Med. J. Kagoshima Univ., Vol. 62, No.3, 27–35, January, 2011

Significance of High Mobility Group Box-1 (HMGB1) Expression in Hepatocellular Carcinoma

Shinichi Ueno, Kiyokazu Hiwatashi, Masahiko Sakoda, Satoshi Iino, Hiroshi Kurahara, Koji Minami, Kei Ando, Kosei Maemura, Yukou Mataki, Masataka Matsumoto, Tetsuhiro Owaki, Masaki Kitazono, Sumiya Ishigami, Hiroyuki Shinchi,* Shoji Natsugoe

Department of Surgical Oncology and Digestive Surgery, Field of Oncology, Course of Advanced Therapeutics, *School of Health Sciences, Kagoshima University Faculty of Medicine, Kagoshima University Graduate School of Medicine and Dental Sciences, Kagoshima, Japan.

Abstract

Background: High mobility group box-1 (HMGB1) induces the release of proinflammatory cytokines and chemokines as a late-acting mediator of inflammation. Hepatocellular carcinoma (HCC) is a typical inflammation-related cancer, however, little is known about the relationship between HCC and HMGB1 and its receptor RAGE (receptor for advanced glycation end products). We studied the clinicopathological relevance of the expression level of HMGB1 and the effect of HMGB1 expression on the characteristics of HCC.

Methods: Samples from the 36 HCC patients including 7 with positive hepatitis B surface antigen and 21 with hepatitis C antibody were studied. Twenty-four patients had chronic active hepatitis and 4 had liver cirrhosis. The expression of HMGB1 was assessed in paired cancerous and noncancerous tissues with HCC, using reverse-transcription polymerase chain reaction (RT-PCR), and Western blotting. Quantitative RT-PCR data were analyzed relation to clinicopathological features. As a control study, 7 normal liver samples were collected from patients other than HCC.

Results: The expression of HMGB1 mRNA was lower in normal liver than in noncancerous tissue and the highest in HCC, although the differences were not significant. Furthermore, in HCC, it was high in well- and moderately differentiated tumors but declined as tumors dedifferentiated to poorly differentiated HCC ($p=0.033$). The expression level was inversely correlated with tumor recurrence ($p=0.036$). No significant correlations were observed between the expression levels and well-known prognostic factors of HCC (e.g. portal invasion and intrahepatic metastasis).

Conclusion: In HCC, HMGB1 expression level correlated inversely with the patient's prognosis. The HMGB1 mRNA expression level is similar to the level we reported previously in a study on the clinicopathological relevance of the level of RAGE in HCC. RAGE-HMGB1 interaction in HCC might work in the early stage of tumorigenesis more than in the stage of cancer development.

Key words: hepatocellular carcinoma (HCC), high mobility group box-1 (HMGB1), reverse transcription-polymerase chain reaction (RT - PCR)

Correspondence to: Shinichi Ueno, MD & PhD.
E-mail: ueno1@m.kufm.kagoshima-u.ac.jp
Requests for reprints : Shinichi Ueno, Departments of Surgical Oncology and Digestive Surgery, Field of Oncology, Course of Advanced Therapeutics, Kagoshima University Graduate School of Medicine and Dental Sciences, 8-35-1, Sakuragaoka, Kagoshima 890-8520, Japan.
Tel: 81-99-275-5361; Fax: 81-99-265-7426 ;
E-mail : ueno1@m.kufm.kagoshima-u.ac.jp

Introduction

Although many cancers arise from chronic inflammation, the relationships between carcinogenesis, cancer promotion, and its molecular characteristics remain poorly understood. Hepatocellular carcinoma (HCC), which typifies inflammation-related tumors, is one of the most common malignancies in the world, especially in Asia and Africa. Japan has high incidences of chronic viral hepatitis, cirrhosis, and HCC. The details of inflammatory activity at the molecular level may be relevant to the prevention of hepatocarcinogenesis and cancer promotion.

High mobility group box-1 (HMGB1) protein was first recognized as one of a group of chromatin-associated proteins with high contents of acidic and basic amino acids, and is composed of three domains[1]. Its function as a cytokine-like factor is activated on translocation from the cytoplasm into the nucleus, where it binds to DNA and regulates transcription[2]. HMGB1 functions as a late mediator in various inflammatory processes[3]. In its role as a late-acting mediator of inflammation, HMGB1 induces the release of proinflammatory cytokines and chemokines such as interleukin (IL)-12, IL-6, IL-1α, IL-8, TNF-α and IL-8[4-6] by stimulating toll-like receptor ligands or LPS, TNF-α, IL-1β[7]from macrophages[8, 9], dendritic cells[10], and natural killer cells[11]. These cytokines cause disease states, including cancer[12-22], sepsis[9], arthritis[23], Alzheimer's disease[24], and ischemia-reperfusion injury[25].

Several receptors for HMGB1 have been identified, such as receptor for advanced glycation end products(RAGE), Toll-like receptor (TLR) 2, and TLR4[26] and thrombomodulin[27]. Blockade of RAGE-HMGB1 interaction decreased tumor growth and metastasis, and suppressed activation of p44/p42, p38, and SAPK/JNK MAP kinases[28], whereas it induced phosphorylation of extracellular signal-regulated kinase (ERK) and nuclear translocation of nuclear factor (NF)- KB[29]. TLR 2 and TLR4 are also involved in HMGB1-induced NF- KB activation and neovascularization [25, 30, 31]. These molecular effectors mechanisms are linked to cancer proliferation and invasion and the expression of matrix metalloproteinases.

Promotion of cancer by HMGB1-RAGE interaction was demonstrated in various cancers, such as gastric cancer[12], colon cancer[13, 14], breast cancer[15, 16], melanoma[17-20], and prostate cancer[21]. We previously reported that the expression of mRNA for RAGE, the representative receptor of HMGB1, was lower in normal liver than in the liver of patients with hepatitis and higher in patients with hepatocellular carcinoma (HCC)[22]. The expression level was high in well-differentiated and moderately differentiated tumors but decreased as tumors dedifferentiated to poorly differentiated HCC. We showed that RAGE mRNA was upregulated in carcinogenesis but downregulated during the development of HCC. Moreover, we found that HCC cell lines resistant to hypoxia had higher levels of RAGE expression and RAGE transfectant also significantly prolonged survival under hypoxia. Thus, RAGE-HMGB1 interaction in HCC may also work in the early stage of tumorigenesis and be different from the potential mechanism for other cancers.

In this study, we examined the clinicopathological relevance of the level of HMGB1 expression in patients with HCC, and revealed that it was closely similar to the clinicopathological relevance of the level of RAGE expression, as we previously reported in HCC.

Materials and methods

Human samples

From March 2000 to September 2005, 65 patients with primary HCC were treated surgically in the Department of Surgical Oncology and Digestive Surgery, Kagoshima University School of Medicine. Of these 65 patients, 12 who had Diabetes Mellitus and 6 who underwent preoperative therapy were excluded from the study. Further 11 patients were excluded because their RNA samples were degraded. Samples from the remaining 36 patients (30 men and 6 women with a mean age of 67.1 years) were included in the study. As shown in Table 1, 7 patients (19.4%) were positive for hepatitis B surface antigen and 21 (58.3%) were positive for the antibody to hepatitis C virus. Eight patients (22.2%) were negative for both of these viruses. Twenty-four patients had chronic active hepatitis and four had liver cirrhosis. The other 8 patients showed non- or mild-inflamed liver such as chronic persistent hepatitis. The mean tumor size was 49.7 mm (range, 16-150mm). The histological grade of each tumor was determined according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer (The Liver Cancer Study Group of Japan, 2000). Four tumors (11.1%) were well-differentiated HCC, 28 (77.8%) moderately differentiated HCC and 4 (11.1%) poorly differentiated HCC. Postoperative tumor

recurrence was observed in 11 patients (30.6%). As a control study, 7 normal liver samples were collected from patients with benign or metastatic liver tumors. Before tissue acquisition, each patient provided written informed consent in a form recognized by the ethical committees of Kagoshima University School of Medicine.

Immunoblot analysis

Samples of protein were prepared according to the Santa Cruz protocol. Lysates (10g) were subjected to immunoblot analysis using a 12.5% SDS-polyacrylamide gel followed by electrotransfer onto nitrocellulose filters. The filters were immunoreacted with anti-HMGB1 antibody (BD Biosciences, Tokyo, Japan) and then incubated with peroxidase-conjugated anti-goat IgG (Medical and Biological Laboratories, Nagoya, Japan). The immune complex was visualized using the ECL Western blot detection system (Pierce, Rockford, IL, USA). The amount of β-actin as an internal control was also examined using a specific antibody (Cytoskeleton Inc., Denver, CO, USA). At least three independent experiments were performed.

Quantitative RT-PCR

For reverse transcription-polymerase chain reaction (RT-PCR) and real-time quantitative PCR, total RNA was extracted from 30 mg frozen tissue using Total RNA Mini (Viogene, CA, USA). For cDNA synthesis, RNA samples (1 μg) were converted to cDNA by reverse transcription using random primers (Takara, Siga, Japan) according to the manufacturer's instructions. To estimate quantitatively the mRNA expression levels of several genes, PCR amplification was performed using a LightCycler instrument system (Roche, Mannheim, Germany) and the LightCycler-FastStart DNA Master SYBR green I kit (Roche). Primers were as follows: HMGB1 5'-GCT CAG AGA GGT GGA AGA CCA-3' and 5'-GGT GCA TTG GGA TCC TTG AA-3' [21], GAPDH 5'-TTG GTA TCG TGG AAG GAC TCA-3' and 5'-TGT CAT CAT ATT TGG CAG GTT T-3'. Amplification was carried out in 20 μL reactions containing 4 mM $MgCl_2$, 2 μL of primer, 2 μL of LightCycler-FastStart DNA Master SYBR green I reagent, and 2 μL of cDNA. Reaction conditions were as follows: initial incubation at 95°C for 10 min followed by 50 cycles at 95°C for 10 s for denaturation, 54°C for 10 s for annealing of HMGB1 primers, and 60°C for 10 s for annealing of GAPDH primers, and 72°C for 10 s for

Table 1. Background of patients.

Gender	
Male	30 (83%)
Female	6 (17%)
Mean age	67.2 y.o.
Virus type	
B	7 (19.4%)
C	21 (58.3%)
none	8 (22.2%)
Background of livers	
Chronic active hepatitis	24 (60.7%)
Liver cirrhosis	4 (11.1%)
Non- or mild-inflamed liver	8 (22.2%)
Mean tumor size	49.7mm
Histological grade	
Well	4 (11.1%)
Moderately	28 (77.8%)
poorly	4 (11.1%)
Post operative tumor reccurence	11 (30.6%)

Table 2. Relationship between tumor HMGB1 expression and clinico-pathologic features.

Factors		N	HMGB1 mRNA expression mean±SD	P-value
Gender				
	male	30	1.066±0.278	0.487
	female	6	1.150±0.180	
Age				
	less than 65	10	0.996±0.284	0.200
	more than 65	26	1.124±0.252	
Virus				
	B	7	1.037±0.282	0.629 / 0.740
	C	21	1.091±0.246	0.994
	none	8	1.090±0.322	
Tumor size (mm)				
	less than 30	25	1.095±0.273	0.614
	morethan 30	11	1.046±0.250	
Portal invasion				
	absent	22	1.097±0.238	0.646
	present	14	1.055±0.308	
Intrahepatic metastasis				
	absent	27	1.058±0.272	0.380
	present	9	1.148±0.240	
Gross classification				
	Localized type	21	1.073±0.248	0.854
	Invasive type	15	1.090±0.293	
Differentiation				
	Well	4	1.249±0.158	0.114 / 0.033*
	Mode	28	1.075±0.275	0.806
	poor	4	0.949±0.216	
Japanese TNM Stage				
	I,II	16	1.111±0.267	0.439
	III,IV	20	1.042±0.263	
PIVKA Ⅱ				
	Normal	9	1.099±0.263	0.814
	high	25	1.074±0.279	
AFP				
	Normal	12	1.116±0.273	0.574
	high	24	1.063±0.263	
Recurrence				
	absent	25	1.119±0.258	0.036*
	present	11	0.941±0.244	

extension. Melting curves were obtained according to the protocol under the following conditions: 0 s denaturation period at 95°C, starting temperature of 65°C, final temperature 95°C, and rate of temperature increase 0.1°C s^{-1}. The quantitative value of the target gene in each sample was normalized using GAPDH expression as an internal control. The quantitative RT-PCR assay was carried out twice and the mean value was calculated. Finally, the mRNA expression ratio of cancerous (C) to non-cancerous (N) tissues was calculated using the following formulae: R = log{target gene (C)/GAPDH (C)}, R = log{target gene (N)/GAPDH (N)}. These experiments were carried out twice to confirm reproducibility.

Statistical analysis

Statistical analysis was performed using the JMP IN version 5.1.2 software system (SAS Institute Inc., Cary, NC, USA). Values of mRNA expression were log-transformed before statistical analysis. The relationships between HMGB1 mRNA expression levels and clinicopathological features were evaluated using the Student's *t*-test and the Mann-Whitney U-test, as

appropriate. A p-value of less than 0.05 was considered statistically significant.

Results

HMGB1 expression in HCC

Using Western blotting and RT-PCR, protein and mRNA expression of HMGB1 were examined in normal liver and noncancerous and cancerous tissues from three cases (Fig. 1). All three cases showed expression of HMGB1 protein and mRNA in these tissues.

Quantitative RAGE mRNA expression in HCC and noncancerous lesions

We compared the quantitative expression of RAGE mRNA in paired cancer and noncancerous tissues from 36 cases and normal liver from seven cases. The mean value in cancerous tissues was higher than those in noncancerous tissues and normal liver tissues (HCC, 1.080 ± 0.26; noncancerous tissue, 0.99 ± 0.34; normal liver, 0.91 ± 0.11), although the differences were not significant (Fig.2).

Relationship between HMGB1 mRNA expression and clinicopathological features

To elucidate the biological significance of HMGB1 expression in HCC, we compared the levels of HMGB1 mRNA expression with the clinicopathological features of 36 patients. As shown in Table 2, we noted significant differences in HMGB1 mRNA expression in association with tumor differentiation and postoperative recurrence. The level of HMGB1 mRNA expression was higher in well-differentiated tumors than in poorly-differentiated tumors (p=0.033). The level showed an inverse correlation with the presence/absence of recurrence (p=0.036). These two findings were similar to results in our previous report comparing the levels of RAGE mRNA expression with the clinicopathological features of 36 patients of HCC. There were no significant differences in other factors.

Discussion

When inflammatory events such as injury, infection, or cell necrosis occurs, HMGB1 is secreted and induces the release of proinflammatory cytokines and chemokines such as p44/p42, p38, SAPK/JNK MAP kinases, ERK, and NF- kB [28]. Therefore, we hypothesized that HMGB1 may play an important role in the occurrence and development of HCC, which typifies inflammation-related tumors caused by viral or alcoholic hepatitis. We previously reported the clinicopathological relevance of the level of expression of RAGE mRNA, the counter-receptor for HMGB-1, in patients with HCC [22]. In that study, we noted significant differences in RAGE mRNA expression according to gender, age, the level of protein induced by vitamin K absence or its antagonist (PIVKA-II), tumor differentiation, and the postoperative recurrence of HCC. In particular, the level of RAGE mRNA expression was higher in well- or moderately differentiated tumors than in poorly-differentiated tumors, and this result was confirmed by immunohistochemical examination. Moreover, the levels of RAGE mRNA showed an inverse correlation with the presence of recurrence.

In the present study, comparing the quantitative expression of HMGB1 mRNA in paired cancerous and non-cancerous tissues of 36 cases, HCC tissues showed higher expression than non-cancerous tissues, although not significant. (Fig. 2) The mean values of RAGE mRNA expression in cancerous (P=0.08) and non-cancerous tissues were also higher than that in normal liver tissues (i.e. non-inflamed liver from benign or metastatic liver tumor patients). Furthermore, in patients with HCC, the HMGB1 level of mRNA expression differed significantly with regard to tumor differentiation, and postoperative recurrence. HMGB1 mRNA was expressed at lower level in poorly-differentiated HCC than in well-differentiated HCC (p = 0.033). The levels of HMGB1 mRNA showed a negative correlation with the presence of the recurrence (p=0.036). These results were remarkably similar to those of our previous study of the clinicopathological relevance of the level of RAGE expression in patients with HCC [22]. Once cancer is established, HCC dedifferentiates step by step to a more malignant histology, from well- or moderately differentiated to poorly differentiated HCC. In our results, when HCC is established (and is well-differentiated), levels of HMGB1 and RAGE expression are high; this may reflect elevated tumor activity.

Evidence from clinical studies indicates that well-differentiated HCC consists of hypovascular tumors primarily fed by the portal vein system, while moderately and poorly differentiated HCC consists of hypervascular tumors primarily fed by arterial blood[33-35]. Considering the level of RAGE mRNA expression in each stage of differentiation and *in vitro* results, we concluded that RAGE has a function in resistance to hypoxia. HMGB1

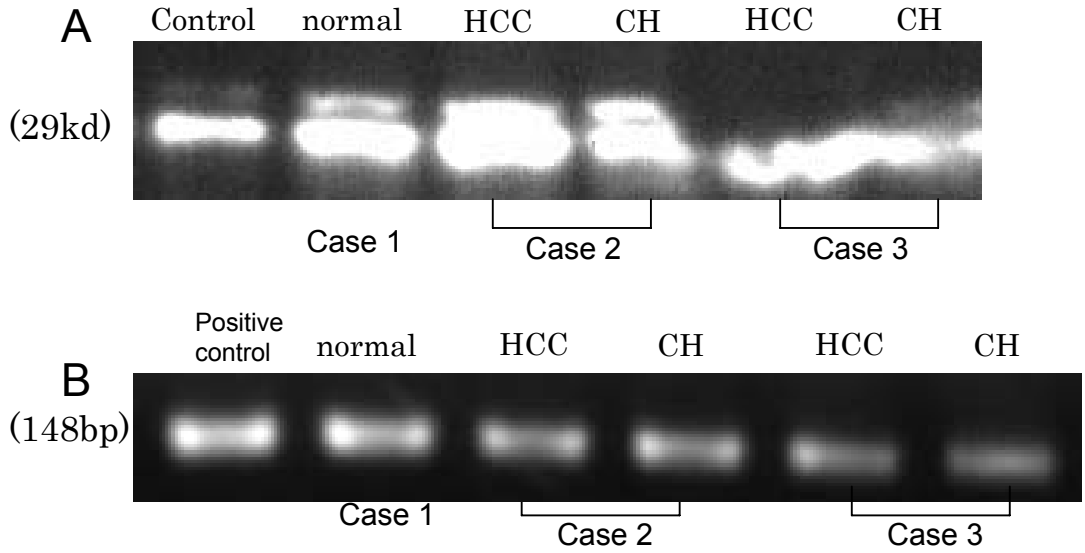

Fig. 1. HMGB1 expression as determined by Western blotting (A) and RT-PCR (B)
normal, normal liver; CH, chronic hepatitis.

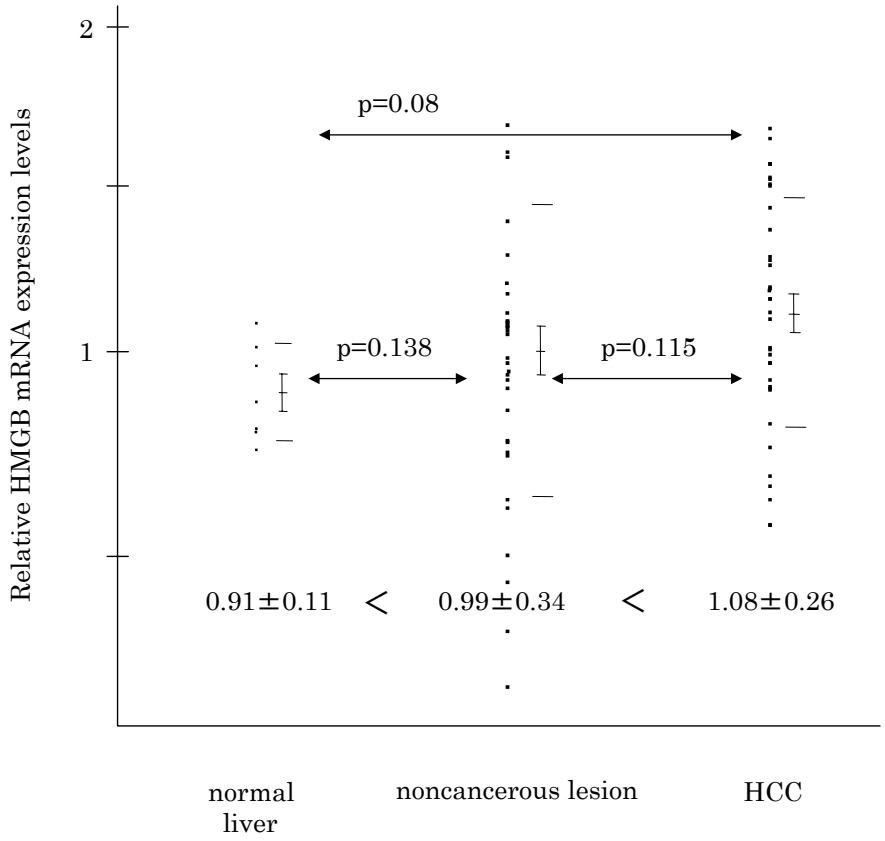

Fig. 2. HMGB1 mRNA expression in normal liver (n=7) and paired cancer and noncancerous tissues (n=36).

might also be related to hypoxia resistance leading to the induction of angiogenic cytokines such as IL-8. HMGB1-RAGE interaction undoubtedly plays a vital role in HCC.

When the liver is injured, such as through hepatitis, hepatocyte death induces the activation of Kupffer cells to produce cytokines that promote regeneration, and the organ regenerates through the compensatory proliferation of hepatocytes[36]. However, recent studies of the role of NF-kB in HCC demonstrated that NF-kB activation in hepatocytes prevented the development of HCC[36, 37]. The liver is an organ that has the ability to regenerate, and the actions of cytokines and transcriptional factors may differ between the liver and other organs. Our demonstration of inverse correlations of HMGB1 expression with malignant histology and tumor recurrence might reflect some special characteristics of the liver. HMGB1 expression may be different in other cancers. The interaction of HMGB1 and its receptors such as RAGE and TLR-4 in cancer and its interstitial cells (e.g. macrophage) is still poorly understood, and it is expected that the resolution of their molecular mechanisms will lead to new cancer therapies.

References

[1] Goodwin GH, Sanders C, Johns EW. A new group of chromatin-associated proteins with a high content of acidic and basic amino acids. Eur J Biochem 1973; 38: 14-19.

[2] Bianchi ME. Significant (re)location: how to use chromatin and/or abundant proteins as messages of life and death. Trends Cell Biol 2004; 14: 287-293.

[3] Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005; 201: 1135-1143.

[4] Dimov SI, Alexandrova EA, Beltchev BG. Differences between some properties of acetylated and nonacetylated forms of HMG1 protein. Biochem Biophys Res Commun 1990; 166: 819-826.

[5] Rouhiainen A, Kuja-Panula J, Wilkman E, Pakkanen J, Stenfors J, Tuominen RK, et al. Regulation of monocyte migration by amphoterin (HMGB1). Blood 2004; 104: 1174-1182.

[6] Messmer D, Yang H, Telusma G, Knoll F, Li J, Messmer B, et al. High mobility group box protein 1: an endogenous signal for dendritic cell maturation and Th1 polarization. J Immunol 2004; 173: 307-313.

[7] Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat Rev Immunol 2005; 5: 331-342.

[8] Bonaldi T, Talamo F, Scaffidi P, Ferrera D, Porto A, Bachi A, et al. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. Embo J 2003; 22: 5551-5560.

[9] Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999; 285: 248-251.

[10] Dumitriu IE, Baruah P, Valentinis B, Voll RE, Herrmann M, Nawroth PP, et al. Release of high mobility group box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products. J Immunol 2005; 174: 7506-7515.

[11] Semino C, Angelini G, Poggi A, Rubartelli A. NK/iDC interaction results in IL-18 secretion by DCs at the synaptic cleft followed by NK cell activation and release of the DC maturation factor HMGB1. Blood 2005; 106: 609-616.

[12] Kuniyasu H, Oue N, Wakikawa A, Shigeishi H, Matsutani N, Kuraoka K, et al. Expression of receptors for advanced glycation end-products (RAGE) is closely associated with the invasive and metastatic activity of gastric cancer. J Pathol 2002; 196: 163-170.

[13] Kuniyasu H, Chihara Y, Kondo H. Differential effects between amphoterin and advanced glycation end products on colon cancer cells. Int J Cancer 2003; 104: 722-727.

[14] Kuniyasu H, Chihara Y, Takahashi T. Co-expression of receptor for advanced glycation end products and the ligand amphoterin associates closely with metastasis of colorectal cancer. Oncol Rep 2003; 10: 445-448.

[15] Flohr AM, Rogalla P, Meiboom M, Borrmann L, Krohn M, Thode-Halle B, et al. Variation of HMGB1 expression in breast cancer. Anticancer Res 2001; 21: 3881-3885.

[16] Brezniceanu ML, Volp K, Bosser S, Solbach C, Lichter P, Joos S, et al. HMGB1 inhibits cell death in yeast and mammalian cells and is abundantly expressed in human breast carcinoma. FASEB J 2003; 17: 1295-1297.

[17] Poser I, Bosserhoff AK. Transcription factors

involved in development and progression of malignant melanoma. Histol Histopathol 2004; 19: 173-188.

[18] Poser I, Golob M, Buettner R, Bosserhoff AK. Upregulation of HMG1 leads to melanoma inhibitory activity expression in malignant melanoma cells and contributes to their malignancy phenotype. Mol Cell Biol 2003; 23: 2991-2998.

[19] Golob M, Buettner R, Bosserhoff AK. Characterization of a transcription factor binding site, specifically activating MIA transcription in melanoma. J Invest Dermatol 2000; 115: 42-47.

[20] Jacob K, Wach F, Holzapfel U, Hein R, Lengyel E, Buettner R, et al. In vitro modulation of human melanoma cell invasion and proliferation by all-trans-retinoic acid. Melanoma Res 1998; 8: 211-219.

[21] Ishiguro H, Nakaigawa N, Miyoshi Y, Fujinami K, Kubota Y, Uemura H. Receptor for advanced glycation end products (RAGE) and its ligand, amphoterin are overexpressed and associated with prostate cancer development. Prostate 2005; 64: 92-100.

[22] Hiwatashi K, Ueno S, Abeyama K, Kubo F, Sakoda M, Maruyama I, et al. A novel function of the receptor for advanced glycation end-products (RAGE) in association with tumorigenesis and tumor differentiation of HCC. Ann Surg Oncol 2008; 15: 923-933.

[23] Taniguchi N, Kawahara K, Yone K, Hashiguchi T, Yamakuchi M, Goto M, et al. High mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid arthritis as a novel cytokine. Arthritis Rheum 2003; 48: 971-981.

[24] Takata K, Kitamura Y, Kakimura J, Shibagaki K, Tsuchiya D, Taniguchi T, et al. Role of high mobility group protein-1 (HMG1) in amyloid-beta homeostasis. Biochem Biophys Res Commun 2003; 301: 699-703.

[25] Tsung A, Hoffman RA, Izuishi K, Critchlow ND, Nakao A, Chan MH, et al. Hepatic ischemia/reperfusion injury involves functional TLR4 signaling in nonparenchymal cells. J Immunol 2005; 175: 7661-7668.

[26] Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, et al. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem 2004; 279: 7370-7377.

[27] Abeyama K, Stern DM, Ito Y, Yoshimoto Y, Tanaka M, Uchimura T, et al. The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel antiinflammatory mechanism. J Clin Invest 2005; 115: 1267-1274.

[28] Taguchi A, Blood DC, del Toro G, Canet A, Lee DC, Qu W, et al. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature 2000; 405: 354-360.

[29] Yang H, Wang H, Czura CJ, Tracey KJ. The cytokine activity of HMGB1. J Leukoc Biol 2005; 78: 1-8.

[30] Ulloa L, Messmer D. High-mobility group box 1 (HMGB1) protein: friend and foe. Cytokine Growth Factor Rev 2006; 17: 189-201.

[31] Mitola S, Belleri M, Urbinati C, Coltrini D, Sparatore B, Pedrazzi M, et al. Cutting edge: extracellular high mobility group box-1 protein is a proangiogenic cytokine. J Immunol 2006; 176: 12-15.

[32] Frantz S, Vincent KA, Feron O, Kelly RA. Innate immunity and angiogenesis. Circ Res 2005; 96: 15-26.

[33] Hayashi M, Matsui O, Ueda K, Kawamori Y, Kadoya M, Yoshikawa J, et al. Correlation between the blood supply and grade of malignancy of hepatocellular nodules associated with liver cirrhosis: evaluation by CT during intraarterial injection of contrast medium. AJR Am J Roentgenol 1999; 172: 969-976.

[34] Ueda K, Terada T, Nakanuma Y, Matsui O. Vascular supply in adenomatous hyperplasia of the liver and hepatocellular carcinoma: a morphometric study. Hum Pathol 1992; 23: 619-626.

[35] Matsui O, Kadoya M, Kameyama T, Yoshikawa J, Takashima T, Nakanuma Y, et al. Benign and malignant nodules in cirrhotic livers: distinction based on blood supply. Radiology 1991; 178: 493-497.

[36] Luedde T, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, et al. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. Cancer Cell 2007; 11: 119-132.

[37] Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 2005; 121: 977-990.

肝細胞癌におけるHigh mobility group box-1（HMGB1）発現の意義

上野　真一，樋渡　清司，迫田　雅彦，飯野　　聡，蔵原　　弘，南　　幸次，安藤　　慶，前村　公成，
又木　雄弘，松本　正隆，大脇　哲弘，北薗　正樹，石神　純也，新地　洋之*，夏越　祥次

鹿児島大学大学院医歯学総合研究科腫瘍制御学消化器外科
*鹿児島大学医学部保健学科

High mobility group box-1（HMGB1）は炎症の晩期活動性メディエーターとして，炎症性サイトカインやケモカインの分泌を誘導する．肝細胞癌（HCC）は典型的な炎症関連癌であるが，HCCとHMGB1ならびにそのレセプターRAGEの関係についてはほとんど知られていない．HCC性状とHMGB1発現の関連について検討した．

36症例（B型肝炎７例とC型肝炎21例を含む）のHCC サンプルについて検討した．24症例で慢性活動性肝炎，４症例で肝硬変が認められた．癌部・非癌部のHMGB1発現について，RT-PCRとWestern blottingを用いて検討し，定量的RT-PCRデータについては臨床病理学的因子と対比した．またHCCと関連のない７症例の正常肝をコントロールとした．

HMGB1mRNAの発現は，明らかな有意差は認められなかったものの，正常肝，HCC非癌部，HCCとなるにつれて上昇した．さらに，HCCにおいては，高～中分化癌で高く，低分化癌で下降した（P=0.033）．その発現レベルは術後癌再発率と逆相関した．その他のHCCのよく知られた予後因子（門脈浸潤や肝内転移）との明らかな関連はみられなかった．

HCCにおけるHMGB1発現は患者予後とは逆相関しており，われわれが以前報告したHCCにおけるRAGE発現と類似している．RAGE-HMGB1系は，癌進展期よりは腫瘍形成期により働いているかもしれない．