Table 3 Results of IFN-γ ELISpot assay in patients in whom HCCs were not detected after therapy

Patient no.	SFC before treatment (/10^5 $CD8^+$ T-cells)	SFC after treatment (/10^5 $CD8^+$ T-cells)	Recurrence-free interval (month)
1	0	0	5
2	15	31	10
3	12	15	5
4	159	130	26
5	58	4	12
6	5	99	29[a]
7	15	17	7
8	20	41	7
9	135	9	12
10	1	6	3
11	8	9	6
12	10	57	15
13	34	42	13[a]
14	6	4	12[a]
15	23	8	9
16	59	37	12
17	12	29	23
18	161	72	24
19	18	4	15
20	25	44	23[a]

SFC Spot-forming cells

[a] These patients had no recurrence detected by ultrasonography, enhanced CT, and/or MRI after treatment

($P = 0.005$, 0.007, and 0.001, respectively). When univariate analysis of prognostic factors for the HCC-free interval was performed, only platelet count ($P = 0.027$; Fig. 1a), prothrombin time ($P = 0.030$; Fig. 1b), and the number of SFCs after treatment ($P = 0.004$; Fig. 1c) were found to be significant. Child-Pugh class A tended to prolong the HCC-free interval, although this was not significant ($P = 0.066$). The other factors, including the number of SFCs before treatment ($P = 0.407$), ALT level ($P = 0.644$), albumin level ($P = 0.488$), total bilirubin level ($P = 0.340$), HCC size ($P = 0.756$), HCC number ($P = 0.486$), and the procedure used for HCC treatment (RFA or TACE, $P = 0.481$), did not affect HCC-free survival, as confirmed by the log-rank test.

Multivariate analysis shows that the magnitude of TAA-specific $CD8^+$ T-cell responses correlates with the HCC-free interval after treatment in patients who have no detectable HCC after therapy

In a further analysis of the 20 patients with HCC who were treated by RFA or TACE and in whom no HCC was detectable 1 month after treatment, we performed multivariate analysis using a Cox proportional hazards model. On multivariate analysis, only the magnitude of TAA-specific $CD8^+$ T-cell responses (≥40 TAA-specific cells/10^5 $CD8^+$ T-cells) was the only significant prognostic factor for a prolonged tumor-free period after treatment

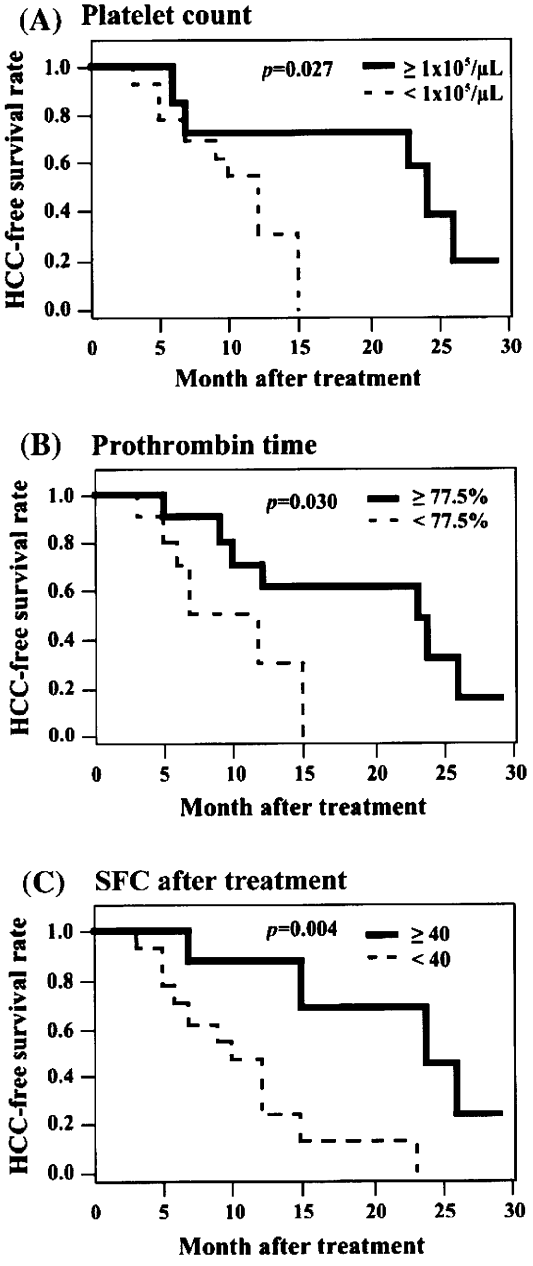

Fig. 1 Kaplan–Meier curves of HCC-free survival rate. In univariate analysis, platelet count, prothrombin time, and the tumor-associated antigen-specific $CD8^+$ T-cell response were found to be prognostic factors for the HCC-free period after treatment. Kaplan–Meier curves representing the relationship between month after treatment (HCC-free interval) and HCC-free survival rate were grouped by **a** platelet count, **b** prothrombin time, and **c** spot-forming cells (*SFCs*) specific for tumor-associated antigens after treatment

Table 4 Multivariate analyses of prognostic factors for tumor-free interval

Variable	Hazard ratio	95% Confidence limit	*P* value
Platelet count			
$\geq 1 \times 10^5/\mu L$	0.916	0.326–2.020	0.843
$< 1 \times 10^5/\mu L$	1.000		
Prothrombin time			
≥77.5%	0.455	0.094–1.390	0.177
<77.5%	1.000		
Child-Pugh class			
A	1.464	0.539–6.813	0.493
B	1.000		
Spot-forming cells after treatment			
≥40	0.342	0.079–0.866	0.022
<40	1.000		

(hazard ratio 0.342, $P = 0.022$), as shown in Table 4. Therefore, the results suggest that TAA-specific CTLs detected after treatment are able to suppress the occurrence or recurrence of HCC in patients with no detectable HCCs after treatment.

Discussion

To determine whether TAA-specific CTLs suppress the occurrence or recurrence of HCC, we investigated the relationship between the magnitude of TAA-specific $CD8^+$ T-cell responses and the HCC-free interval in patients who had no detectable viable HCC 1 month after treatment for HCC. We found that potent TAA-specific $CD8^+$ T-cell responses, as observed 1 month after treatment for HCC, led to a prolonged HCC-free interval.

An HLA-A24-restricted MAGE-1 peptide-specific CTL line was established in a patient with metastatic melanoma [18], and an NY-ESO-1 DNA vaccine induced both antigen-specific effector $CD4^+$ and/or $CD8^+$ T-cell responses in most patients who did not show detectable pre-vaccination immune responses [19]. In addition, HLA-A2- and HLA-A24-restricted GPC3-derived peptide vaccine induced specific CTLs in mice [20]. In this study, we selected GPC3, MAGE-1, and NY-ESO-1 to monitor antigen-specific $CD8^+$ T-cell responses against HCC because they had been reported to be expressed commonly and frequently in HCC tissues [7, 11–13], and thus the combination of these TAAs would cover most HCCs. Among the 20 patients enrolled in the present study, 16 (80%) showed positive $CD8^+$ T-cell responses (10 or more SFCs) against the TAAs before and/or after the treatment. Although we did not examine the expression of TAAs in the HCC tissues, it would be expected that at least one of these three TAAs will be expressed in HCCs in patients who have a positive $CD8^+$ T-cell response against TAAs.

In patient 10, HCC recurrence was detected 3 months after treatment. Insufficient treatment or the pre-existence of intrahepatic metastases might be considered in a patient in whom HCCs are undetectable 1 month after treatment, but are detected within a few months after treatment. We expected that TAA-specific CTLs induced by treatment would suppress the development of a small HCC, which is not easily detected by conventional methods of examination. Thus, we enrolled and analyzed all patients in whom no HCC was detectable by ultrasonography, CT, and/or MRI 1 month after treatment, even if a recurrent or metastatic HCC was detected within a few months after treatment.

It is of interest whether tumor destruction by local HCC treatment would induce immune responses against HCCs. Apoptotic tumor cells are capable of inducing tumor-specific immune responses [21]. Dendritic cells, representing antigen-presenting cells, around damaged tumor cells take up tumor antigen released from the tumor cells and then migrate into draining lymph nodes [22]. There, they mature and stimulate tumor-specific helper T-cells and CTLs. In turn, the effector cells migrate into the tumor tissue and attack the tumor cells [23]. Tumor-specific immune responses were induced by a combination of direct dendritic cell injections into the HCC and radiation therapy that might induce tumor destruction [3]. When we compared TAA-specific $CD8^+$ T-cell responses before HCC treatment and those after treatment, about half of the patients (55%) showed an increased frequency of TAA-specific $CD8^+$ T-cells, which might have been induced by the treatment. However, the increase in TAA-specific CTLs did not affect the recurrence-free interval. Rather, it was the magnitude of TAA-specific $CD8^+$ T-cell responses after the treatment itself that affected the recurrence-free interval. Even if the frequency of these CTLs seemed to be decreased after treatment, they might infiltrate the liver. Furthermore, new CTLs other than pre-existing CTLs might be induced by the treatment because many TAA peptides recognized by CTLs were different between before and after the treatment. Although some patients showed a potent TAA-specific $CD8^+$ T-cell response before treatment, SFC before treatment did not correlate with the recurrence-free interval. We believe that TAA-specific CTLs are not able to control a large tumor burden by itself. As HCCs enlarge, they may secret immune suppressive factors such as TGF-β [24] and/or IL-10 [25] and modify gene expression of TAAs [26]. We speculate that TAA-specific CTLs detected after the treatment, but not detected before the treatment may be able to control HCCs. Otherwise, TAA-specific CTLs detected before the

treatment may be able to destroy a small HCC that was not detected by conventional examinations.

The ELISpot assay is a convenient means of detecting antigen-specific $CD8^+$ T-cells in a variety of diseases. We have detected HCV-specific $CD8^+$ T-cell responses in patients with acute HCV infection using this method and identified 6 new epitopes within the HCV protein [17]. In fact, we identified a novel GPC3-specific CTL epitope using this method (unpublished observation). At present, we are trying to identify more CTL epitopes among these TAAs that will be used as cancer vaccines.

In this study, we used peptide mixtures to stimulate $CD8^+$ T-cells. This procedure may mask responses to individual peptides because a peptide that interacts only weakly with HLA molecules is unable to attach to the molecule if the mixture contains 1 peptide with a high affinity for the same molecule. However, such a weak peptide would not contribute to tumor immune responses because of its weak interaction with the HLA molecules. Thus, we ignored this issue in this study.

Recurrence and multicentric carcinogenesis are major factors in determining the prognosis of HCC, and several treatments have been tried for the prevention of recurrence. IFN therapy [27, 28], treatment with acyclic retinoid therapy [29, 30], and adoptive immunotherapy [31] have been reported as effective in suppressing HCC recurrence. Preoperative hepatic function influenced early HCC recurrence in patients in whom small HCCs were resected [32]. This is consistent with our result that prothrombin time, reflecting hepatic function, affected the recurrence-free interval in the univariate analysis. In our study, higher platelet counts also contributed to a longer recurrence-free interval in the univariate analysis. In the multivariate analysis, however, only the magnitude of TAA-specific $CD8^+$ T-cell responses remained as an independent factor contributing to a longer recurrence-free interval.

Although the size and number of HCCs were reported to affect the period of HCC-free survival (recurrence) in patients with HCC treated by hepatic resection [33], they are not significant factors affecting the recurrence-free interval. Further investigation, such as the accumulation of analyses of HCC patients, is needed to clarify this issue. Sixteen out of 20 patients without detectable HCC 1 month after treatment had recurrent or metastatic HCCs during the observation period in this study. Our results suggest that the maintenance of strong TAA-specific $CD8^+$ T-cell responses for a long period may lead to a longer recurrence-free state. A long-term observation of TAA-specific immune responses should also be performed in any future study.

The results of our study suggest that strong TAA-specific $CD8^+$ T-cell responses would suppress HCC recurrence in patients with HCC who are treated by RFA or TACE and in whom any HCC is undetectable by ultrasonography, CT, and/or MRI 1 month after treatment. Since recurrence and intrahepatic metastasis are major risk factors influencing the prognosis of patients with HCC, immunotherapy to induce TAA-specific $CD8^+$ T-cells, such as a peptide vaccine, should be considered for clinical application in patients with HCC after local therapy.

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Role of Hepatoma-derived Growth Factor (HDGF) in hepatocyte proliferation and differentiation

Hirayuki Enomoto, Hideji Nakamura and Shuhei Nishiguchi

Division of Hepatobiliary and Pancreatic Medicine, Department of Internal Medicine, Hyogo College of Medicine, Mukogawa-cho 1-1, Nishinomiya, Hyogo 663-8501, Japan.

Abstract

Hepatoma-derived growth factor (HDGF) is a novel growth factor, which was identified from the conditioned medium of Huh-7 hepatoma cells. HDGF is a unique growth factor which is both a secreted protein and a nuclear protein. We have investigated the roles of HDGF in hepatocyte growth and differentiation. HDGF is highly expressed in the fetal liver and stimulates the growth of immature hepatocytes, suggesting that HDGF participates in the proliferation of hepatocyte during liver development. In rodent models of liver regeneration, HDGF expression was induced in parenchymal hepatocytes, showing the possible involvement of HDGF in the proliferation of mature hepatocytes. HDGF stimulates the

Correspondence: : Hirayuki Enomoto, Division of Hepatobiliary and Pancreatic Medicine, Department of Internal Medicine, Hyogo College of Medicine, Mukogawa-cho 1-1, Nishinomiya, Hyogo 663-8501, Japan; E-mail:enomoto@hyo-med.ac.jp

proliferation of various hepatoma cells, and HDGF expression in HCC (hepatocellular carcinoma) is closely related to the disease-free and overall survival of patients, indicating that HDGF expression could be a prognostic factor for the disease. Transgenic expression of HDGF in the liver of mice resulted in the partial maturational disturbance of hepatocytes during the post-natal stage, thus suggesting the inhibitory role of HDGF in the hepatocyte differentiation. Our findings have shown that HDGF promotes the proliferation of both normal and malignant hepatocytes In addition, HDGF has a suppressive role in the differentiation of hepatocytes. We conclude that HDGF is a unique growth factor, which has important roles in both hepatocyte proliferation and differentiation.

Introduction

In the normal adult liver, most hepatocytes stay in a quiescent state, and the cell turnover is extremely low. However, hepatocytes actively proliferate under certain situations such as liver development, regeneration and carcinogenesis (1,2). In the developing liver, immature hepatocytes decrease their proliferative activity, acquiring the highly specialized functions of mature hepatocytes. In contrast, malignant hepatic cells acquired unregulated growth capacity, losing the characteristics of mature hepatocytes. Therefore, the proliferative activity of hepatocytes depends on the cellular conditions, and it is usually in opposition to the degree of the cellular differentiation. Clarifying the molecular mechanisms that regulate hepatic cell proliferation and differentiation would provide us with important knowledge for the understanding of liver development, regeneration and carcinogenesis.

We have identified a novel factor, hepatoma-derived growth factor (HDGF) from the human hepatoma-derived cell line Huh-7, which autonomously proliferates in serum-free chemically defined medium (3, 4). We have shown that HDGF participates in liver development as an important growth stimulating factor for fetal hepatocytes (5-7). In addition, we found that the expression of HDGF was induced during liver regeneration, thus suggesting a functional role of HDGF in the proliferation of mature hepatocytes (8). Furthermore, we have shown that HDGF is a growth factor for hepatoma cells, and that HDGF expression is closely associated with the prognosis of patients with HCC (hepatocellular carcinoma) (9). We recently generated a HDGF transgenic mouse and reported that HDGF inhibited hepatocyte differentiation in these mice (10). In this article, we describe the characteristics of this novel growth factor and its possible roles in hepatocyte proliferation and differentiation.

HDGF and its dual characteristics as a secreted protein and a nuclear protein

HDGF is a 26kDa heparin-binding acidic glycoprotein, which we identified from the conditioned medium of the human hepatoma-derived cell line, Huh-7 (3, 4). In addition, several groups including ours have found 4 additional novel genes, HDGF-related proteins (HRPs) (11-13). These proteins (named HRP1- HRP4) share a highly homologous N-terminal amino region consisting of about 100 amino acids, which we call the "HATH (homologous to the amino terminus of HDGF) region". Besides HRP proteins, Lens epithelium-derived growth factor (LEDGF), which was reported to be a survival factor for lens epithelium, contains a HATH region and is regarded as a member of the HDGF family (14). HDGF was originally purified from the conditioned medium of hepatoma cells, however, HDGF mRNA is ubiquitously expressed in adult non-cancerous tissues, and the exogenous administration of HDGF stimulates the proliferation of various types of cells, including fibroblast, vascular smooth muscle cells, as well as several hepatoma cell lines (3-5, 15). These findings suggest that HDGF therefore plays some functional roles in these normal tissues as well as the proliferation of cancer cells.

Although HDGF was originally identified from the conditioned media of cultured cells, the primary sequence of the HDGF protein was lacking the hydrophobic sequence which functioned as a signal peptide. However, previous studies have reported that HDGF can be detected in the conditioned media of various types of cells (3, 4, 15, 16), thus suggesting that HDGF is likely secreted via a process different from the classical secreting pathway of the Golgi system. Recently, amino acid residues 81-100 in the HATH region have been reported as a possible receptor-binding site (17), and we have found the putative receptor of HDGF (Liu et al, in submission). Furthermore, exogenously administration of HDGF protein activated the Erk/MAPK signaling pathway in both in hepatoma cells and endothelial cells (15, 18). These recent findings indicate that HDGF functions via a receptor-mediated signal transduction pathway to act as a growth stimulating factor.

While suggestive receptor-mediated signaling of HDGF has been reported, we noted that the HDGF protein contains 2 putative nuclear localization signals (NLSs) (4, 19). The first NLS (NLS1) resides in the HATH region and the second NLS (NLS2) is in the gene-specific region, and previous studies have shown that HDGF is detected in the nucleus as well as in the cytoplasm, implying that HDGF could have some characteristics of a nuclear protein. We have shown that HDGF can be transported to the nucleus by means of the NLSs, and

that the nuclear translocation is important for the growth stimulating activity of HDGF (19). Regarding the function of HDGF in the nucleus, recent reports have suggested that the HATH region serves as a DNA binding domain. The HATH regions of HDGF family contain a PWWP motif, which was first reported in a candidate gene WHSC1 for Wolf-Hirschhorn syndrome (20, 21). HDGF and HRPs form one of the gene families with PWWP motif, and an NMR analysis has revealed that the PWWP domain of HDGF has a characteristic of hydrophobic cavity, suggesting that HDGF binds to some component of chromatin through this cavity (22). Furthermore, HDGF has been proved to function as a transcriptional repressive factor through the binding to a conserved DNA sequence in the promoter regions of target genes, and the putative DNA binding site is considered to reside in the PWWP domain (23). These findings suggest that the PWWP motif of the HDGF protein acts as a DNA binding domain.

Based on these findings, we consider HDGF to be a unique growth factor that has dual characteristics; thereby acting as a secreted protein and as a nuclear protein.

HDGF in Hepatocyte Proliferation

HDGF and fetal hepatocyte proliferation

Unlike mature hepatocytes in the adult liver, immature hepatocytes in the fetal stage can autonomously proliferate *in vitro*, even in the absence of any growth factors (24, 25). However, the growth regulation of fetal hepatocytes has been poorly understood. During liver development, HDGF was highly expressed in immature fetal hepatocytes, especially in the mid-gestation stage, and its expression was dramatically decreased near birth (6). Using an *in vitro* model of hepatocyte maturation, we demonstrated that HDGF expression in hapatocytes decreased with cellular differentiation, suggesting that HDGF was related to the hepatocyte proliferation. Indeed, exogenous administration of recombinant HDGF stimulated the growth of primary cultured fetal hepatocytes. Furthermore, the reduction of HDGF by an antisense adenovirus suppressed the proliferation of fetal hepatocytes. The growth inhibitory effect of the HDGF antisense virus was reversed by the exogenous administration of recombinant HDGF (6). These findings strongly suggest that HDGF is associated with the proliferation of hepatocytes during liver development.

Several growth factors such as EGF (Epidermal growth factor), HB-EGF (heparin-binding EGF-like growth factor), TGF-α (transforming growth factor-α) and HGF (hepatocyte growth factor) have been shown to play significant roles in hepatocyte proliferation (1, 2). The expression levels of

these factors and their receptor in the fetal stage were lower than those observed in the post-natal stage, thus suggesting that these growth factors and their signals could have more significant roles during the post-natal stage compared with the fetal stage. In contrast, HDGF expression in the developing liver was highly detected during the mid-gestation stage and was markedly decreased with hepatocyte maturation. Despite the normal development of HDGF-null mice, possibly as a result of the functional redundancy that has been considered to exist among HDGF and its related genes (26), HDGF is presumed to participate in liver development as a unique growth stimulating factor for fetal hepatocytes.

HDGF and adult hepatocyte proliferation

Although adult hepatocytes are cells which rarely replicate in their normal state, their proliferative capacity appears in the regenerating liver such as after hepatectomy or drug-induced hepatic injury. Many growth factors have been reported to be involved in the liver regeneration (1, 2). For example, EGF is a paracrine or endocrine growth factor that has growth stimulating effects on primary cultured hepatocyets. TGF-α is a growth factor which is induced in hepatocytes after partial hepatectomy and promotes their growth in an autocrine manner. HB-EGF is a paracrine growth factor which stimulates DNA synthesis of primary cultured rat adult hepatocytes and is induced in non-parenchymal cells during liver regeneration. HGF is the most potent growth stimulating factor for mature hepatocytes and HGF promotes hepatocyte replication by a paracrine or endocrine mechanism. The concentrations of plasma HGF increase within 1hour after partial hepatctomy, and the phosphorylation of the HGF receptor (c-Met) is observed before the DNA synthesis, suggesting an important role of HGF/c-Met system in liver regeneration (27). In addition, other growth factors such as aFGF (28), bFGF (29) and VEGF (30) have been suggested to be involved in liver regeneration, but their roles are still unclear.

Although the expression level of HDGF in non-proliferative hepatocytes of the adult liver is lower than fetal hepatocytes, we considered that HDGF expression could be induced in the proliferative hepatocytes of the regenerating liver. Therefore, we examined the expression patterns of HDGF in two different liver regeneration models (8). In the CCl_4-treated liver, HDGF expression was up-regulated, and a single peak was observed prior to the DNA synthesis peak. HDGF expression was also induced in the hepatectomized liver, and its peak induction was also detected before the peak of DNA synthesis. The HDGF expression in the regenerating liver was

predominantly induced in hepatocytes, but barely increased in non-parenchymal cells. Our findings showed that the HDGF expression to increase in parenchymal hepatocytes before DNA synthesis in the regenerating liver, suggesting that HDGF acts as an autocrine factor promoting liver regeneration. These findings suggest that HDGF plays a major role in liver regeneration, especially in the hepatocyte proliferation.

HDGF and hepatoma cell proliferation

As described above, we have purified HDGF from the conditioned media of Huh-7 hepatoma cells and demonstrated that HDGF participates in the proliferation of both fetal and adult non-transformed hepatocytes. However, malignant transformation is an important event which induces active proliferation of hepatocytes, and our original purpose of finding HDGF was to identify a novel growth factor involved in the proliferation of hepatoma cells. Therefore, we investigated the role of HDGF in the proliferation of hepatic cancer cells.

As expected, HDGF is expressed in various hepatoma cell lines such as Huh-7, HLF and HepG2, and HDGF stimulates the proliferation of these cells. In addition, endogenous overexpression of HDGF significantly increases the cell number and DNA synthesis of hepatoma cells (19), whereas antisense treatment targeting HDGF reduced the cellular proliferation (31). These findings strongly suggested that HDGF has a pivotal role in the proliferation of hepatoma cells. Therefore, we further examined the relationship of HDGF expression to the prognosis of the patients with hepatocellular carcinoma (HCC).

The expression level of HDGF was strongly associated with the prognosis of heptocellular carcinoma and higher expression of HDGF resulted in more unfavorable prognosis (9, 32). In our study, high expression of HDGF was more frequently observed in well-differentiated carcinomas than in poorly-differentiated carcinomas (9). However, another group reported that HDGF was expressed more highly in poorly-differentiated HCC than in well-differentiated HCC (32). Although we cannot fully explain this discrepancy, it may depend on the differences between the anti-HDGF antibodies used in these studies. Despite the inconsistency, both studies reported that the HCC patients with a higher HDGF expression showed an earlier recurrence and a poorer overall survival rate than those with a lower level of expression. Furthermore, both univariate and multivariate analyses revealed a significantly poorer disease-free and overall survival in patients with higher HDGF expression after a resection of HCC (9, 32). These findings suggest that HDGF could be a prognostic

factor for the disease-free and overall survival of patients with HCC.

HDGF in Hepatocyte Differentiation

In the previous study, we used an *in vitro* culture system, which recapitulated the maturational process of hepatocytes ranging from mid-gestation to the newborn stage. Using the culture system, we have demonstrated that HDGF expression is high in immature hepatocytes and markedly decreased with cellular differentiation (6). However, we could not clarify the functional role of HDGF in the hepatocyte differentiation using the *in vitro* model. On the other hand, Lepourcelet et al (33) have reported that overexpression of HDGF showed inhibitory effects on epithelial cellular maturation, suggesting a suppressive role of HDGF in cellular differentiation. Therefore, we generated transgenic mice which overexpressed HDGF in hepatocytes under the transcriptional control of the mouse albumin promoter/enhancer, and investigated the effects on hepatocyte differentiation *in vivo* (10). The HDGF transgenic mice did not have any apparent morphological abnormalities in the liver. However, their gene expression patterns showed the possibility that the maturational process of hepatocytes during the post-natal stage was partially disturbed. In light of these findings, the HDGF expression may therefore be important for sustaining the characteristics of immature cells, and it may also be associated with the high growth activity of cancer cells.

Recently, Lee et al (34) reported that patients with HCC who had a gene expression pattern similar to fetal hepatoblasts showed a poor prognosis. The gene expression pattern was characterized by expression of markers of oval cells (hepato-cholangio progenitor cells), thus suggesting that the HCC of this subtype may originate from immature hepatic progenitor/stem cells. As described above, two groups including our group have shown a high expression of HDGF to be closely associated with a poor prognosis for HCC patients. Although we believe that such a poor prognosis depends on the growth stimulating effects of HDGF on HCC cells, HDGF may promote the proliferation of hepatic progenitor/stem-derived cells and thereby cause a poor prognosis, because HDGF can function as a growth factor for immature fetal hepatocytes. Therefore, clarifying the role of HDGF in hepato-cholangio progenitor cells (like oval cells) should lead to the development of a new therapeutic strategy for HCC.

Conclusion

HDGF is a novel nuclear/growth factor belonging to a new gene family. HDGF not only promotes hepatocyte proliferation

but it also suppresses the differentiation of hepatocytes, thus indicating that HDGF participates in both hepatocyte growth and differentiation. Clarifying the functional role of HDGF will provide further insights into the molecular mechanisms of liver development, regeneration and carcinogenesis.

Acknowledgements

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別刷

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自己免疫性肝炎に発症した肝細胞癌にUFT-E投与が有効であった1例

榎本　平之[*1]　石井　昭生[*1]　今西　宏安[*1]　斎藤　正紀[*1]　田中　弘教[*1]
岩田　恵典[*1]　坂井　良行[*1]　岩井　孝史[*1]　楊　和典[*1]　高嶋　智之[*1]
吉川　昌平[*1]　曾澤　信弘[*1]　山本　晃久[*1]　池田　直人[*1]　下村　壯治[*1]
飯島　尋子[*1]　廣田　誠一[*2]　西口　修平[*1]

[*1] 兵庫医科大学病院・内科・肝・胆・膵科
[*2] 同　・病理部

癌と化学療法社

● 症　例 ●

自己免疫性肝炎に発症した肝細胞癌にUFT-E投与が有効であった1例

榎本　平之[*1]　石井　昭生[*1]　今西　宏安[*1]　斎藤　正紀[*1]　田中　弘教[*1]
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飯島　尋子[*1]　廣田　誠一[*2]　西口　修平[*1]

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A Case of Hepatocellular Carcinoma with Autoimmune Hepatitis Showing Marked Reduction of Tumors by Oral Administration of UFT-E: Hirayuki Enomoto[*1], Akio Ishii[*1], Hiroyasu Imanishi[*1], Masaki Saito[*1], Hironori Tanaka[*1], Yoshinori Iwata[*1], Yoshiyuki Sakai[*1], Takashi Iwai[*1], Kazunori Yoh[*1], Tomoyuki Takashima[*1], Shohei Yoshikawa[*1], Nobuhiro Aizawa[*1], Teruhisa Yamamoto[*1], Naoto Ikeda[*1], Soji Shimomura[*1], Hiroko Iijima[*1], Seiichi Hirota[*2] and Shuhei Nishiguchi[*1]
([*1]*Division of Hepatobiliary and Pancreatic Medicine, Dept. of Internal Medicine, and* [*2]*Dept. of Surgical Pathology, Hyogo College of Medicine*)

Summary

We report a case of hepatocellular carcinoma (HCC) in association with autoimmune hepatitis (AIH). In May 2003, a 66-year-old man was admitted to our hospital because of acute liver dysfunction. He was diagnosed with AIH, and his liver function was normalized by oral administration of the corticosteroid. In July 2007, when he was admitted for the treatment of bacterial pneumonia, two liver tumors (S4: ϕ4 cm and S2: ϕ1 cm) were revealed by abdominal CT scan, and the serum level of AFP was high. According to the findings of imaging diagnosis and laboratory data, the patient was diagnosed as having HCC. Since the standard invasive therapies of HCC were not accepted by the patient and his family, he was treated by oral administration of UFT-E (tegafur/uracil: 200 mg/day). Three months after the initiation of administration, CT scan showed a remarkable reduction of the tumors, and his serum AFP level was decreased to the normal range. This case shows that HCC develops in an AIH patient even if liver function is maintained in the normal range. It also suggests the clinical usefulness of UFT-E in the management of HCC given the difficulty of treatment by the standard therapies. **Key words**: Hepatocellular carcinoma, Autoimmune hepatitis, UFT-E (*Received Jul. 29, 2009/Accepted Oct. 9, 2009*)

要旨　症例は70歳，男性。2003年66歳時に急性肝障害のため入院し，自己免疫性肝炎（autoimmune hepatitis: AIH）と診断され，副腎皮質ステロイド投与で肝機能は改善していた。2007年7月，肺炎のため入院した際の検査にて腹部CTで肝S4に径4 cmとS2に径1 cmのSOLを認め，またAFPも高値を示した。画像および血液検査所見から肝細胞癌（HCC）と診断したが，全身状態不良で侵襲的治療の同意が得られずUFT-E 200 mgの投与を行った。治療開始後3か月には腫瘍は著明に縮小し，AFPは正常化した。本症例は，肝機能の正常化が維持されたAIH患者にもHCCが発症し得ることを示している。さらに本例からは，標準的な治療が困難なHCCに対するUFT-Eの有効性も示唆される。

はじめに

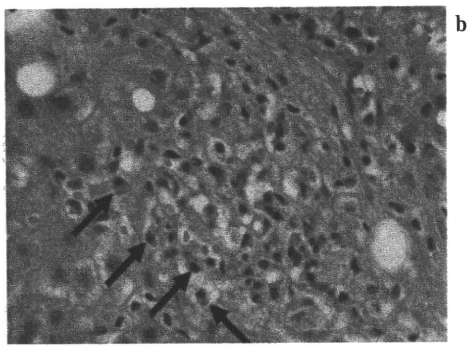
自己免疫性肝炎（autoimmune hepatitis: AIH）には肝細胞癌（HCC）の合併はまれといわれている。今回われわれは男性AIH患者にHCCを発症し，経口抗癌剤UFT-E投与が有効であった1例を経験したので報告する。

[*1] 兵庫医科大学病院・内科・肝・胆・膵科
[*2] 同　・病理部

連絡先: 〒663-8501 西宮市武庫川町1-1　兵庫医科大学病院・内科・肝・胆・膵科
榎本　平之

a

生化学・血清検査		ウイルスマーカー	
AST	238 IU/L	IgM-HA Ab	(−)
ALT	498 IU/L	HBs-Ag	(−)
ALP	269 IU/L	IgM-HBc Ab	(−)
LDH	155 IU/L	HBc-Ab	(−)
T-Bil	0.9 mg/dL	HCV-Ab	(−)
TP	6.9 g/dL	HCV-RNA	(−)
Alb	3.9 g/dL	免疫学的検査	
Cr	0.8 mg/dL	IgG	1,586 mg/dL
BUN	7 mg/dL	抗核抗体	×160 (Speckled)
Na	139 mEq/L	抗平滑筋抗体	×20
K	4.5 mEq/L	P-ANCA	(−)
Cl	102 mEq/L	C-ANCA	(−)
CRP	0.9 mg/dL	抗 SS-A 抗体	(+)
		抗 SS-B 抗体	(−)



b

図 1

a: AIH 診断時検査成績。トランスアミナーゼの上昇を認め，ウイルスマーカーは陰性であった。抗核抗体，抗平滑筋抗体，抗 SS-A 抗体が陽性であった。

b: 肝生検組織（HE 染色）。piecemeal necrosis を伴う慢性肝炎像で，リンパ球とともに形質細胞の浸潤（矢印）が認められた。

Ⅰ．症　例

患者: 70 歳，男性。

現病歴: 2003 年，心房細動および COPD による慢性呼吸不全にて近医加療中に肝障害を生じ当科紹介となった。血液検査・肝生検像を含め AIH と診断し，ステロイドホルモンの投与にて肝機能は安定して退院した。2007 年 4 月，腹部エコーで肝 S4 に径 1 cm の SOL が疑われるも，全身状態不良のため経過観察となっていた。7 月，当院に肺炎で入院した際の腹部 CT で肝 S4 に径 4 cm 大の SOL を認め当科紹介となった。

家族歴，既往歴: 特記すべきことなし。

輸血歴: なし。

入院時現症: 眼瞼結膜に軽度の貧血を認めるが，他に特記すべき所見は認めなかった。

AIH 診断時検査成績: トランスアミナーゼの上昇を認めるが，ウイルスマーカーは陰性であった（図 1a）。IgG は正常範囲内であるが，抗核抗体 160 倍，抗平滑筋抗体 20 倍と陽性を示し，抗 SS-A 抗体も陽性であった。肝生検像ではリンパ球とともに形質細胞の浸潤を伴う慢性肝炎像を呈し，軽度の piecemeal necrosis，ロゼット形成様の病変も認められた（図 1b）。血液データと併せて国際診断基準 16 点で，AIH と診断した。

HCC 発症時検査成績: ステロイドによりトランスアミナーゼは持続正常化していたが，一方腫瘍マーカー AFP の上昇が認められた。なお PT 低下と PIVKA-Ⅱの上昇は，心房細動に対する warfarin 内服の影響と考えられた（図 2a）。腹部造影 CT では，S4 に径 4 cm，S2 に径 1 cm の SOL を認め，早期動脈相での濃染と，後期相で wash out の所見を認めた（図 2b，c）。以上の結果より AIH に合併した HCC と診断したが，入院中に不整脈からショック状態となるなど全身状態不安定で，HCC への治療には同意が得られず無治療で退院された。その後も呼吸器感染による入退院を繰り返し，半年余り後の 2008 年 3 月には S4 と S2 の病変は，各々 9 cm と 4 cm まで増大した。そのころより肝動脈塞栓術を含む侵襲的治療を依然として拒みつつも，一方で腫瘍増大への不安を訴えるようになった。そこで患者および家族と相談したところ，副作用が少なく外来での治療継続が可能である UFT-E（tegafur・uracil 配合）の内服治療を希望されたため 200 mg/日投与を開始した。AFP 値は投与前の 110,000 ng/mL 台から 2 か月後には約 52 ng/mL まで著減し，その後正常化した（図 3a）。また腹部 CT 検査では S4, S2 いずれの病変も腫瘍の縮小がみられた（図 3b）。UFT-E の内服による副作用は特になく，肝機能の変動もなく継続投与可能であり，現在も加療継続中である。

Ⅱ．考　察

従来 AIH には，HCC の合併はまれといわれてきた。しかしながらステロイド投与による予後改善と長期観察例の増加により，合併例の報告が増加している。

西村らの検討[1]によれば，2007 年までに 68 例の報告がなされており，発症年齢は 19〜89 歳で平均は約 60 歳であった。女性患者が多く，本例のような男性 AIH 患者での肝癌合併は 15 例と少数であった。AIH からの発癌は発見時には腫瘍が大きく，また進行した肝硬変のため予後不良例が多く，診断から死亡までの期間は平均 10.1 か月にしかすぎない。また HCC 発症に関連する因

a

末梢血		生化学・血清検査		腫瘍マーカー	
RBC	$330\times10^4/mm^3$	AST	14 IU/L	CEA	5.0 ng/mL
Hb	9.7 g/dL	ALT	15 IU/L	CA19-9	17.9 U/L
WBC	$8,460/mm^3$	ALP	193 IU/L	AFP	193.0 ng/mL
Neut	65%	LDH	164 IU/L	PIVKA-Ⅱ	28,600 mAU/mL
Lypho	27%	T-Bil	0.3 mg/dL	検尿	
Mono	8%	TP	6.7 g/dL	潜血	(−)
Eosino	0%	Alb	3.7 g/dL	蛋白	(−)
Baso	0%	Cr	1.3 mg/dL	沈渣	異常なし
Plt	$30.5\times10^4/mm^3$	BUN	7 mg/dL		
止血		UA	7.7 mEq/L		
PT	59.9%	Na	139 mEq/L		
PT-INR	1.45	K	4.5 mEq/L		
		Cl	102 mEq/L		
		CRP	0.9 mg/dL		

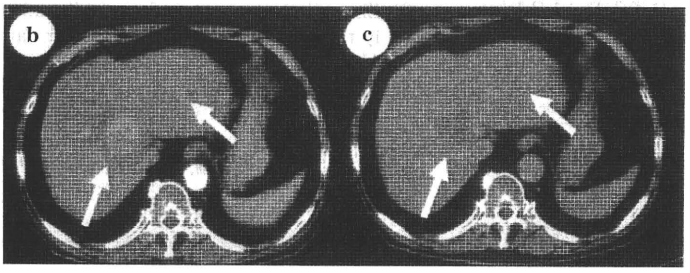


図 2
a: HCC 発症時検査成績。トランスアミナーゼは正常化していたが，腫瘍マーカー AFP の上昇が認められた。
b, c: 腹部造影 CT。S4 に径 4 cm，S2 に径 1 cm の SOL を認め（矢印），早期相での濃染（b）と，後期相で wash out（c）の所見を認めた。

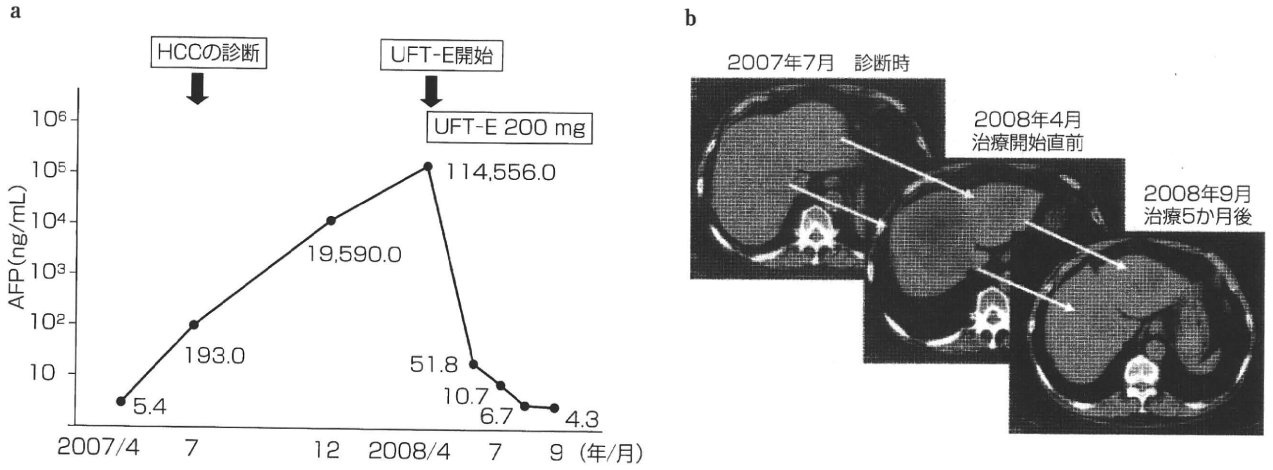


図 3　臨床経過
a: UFT-E 200 mg/日投与開始後 AFP 値は著減し，その後正常化した。
b: 腹部 CT 検査では S4，S2 いずれの病変も腫瘍の縮小が認められた。

子としてウイルス性肝炎の合併，肝硬変への進展，ステロイドを含む免疫抑制剤による腫瘍免疫の低下などが考えられている[2]。

本例では肝組織中での HBV-DNA，HCV-RNA の検討はできていないが，血液検査所見からは肝炎ウイルスの関与は否定的と考えられる。また 2003 年の組織所見は F2 stage で，その後トランスアミナーゼは正常値が維持されていたため著しい肝線維化の進展も考えにくく，ステロイド投与による免疫抑制が発癌に関与した可能性が考えられる。ただしステロイド使用に関する過去

の検討では[2]，AIH診断からHCC発症までの期間は，ステロイド未使用例で平均6.4年，一方ステロイド使用例では平均11.3年であり，ステロイドが発癌に抑制的に働く可能性も指摘されており，本例での発癌に関する明確な機序は不明である。

近年HCCに対する化学療法は分子標的薬を中心に開発が進み，海外で標準治療の一つとされる[3]sorafenibが2009年5月よりわが国でも保険適応となった。本例への治療開始当時は，十分なエビデンスに基づき推奨される全身化学療法は存在しなかったが，以前より進行HCCに対しUFT-E単独投与の有効性はしばしば報告されている[4,5]。UFT-E（あるいはUFTカプセル）の内服による副作用の頻度は高くないが，食欲不振（3.8%），悪心（2.4%），嘔吐（1.1%），下痢（1.5%）などの消化器症状，白血球減少（3.1%），血小板減少（1.1%），貧血（0.8%）などの血液障害，肝障害（1.8%），色素沈着（0.7%）などがあげられている[6]。本例では副作用の発現なく発症2年以上経過した現在も生存中であり，UFT-E内服は生命予後改善に貢献したと考えられる。

結　語

男性AIH患者に発症したまれなHCCの1例を経験した。ステロイドによって良好な炎症のコントロールが得られても，肝発癌の可能性を念頭におく必要があると考えられた。また治療困難なHCC症例に対するUFT内服は，一つの選択肢に成り得ると思われた。

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Changing Trends in Hepatitis C Infection over the Past 50 Years in Japan

Hobyung Chung Taisuke Ueda Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan

Key Words

Chronic hepatitis · Hepatitis C virus · Hepatocellular carcinoma · Injecting drug user · Mortality rate · Post-transfusion hepatitis

Abstract

In Japan, hepatocellular carcinoma (HCC) is the fourth leading cause of death in males and the fifth in females. Hepatitis C virus (HCV) is a major cause of HCC in Japan, with 70% of cases being HCV related. HCV genotype 1b, the most prevalent subtype in Japan, started to spread in the 1930s among injecting drug users (IDUs) during and after World War II or through medical procedures such as blood transfusion and use of contaminated syringes. The prevalence of HCV infection is much lower in the current younger generation compared with that in the older generation, particularly those aged >55 years (0.1–0.2% vs. ≥ 2%). Therefore, the total number of patients with HCV infection is estimated to decrease, even though sporadic HCV transmission is mainly seen among young IDUs. Of note, HCV genotype 2 seems to be spreading among IDUs, but the response to antiviral therapy in these patients seems to be better than that in older patients, irrespective of the genotype. Although the number of patients who die because of HCC has steadily increased over the last 50 years, the incidence of HCC is now decreasing, mainly because of the decreased prevalence of HCV-related HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer death. The prevalence of HCC in Japan has increased over the past 50 years and more than 30,000 patients die because of HCC every year, accounting for rates of death of 36.3 and 17.5 per 100,000 males and females, respectively [1]. The main causes of HCC in Japan are hepatitis C virus (HCV) and hepatitis B virus (HBV); nearly 70% of HCC cases are caused by HCV [1]. This situation differs from that in other Asian countries where HBV-related HCC is more common, and the situation in Japan is more similar to that in Western countries [2, 3].

Japan has one of the highest endemic rates of HCV infection. The number of patients with HCV infection is estimated to be about 2,000,000 in Japan; 70% of patients are infected with HCV genotype 1b, 20% with genotype 2a, and the rest with genotype 2b. The prevalence of HCV infection is closely related to age: the number of HCV carriers increases with age and the prevalence of HCV infection is much higher in people aged over 55 years [4].

Here, we review the history and current status of HCV infection in Japan and predict the future changes in rates of infection and HCV-related mortality.

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Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp