MULTIPLE ROLES OF HMGB1 IN CANCER CELLS

YI LUO, HIROKI KUNIYASU
Department of Molecular Pathology, Nara Medical University
Received November 8, 2011

Abstract : HMGB1 is a non-histone chromosomal protein, a secretory protein binding to the receptor for advanced glycation end products in cancer cells and monocyte-lineage immune cells. HMGB1 enhances proliferation, motility, invasion, and survival of cancer cells. HMGB1 associated with DNA repair of anti-cancer drug-induced DNA damage. Importantly, HMGB1 is released from necrotic cancer cells and induces re-growth of the remnant cancer cells. In contrast, HMGB1 induces apoptosis in monocyte-lineage immune cells and inhibits tumor-infiltrating macrophages and dendritic cells, lymph node sinus macrophages, liver Kupffer cells to attenuate anti-cancer immune responses and anti-metastatic organ defense.

Key words : cell growth, apoptosis, macrophage, necrosis, metastasis

INTRODUCTION

High motility group box (HMGB)-1 is a multifunctional protein possessing diverse biological activities in normal cells. The roles of HMGB1 in cancer are also diverse and can be divided into 2 categories: its direct effect on cancer cells, and its effect on host immunity. HMGB1 provides pro-tumoral and anti-immune effects in the cells expressing the receptor for advanced end glycation products (RAGE). Both cancer cells and monocyte-lineage cells express RAGE; however, the effect is completely different between the two cells. Essentially, HMGB1 accelerates the metastasis of cancer cells. In this review article, we describe the roles of HMGB1 in cancer and immunity, and anti-cancer drug and tumor re-growth after anti-cancer treatment. The significant roles of HMGB1 in cancer suggest that HMGB1 is an excellent molecular target for cancer treatment, especially anti-metastatic therapeutics. These roles of HMGB1 after all provide resistance to anti-cancer drugs.

HMGB1

The high mobility group box 1 (HMGB1) protein is one of several non-histone chromosomal proteins found in eukaryotic cells[1-3]. HMGB1 is isolated as a cytosolic 30-kDa protein from fetal brain tissue[4, 5] and is associated with neurite outgrowth[2, 3]. As a nuclear protein, HMGB1 binds to DNA, participating in multiple processes such as transcription, replication, recombination, DNA repair, and genomic stability[6].

In the cytoplasm, HMGB1 is associated with cell motility as observed in the outgrowing neurites. At the leading edge of the motile cell, HMGB1 accelerates formation of filopodia as well as actin-polymer formation[2]. The mechanism of HMGB1-dependent cell migration in cancer cells is considered to be similar to that of outgrowing neurites. HMGB1 is expressed in immature cells and malignant cells at high levels and it plays a major role in controlling cell migration activity[7]. The DNA-binding capacity of HMGB1 provides a role as a DNA

chaperon. In the innate immune system, HMGB1 is the effective DNA sensor presenting the DNA to toll-like receptor (TLR)[8]. Recently, endogenous HMGB1 is revealed to activate an autophagy signal, which promotes cell survival[9].

HMGB1 is secreted from activated monocytes, macrophages, and NK cells, and acts extracellularly as a proinflammatory cytokine. HMGB1 expression and secretion is upregulated in response to stimulation of cells by proinflammatory cytokines, endotoxin, and oxidative stresses in macrophages[10-13]. Cancer cells also overexpress and secrete HMGB1 by stimulation of growth factors, cytokines, and cellular stresses involving advanced glycation end products (AGE) and deoxycholic acid[14-17]. Secreted HMGB1 activates RAGE as a ligand to induce cell growth, motility, invasion, and angiogenesis as will be described below.

HMGB1 receptor

The HMGB1 receptor, RAGE is purified from bovine lung endothelial extract as receptor of AGE[18]. RAGE is a member of cell surface receptors belonging to the immunoglobulin superfamily[19-22]. RAGE is closely associated with cell growth, cell invasion through mitogen-activated protein (MAP) kinase activation, and matrix metalloproteinase (MMP)-2 and -9 expression in glioma cells[22]. RAGE upregulation is found in colon and oral carcinogenesis in rodents[16, 23].

The co-expression of HMGB1 and RAGE is pivotal for accelerating tumor metastasis and poor prognosis in glioma, gastric, colorectal, and prostate cancer[15, 22, 24-26]. Gastric and colon cancer cells show concurrent expression of HMGB1 and RAGE, which is closely associated with the autocrine/paracrine regulation of cell motility and invasion of cancer cells[15, 24-26]. Metastatic prostate cancer cases show HMGB1 induction in prostatic stromal cells. Concurrence of RAGE expression in tumor cells and HMGB1 expression in stromal cells accelerate cancer metastability[15].

In contrast, high-level expressions of RAGE and HMGB1 are found in normal lung tissue and non-small cell lung cancer, which, in contrast with other cancer, is associated with tissue differentiation and good prognosis[27, 28]. RAGE is also associated with myogenic differentiation of myoblasts and rhabdomyosarcoma, which is associated with reduction of malignant phenotypes of the disease[29, 30].

HMGB1 secretion

HMGB1 is released by both active and passive processes. HMGB1 is actively transported from the nucleus to the cytoplasm following detachment from loosened chromosomes by histone acetylation[17]. And recent studies have shown that it shuttles between the nucleus to the cytoplasm through hyperacetylation and phosphorylation in macrophages, and is monomethylated at Lys42 in neutrophils. HMGB1 is released from necrotic cells by passive diffusion[31]. However, HMGB1 is not released from tightly packed nuclei of apoptotic cells and triggers inflammation..

HMGB1 intracellular signals

The interaction of HMGB1 with RAGE also activates the intracellular signaling pathway of MAP kinase. Consequently, RAGE activates GTPases, Ras, Cdc42, Rac, Rho, and MMP-2/-9

[3, 22]. RAGE expression is associated with cell invasion[24, 26] and it is suggested that type IV collagenase activation may be one mechanism for enhancement of the invasive capacity of cancer cells. RAGE activation induces cell growth through MAP kinase signaling[22]. RAGE activation is also associated with induction of inducible nitric oxide synthase (iNOS), nuclear factor (NF) kB activation, and Bcl-2 production[32]. NFkB activation is associated with HMGB1-dependent chemotaxis[33].

HMGB1 and angiogenesis

Intracellular signaling pathways of RAGE induce vascular endothelial cell growth factor (VEGF) expression and activate NF-kB in vascular endothelial cells[34]. Activated RAGE induces VEGF expression transcriptionally through activation of NFkB, AP-1, and hypoxia-inducible factor (HIF)-1a[34, 35], which is also associated with complications in diabetes, such as diabetic retinopathy[36]. There is a difference of VEGF induction between AGE and HMGB1[26]. AGE-BSA has a more pronounced effect on VEGF expression than HMGB1 in colorectal cancer cell lines. In our studies, HMGB1 induced the secretion of VEGF but not that of VEGF-C in human oral squamous cell carcinoma (OSCC) cell lines[37]. VEGF-C and VEGF-D are associated with lymph node metastasis[38]. Differential induction of VEGF from VEGF-C through activation of RAGE by HMGB1 may explain why RAGE expression is not associated with lymphangiogenesis. Lymph node metastasis of cancer is strongly associated with lymphangiogenesis[39].

HMGB1 in anti-cancer immunity

HMGB1 is associated with a significant reduction of intratumoral macrophage infiltration in metastatic colon cancer[40]. HMGB1 induces growth inhibition in rat peritoneal macrophages, U937 human monocytic cells, and human alveolar macrophages, and induces apoptotic death with phosphorylation of JNK and Rac1, and upregulation of caspase-3 and caspase-9[41, 42]. JNK is associated with apoptotic signals transmitted by Rac1/Cdc42[29, 30, 43].

Tumor-associated macrophages also have anti-cancer effects[44]. In clinical studies, colon cancer patients with high-level macrophage infiltration show less invasion and metastasis than those with low-level macrophage infiltration[45]. Depletion of tumor-infiltrating macrophages is closely associated with advanced stages of human colon cancer and with metastatic ability in a mouse colon cancer model[40]. Dukes B CRC cases with macrophage-cancer cell contact were found, whereas Dukes C cases showed no such contact. HMGB1 expression is associated with macrophage depletion in colon cancer tissues[40].

Lymph sinus macrophages and liver Kupffer cells (KCs) participate in the immune response of the organs against metastatic cancer cells. Sinus macrophages and KCs mediate the phagocytosis of cancer cells attached to the sinus wall in order to inhibit their metastasis [46, 47]. In CRC cases, macrophage numbers in the regional lymph nodes are decreased in both non-metastasized and metastasized nodes in Dukes C cases, whereas macrophage numbers in Dukes B nodes are higher[48]. Nodal HMGB1 concentration is higher in Dukes C nodes than that in Dukes B nodes; this is inversely correlated with macrophage numbers. Nodal HMGB1 concentration is correlated with HMGB1 concentration and lymph vessel density found in the primary tumors[48]. High concentration of HMGB1 is reported in effusions from cancer

patients. These data indicate that HMGB1 secreted from primary tumors is delivered to the regional lymph nodes and decreases the number of macrophages to weaken the anti-metastatic defense of the lymph nodes in patients with CRCs.

In a nude mouse liver metastasis model, the cecal administration of HMGB1 decreased the number of KCs and increased the embedment of colon cancer cells in a dose-dependent manner[49]. HMGB1 is secreted from primary tumors of colon cancer and delivered to the liver through portal blood flow. Following this, HMGB1 inhibits KCs to accelerate liver metastasis of colon cancer. In clinical studies, higher HMGB1 concentrations are found in the primary tumors and metastatic foci, and fewer KCs are found in Dukes D cases than in Dukes C cases. The portal blood HMGB1 concentrations are higher in Dukes D cases than in Dukes C cases, and we have shown that the concentration of HMGB1 in the portal blood is strongly correlated with the concentration of HMGB1 in the primary tumors[49]. As a result, HMGB1 affects the host immunity in the metastasis-target organs in a humoral manner. Large amounts of secreted HMGB1 can affect remote organs such as the target organs of metastases from CRCs.

Dendritic cells (DCs) play a crucial role in host immune response to various extrinsic microorganisms and also to cancer cells[50]. Dendritic cell densities in primary tumors and metastatic tumors are suppressed[51]. Indeed, nodal metastasis-positive colon cancer cases show higher HMGB1 concentrations in lymph nodes and primary tumor tissues, and fewer dendritic cell numbers[42]. HMGB1 produced by colon cancer cells resulted in a suppression of nodal dendritic cells to attenuate host anti-cancer immunity. HMGB1 results in activation of monocytes and dendritic cells; however, high concentrations of HMGB1 result in a death signal to dendritic cells, as found on macrophages[42]. Mouse peritoneal macrophage-derived dendritic cells (PMDDCs) treated with HMGB1 show a decrease in cell number in a dose-dependent manner. HMGB1-treated PMDDCs show apoptosis and increased levels of phosphorylated JNK, and intraperitoneal administration of HMGB1 decreased splenic dendritic cells in C57BL mice[42].

HMGB1 may provide cancer cells with the advantages of cancer progression and suppression of host immunity; therefore, further examination of the role of HMGB1-induced macrophage apoptosis in cancer may provide novel therapeutic targets against these diseases.

HMGB1 and anti-cancer drugs

They bind with high affinity to specific structural distortions in the double helix such as synthetic four way junctions and adducts that are formed in DNA modified by the anti-tumor drug cisplatinum and UV light[52, 53]. DNA bound HMGB1 plays a role in DNA repair providing drug resistance to platinum derivatives in cancer cells[54].

HMGB1 is passively secreted from necrotic cells. We confirmed that necrosis inducers, such as doxorubicin (DXR) increase HMGB1 concentration in the cultured medium. In contrast, apoptosis inducers, such as trichostatin A (TSA) do not increase HMGB1 in the cultured medium. In a mouse tumor model of bilateral scapular subcutaneous tumors, induction of necrosis at one tumor by DXR enhances growth of the contralateral tumor. In contrast, induction of apoptosis at one tumor by TSA does not affect growth of the contralateral tumor. Moreover, in mouse liver and lung metastasis models with one

subcutaneous tumor, induction of necrosis at the subcutaneous tumor by DXR increases metastasis to the liver and lung. The enhancement of metastasis is abrogated by administration of anti-HMGB1 antibody. These findings suggest that HMGB1 enhances growth of the remnant cancer cells to increase the tumor relapse and metastasis. The pro-apoptotic but not pro-necrotic anti-cancer drugs are needed to avoid HMGB1-induced cancer relapse and metastasis.

Conclusion

Resistance to anti-cancer drugs is provided primarily by abrogation of the pharmacological mechanism of the drugs. Multiple drug resistance (MDR) gene product, P glycoprotein reduced intracellular drug concentration by pumping out the drug. Drug resistance is provided secondarily by enhancement of tumor survival and reduction of anti-cancer immunity. HMGB1 accelerates drug resistance of cancer cells by increase of DNA repair, suppression of anti-cancer immunity, and enhancement of survival and growth of cancer cells. In this context, HMGB1 is a pivotal anti-cancer drug resistant factor. To increase the efficacy of anti-cancer treatment, HMGB1 is a relevant target.

REFERENCES

1) **Lee, K. L., Pentecost, B. T., D'Anna, J. A., Tobey, R. A., Gurley, L. R.** and **Dixon, G. H.** : Characterization of cDNA sequences corresponding to three distinct HMG-1 mRNA species in line CHO chinese hamster cells and cell cycle expression of the HMG-1 gene. Nucleic Acids Res **15** : 5051-5068, 1987.
2) **Rauvala, H.** and **Pihlaskari, R.** : Isolation and some characteristics of an adhesive factor of brain that enhances neurite outgrowth in central neurons. J Biol Chem **262** : 16625-16635, 1987.
3) **Parkkinen, J., Raulo, E., Merenmies, J., Nolo, R., Kajander, E. O., Baumann, M.,** et al. : Amphoterin, the 30-kDa protein in a family of HMG1-type polypeptides. Enhanced expression in transformed cells, leading edge localization, and interactions with plasminogen activation. J Biol Chem **268** : 685-691, 1993.
4) **Tanji, N., Markowitz, G., S., Fu, C., Kislinger, T., Taguchi, A., Pischetsrieder, M.,** et al. : Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. J Am Soc Nephrol **11** : 1656-66, 2000
5) **Merenmies, J., Pihlaskari, R., Laitinen, J., Wartiovaara, J.** and **Rauvala, H.** : 30-kDa heparin-binding protein of brain (amphoterin) involved in neurite outgrowth. Amino acid sequence and localization in the filopodia of the advancing plasma membrane. J Biol Chem **266** : 16722-9, 1991.
6) **Stros, M.** : HMGB proteins: Interactions with DNA and chromatin. Biochim Biophys Acta **1799** : 101-13, 2010.
7) **Fages, C., Nolo, R., Huttunen, H., J., Eskelinen, E.** and **Rauvala, H.** : Regulation of cell migration by amphoterin. J Cell Sci **113** : 611-620, 2000.
8) **Tesniere, A., Ghiringhelli, F., Joza, N., Panaretakis, T., Kepp, O., Schlemmer, F., Zitvogel, L.** and **Kroemer, G.** : Immunogenic cancer cell death: a key-lock paradigm. Curr Opin Immunol **20** : 504-511, 2008
9) **Tang, D., Kang, R., Livesey, K., M., Cheh, C., W., Farkas, A., Loughran, P.,** et al. : Endogenous HMGB1 regulates autophagy. J Cell Biol **190** : 881-92, 2010.
10) **Czura, C., J., Wang, H.** and **Tracey, K., J.** : Dual roles for HMGB1: DNA binding and cytokine. J Endotoxin Res **7** 315-21, 2001.
11) **Degryse, B., Bonaldi, T., Scaffidi, P., Muller, S., Resnati, M., Sanvito, F.,** et al. : The high mobility

group (HMG) boxes of the nuclear protein HMG1 induce chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. J Cell Biol **152** : 1197-206, 2001.

12) **Wang, H., Bloom, O., Zhang, M., Vishnubhakat, J., M., Ombrellino, M., Che, J.,** et al. : Science **285** : 248-251, 1999.

13) **Tang, D., Kang, R., Zeh, H., J.** and **Lotze, M., T.** : High-mobility group box 1, oxidative stress, and disease. Antioxid Redox Signal **14** 1315-35, 2010.

14) **Sasahira, T., Sasaki, T.** and **Kuniyasu, H.** : Interleukin-15 and transforming growth factor a are associated with depletion of tumor-associated macrophages in colon cancer. J Exp Clin Cancer Res **24** : 69-74, 2005.

15) **Kuniyasu, H., Chihara, Y., Kondo, H.** and **Ohmori, H., Ukai, R.** : Amphoterin induction in prostatic stromal cells by androgen deprivation is associated with metastatic prostate cancer. Oncol Rep **10** : 1863-1868, 2003.

16) **Ohmori, H., Luo, Y., Fujii, K., Sasahira, T., Shimomoto, T., Denda, A.,** et al. : Dietary linoleic acid and glucose enhances azoxymethane-induced colon cancer and the metastasis through the expression of high mobility group box 1. Pathobiol **77** : 210-217, 2010.

17) **Fujii, K., Luo, Y., Sasahira, T., Denda, A., Ohmori, H.** and **Kuniyasu, H.** : Co-treatment with deoxycholic acid and azoxymethane accelerates the secretion of HMGB1 in IEC6 intestinal epithelial cells. Cell Prolif **42** : 701-709, 2009.

18) **Schmidt, A., M., Vianna, M., Gerlach, M., Brett, J., Ryan, J., Kao, J.,** et al. : Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. J Biol Chem **267** : 14987-97, 1992.

19) **Kostova, N., Zlateva, S., Ugrinova, I.** and **Pasheva, E.** : The expression of HMGB1 protein and its receptor RAGE in human malignant tumors. Mol Cell Biochem **337** : 251-8, 2010.

20) **Sasahira, T., Akama, Y., Fujii, K.** and **Kuniyasu, H.** : Expression of receptor for advanced glycation end products and HMGB1/amphoterin in colorectal adenomas. Virchows Arch **446** : 411-415, 2005.

21) **Yan, S., F., Ramasamy, R.** and **Schmidt, A., M.** : Mechanisms of disease: Advanced glycation end-products and their receptor in inflammation and diabetes complications. Nat Clin Pract Endocrinol Metab **4** : 285-93, 2008.

22) **Taguchi, A., Blood, D., C., del Toro, G., Canet, A., Lee, D., C., Qu, W.,** et al. : Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases.Nature **405** : 354-360, 2000.

23) **Yamamoto, K., Kitayama, W., Denda, A., Sasahira, T., Kuniyasu, H.** and **Kirita, T.** : Expression of receptor for advanced glycation end products during rat tongue carcinogenesis by 4-nitroquinoline 1-oxide and effect of a selective cyclooxygenase-2 inhibitor, etodolac. Pathobiology **73** : 317-24, 2006.

24) **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 **196** : 163-70, 2002.

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

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

27) **Schraml, P., Shipman, R., Colombi, M.** and **Ludwig, C., U.** : Identification of genes differentially expressed in normal lung and non-small cell lung carcinoma tissue. Cancer Res **54** : 5236-5240, 1994.

28) **Bartling, B., Hofmann, H., S., Weigle, B., Silber, R.** and **E., Simm, A.** : Down-regulation of the receptor for advanced glycation end-products (RAGE) supports non-small cell lung carcinoma. Carcinogenesis **26** :

293-301, 2005.

29) **Riuzzi, F., Sorci, G.** and **Donato, R.** : The amphoterin (HMGB1)/receptor for advanced glycation end products (RAGE) pair modulates myoblast proliferation, apoptosis, adhesiveness, migration, and invasiveness. Functional inactivation of RAGE in L6 myoblasts results in tumor formation in vivo. J Biol Chem **281** : 8242-8253, 2006.

30) **Riuzzi, F., Sorci, G.** and **Donato, R.** : RAGE expression in rhabdomyosarcoma cells results in myogenic differentiation and reduced proliferation, migration, invasiveness, and tumor growth. Am J Pathol **171** : 947-961, 2007.

31) **Scaffidi, P., Misteli, T.** and **Bianchi, M., E.** : Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature **418** : 191-5, 2002.

32) **Huttunen, H., J., Kuja-Panula, J., Sorci, G., Agneletti, A., L., Donato, R.** and **Rauvala, H.** : Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. J Biol Chem **275** : 40096-40105, 2000.

33) **Penzo, M., Molteni, R., Suda, T., Samaniego, S., Raucci, A., Habiel, D., M.,** et al. : Inhibitor of NF-kb kinases a and b are both essential for high mobility group box 1-mediated chemotaxis. J Immunol **184** : 4497-4509, 2010.

34) **Okamoto, T., Yamagishi, S., Inagaki, Y., Amano, S., Koga, K., Abe, R.,** et al. : Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin.FASEB J **16** : 1928-1930, 2002.

35) **Treins, C., Giorgetti-Peraldi, S., Murdaca, J., Van Obberghen, E.** : Regulation of vascular endothelial growth factor expression by advanced glycation end products. J Biol Chem **276** : 43836-43841, 2001.

36) **Yamamoto, Y., Yamagishi, S., Yonekura, H., Doi, T., Tsuji, H., Kato, I., et al.** : Roles of the AGE-RAGE system in vascular injury in diabetes. Ann N Y Acad Sci **902** : 163-170, 2000.

37) **Sasahira, T., Kirita, T., Oue, N., Bhawal, U., K., Yamamoto, K., Fujii, K.,** et al. : Cancer Sci **99** : 1806-1812, 2008.

38) **Achen, M., G.,** and **Stacker, S., A.**: Molecular control of lymphatic metastasis. Ann N Y Acad Sci **1131** : 225-234, 2008.

39) **Sundar, S., S.,** and **Ganesan, T., S.** : Role of lymphangiogenesis in cancer. J Clin Oncol **25** : 4298-4307, 2007.

40) **Kuniyasu, H., Sasaki, T., Sasahira, T., Ohmori, H.** and **Takahashi, T.** : Depletion of tumor-infiltrating macrophages is associated with amphoterin expression in colon cancer. Pathobiology **71** : 1 29-136, 2004.

41) **Kuniyasu, H., Yano, S., Sasaki, T., Sasahira, T., Sone, S.** and **Ohmori, H.** : Colon cancer cell-derived high mobility group 1/amphoterin induces growth inhibition and apoptosis in macrophages. Am J Pathol **166** : 751-760, 2005.

42) **Kusume, A., Sasahira, T., Luo, Y., Isobe, M., Nakagawa, N., Tatsumoto, N.,** et al. : Suppression of dendritic cells by HMGB1 is associated with lymph node metastasis of human colon cancer. Pathobiology **76** : 155-162, 2009.

43) **Embade, N., Valeron, P., F., Aznar, S., Lopez-Collazo, E.** and **Lacal, J., C.** : Apoptosis induced by Rac GTPase correlates with induction of FasL and ceramides production. Mol Biol Cell **11** : 4347-4358, 2000.

44) **Kikkawa, H., Imafuku, H., Tsukada, H.** and **Oku, N.** : Possible role of immune surveillance at the initial phase of metastasis produced by B16BL6 melanoma cells. FEBS Lett **467** : 211-216, 2000.

45) **Hermanowicz, A., Gibson, P., R.** and **Jewell, D., P.** : Tumour related inhibition of macrophage chemotaxis in patients with colon cancer. Gut **28** : 416-422, 1987.

46) **Gannon, P., O., Alam Fahmy, M., Begin, L., R., Djoukhadjian, A., Filali-Mouhim, A., Lapointe, R.,** et al. : Presence of prostate cancer metastasis correlates with lower lymph node reactivity. Prostate **66** : 1710-1720, 2006.

47) **Timmers, M., Vekemans, K., Vermijlen, D., Asosingh, K., Kuppen, P., Bouwens, L.,** et al. : Interactions between rat colon carcinoma cells and Kupffer cells during the onset of hepatic metastasis. Int J Cancer **112** : 793–802, 2004.

48) **Moriwaka, Y., Luo, Y., Ohmori, H., Fujii, K., Tatsumoto, N., Sasahira, T.,** et al. : HMGB1 attenuates anti-metastatic defense of the lymph nodes in colorectal cancer. Pathobiology **77** : 17–23, 2010.

49) **Luo, Y., Ohmori, H., Fujii, K., Moriwaka, Y., Sasahira, T., Kurihara, M.,** et al. : HMGB1 attenuates anti-metastatic defense of the liver in colorectal cancer. Eur J Cancer **46** : 791–799, 2010.

50) **Wertel, I., Barczynski, B.** and **Kotarski, J.** : The role of dendritic cells in cytotoxic immune response regulation in ovarian cancer micro-environment. Front Biosci **13** : 2177–2190, 2008.

51) **Shunyakov, L., Ryan, C., K., Sahasrabudhe, D., M.** and **Khorana, A., A.** : The influence of host response on colorectal cancer prognosis. Clin Colorectal Cancer **4** : 38–45, 2004.

52) **Bianchi, M., E., Beltrame, M.** and **Paonessa, G.** : Specific recognition of cruciform DNA by nuclear protein HMG1. Science **243** : 1056–1059, 1989.

53) **Pasheva, E., A., Pashev, I., G.** and **Favre, A.** : Preferential binding of high mobility group 1 protein to UV-damaged DNA. Role of the COOH-terminal domain. J Biol Chem **273** : 24730–24736, 1998.

54) **Chaney, S., G.** and **Vaisman, A.** : Specificity of platinum-DNA adduct repair. J Inorg Biochem **77** : 71–81, 1999.