Cytosolic HMGB1 Expression in Human Renal Clear Cell Cancer Indicates Higher Pathological T Classifications and Tumor Grades

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Purpose: High mobility group box (HMGB) proteins are nuclear nonhistone chromosomal proteins that bend DNA, bind preferentially to distorted DNA structures, and promote the assembly of site-specific DNA binding proteins. Recent reports indicate that HMGB1 functions as a proinflammatory cytokine. Here, we studied expressions of HMGB1 and HMGB2 in human renal cancer.

Material and Methods: Immunohistological expressions of HMGB1 and HMGB2 were assessed in 39 surgically resected human renal cancer specimens.

Results: HMGB1 was expressed in the nucleus in 37 out of 39 (94.9%) renal clear cell cancers, while its expression in the cytosol was noted in 19 cases (48.7%). Cytosolic HMGB1 is expressed more frequently in cancers beyond the pT1b classification than in those at the pT1a classification. Higher tumor grades (≥ G2) were also significantly linked with the cytosolic expression of HMGB1. HMGB2 was expressed in the nucleus in 35 of 39 (89.7%) renal clear cell cancers, while its expression in the cytosol was observed in only 7 case (17.9%). Linkage between cytosolic expression of HMGB2 and T classifications was weakly observed, while that between nuclear expression and T classifications was not.

Conclusion: HMGB1 expressed in the cytoplasm may be an effective marker indicating higher T classifications and tumor grades.

keywords: kidney neoplasms; gene expression; HMGB1 protein; genetics; neoplasm invasiveness

INTRODUCTION

High mobility group box (HMGB) proteins are nuclear nonhistone chromosomal proteins which bend DNA, bind preferentially to distorted DNA structures, and promote the assembly of site-specific DNA binding proteins leading to the regulation of gene transcription.[1] HMGB1 consists of 215 amino acids and is markedly conserved among species.[2] HMGB1, as well as HMGB2 and HMGB3 of the same family, is structurally composed of a bipolar structure of two L-shaped homologous DNA-binding sequences termed box A and box B, and a highly negatively charged C terminus.[3] Each of the two conserved HMG box domains forms three alpha-helices that fold into a sequence-nonspecific DNA-binding module recognizing the DNA minor groove. HMGB1 has been regarded as a ubiquitous nuclear protein with an architectural function, although HMGB2 and HMGB3 expressions are relatively restricted. However, recent reports indicate that HMGB1 expression can be outside of the nucleus, and so it is not a housekeeping protein but has a dual function,[4] a cytokine in addition to a nuclear protein.

In certain cell types, extranuclear and extracellular HMGB1 as a potent proinflammatory cytokine plays important roles in inflammation, cell migration, and tumor proliferation/invasion.[5,6] Although HMGB1 was first believed to be released by necrotic cells, not by apoptotic cells,[7] its release from cells can occur during the course of apoptosis and autophagy as well as necrosis with the release process varying with the cell type.[8,9] The receptor for advanced glycation end products (RAGE), Toll-like receptor-2 (TLR-2), and Toll-like receptor-4 (TLR-4) are known as receptors of HMGB1.[10] The autocrine loop consisting of HMGB1 and RAGE modulates the maturation of human plasmacytoid dendritic cells.[11]

Extracellular HMGB1 is an inflammation mediator and a danger signal.[12] The release of HMGB1 by necrotic cells as well as activated macrophages or monocytes triggers inflammation, and HMGB1-negative necrotic cells have a markedly reduced ability to promote inflammation.[7] The HMGB1 B box domain induces dendritic cell maturation and Th1 cell polarization, enhancing immune reactions.[13] On the contrary, Kusume and associates reported that HMGB1 produced by colon cancer cells disturbed anti-cancer immunity in the host by suppressing nodal dendritic cells.[14]

The increased expression of HMGB1, particularly in conjunction with RAGE, has been reported for several different tumors, including colon cancer,[14] breast cancer,[15] melanoma,[16] prostate cancer,[17,18] and gastrointestinal stromal tumors,[19] often indicating invasiveness, metastasis, and a poor prognosis.[20,21] Lung cancer is an exception where the loss of HMGB1-RAGE-mediated regulation is associated with increased aggressiveness of tumor behavior.[22] On the contrary, a role for HMGB1 in the error-free repair of DNA lesions is indicated with its absence leading to increased mutagenesis, decreased cell survival, and altered chromatin reorganization after DNA damage.[23]

HMGB2 has not been reported to come out of the nuclus or cells differently from HMGB1. Ubiquitous expressions of HMGB1 and HMGB2 have the potential to regulate the transcriptional activity of different members of the p53 family in cell-specific and promotor-specific manners in vivo.[24] Both HMGB1 and HMGB2 are sensors of DNA damage inducing a p53-mediated DNA damage response, abrogation of which increased chemoresistance in some cancer cell lines.[25]

Renal cancer can be cured only by surgical excision, i.e. radical or partial nephrectomy. Radiotherapy and conventional chemotherapy are basically ineffective. Immunotherapy by administrating interferon-a and/or interleukin-2 has been used for a long time for the treatment of metastatic or unresectable renal cancer with a limited response rate of less than 20%. Now, molecular targeting drugs such as sunitinib, sorafenib, everolimus, and temsilolimus are replacing immunotherapy. Here, we have studied histological expressions of HMGB1 and HMGB2 in surgically resected human renal cancer specimens, and compared them with the clinicopathologic features. HMGB1 expression localized in the cytopasm of renal clear cell cancer was correlated with poor pathological characteristics.

MATERIAL AND METHODS

Ethics statement

This study was conducted in accordance with the Helsinki declaration after approval by the Ethical Committee of Kanto Rosai Hospital. The committee approved the use of the oral consent documented in the chart for each patient, as the study

Table 1. Patient and tumor profiles.

Diagnostic Index	Values
Age at renal surgery, years	Median 63 (34-81)
Sex	
Male	28
Female	11
Tumor grades	
G1	12
G2	18
G3	8
G4	1
pT classifications	
pT1a	22
pT1b	10
pT2	3
pT3a	1
pT3b	3
Metastasis at renal surgery	
M0	36
M1	3
Metastasis after renal surgery	2

MRI indicates magnetic resonance imaging; and CI, confidence interval.

was retrospective and non-randomized. Oral informed consent was obtained from all participants involved in the study. Written consent was not obtained based on the judgment of the Ethical Committee. The process of obtaining oral consent was documented in the individual electronic chart used in Kanto Rosai Hospital, and the Ethical Committee approved this consent procedure.

Study design

Consecutive thirty-nine patients with pathologically confirmed renal clear cell carcinoma in Kanto Rosai Hospital between January, 2009 and August, 2012 were enrolled in this study. Paraffin-embedded sections of specimens of renal cancers excised by radical or partial nephrectomy were histochemically stained with anti-human mouse monoclonal HMGB1 antibody (Abcam, AB80246) or anti-human mouse monoclonal HMGB2 antibody (Abgent, AT2387a) as primary antibodies essentially following the manufacturers' instructions. Expressions of HMGB1/2 were determined by a single pathologist (AT). Basically, expressions of HMGB1/2

Table 2. Nuclear HMGB expression classified by pT classification and tumor grade.

	HMGB1-	HMGB1+	HMGB2-	HMGB2+
pT1a	0	22	1	21
≥ pT1b	2	15	3	14
	$P = .1835$		$P = .2998$	
G1	0	12	2	10
≥ G2	2	25	2	25
	$P = 1.0000$		$P = .5733$	

P values by the two-tailed Fisher's Exact test.

in more than 30% tumor cells on sections were judged as positive.

Statistical analyses

HMGB1/2 expressions in different pathological T classifications and tumor grades (the Fuhrman grading system) were statistically analyzed using the two-tailed Fisher's Exact test.

RESULTS

Patient and tumor profiles are presented in Table 1. Representative microphotographs of nuclear and cytosolic HMGB1 expression are shown in Figure 1, while those of HMGB2 are in Figure 2. In the present study, HMGB1 was expressed in the nucleus in 37 out of 39 (94.9%) renal clear cell cancers, while its expression in the cytosol was noted in 19 out of 39 cases (48.7%). As shown in Table 2, nuclear HMGB1 is expressed in all pT1a renal cancers and almost all renal cancers whose pT classifications are above pT1b. On the contrary, cytosolic HMGB1 is expressed more frequently in cancers with classifications above pT1b than in those at the pT1a classification (Table 3). In addition, higher tumor grades (≥ G2) were significantly linked with cytosolic expression of HMGB1 (Table 3), but not with its nuclear expression (Table 2).

HMGB2 was expressed in the nucleus in 35 of 39 (89.7%) renal clear cell cancers, while its expression in the cytosol (Figure 2) was only noted in 7 cases (17.9%). Linkage between cytosolic expression of HMGB2 and T classifications

Table 3. Cytosolic HMGB expression classified by pT classification and tumor grade.

	HMGB1-	HMGB1+	HMGB2-	HMGB2+
pT1a	17	5	21	1
≥ pT1b	3	14	11	6
	P = .0003		*P* = .0301	
G1	10	2	11	1
≥ G2	10	17	21	6
	P = .0138		*P* = .4026	

P values by the two-tailedFisher's Exact test.

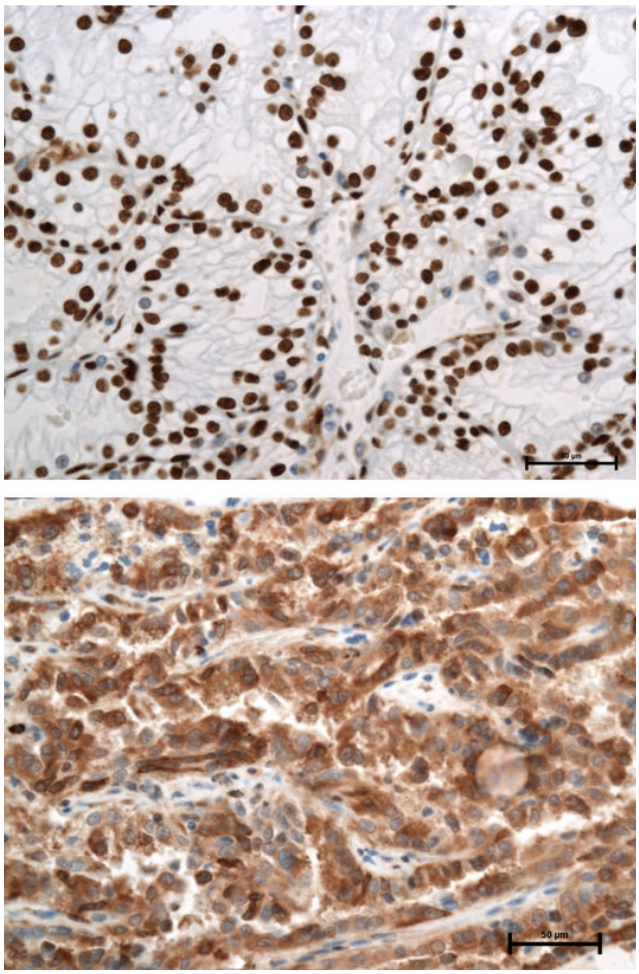

Figure 1. Representative HMGB1 staining in renal clear cell cancer. Top: nuclear staining +, cytoplasmic staining -, Bottom: nuclear staining -, cytoplasmic staining +. Bar=50 µm.

was weakly observed (Tables 3), while that between nuclear expression and T classifications was not (Table 2). Linkage between cytosolic/nuclear expression of HMGB2 and tumor grades was not observed (Tables 2 and 3).

DISCUSSION

HMGB1/2 expression in renal cancer has not previously been reported. In the present study, renal clear cell cancer basically expressed HMGB1 as a nuclear protein at any tumor stage and grade. Thus, nuclear HMGB1 may be engaged in the regular intranuclear functioning of HMGB, such as the assembly of proteins, gene transcription, and DNA repair. As tumor stages and grades progress, HMGB1 is expressed not only in the nucleus but also the cytosol. This may indicate that HMGB1 released from cancer cells functions as a proinflammatory cytokine in renal cancers of higher stages and grades. Otherwise, HMGB1 released from them may suppress antitumor immunity, leading to more invasiveness, metastasis, and a poorer prognosis. In most of the previous reports showing overexpression of HMGB1 in cancers, localization of HMGB1 in cancer cells was not necessarily described clearly,[14-18] as the experiments were done utilizing proteins or mRNAs extracted from materials. A report by Kostova and associates[20] uniquely revealed the localization of HMGB1 in cancer tissues by immunohistochemistry, in which ductal breast, colorectal, and hepatocellular cancer all exhibited perinuclear localization of HMGB1 in moderately differentiated carcinomas, while they showed whole nuclear localization in less differentiated ones. Additionally, gastrointestinal stromal tumors with KIT mutation were reported to show nuclear and cytoplasmic expression of HMGB1, but those without KIT mutation did not express HMGB1 at all.[19] Our study may be the first to show that the cytoplasmic expression of HMGB1 in cancer cells is clearly linked with the poorer pathological characteristics as T classifications and tumor grades. Actually, two cases where distant metastasis occurred after radical nephrectomy as well as a case with multiple lung metastases at nephrectomy were all HMGB1-positive in the cytoplasm of cancer cells in nephrectomy specimens.

In the present study, HMGB2 expression in renal clear cell cancer was mainly in the nucleus and less frequently in the cytosol. It has not been definitely reported that HMGB2 is expressed extranuclearly and secreted extracellularly, but

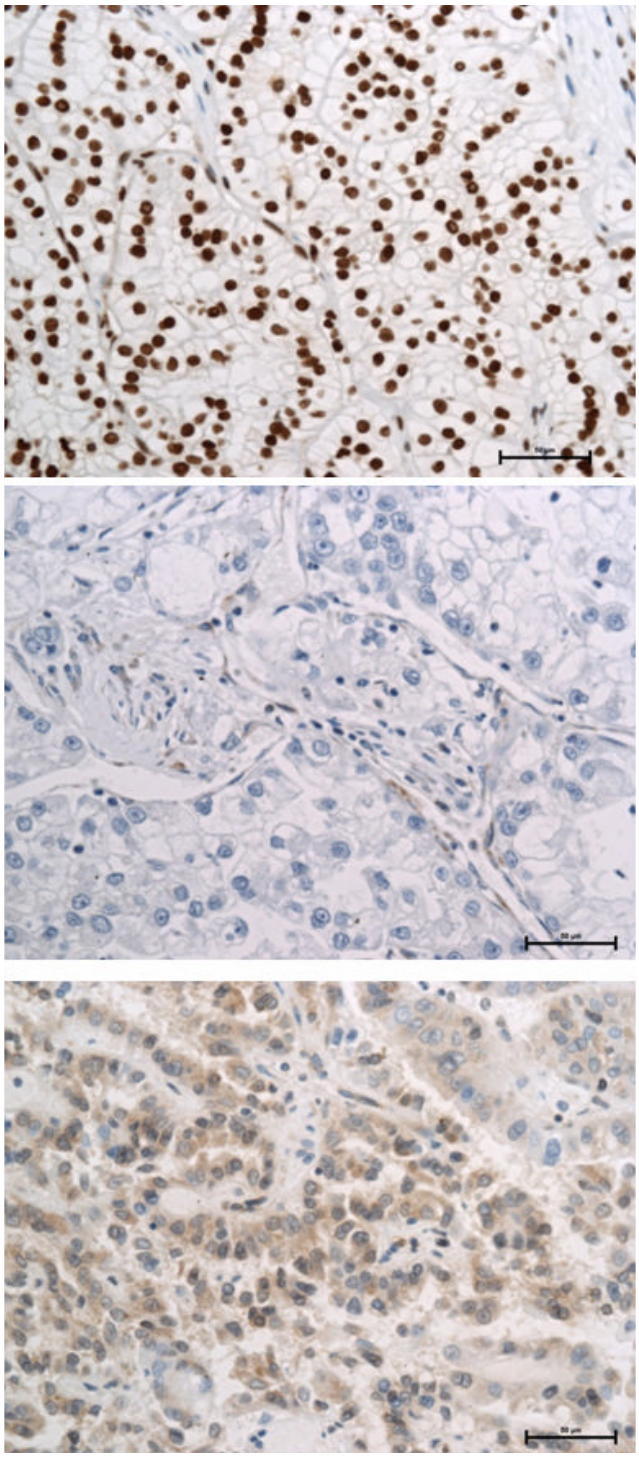

Figure 2. Representative HMGB2 staining in renal clear cell cancer. Top: nuclear staining +, cytoplasmic staining -, Middle: nuclear staining -, cytoplasmic staining -, Bottom: nuclear staining -, cytoplasmic staining +. Bar=50 µm.

HMGB2 is clearly localized in the cytoplasm of renal cancer cells on occasion, as shown in Figure 2. Thus, it can be hypothesized that HMGB2 is also secreted from cells and functions as a cytokine as HMGB1. There was actually weak linkage between cytosolic expression of HMGB2 and T classifications similarly with HMGB1. A role of HMGB2 in cancer biology needs to be further investigated.

CONCLUSION

HMGB1 expressed in the cytoplasm may be an effective marker indicating higher T classifications and tumor grades.

CONFLICT OF INTEREST

None declared.

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