, 39, 40]. In the present study, SVR emerged as an independent risk factor for the development of HCC, while non-SVR was not. However, the cumulative incidence rate of HCC in patients with non-SVR was lower than that in those without IFN therapy. These results suggest that the use of IFN in patients with HCV-related liver disease may be beneficial in preventing the development of HCC. Several Japanese cohort studies have demonstrated that IFN therapy reduces the incidence of HCC, not only in sustained virological responders but also in transient responders who have failed to eliminate HCV [6, 41–45]. In cirrhotic patients, Nishiguchi et al. [39] reported that the relative risk of patients with IFN-alpha treatment developing HCC was 0.067 in comparison with the control

group. In contrast, Valla et al. [46] could not prove any significant benefit for the prevention of HCC between patients with and without IFN treatment. Camma et al. [47] suggested a slight preventive effect of IFN on HCC development in patients with HCV-related cirrhosis. Shiffman et al. [48] have reported that continuous IFN therapy led to a decline in hepatic fibrosis despite the persistence of viremia. In addition, there are case reports that IFN therapy reduced AFP levels in virological nonresponders [49]. Murashima et al. [50] showed that IFN therapy, but not Strong Neo-Minophagen C (SNMC) (Glycyrrhizin, Tokyo, Japan), universally reduced basic AFP levels. In an in vitro study of the effects of IFN on an HCC cell line, IFN exhibited antitumor effects [51]. Taken together, these findings suggest that AFP levels may be useful for predicting the development of HCC during IFN-based treatments, including long-term low-dose IFN therapy.

There have been several reports on the relationship between chronological trends in platelet counts, AST or AFP levels, and the development of HCC [11, 26, 27, 52–54]. Tarao et al. [52, 53] showed that in patients with HCV-related cirrhosis, those with persistently high serum ALT levels had a high risk of developing HCC and multicentric carcinogenesis, whereas those with persistently low ALT levels faced a very low risk. Likewise, the dynamics of AFP levels in patients with chronic HCV infection may be useful to estimate the risk of developing HCC. Recently, Bruce et al. [32] found serial measurements of AFP helpful in identifying persons with advanced fibrosis. They used an AFP level of 8 ng/mL, the test manufacturer's upper limit of normal, as the evaluation of the risk of development of HCC. It is not certain whether or not AFP would be a risk factor of HCC development in patients with chronic liver disease of etiologies other than persistent HCV infection. Velazquez et al. [55] reported that an AFP level of >5 ng/mL at study entry was associated with the development of HCC in their univariate analysis but not in their multivariate analysis. They speculated that this could have been because the main causative factor of liver cirrhosis in their series was alcohol. Taken together, the findings of various studies suggest that the baseline AFP level may be more reliable as a predictive factor for the development of HCC in patients with HCV-related liver disease than in those with liver disease of other etiologies.

In conclusion, AFP is a noninvasive predictive marker for the development of HCC in patients infected with HCV. The present study indicates that not only high AFP levels (≥20 ng/mL) but also slightly elevated AFP levels, between 6 and 20 ng/mL, could indicate substantial risks for the development of HCC, complementing the fibrosis stage. In contrast, AFP levels of <6 ng/mL indicate a low risk of HCC development, irrespective of the liver fibrosis stage. IFN therapy significantly reduces the risk of the

development of HCC, especially in patients with an SVR to the therapy.

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Pilot Study of Systemic Combination Therapy with S-1, an Oral Fluoropyrimidine, and Cisplatin for Hepatocellular Carcinoma with Extrahepatic Metastases.

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KEY WORDS:
Hepatocel-
lular carcinoma;
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metastases; S-1;
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ABBREVIATIONS:
Cisplatin (CDDP);
Hepatocellular
Carcinoma (HCC);
Complete Response
(CR), Partial
Response (PR);
Stable Disease (SD);
Progressive Disease
(PD); Computed
Tomography (CT);
Magnetic Resonance
Imaging (MRI); Radi-
ofrequency Ablation
(RFA); Transcatheter
Arterial Chemoem-
bolization (TACE);
Radiotherapy (RT);
Hepatic Arterial Infu-
sion Chemotherapy
(HAIC); Portal Vein
Tumor Thrombosis
(PVTT); 5-fluor-
ouracil (5-FU);
Interferon (IFN);
5-chloro-2,4-
dihydroxypyridine
(CDHP); Potassium
Oxonate (Oxo); Di-
hydropyrimidine
Dehydrogenase
(DPD); Thymidylate
Synthase (TS);
5-FU-derived 5-
fluoro-2'-deoxyu-
ridine-5'-mono-
phosphate (FdUMP);
Performance Status
(PS); -fetoprotein
(AFP); Lens Culinaris
Agglutininreac-
tive Fraction of AFP
(AFP-L3) ; des-
carboxy prothrombin
(DCP); National
Cancer Institute
Common Toxicity
Criteria (NCI-CTC)

ABSTRACT

Background/Aims: The aim of this pilot study was to elucidate the efficacy and safety of systemic combination therapy with S-1 and cisplatin (CDDP) for hepatocellular carcinoma (HCC) patients with extrahepatic metastases.
Methodology: Sixteen patients were enrolled in this pilot study. Two weeks of combination therapy represented one cycle, followed by two-to-four weeks rest. In each cycle, S-1 was administrated orally at 80-120 mg (depending on body surface area) every day and cisplatin was administrated intravenously at 60 mg/m² on day 8. Response, overall survival and adverse effects were assessed.
Result. No patient had intrahepatic HCC and all patients had a class A Child-Pugh score. Regarding overall response, 2 (13%), 0 (0%), 5 (31%), and 9 (56%) patients showed complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD), respectively, giving an overall response rate of 13% (2/16). The overall survival rate at 12 months was 77%. With regard to NCI-CTC grade-3 adverse reactions, 2 (13%), 2 (13%), and 6 (38%) patients developed nausea, anorexia, and neutropenia, respectively. No grade-4 adverse reaction or toxicity-related death occurred.
Conclusion. S-1/CDDP is a potentially safe and effective combination therapy for HCC patients with extrahepatic metastases.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers and causes of cancer death worldwide (1-3). Development of new diagnostic techniques, such as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and angiography, and advancements in therapeutic modalities such as surgical resection, radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE), radiotherapy (RT), and chemotherapy by intra-arterial infusion via implantable drug delivery systems have gradually improved the prognosis of HCC patients (4-10). Nevertheless, the prognosis for advanced HCC patients with extrahepatic metastases remains poor (11-14). Several investigators have suggested the use of combination systemic chemotherapies such as 5-fluorouracil (5-FU)/cisplatin (CDDP), 5-FU with mitoxantrone and CDDP, tegafur-uracil /interferon (IFN), and 5-FU/IFN for advanced HCC with extrahepatic metastases (15-18). Of note, every regimen is based on fluoropyrimidine, with concomi-

tant IFN or CDDP playing synergistic roles as modulators of fluoropyrimidine. Recently, we and other investigators reported the efficacy of combination chemotherapy with S-1/IFN for HCC with extrahepatic metastases (19, 20). S-1 consists of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo), and has greater efficacy and fewer side effects than 5-FU (21). Meanwhile, the efficacy of 5-FU/CDDP via hepatic arterial infusion chemotherapy (HAIC) has been reported for advanced HCC with portal vein tumor thrombosis (PVTT) (22-24), and it is therefore possible that systemic combination therapy with S-1 and CDDP (S-1/CDDP) is effective against HCC with extrahepatic metastases. In the present study, we assessed the efficacy and safety of S-1/CDDP for HCC patients with extrahepatic metastases.

METHODOLOGY

Study design and eligibility

This pilot study had the following enrolment criteria: HCC with extrahepatic metastases; ab-

sence of intrahepatic HCC; no HCC vascular invasion; age 20 years or older; Eastern Cooperative Oncology Group performance status (PS) 0 or 1 (25); Child-Pugh class A; serum total bilirubin <2.0 mg/dL; leukocyte count >3,000/mL; platelet count >50,000/mL; serum creatinine <1.2 mg/dL; at least 4 weeks since any previous treatment for HCC; no recent history of upper gastrointestinal bleeding; no history of heavy alcohol abuse; and no other serious medical conditions that would interfere with participation in this study. All patients provided written informed consent and the study was approved by the Institutional Review Board of Hiroshima University.

Diagnosis of hepatocellular carcinoma

Extrahepatic metastases were diagnosed by one or a combination of CT, MRI, bone scintigraphy, X-ray, or positron emission tomography with ^{18}F-fluorodeoxyglucose. Further, α-fetoprotein (AFP), lens culinaris agglutininreactive fraction of AFP (AFP-L3) and des-γ-carboxy prothrombin (DCP) were measured. We excluded other malignancies (e.g. gastric cancer, colon cancer, lung cancer, etc.) by one or a combination of various imaging modalities, serological tumor markers, or pathological examination. First, upper and lower gastrointestinal endoscopies were performed; then, several tumor markers (e.g. carcinoembryonic antigen, carbohydrate antigen 19-9, cytokeratin 19 fragment antigen 21-1, pro-gastrin-releasing peptide, etc.) were checked; and lastly, pathological examination was performed where possible.

Treatment protocol

Two weeks represented one cycle of treatment.

In each cycle, S-1 was administered orally at a dose of 80-120 mg daily (depending on body surface area: <1.25 m², 80 mg; 1.25-1.5 m², 100mg; ≥1.5 m², 120 mg), and CDDP was administered intravenously at a dose of 60 mg/m² on day 8. Each treatment cycle was followed by a two-to-four-week rest period of no treatment, determined by the time taken to recover from adverse reactions as assessed by the National Cancer Institute Common Toxicity Criteria (NCI-CTC) (version 3.0), with the next treatment undertaken once the NCI-CTC adverse reaction grade improved to 0 or 1. When complete response (CR) was observed, S1/CDDP was finished. When partial response (PR) was observed, S1/CDDP was repeated over several cycles. When stable disease (SD) or progressive disease (PD) was observed after more than two course of S1/CDDP, S1/CDDP was finished and other therapeutic modalities such as surgery, TACE, RT, another systemic chemotherapy or symptomatic treatment were considered.

Evaluation

Response of HCC patients with extrahepatic metastases to S1/CDDP combination therapy was assessed by contrast-enhanced CT after two courses and then every three months, with all patients assessed within two-to-three months of commencing therapy. In addition to overall response and response of extrahepatic metastases were also separately assessed. These responses were defined according to the Response Evaluation Criteria in Solid Tumors (RECIST) (26). CR was defined as complete disappearance of all target/non-target lesions and no appearance of other lesions, with confirmation of CR performed four weeks after

TABLE 1 Baseline Characteristics of HCC Patients with Extrahepatic Metastases

case	Age	Gender	PS	Etiology	Child-pugh score (point)	previous treatment	site of extrahepatic metastases
1	76	F	0	HCV	5	surgery	lymph node
2	61	M	0	HBV	5	surgery	lung
3	62	M	0	NBNC	5	surgery	lung
4	78	M	0	HCV	6	surgery	lung, adrenal gland, muscle
5	70	M	0	HCV	6	TACE	lymph node, peritoneal
6	78	M	0	HCV	6	TACE	lung, lymph node
7	71	M	0	HCV	5	RFA	lung
8	64	M	0	HCV	6	surgery	lung
9	67	F	1	HCV	6	surgery	lung
10	87	M	1	NBNC	5	TACE	lung
11	77	M	0	HCV	5	surgery	lymph node
12	70	M	0	HCV	5	surgery	lung
13	74	F	1	NBNC	6	HAIC	lung, lymph node
14	60	M	0	HBV	5	surgery	lung
15	59	M	1	NBNC	6	surgery	lung, bone, lymph node
16	69	M	1	NBNC	5	surgery	lung

the first evaluation and normalization of AFP and DCP also needed for confirmation. PR was defined as at least a 30% decrease in the sum of each target lesion's longest diameter with this sum at baseline as the reference. PD was defined as at least a 20% increase in the sum of each target lesion's longest diameter, and SD was defined as meeting neither PR nor PD criteria. Adverse reactions were assessed every week during treatment using the NCI-CTC.

Statistical analysis

Statistical analysis was performed on 1 December 2009 using the SPSS program (version 11, SPSS Inc., Chicago, IL). Cumulative survival rate was calculated from the commencement of combination therapy and assessed by the Kaplan-Meier life-table method, with differences evaluated by the log rank test. Statistical significance was defined as a p value less than 0.05. In this study we assessed response, survival, and safety of S-1/CDDP combination therapy for HCC patients with extrahepatic metastases.

RESULTS

Patients

From January 2008 to July 2009, 16 patients with extrahepatic HCC metastases were enrolled in the pilot study, and their baseline characteristics are listed in **Table 1**. The median age was 70 years (range, 59-87). A total of 11 patients had received previous hepatectomy with curative intent for HCC, 3 had received previous TACE, 1 had received previous RFA, and 1 had received previous HAIC. When these therapies were performed, all patients did not have extrahepatic metastases. A total of 13 patients had HCC with pulmonary metastases, 7 had lymph node metastases, 1 had bone metastases, 1 had peritoneal metastases, 1 had adrenal gland metastasis, and 1 had muscle metastasis, with 5 patients having multiple organ metastases. Three patients received pathological diagnosis of extrtahepatic metastases. None had intrahepatic HCC.

Response

A total of 14 patients were treated with 2 courses and 2 with 3 courses. The clinical outcomes are listed in **Table 2**. With regard to overall response, 2 (13%), 0 (0%), 5 (31%), and 9 (56%) patients showed a CR, PR, SD, and PD, respectively, giving an objective response (CR+PR) of 13% (2/16). With regard to response of extrahepatic HCC, 2 (13%), 1 (6%), 5 (31%), and 8 (50%) patients showed a CR, PR, SD and PD, respectively, giving an objective response rate for extrahepatic metastases of 19% (3/16). Although one patient (patient no.5, **table 2**) showed PR with regard to extrahepatic HCC, the patient showed recurrence of intrahepatic HCC. Thus, overall response of the patient was PD.

Survival

The cumulative survival rates at 6, 12 and 18 months were 86%, 77% and 61%, respectively (**Figure 1**). The cumulative survival rate of patients who showed CR at 18 months was 100%, while the cumulative survival rates of patients who showed SD/PD at 6, 12 and 18 months were 84%, 72% and 48%, respectively. There was no significant difference between the cumulative survival rates of patients showing CR and those showing SD/PD (p = 0.2625) (**Figure 2**). At the time of analysis, 12 patients were still alive and 4 had died from their disease, with 2 dying of respiratory failure secondary to progression of pulmonary HCC metastases, 1 dying of recurrence of intrahepatic HCC, and 1 dying of brain hemorrhage by rupture of brain metastases. The brain metastases had not been observed at the start of S1/CDDP.

FIGURE 1 Cumulative survival rates of HCC patients treated with systemic S-1 and cisplatin combination chemotherapy.
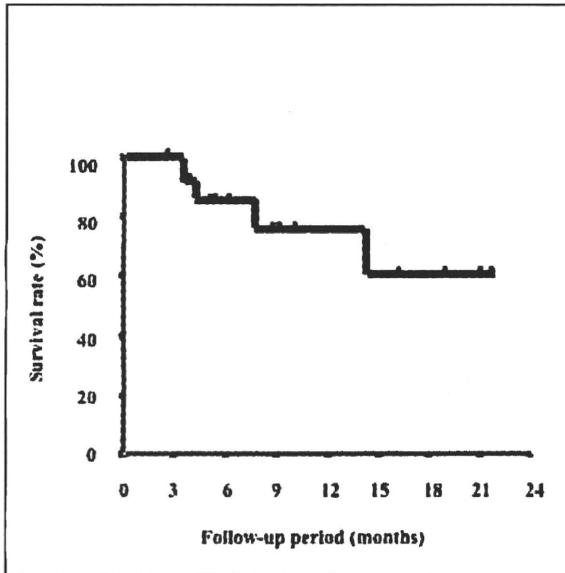
FIGURE 2 Comparison of the cumulative survival rates among patients showing CR with that of those showing SD/PD. No significant difference was observed (P = 0.2625).
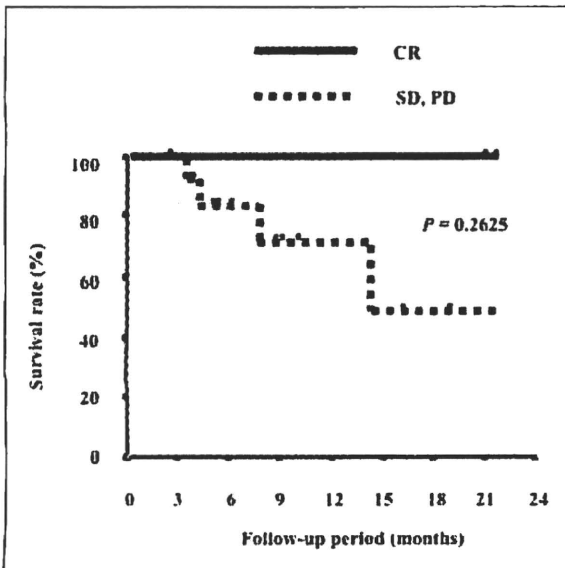
TABLE 2. Clinical Outcomes of HCC Patients with Extrahepatic Metastases Trated with S-1/CDDP Combination Therapy

Case	Number of treatment cycle	Response overall	extrahepatic metastases	AFP (ng/ml)		AFP-L3 (%)		DCP (mAU/ml)		adjuvant therapy	survival period (months)	out come
				pre	post	pre	post	pre	post			
1	3	CR	CR	37.2	10.4	52.4	<0.5	21	19	-	22	alive
2	2	CR	CR	2960	7.5	40	<0.5	19	26	-	21	alive
3	2	PD	PD	145.6	244.7	<0.5	<0.5	30	27	RT for brain metastases	19	alive
4	3	SD	SD	6.1	5.7	<0.5	<0.5	5449	1080	RT for muscle metastases	16	alive
5	2	PD	PR	1969	1953	21.6	28.8	4556	1778	S1/IFN, TACE	14	dead
6	2	PD	PD	49.7	20	24.6	23.2	59	100	S1/IFN	10	alive
7	2	PD	PD	139.2	206.3	<0.5	<0.5	2594	3556	surgery for lung metastases	9	alive
8	2	SD	SD	795.4	788	67	66.2	1869	2116	HAIC	9	alive
9	2	PD	PD	105.6	192	24.2	22.9	26	14	S1/IFN	8	dead
10	2	PD	PD	<5	<5	<0.5	<0.5	7327	4284	-	6	alive
11	2	SD	SD	24	26.5	<0.5	<0.5	2761	1784	surgery for lung metastases	5	alive
12	2	SD	SD	10.4	7.1	<0.5	<0.5	2024	1967	S1/IFN	5	alive
13	2	PD	PD	56300	46480	32.7	37.9	194400	129300	-	4	dead
14	2	SD	SD	12.3	11.4	<0.5	<0.5	87	132	S1/IFN	4	alive
15	2	PD	PD	24	17.8	49.1	38.7	10	15	-	4	dead
16	2	PD	PD	<5	5.8	<0.5	<0.5	703	3743	-	3	alive

Adjuvant therapy

Fourteen patients resulted in SD or PD after 2 or 3 courses of S1/CDDP and S1/CDDP was suspended. As adjuvant therapy, 2 patients received surgical resection for pulmonary metastases, 2 received RT for extrahepatic metastases (brain and muscle), 4 received S-1/IFN, 1 received S-1/IFN and TACE, 1 received HAIC, and 4 received symptomatic treatment.

Adverse reactions

Table 3 shows adverse reactions. Concerning bone marrow toxicities, NCI-CTC grade-3 leukopenia, neutropenia, anemia, and thrombocytopenia were observed in 1 (6%), 6 (38%), 1 (6%), and 2 (13%) patients, respectively, with no patients showing grade-4 bone marrow toxicities requiring administration of granulocyte colony-stimulating factor and blood transfusion.

DISCUSSION

There is no standard therapeutic regimen for HCC with extrahepatic metastases. We previously reported that although the majority of advanced HCC patients with extrahepatic metastases should be treated for intrahepatic HCC, selected patients with good hepatic reserve and well-controlled intrahepatic HCC could undergo treatment for extrahepatic metastases(14). S-1 is a novel oral anticancer medication consisting of tegafur, a 5-FU prodrug; CDHP, an inhibitor of dihydropyrimidine dehydrogenase (DPD) which is a metabolic enzyme for 5-FU; and Oxo, a reversible competitive inhibitor of orotate phosphoribosyl transferase that works to decrease GI toxicity by inhibiting the phosphorylation of 5-FU in the GI tract(21). We and other investigators previously reported that S-1/IFN was potentially effective for HCC patients with extrahepatic metastases (19,20), and 5-FU/IFN and 5-FU/CDDP was reported to show similar anticancer effects in HCC patients with PVTT when administered as HAIC (22-24,27-31). We therefore postulated that S-1/CDDP may have anticancer potential for HCC patients with extrahepatic metastases, and consequently performed this pilot study of S-1/CDDP for patients with advanced HCC with extrahepatic metastases and without intrahepatic HCC. Here, we found an overall response rate and response rate of extrahepatic metastases of 13% and 19%, respectively, and a 1-year survival rate of 77%. These results therefore indicate that S-1/CDDP has anticancer potential for HCC patients with extrahepatic metastases.

CDDP synergistically modulates 5-FU by increasing the availability of reduced folate, an essential cofactor for the formation of the tight ternary complex formed between thymidylate synthase (TS) and 5-FU-derived 5-fluoro-2'-deoxyuridine-5'-

TABLE 3 Adverse Reactions (CTCAE v.3.0) During and After S-1/CDDP Combination Therapy

Adverse reaction	Grade 1	Grade 2	Grade 3	Grade 4	Patients with grade 3 or 4 (%)
Leukopenia	4	8	1	0	6
Neutropenia	3	5	6	0	38
Anemia	11	1	1	0	6
Thrombocytopenia	8	6	2	0	13
Fever	4	0	0	0	0
Fatigue	5	3	0	0	0
Anorexia	2	4	2	0	13
Nausea	3	4	2	0	13
Stomatitis	2	1	0	0	0
Dermatitis	0	0	0	0	0

monophosphate (FdUMP), resulting in enhancement of 5-FU's antitumor effects (32,33). Reports have demonstrated the effectiveness of S-1/CDDP combination therapy for unresectable gastric cancer and lung cancer (34,35), with the SPIRITS trial showing an extended overall survival in advanced gastric cancer patients when compared to S-1 alone. These results therefore demonstrated the additional survival benefit of adding CDDP to a S-1 chemotherapy regimen (34).

Previously, we reported on the efficacy of S-1/IFN combination therapy in 29 HCC patients with extrahepatic metastases, showing a 40% 1-year survival rate (19). Although the patients in this previous report had intrahepatic primary tumors at various disease states, 8 patients with controlled intrahepatic primary tumors showed a 71% 1-year survival rate. These results therefore offer a similar pattern to the present study; however, it remains unclear which of S-1/CDDP or S-1/IFN is more effective for individual HCC patients with extrahepatic metastases. Although both CDDP and IFN are synergistic modulators of fluoropyrimidine, their underlying mechanisms of action in this regard are different(32,33,36,37), suggesting a patient who is unresponsive to one combination therapy may be responsive to another. We previously reported on a HCC patient with pulmonary metastases who achieved PR by systemic 5-FU/IFN after showing PD by 5-FU/CDDP (38). Several studies have nevertheless attempted to identify predictors of response to these combination chemotherapy regimens. For example, Ota et al (28) and Noda et al (39) reported that response to 5-FU/IFN correlated positively with the expression levels of type I interferon receptor 2 and negatively with that of epithelial cell ad-

hesion molecule. Kogure et al. (40) reported that low DPD mRNA levels could predict an improved response of human hepatoma cell lines to 5-FU/CDDP, while Nishiyama et al (41)showed that after exposure to 5-FU/CDDP, the expression levels of DPD, multidrug resistance-associated protein (MRP), glutathione S-transferase π (GSTπ), and TS gene correlated positively with drug resistance in human gastrointestinal cancer cell lines. Further studies are still needed to identify factors that could predict whether IFN or CDDP is more the suitable modulator of fluoropyrimidine in individual patients.

Recently, the SHARP trial showed that sorafenib, a multi-kinase inhibitor, conferred a survival benefit for patients with unresectable HCC (42); however, the response rate was only 2% and CR was not observed, while in the present study we showed 2 patients (13%) obtained CR after 2 and 3 courses of S-1/CDDP. Further, although they did not receive further S-1/CDDP therapy, these 2 patients were continuing to maintain a CR for 22 and 21 months at the time of analysis. S-1/CDDP may therefore provide complete remission of extrahepatic HCC metastases and confer long-term survival. Thus, S-1/CDDP for HCC patients with extrtahepatic metastases might be considered prior to sorafenib.

In the present study, most patients did not received pathological diagnosis of extrahepatic metastases. They were diagnosed by changing of image findings and tumor markers. When new extrtahepatic lesions appeared, if intrahepatic HCCs were absence and HCC related tumor markers elevated, the new lesions were diagnoses as extrtahepatic HCC.

In the present study, although 38% (6/16) of patients showed grade-3 neutropenia, other bone marrow toxicities were generally mild. Meanwhile, 13% (2/16) of patients experienced grade-3 vomiting and nausea, and as this occurred within 1 day of CDDP administration. This adverse reaction was therefore considered mainly a result of the CDDP. When administering 5-FU/CDDP as HAIC, CDDP was given as a daily low dose (10 mg/day) to reduce adverse reactions (22-24), and therefore a trial of daily low dose CDDP in S-1/CDDP combination therapy to reduce these adverse reactions is also warranted.

In conclusion, although there is no standard therapeutic regimen, S-1/CDDP is a potentially safe and effective combination therapy for HCC with extrahepatic metastases. Further studies into S-1/CDDP for HCC patients with extrahepatic metastases are needed to evaluate any survival benefits and further elucidate factors that may determine which of S-1/CDDP or S-1/IFN is the more suitable combination therapy for individual patients.