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HMGB1 attenuates anti-metastatic defence of the liver in colorectal cancer ☆

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ABSTRACT

High mobility group box (HMGB) 1 induces apoptosis of monocyte-lineage cells. We examined the effect of HMGB1 on Kupffer cells (KCs). In 50 Dukes C and 12 liver-metastasised Dukes D colorectal cancers (CRCs), higher HMGB1 concentration in the primary tumours and metastatic foci, and fewer KCs were found in Dukes D cases than in Dukes C cases. The portal blood HMGB1 concentration was higher in Dukes D cases than in Dukes C cases. HMGB1 induced growth inhibition and apoptosis in mouse KCs in a dose-dependent manner, which was associated with the phosphorylation of c-Jun N-terminal kinase (JNK). JNK inhibition and knockdown of HMGB1 receptor abrogated growth inhibition and apoptosis. In a nude mouse liver metastasis model, the caecal administration of HMGB1 decreased the number of KCs and increased the embedment of Colo320 CRC cells in a dose-dependent manner. *HMGB1* transfection increased the liver metastasis of Colo320 cells, and the metastasis was inhibited by anti-HMGB1 antibody administration. These results suggest that HMGB1 secreted from primary tumours decreases the number of KCs and attenuates the anti-metastatic defence of the liver in patients with CRCs.

1. Introduction

Liver metastasis is one of the critical conditions of colorectal cancer (CRC), which determines the disease prognosis and the quality of patients' lives. One-fourth of invasive CRCs show liver metastasis at the operation and/or disease recurrence with liver metastasis after operation.[1] One-third of CRC patients then died from liver metastasis.[2] These findings suggest that the control of the liver metastasis is the relevant matter for conquering CRCs. CRC is the fourth leading cause of Japanese cancer death and is still increasing.[3] The liver metastasis of CRC is a major target of anti-cancer tactics.

In CRC metastasis, many molecular markers are reported. CD10, a neutral endopeptidase, is frequently expressed in CRCs with liver metastasis (Fujimoto, 2005 #203).[4] CD10 degrades methionine-enkephalin to escape from the cancer inhibitory effect.[5] Regenerating gene type IV (Reg IV) is a secretory small protein, which enhances cancer cell survival

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by the induction of Bcl-2, survivin, phosphorylated AKT and phosphorylated epidermal growth factor receptor (EGFR).[6] Reg IV expression is associated with delayed liver metastasis of CRCs.[7] High mobility group box 1 (HMGB1) activates a specific membrane receptor on cancer cells (receptor for advanced glycation end products; RAGEs) to accelerate cancer cell growth, motility, invasion, survival and subsequently cancer metastasis in CRCs.[8,9]

Well known as metastasis is a highly selective process that consists of a series of sequential and interrelated steps based on cancer-host relationship.[10] Macrophages are responsible for the anti-cancer host immune responses. We reported that the decrease of intratumoural macrophage infiltration is significantly associated with lymph node metastasis of CRCs.[11] HMGB1 is a significant modulator of macrophages in cancer and inflammation.[12–14] HMGB1 activates macrophages in response to lipopolysaccharide[12] and increases the secretion of the inflammatory cytokines interleukin (IL)-1β, interferon-γ and tumour necrosis factor (TNF)-α. The HMGB1-induced macrophage activation worsens septic shock, systemic inflammatory response syndrome and rheumatoid arthritis.[15] A high level of intratumoural HMGB1 in CRC induces apoptosis by activating c-Jun N-terminal kinase (JNK) in tumour-associated macrophages (TAMs).[14] HMGB1-induced TAM inhibition enhances CRC metastasis.[11] HMGB1 delivered to regional lymph nodes inhibited monocyte-dendritic cells and sinus macrophages.[16,17]

In the present study, we attempted to reveal that the inhibitory effects of CRC-derived HMGB1 on Kupffer cells (KCs) in the liver, and to show that these effects result in metastasis.

2. Materials and methods

2.1. Surgical specimens

Formalin-fixed, paraffin-embedded archival surgical specimens from 62 patients with primary colon adenocarcinomas that had invaded the subserosal layer were selected from the Nara Medical University Hospital and the Miyoshi Central Hospital (Table 1). Of 62 cases, 50 were stage C (any cases with lymph node metastasis; all cases invaded into subserosal layer) and 12 were stage D (any case with or without lymph node metastases but with distant metastases; all cases metastasized to the liver). In all cases, fresh tissues were obtained from primary tumours and lymph nodes. In 8 Dukes C cases and 8 Dukes D tissues, the liver tissues were obtained. The tissues were frozen by liquid nitrogen and kept at −80 °C. Because a written informed consent was not obtained, identifying information for all samples was removed before analysis for strict privacy protection; the procedure was in accordance with the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government.

2.2. Immunohistochemistry

Consecutive 4-μm sections were immunohistochemically stained using the immunoperoxidase technique described previously.[18] Antibodies to HMGB1 (Upstate Biotechnology Inc., Lake Placid, NY), CD68 (DAKO Corp., Carpintaria, CA) and ED2 (BMA Biomedicals, Augst, Switzerland) were used at a concentration of 0.5 μg/ml. Secondary antibodies (Medical and Biological Laboratories, Nagoya, Japan) were used at a concentration of 0.2 μg/ml. The specimens were colour-developed with diamine benzidine hydrochloride (DAKO). Meyer's haematoxylin (Sigma Chemical Co., St. Louis, MO) was used for counterstaining. CD68- and ED2-positive cells were counted from 1000 cells observed by microscopic examination.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Frozen tissues of the primary tumours, lymph nodes and livers were homogenised by 40 strokes of Dounce's pestol B in Dounce buffer (10 mM Tris–HCl pH 8.0, 1 mM $MgCl_2$, 0.25 M sucrose). The homogenate was centrifuged by 1000*g* for 10 min at 4 °C. The supernatant was saved and mixed with the same amount of 2× lysis buffer (100 mM Tris–HCl, pH 7.4, 300 mM NaCl, 10 mM ethylene diamine tetra acetic acid, 100 μg/ml phenylmethylsulphonyl fluoride, 2 μg/ml aprotinin, 1.0% w/w Nonidet P-40). The mixture was used for ELISA. The nuclear pellets were discarded to avoid the contamination of nuclear HMGB1. Concentrations of HMGB1 were detected by HMGB1 ELISA kit (Shinotest, Tokyo, Japan) according to the provider's instruction.

2.4. Separation of Kupffer cells

BALB/c mice (6-weeks old, male, Japan SLC Inc., Shizuoka, Japan) were used for Kupffer cell separation. Resected mice liver was perfused with Hanks' balanced salt solution (HBSS, Sigma) from portal vein cannulated by a 27-gauge needle. After the washout of blood, tissue lysis solution (HBSS containing 0.05% v/v collagenase (Wako Pure Chemical, Osaka, Japan), 0.005% v/v DNase (Sigma) and 0.5% v/v dispase (Sigma) was injected into the vein. The liver was perfused with 200 ml of the tissue lysis solution, which was collected and centrifuged by 300*g* for 5 min at room temperature. The cell pellet was suspended with Dulbecco's modified essential medium (DMEM, Sigma) and washed twice. Cells (1000 cells/ml) were exposed to rat IgG anti-F4/80 mAb (MCAP 497; Serotec Ltd., Oxford, UK) at 10 μg/ml for 1 h on ice. The cells then were washed three times in DMEM, and magnetic beads (Dynal, Lake Success, NY) coated with sheep anti-rat IgG were added for cell isolation as suggested by the manufacturer. Cells adhered with the magnetic beads were washed three times with phosphate buffered saline (PBS, Sigma) and used for immunoblotting.

2.5. Immunoblot analysis

Whole-cell lysates were prepared as described previously.[9] Fifty-microgram lysates were subjected to immunoblot analysis in 12.5% w/w sodium dodecyl sulphate-polyacrylamide gels followed by electrotransfer to nitrocellulose filters. The filters were incubated with primary antibody and then with peroxidase-conjugated IgG antibody (Medical and Biological Laboratories). A γ-tubulin antibody was used to assess the levels of protein loaded per lane (Oncogene Research Products, Cambridge, MA). The immune complex was visualised with an ECL Western-blot detection system (Amersham, Aylesbury, UK). Antibodies for ED2 (BMA Biomedicals), mouse albumin

Table 1 – Cases of human colorectal cancer.

Stage[a]	Dukes C	Dukes D	P value
Metastasis	Lymph nodes	Liver	
Number	50	12	
Sex (M:F)	32:18	6:6	NS
Age	51–92	53–78	NS
Location			
Caecum	2	1	
Right colon	15	3	
Left colon	22	5	
Rectum	11	3	NS
Size (mm)	45 (25–68)	44 (35–60)	NS
Depth of invasion			
Muscularis propria	3	0	
Subserosa	26	7	
Serosa exposed	21	5	NS
Nodal metastasis			
0	0	0	
1–3 nodes	29	5	
>3 nodes	21	7	NS

[a] Pathological stage was determined according to the TNM classification.[31]

(Bethyl Laboratories Inc., Montgomery, TX), receptor for advanced glycation products (RAGE, clone C-20), JNK1 (p46), phosphorylated JNK, p38 (clone A-12) and phosphorylated p38 (clone D-8) (Santa-Cruz Biotechnology, Santa-Cruz, CA) were used for primary reaction.

2.6. Assessment of cell growth and apoptosis

Cells were seeded at a density of 10,000 cells per well in 24-well tissue culture plates. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma) was added to the culture medium at a concentration of 25 μg/ml for 30 min. After removing all the medium, dimethylsulphoxide (DMSO, 1 ml) was added to dissolve formazan pigment, and 200 μl of the DMSO was examined at 540 nm. The experiments were performed in triplicate. Apoptosis was assessed by staining with Hoechst33258 fluorescent dye (Wako). Number of apoptotic cells was counted by the observation of 1000 cells.

2.7. Cell culture and reagents

U937 monocytic leukaemia cell line was purchased from Dainihon Pharmaceutical Co., Tokyo, Japan. Colo320 human colon cancer cell line was a kind gift from Dr. Wataru Yasui (Hiroshima University). Cells were maintained in DMEM containing foetal bovine serum (Sigma) under the conditions of 5% CO_2 in air at 37 °C. Macrophage differentiation of U937 cells was induced by incubation with 10 ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma) for 5 days, after which floating cells were removed by rinsing with PBS.[14] Differentiated U937 cells (PMA-U937 cells) attached to the dishes were used in further studies. For cell labelling, PKH26 chemiluminescence dye (Zynaxis Inc., Malvern, PA) was used according to the provider's instruction.[14] Infiltration of labelled cells was observed at 480 nm with a fluorescence microscope. Cells were treated with p38 inhibitor (SB239063, Sigma), and JNK inhibitor (SP600125, Biomol, Humberg, Germany) for 24 and 48 h.

2.8. HMGB1 transfectant

For constitutive expression of HMGB1, cDNA was synthesised from RNA extracted from U937 cells. The cDNA was amplified by PCR and sub-cloned into pcDNA3.1 (Invitrogen Corp., Carlsbad, CA). Colo320 cells were transfected HMGB1-pcDNA3.1 with Lipofectamine transfection reagent (Invitrogen). Transfectant was selected by treatment with G418 (100 μg/ml) for 5 weeks. Cells with the highest HMGB1 expression were used for further examination (designated as Colo320H).

2.9. Animal model

BALB/c nu-nu athymic mice (5-weeks old, male) were purchased from Japan SLC Inc. The mice were maintained according to the current regulations and standards of the Ministry of Health, Labour and Welfare, Japanese Government. Colo320 or Colo320H cells were briefly trypsinized and washed with HBSS three times. The cells suspended by HBSS were injected into the spleen by 1×10^6 in 50 μl HBSS in each mouse. For examining cancer cell embedding (Fig. 3), hrHMGB1 (Abnova Corporation, Taipei City, Taiwan, 20 μg/mouse) was injected into the caecum wall. For examining liver metastasis (Fig. 3), hrHMGB1 (100 μg/mouse), PBS (20 μl/mouse), anti-HMGB1 antibody (Abcam, Cambridge, UK, mouse monoclonal, 10 μg/mouse) or control mouse serum (DAKO, 20 μg/mouse) was injected into the peritoneal cavity twice in each week for 5 weeks. And then, the mice were sacrificed to count the number and size of metastatic foci in the liver. Metastatic tumours were observed macroscopically before fixation, and the number of the foci and each size of the focus were measured.

2.10. Statistical analysis

Statistical analyses of experimental data were done by Mann–Whitney *U* test, ANOVA test and chi-square test. Statistical significance was defined as a two-sided *P* value of less than 0.05.

3. Results

3.1. Number of Kupffer cells and liver metastases in patients with CRC

Kupffer cells play an important role in the prevention of liver metastasis by inhibiting the embedment of cancer cells in the liver sinusoids.[19] We therefore determined the number of CD68-positive Kupffer cells in the metastatic tumours of Dukes C cases and Dukes D cases with liver metastasis (Fig. 1a and b). The number of Kupffer cells was significantly lower in the latter than in the former (Fig. 1c; $P = 0.0002$). The tissue HMGB1 concentration in both primary and metastatic liver tumours was higher in Dukes D cases than in Dukes C cases (Fig. 1d and e; $P < 0.0001$ and $P = 0.0006$, respectively). In 6 Dukes C and 5 Dukes D tumours, portal bloods were sampled from peripheral veins of the mesocolon. The portal blood HMGB1 concentration in Dukes D patients (88.8 ± 10.7 μg/ml) was higher than that in Dukes C patients (28.5 ± 9.6 μg/ml)(Fig. 1f; $P = 0.0043$). The HMGB1 concentration in the portal blood was well correlated with that in the primary tumours in both Dukes C and D cases (Fig. 1g; Spearman $r = 0.8887$, $P = 0.0199$, and Spearman $r = 1.000$, $P = 0.0167$, respectively).

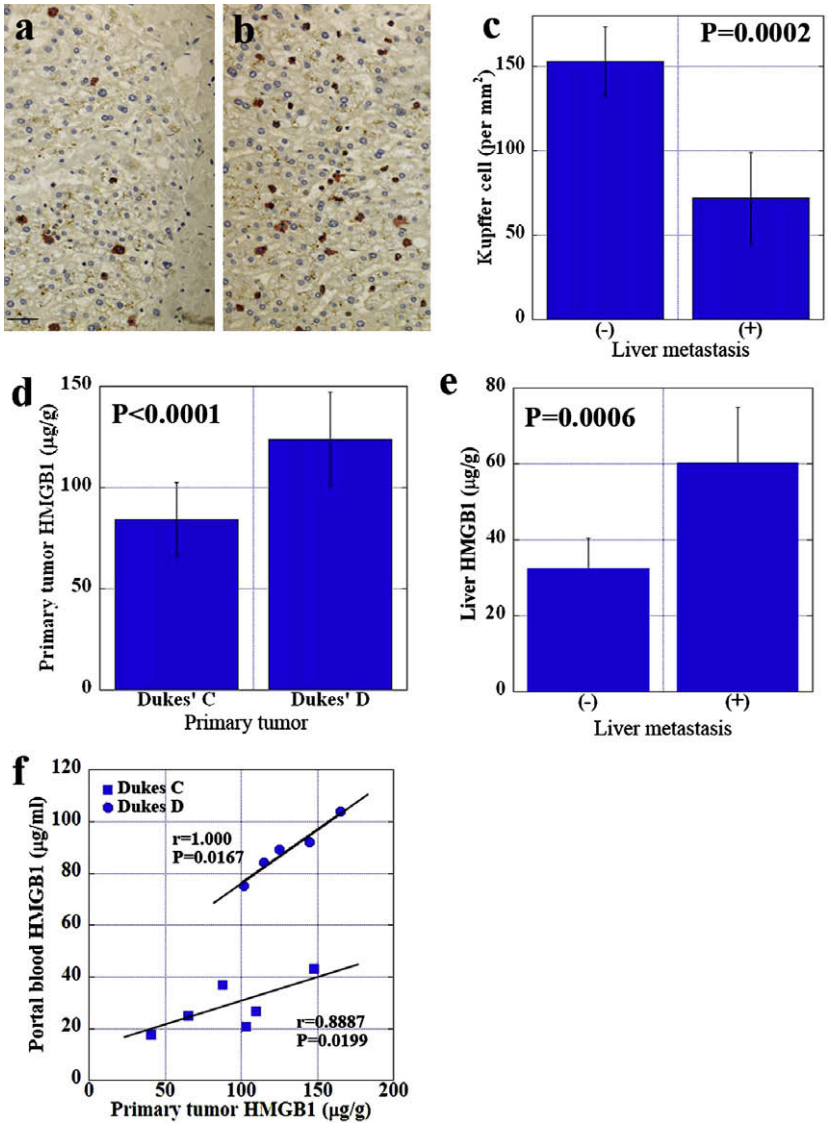

Fig. 1 – Kupffer cells in the livers of patients with CRCs. (a) and (b) Immunostaining of CD68-positive Kupffer cells in the liver of Dukes D (a) and Dukes C (b) cases. Bar 50 μm. (c) Number of CD68-positive Kupffer cells in the non-metastasised livers of Dukes C cases and metastasised livers of Dukes D cases. (d) HMGB1 concentration in primary Dukes C and D tumours. (e) HMGB1 concentration in the livers of Dukes C cases and in the metastatic livers of Dukes D cases. (f) Comparison of the HMGB1 concentrations in the portal blood with that in the primary tumours in Dukes C and D cases. Error bar, SD. *r*, Spearman's *r*.

3.2. *Effect of HMGB1 on mouse Kupffer cells*

We next examined the effects of HMGB1 on mouse Kupffer cells by *in vitro* treatment (Fig. 2). Mouse Kupffer cells separated from BALB/c male mouse confirmed their cell lineage marker (Fig. 2c). They expressed resident macrophage marker ED2 but not hepatocyte marker albumin. Mouse Kupffer cells were treated with HMGB1 to examine the effects on cell growth and apoptosis (Fig. 2a and b). They even showed HMGB1-induced growth suppression and induction of apoptosis in a dose-dependent manner. Comparing PMA-induced human macrophage cells (PMA-U937 cells), which show high sensitivity to HMGB1,[14] mouse Kupffer cells showed lower sensitivity to HMGB1 for both growth inhibition and apoptosis. Mouse Kupffer cells expressed HMGB1-specific receptor, RAGE, which was upregulated by HMGB1 treatment (Fig. 2c). HMGB1 activates MAPK family members in macrophage/monocytic lineage cells.[14,16] We then examined the effect of HMGB1 on MAPK family phosphorylation in mouse Kupffer cells (Fig. 2c). HMGB1 treatment increased phosphorylation form of JNK but not p38. Inhibition of JNK but not of p38 abrogated HMGB1-induced cell decrease (Fig. 2e). RAGE knockdown by siRNA also abrogated the cell growth inhibition by HMGB1 (Fig. 2d and e).

3.3. *Effect of HMGB1 on the number of Kupffer cells in nude mice*

To examine the effect of HMGB1 on Kupffer cells in *in vivo*, we used a mouse liver metastasis model (Fig. 3). HMGB1 was administered on day 1 into the caecum wall. On day 2, we counted the Kupffer cells in the mice (Fig. 3a–c). The HMGB1-injected mice showed significantly fewer ED2-positive Kupffer cells than the control PBS-injected mice ($P = 0.0038$). On day 3, chemiluminescent Colo320 human colon cancer cells were inoculated into the spleen. On day 4, the number of cancer

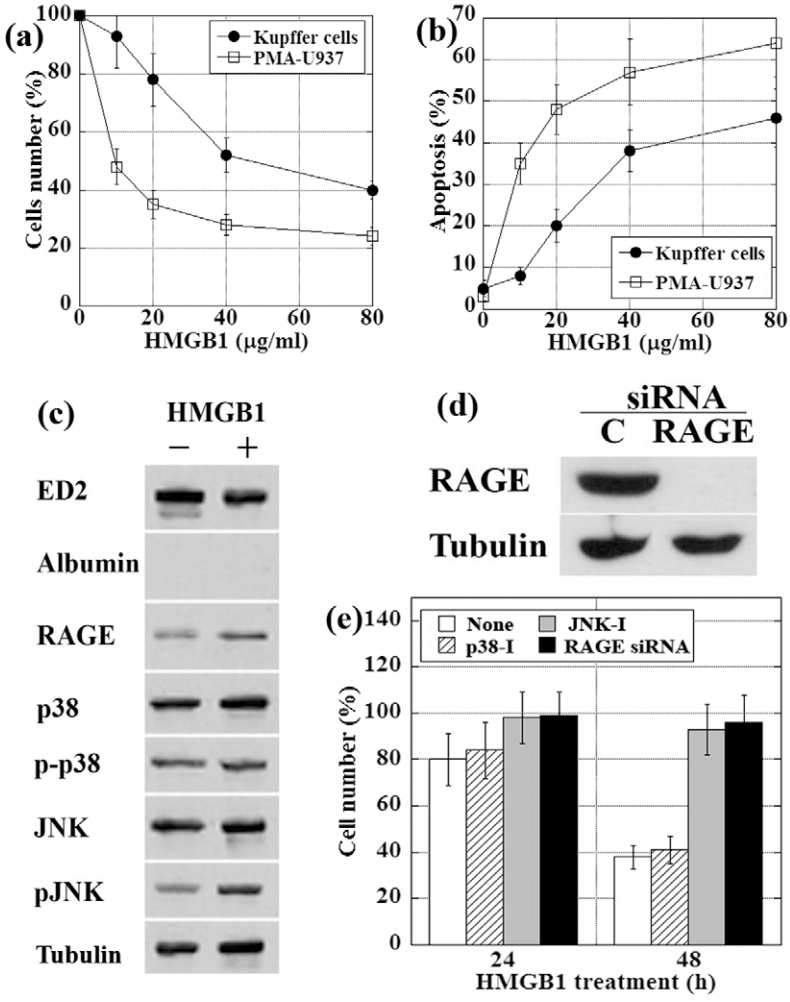

Fig. 2 – Effect of HMGB1 on mouse Kupffer cells. Kupffer cells were separated from mouse liver by perfusion with HBSS containing collagenase, dispase and DNase, and were isolated by F4/80 antibody captured with magnetic beads. (a) and (b) Effects of HMGB1 on cell growth and apoptosis in Kupffer cells. PMA-U937 cells were examined as the control. Cells were treated with HMGB1 for 48 h. (c) Effect of HMGB1 on protein levels was examined by immunoblotting mouse Kupffer cells. (d) Suppression of RAGE expression by siRNA in Kupffer cells. (e) Effect of the inhibition of p38 and JNK, and RAGE knockdown on HMGB1-induced growth suppression in mouse Kupffer cells. Error bar, SD from three independent trials.

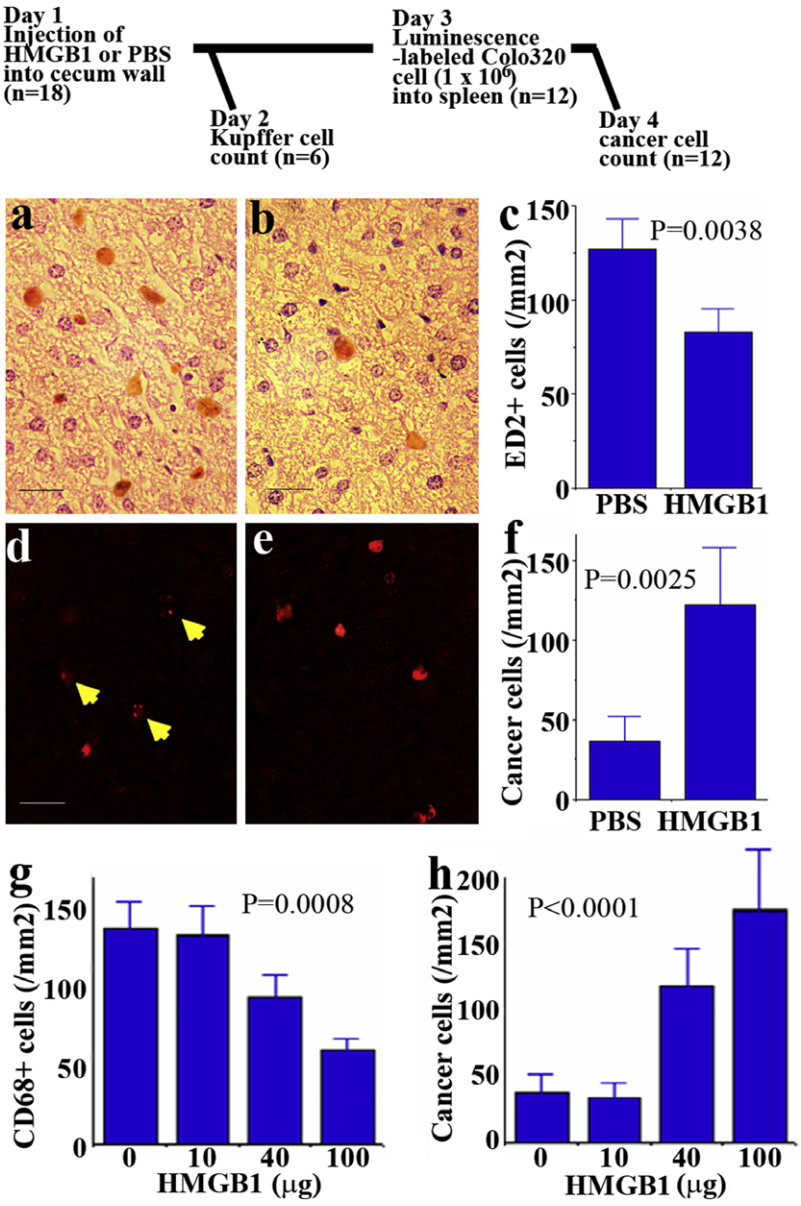

Fig. 3 – Effect of caecal administration of HMGB1 on liver metastasis of Colo320 CRC cells in nude mice. (Top panel) Experimental protocol. (a)–(c) Kupffer cells were detected in the liver by immunostaining with anti-ED2 antibody on day 2. (a) PBS-injected group. (b) HMGB1 (40 μg/mouse)-injected group. Bar 50 μm. (c) Number of ED2-positive Kupffer cells in the mouse livers after the administration of HMGB1 or PBS. (d)–(f) Chemiluminescent cancer cells embedded in the liver were detected using a fluorescence microscope on day 4. (d) PBS-injected group. Arrowhead; apoptotic cells. (e) HMGB1 (40 μg/mouse)-injected group. Bar 50 μm. (f) The number of embedded cancer cells in the mice livers administered with HMGB1 or PBS. (g) and (h) The above-mentioned experiment was repeated with different doses of HMGB1 (10 and 100 mg/mouse). (g) Number of ED2-positive Kupffer cells in the liver. (h) Number of embedded cancer cells. Error bar, SD.

cells embedded in the liver sinusoids was counted under a fluorescent microscope (Fig. 3d–f). Fewer cancer cells were found in PBS-injected mice than in HMGB1-injected mice. Some cancer cells in the PBS-injected mice showed apoptosis. The number of embedded cells was significantly greater in the HMGB1-pretreated mice than in the control mice ($P = 0.0025$). The HMGB1 dosage was compared with the number of Kupffer cells or embedded cancer cells (Fig. 3g and h). By HMGB1 administration, the number of Kupffer cells decreased in a dose-dependent manner ($P = 0.0008$), whereas the number of embedded cancer cells increased in a dose-dependent manner ($P < 0.0001$).

3.4. *Effect of HMGB1 on liver metastasis of Colo320 cells*

Finally, we confirmed the relevance of HMGB1 in liver metastasis (Fig. 4). HMGB1-transfected Colo320 cells (Colo320H) secreted a higher concentration of HMGB1 (Fig. 4a; $P = 0.0016$). We prepared a mouse liver metastasis model by the inoculation of cancer cells into mouse spleens, along with HMGB1 or

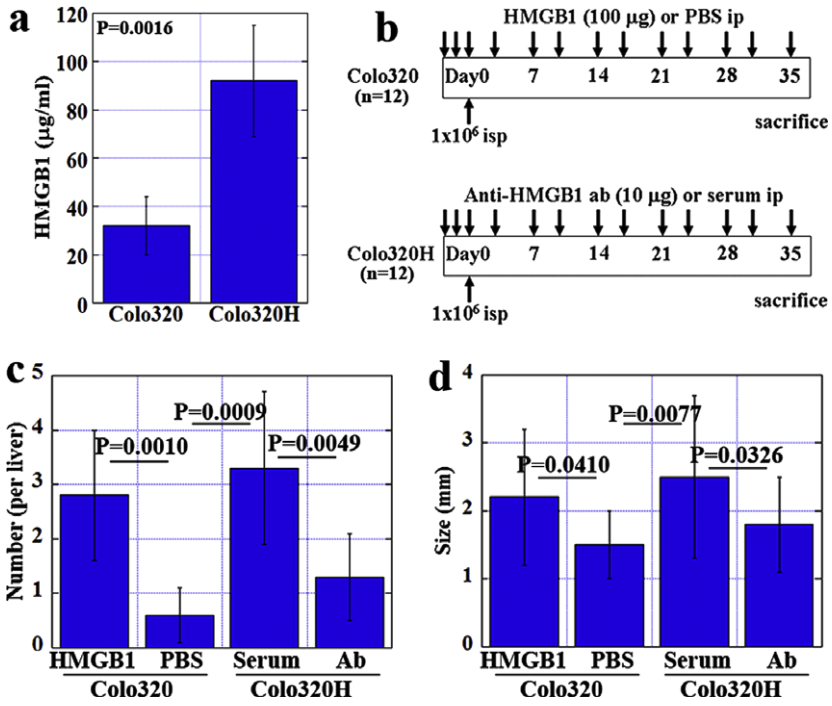

Fig. 4 – Effect of HMGB1 transfection on liver metastasis of Colo320 CRC cells in nude mice. (a) HMGB1 secretion of control Colo320 cells and HMGB1-transfected Colo320H cells. (b) Experimental protocol. A mouse liver metastasis model was prepared by the intrasplenic inoculation of cancer cells. Control mice that had been inoculated with Colo320 were injected with HMGB1 or PBS. In contrast, Colo320H-inoculated mice were injected anti-HMGB1 antibody or control mice serum. (c) Number of metastatic liver tumours. (d) Size of metastatic liver tumours. Ab, anti-HMGB1 antibody. Error bar, SD.

anti-HMGB1 antibody administration (Fig. 4b). After the inoculation, Colo320H cells were found to be more abundant than Colo320 cells and produced larger metastatic foci than Colo320 cells (Fig. 4c and d; $P = 0.0009$ and $P = 0.0077$, respectively). Further, compared to the PBS-injected mice, those inoculated with Colo320 cells showed more abundant and enlarged liver metastatic foci in response to HMGB1 administration ($P = 0.0010$ and $P = 0.0009$, respectively). In contrast, the administration of anti-HMGB1 antibody in the mice inoculated with Colo320H cells decreased the number and size of liver metastatic foci relative to the number and size of liver metastatic foci in the control serum-administered mice ($P = 0.0049$ and $P = 0.0326$, respectively).

4. Discussion

In the present study, we examined the effect of HMGB1 secreted from CRC cells on remote organs; HMGB1 was secreted from the primary tumours of CRC and delivered to the liver through portal blood flow. Then HMGB1 inhibited liver Kupffer cells to accelerate liver metastasis of CRCs. Thus, HMGB1 affects the host immunity in the metastasis-target organs in a humoral manner. Humoral effect of HMGB1 is revealed in severe inflammation.[12] In endotoxin shock, HMGB1 is released to the blood circulation and damages various organs.[15] HMGB1 also decreases the number of sinus macrophages monocytic-dendritic cells in the regional lymph nodes of CRCs.[16,17]

In the metastasis-positive livers, the HMGB1 concentrations were increased, whereas the number of Kupffer cells was proportionately decreased. In our experiments, HMGB1 was detected in the cytosolic/membrane fraction after the removal of nuclei in order to avoid contamination by nuclear HMGB1, which is the major intracellular site of HMGB1.[12] The HMGB1 detected in our experiments was thought to be in a secreted form. HMGB1 is secreted from activated macrophages,[12] and cancer cells.[20] In patients with CRC, cancer cells are a major source of HMGB1. We showed that the concentration of HMGB1 in the portal blood was well correlated with the concentration of HMGB1 in the primary tumours. We also measured serum HMGB1 concentration before and after CRC resection. If there was no remnant cancer, the serum HMGB1 levels would have decreased dramatically (data not shown). HMGB1 acts as a survival factor for cancer cells.[9,13] High concentration of HMGB1 in the portal vein might inhibit cancer cell death during transmigration via blood flow. We showed that the caecal administration of HMGB1 reduces the number of Kupffer cells, which facilitate the implantation of cancer cells from the primary tumour to the liver. HMGB1 also induces angiogenesis, which might support re-growth of embedded cancer cells. These data suggest that HMGB1 secreted from primary CRC tumours might enhance liver metastasis of CRC cells.

Our data showed that the decrease of Kupffer cells induced the metastasis of CRCs. Kupffer cells possess an important role in the anti-metastatic immune response in the metastasis-target organs. Kupffer cells phagocyte embedded cancer cells in the sinusoids and destroy them.[19,21] Sinus macrophages are also a part of an anti-cancer cytokine network involving TNF-α.[22] Kupffer cells express scavenger/lectin receptors, such as type-C lectin, sialoadhesin, CD36, CD163

and MARCO, which is distinct from those expressed in the splenic sinus macrophages.[23,24] These Kupffer cell-specific characteristics are thought to be associated with their anti-metastatic properties.

We have reported that HMGB1 induces apoptosis in monocyte-lineage cells such as macrophages and monocyte-dendritic cells.[14,16] JNK activation by HMGB1 is associated with apoptosis.[14,16] HMGB1 receptor, RAGE is expressed in macrophages,[8] which is upregulated by EGFR activation (data not shown). We confirmed that JNK inhibition and RAGE knockdown abrogated HMGB1-derived cell growth inhibition in Kupffer cells.

RAGE expressed in Kupffer cells is also activated by advanced glycation end-products (AGE), which is associated with liver disorders including diabetes, ischaemia and cirrhosis.[25]

We have previously reported that CRC-related growth factors/cytokines such as TGF-α, IL-15 and the CRC-related carcinogenic promoter deoxycholic acid enhance the secretion of HMGB1.[26–29] These factors are also known to be associated with the progression of CRC, and they directly activate cancer cell proliferation, migration and invasion.[27,28] They also attenuate the anti-metastatic defence in the target organs of metastasis via HMGB1 secretion. In contrast, we reported that KM12C cells, which show low levels of HMGB1 secretion, more pronounced macrophage infiltration, and the low incidence of liver metastasis in comparison with those in a high metastatic subline, KM12SM.[11] We have established a subline (KM12C-C9) from KM12C with high expression of TGF-α.[30] KM12C-C9 tumours, in which macrophages expression lymphatic vessel endothelial hyaluronic acid receptor and VEGF-C are infiltrating, metastasize to the regional lymph nodes. These findings suggest that HMGB1 high-producing CRCs damage the host anti-tumour immunity resulting in liver metastasis, whereas HMGB1 low-producing CRCs with high TGF-α production affect macrophages to secrete lymph-angiogenic factors resulting in lymph node metastasis. Our data suggest that the neutralisation of HMGB1 by anti-HMGB1 antibody inhibits liver metastasis. HMGB1 is expected to be a good marker for CRC metastasis and is a promising target for the inhibition of metastasis.

Conflict of interest statement

None declared.

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