

Original Article

Co-expression of RAGE and HMGB1 is associated with cancer progression and poor patient outcome of prostate cancer

Chu-Biao Zhao^{1*}, Ji-Ming Bao^{1*}, Yong-Jie Lu², Tong Zhao³, Xin-Hua Zhou³, Da-Yong Zheng⁴, Shan-Chao Zhao¹

¹Department of Urology and Medical Center for Overseas Patients, Nanfang Hospital, Southern Medical University, Guangzhou, China; ²Molecular Oncology, Barts Cancer Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; ³Department of Pathology, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China; ⁴Department of Oncology, Nanfang Hospital, Southern Medical University, Guangzhou, China. *Equal contributors.

Received February 26, 2014; Accepted June 6, 2014; Epub July 16, 2014; Published July 30, 2014

Abstract: Receptor for advanced glycation end products (RAGE), along with its ligand high mobility group box 1 (HMGB1), is believed to play an important role in prostate cancer. The aim of this retrospective study was to investigate the expression of RAGE and HMGB1 and their clinical impact on prostate cancer progression and prognosis. The expression of RAGE and HMGB1 was assessed by immunohistochemistry in cancer lesions from 85 confirmed prostate cancer cases. We determined the potential association between the expression level of these two proteins and the clinicopathological features and overall patient survival. RAGE and HMGB1 were expressed in 78.8% (67/85) and 68.2% (58/85) cases of prostate cancer, respectively, and in the majority (54/85) of cases, these two proteins were co-expressed. There was a strong correlation between RAGE and HMGB1 expressions ($P < 0.001$). The expression of RAGE, HMGB1 and their co-expression were all associated with advanced tumor clinical stage ($P < 0.05$ for all). RAGE expression was also associated with the prostate specific antigen (PSA) level ($P = 0.014$). However, neither the individual expression of those genes nor their co-expression was significantly related with age or Gleason score. The co-expression of RAGE and HMGB1 was associated with poor overall survival in patients with stage III and IV prostate cancer ($P = 0.047$). These results suggest that the expression of RAGE and HMGB1 is associated with the progression and poor prognosis of prostate cancer. RAGE and HMGB1 could be new prognostic biomarkers for prostate cancer as well as molecular target for novel forms of therapies.

Keywords: RAGE, HMGB1, prostate cancer, progression, prognosis

Introduction

Prostate cancer is one of the most common cancers in males, especially in aged males. In 2008, prostate cancer was the second most diagnosed cancer and the sixth leading cause of cancer death among males, accounting for 14% of the total new cancer cases and 6% of the death caused by cancer [1]. In China, with the growing average life expectancy, the western life style and diet, and the improved detection rate, the incidence of prostate cancer has shown a steep increase in the last decade [2, 3].

Prostate cancer has a natural course that is different from many other human tumors. Most

early-stage prostate cancers are latent and only approximately 25% of them will become aggressive and life-threatening [4]. However, currently, it is difficult to predict the progression of early stage cancers [4-7], which will have a great impact on therapeutic decisions.

It has been reported that the role of RAGE (receptor for advanced glycation end products) and its ligands play a role in the development and progression of cancer [8-10]. RAGE is a cell surface molecule and multi-ligands receptor, belonging to the immunoglobulin superfamily. It has several ligands, including HMGB1 (high mobility group box 1), several members of the calcium-binding S100 family of proteins, some species of AGEs, and β -sheet fibrillar material

Co-expression of RAGE and HMGB1 in prostate cancer

Table 1. The demography of cancer cases

Characteristic	No. of patients (%)
Age (years)	
<60	10 (11.8)
≥60	75 (88.2)
PSA (ng/ml)	
<20	22 (25.9)
≥20	63 (74.1)
Gleason score	
<7	28 (32.9)
=7	35 (41.2)
>7	22 (25.9)
T stage	
T1-T2	36 (42.4)
T3-T4	49 (57.6)
N stage	
N0	47 (55.3)
N1	38 (44.7)
Distant metastases	
No	44 (51.8)
Yes	41 (48.2)

such as amyloid- β , serum amyloid A, immunoglobulin light chains, transthyretin, and prions, among others [8, 11]. RAGE was first reported in 1992 [12] and has been suggested to be involved in several diseases, including diabetes, cancers, inflammation, cardiovascular disease, chronic kidney disease and Alzheimer's disease [13]. Abnormal expression of RAGE and its ligands has been reported in a number of cancers, including prostatic, colorectal, pancreatic, lung, oral squamous cell cancers and these molecules may be involved in cancer invasion and metastasis [8-10, 14, 15]. HMGB1, also known as amphoterin, is an abundant non-histone component of chromatin [16, 17] and can be released into the extracellular to bind to RAGE [8]. The interaction between RAGE and HMGB1 triggers the activation of key cell signaling pathways, such as NF- κ B, p38, p44/42 MAPKs, resulting in the cancer progression and metastasis [8-10, 18-24].

Overexpression of RAGE and HMGB1 have been observed in prostate cancer [14, 15, 25-29]. Ishiguro et al. found that untreated primary prostate cancer tissue and hormone-refractory prostate cancer tissue showed significantly higher RAGE and HMGB1 mRNA expression than normal prostate tissues. In addition, they detected RAGE and HMGB1

mRNA expression in all three commonly used prostate cancer cell lines, DU145, PC-3 and LNCaP, with DU145 the highest of the three lines [14]. Kuniyasu et al. described that RAGE production was enhanced in metastatic compared to non-metastatic prostate cancer, where it was co-expressed with HMGB1 [15]. We also reported RAGE overexpression at both RNA and protein level in Chinese prostate cancer samples [29]. In this retrospective study, we analyzed the expression of RAGE and HMGB1 in a larger series of Chinese prostate cancer samples using immunohistochemistry (IHC) and explored its relationship with the clinicopathologic parameters and overall survival. We found that the expression of RAGE and HMGB1 was associated with tumor progression and poor prognosis of prostate cancer.

Materials and methods

Samples

A total of eighty-five cases of prostate cancer, including forty-two radical prostatectomy and forty-three needle biopsy samples, were included in this study. Hematoxylin and eosin stained slides from all cases were reviewed to confirm the diagnosis of prostate cancer by two independent pathologists (TZ and XZ). Cases received pre-operative hormone therapy, chemotherapy and radiotherapy, or with a diagnosis of diabetes, chronic kidney, cardiovascular and Alzheimer's disease as well as other cancers had been excluded. Clinical information about the cases was described in **Table 1**. TNM staging was made before any treatment according to American Joint Committee on Cancer (AJCC), 7th edition TNM-system. Prostate specific antigen (PSA) was assessed before biopsy and any treatment. Thirty histologically benign prostatic hyperplasia (BPH) diagnosed in the same period were randomly chosen as controls. This study was approved by Medical Ethics Committee of Nanfang hospital, and the tissue samples were used with the informed written consent from the patients or their family members.

Immunohistochemical staining and scoring

Consecutive 4 μ m sections were cut from formalin-fixed paraffin-embedded tissue blocks. Before IHC staining, histological features in hematoxylin and eosin stained slides were reviewed by pathologists. Sections were immu-

Co-expression of RAGE and HMGB1 in prostate cancer

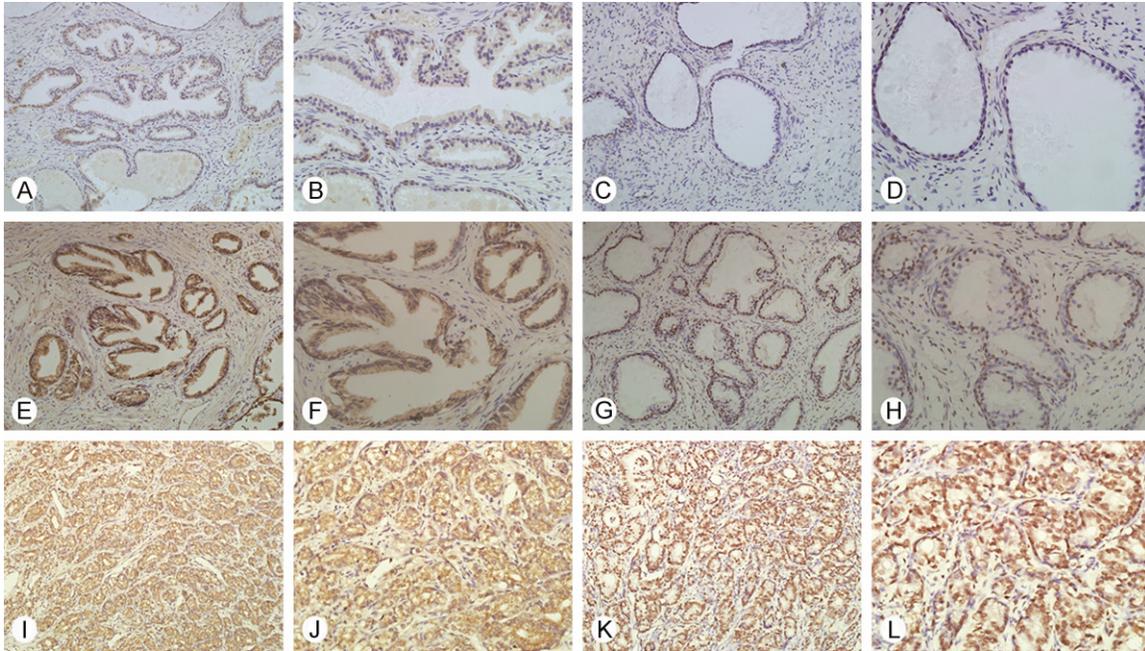


Figure 1. Representative immunohistochemistry images of the expression of RAGE and HMGB 1 in BPH and prostate cancer samples. (A-D) Negative expression of RAGE (A and B) and HMGB1 (C and D) in BPH (case 919347). (E-H) Positive expression of RAGE (E and F) and HMGB1 (G and H) in BPH (case 906875). (I-L) Positive expression of RAGE (I and J) and HMGB1 (K and L) in prostate cancer (case 600491). (A, C, E, G, I, K) $\times 200$; (B, D, F, H, J, L) $\times 400$.

nostained by anti-RAGE and anti-HMGB1 antibodies (Abcam, Cambridge, UK) using the immunoperoxidase technique. Briefly, after deparaffinization in xylene, slides were rehydrated in decreasing concentrations of ethanol and then heated for antigens retrieval. 0.3% H_2O_2 was applied to block the endogenous peroxidase and goat serum albumin (Zymed, South San Francisco, USA) was used to reduce non-specific antibody binding. Anti-RAGE antibody was used at 1:300 and anti-HMGB1 antibody was used at 1:250 dilution. After incubation at 4°C overnight with the primary antibody, slides were briefly washed in PBS and incubated with biotinylated secondary antibody. Then the sections were incubated with Horseradish Peroxidase (HRP) followed by incubations with 3,3'-diaminobenzidine (DAB) working solution for color reactions. Finally, slides were counterstained with hematoxylin and mounted. The primary antibody was replaced by appropriate serum for negative control. Colon cancer and normal liver samples were used as positive control tissues for RAGE and HMGB1, respectively.

All slides stained with RAGE and HMGB1 were assessed by two independent pathologists (TZ

and XZ). The scoring system included the extent and intensity of staining. The extent score was graded as: 0, less than 5% of tumor cells stained; 1, 5 to 25% stained; 2, 25 to 50% stained; and 3, more than 50% stained. The staining intensity was scored as: 0, negative; 1, weak; 2 moderate; 3, strong. Final scores (range, from 0 to 9) were obtained by multiplying staining extents and intensities. Final scores were described based on the following: 0, no expression; 1 to 3, weak expression; 4 to 6, moderate expression; and 7 to 9, strong expression. For statistical analysis, no expression and weak expression were combined and defined as negative expression (-), moderate and strong expressions were combined for positive expression (+).

Statistical analyses

SPSS13.0 software was employed for statistical analysis. Statistical difference of data from categorized groups was assessed by two-tailed χ^2 test. Spearman rank correlation was used to analyze the correlation of RAGE and HMGB1 expression. The Kaplan-Meier method was used to estimate the correlation of overall survival with RAGE, HMGB1 and their co-expres-

Co-expression of RAGE and HMGB1 in prostate cancer

Table 2. Correlation of clinicopathologic parameters with RAGE and HMGB1 expressions

Clinicopathologic parameters		RAGE expression		P value	HMGB1 expression		P value	Co-expression RAGE (+)/HMGB1 (+)		P value
		-	+		-	+		No	Yes	
		Age	<60		1	9		0.681	2	
	≥60	17	58		25	50		29	46	
PSA (ng/ml)	Median	19.25	57.17	0.016	27.9	55.77	0.090	27.90	55.77	0.134
	Mean	30.58	46.43		36.36	46.09		37.71	46.04	
Gleason score	<7	8	20	0.234	13	15	0.090	14	14	0.130
	=7	8	27		10	25		12	23	
	>7	2	20		4	18		5	17	
T stage	T1-T2	13	23	0.004	17	19	0.009	19	17	0.007
	T3-T4	5	44		10	39		12	37	
N stage	N0	15	32	0.007	21	26	0.004	24	23	0.002
	N1	3	35		6	32		7	31	
Distant metastases	No	14	30	0.013	19	25	0.019	21	23	0.026
	Yes	4	37		8	33		10	31	

sion in prostate cancer samples and the significance was estimated using the log-rank test. The level of significance was defined as $p < 0.05$.

Results

Overexpression of RAGE and HMGB1 in prostate cancer

The average diagnostic ages with standard deviations of BPH and prostate cancer patients were 69.63 ± 8.24 and 70.15 ± 9.60 years respectively (**Table 1**) and there was no significant difference between the two groups ($P=0.792$). RAGE staining was detected predominantly in the cytoplasm and membrane and HMGB1 signals were localized in the nucleus and cytoplasm in prostate cancer cells. Positive expressions of RAGE and HMGB1 also could be detected in luminal and basal cells in some BPH tissues but not as strong as in prostate cancer cells (**Figure 1**).

Detailed expression data were presented in **Table 2**. Positive expression of RAGE was detected in 78.8% (67/85) of prostate cancer and 46.7% (14/30) of BPH samples respectively, and it was significantly higher in prostate cancer than BPH cases ($P=0.002$). HMGB1 expression was positive in 68.2% (58/85) of prostate cancer and in 33.3% (10/30) of BPH cases and its expression was also significantly higher in prostate cancer than BPH cases ($P=0.001$). 63.5% (54/85) of prostate cancer

and 13.3% (4/30) of BPH samples expressed both RAGE and HMGB1 with significant difference between prostate cancer and BPH cases ($P < 0.0001$). There was a strong correlation between RAGE and HMGB1 expressions with correlation coefficient of 0.512 ($P < 0.001$).

Correlations of RAGE and HMGB1 expression with advanced clinical stage

High-level expression of both RAGE and HMGB1 was individually significantly associated with T stage, lymph node metastasis and distant metastases (**Table 2**). Furthermore, high level RAGE expression was correlated with high diagnostic PSA level ($P < 0.05$). Both RAGE and HMGB1 expression was not significantly related to the diagnostic age (<60 vs ≥ 60) or Gleason score. The co-expression of RAGE and HMGB1 also showed a significant positive correlation with T stage, lymph node metastasis and distant metastases (**Table 2**).

Correlations of RAGE and HMGB1 expression with patient outcome

To explore the association of RAGE and HMGB1 expression with patient outcome, we only included patients with clinical stage III and IV cancers, due to the short follow-up period and the generally slow progression nature of prostate cancer. These patients received regular treatment according to prostate cancer diagnosis and treatment guideline of China, including

Co-expression of RAGE and HMGB1 in prostate cancer

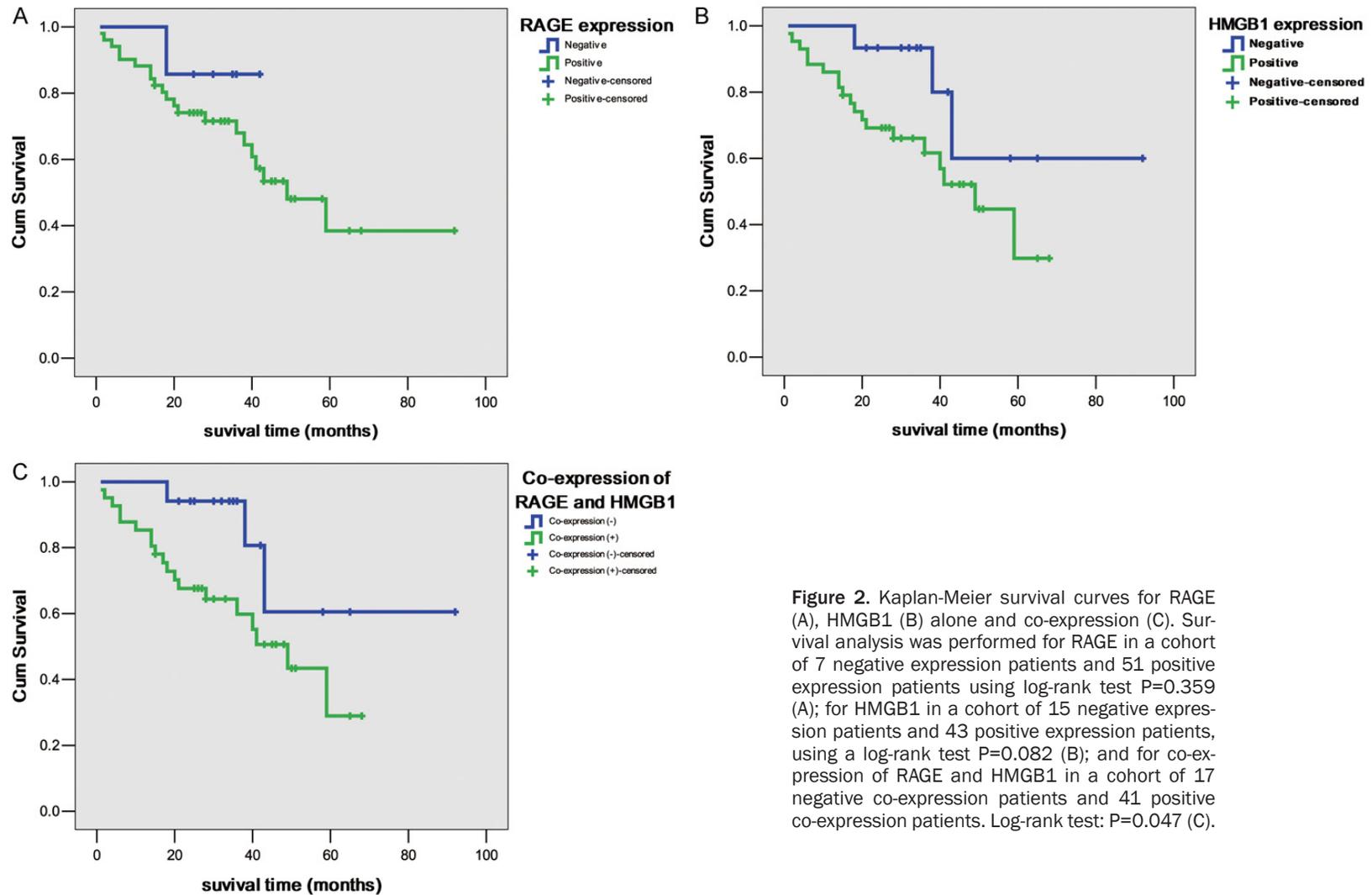


Figure 2. Kaplan-Meier survival curves for RAGE (A), HMGB1 (B) alone and co-expression (C). Survival analysis was performed for RAGE in a cohort of 7 negative expression patients and 51 positive expression patients using log-rank test $P=0.359$ (A); for HMGB1 in a cohort of 15 negative expression patients and 43 positive expression patients, using a log-rank test $P=0.082$ (B); and for co-expression of RAGE and HMGB1 in a cohort of 17 negative co-expression patients and 41 positive co-expression patients. Log-rank test: $P=0.047$ (C).

radical prostatectomy (8 cases), hormonal therapy (52 cases), radiotherapy (5 cases) and chemotherapy (2 cases), single or in combination. Fifty-eight patients with stage III and IV cancers were underwent overall survival analysis, with average follow-up of 47.9 months. Thirty six of the 58 patients were still alive at the end of the follow-up period. In Kaplan-Meier survival analysis, there was statistically significant decreased overall survival rate for cases with co-expression of RAGE and HMGB1 compared with the rest of patients ($P=0.047$). Neither RAGE nor HMGB1 alone was associated with overall survival rate (**Figure 2**).

Discussion

Finding new abnormal gene expression in prostate cancer will help us to predict prognosis and identify novel therapeutic targets. In previous studies, both RAGE and its ligand HMGB1 had been found overexpression in prostate cancer tissue and cell lines [14, 15, 25-29]. In the present study, we analyzed the expression of HMGB1 and RAGE in Chinese prostate cancer samples and correlated them with both clinicopathologic parameters and patient outcome.

We previously demonstrated RAGE overexpression at both RNA and protein level in Chinese prostate cancer samples [29]. In this study, we not only confirmed our previous observation of RAGE overexpression in a larger series of prostate cancer samples, but also detected significantly higher expression levels of HMGB1 and more cases with co-expression of these two proteins in prostate cancer samples compared with BPH cases. High frequency co-expression of HMGB1 and RAGE have been previously reported [14, 15], but not in Chinese prostate cancer.

We found that high-level expression of RAGE and HMGB1 was significantly associated with aggressive features of prostate cancer, including T stage, lymph node metastasis and distant metastases, individually and in combination. While previous studies have showed the correlations of HMGB1 expression with T stage in Chinese prostate cancer [26] and the expression of both proteins with cancer metastasis in Japanese prostate cancer [15], this study firstly demonstrated that the expression level of both

genes are correlated with all the features (T, N and M) associated with a clinical advanced disease.

We also found that high-level RAGE expression was correlated with high diagnostic PSA level, which is consistent with the report of PSA level reduction in LNCaP cells transfected with RAGE RNAi constructs [30]. In a previous report, where HMGB1 protein expression was evaluated in 168 primary prostatectomy tissue samples by IHC, the expression level of HMGB1 was found to be correlated with both Gleason score and preoperative PSA concentration [26]. However, our data show that HMGB1 expression was not associated with Gleason score. While there is a trend that high HMGB1 correlates with high PSA value, it is not statistically significant. This distinctive may due to different samples inclusion criteria or limited sample size in our study. Nevertheless, our data and the previous studies also support deregulation of RAGE and HMGB1 is strongly associated with advanced stage of prostate cancer, which may have impact on patient survival.

Due to the heterogeneity of natural progression of individual prostate cancer cases, it is a major issue to predict the outcome of a prostate cancer diagnosed at an early stage. So far, very limited number of prognostic molecular markers has been identified [31]. Therefore, the most important finding of this study is the association of co-expression of RAGE and HMGB1 with prostate cancer patient outcome. While further investigations with larger number of cases and longer follow-ups are required to confirm our findings, our data indicates the potential to use the co-expression of those proteins as prognostic markers to stratify patient treatment.

There is only one reported study on the prognostic value of HMGB1/ RAGE in prostate cancer and only HMGB1 was included [26]. In that study, HMGB1 protein expression was an independent prognostic factor for biochemical recurrence (BCR)-free survival after radical prostatectomy (RP). In other human cancers, most studies showed that a high-level HMGB1 protein alone or in combination with VEGF-C was linked to poor patient survival rate, including colorectal [32], nasopharyngeal [33] and gastric [34] carcinomas, although it has also been reported that low HMGB1 expression gas-

tric cancer cases had a significantly poorer outcome compared with the high HMGB1 group [35]. In colorectal cancer with liver metastases, it has also been reported that serum HMGB1 level correlated with disease progression after radioembolization therapy [36]. In our study, there is a trend of inverse correlation of HMGB1 expression with patient outcome, but it is not statistically significant ($P=0.082$), potentially due to limited number of cases. However, the co-expression of RAGE and HMGB1 is significantly associated with patient survival, suggesting the expression of those two proteins has additive effect to promote aggressive cancer growth. Co-expression of HMGB1 and RAGE has also been associated with tumor progression and metastasis in gastric [24] and colon cancer [18] as well as glioma [37], although no patient outcome correlation analysis has been done. All those indicated that co-expression of both proteins has a better potential than individually for the potential to be developed as a novel prognostic marker to stratify prostate cancer treatment.

It is still controversial on the association between RAGE expression and patient outcome. Tateno et al. revealed that overexpression of RAGE is related to bad prognosis of esophageal squamous cell carcinoma [38]. However, in colorectal cancer with liver metastases, only HMGB1 but not RAGE expression levels is associated with disease progression after radioembolization therapy [36]. Our data suggest that overexpression of RAGE itself is not associated with bad patient outcome of prostate cancer, but through its effects on prostate cancer progression, it contributes to poor patient outcome in cooperation with HMGB1.

While we showed the clinical correlation between HMGB1/RAGE expression and prostate cancer progression and prognosis, functional studies demonstrated the biological role of those two genes in promoting the survival of prostate cancer cells. Previous studies showed that knockdown of HMGB1 suppressed the growth of LNCaP prostate cancer cells through induction of apoptosis [19] and down-regulation of RAGE expression induced apoptosis and inhibits prostate tumor growth both in vitro and in vivo [30]. Therefore, targeting HMGB1/RAGE pathway may be a novel therapeutic strategy for aggressive prostate cancer.

Conclusion

In summary, this study demonstrates the relationship between RAGE and HMGB1 expression and the disease progression in Chinese prostate cancer. In addition to confirm that RAGE and HMGB1 abnormality are involved in prostate cancer progression, we found that co-expression of RAGE and HMGB1 associated with prostate cancer progression better than each protein individually, thus only co-expression of RAGE and HMGB1 is correlated with poor patient outcome. Therefore, the co-expression of RAGE and HMGB1 has the potential to be used as a prognostic marker to help stratifying prostate cancer into indolent or aggressive groups.

Acknowledgements

This project was supported by National Natural Science Foundation of China (81072092; 81328017), Guangdong Provincial Natural Science Foundation of China (101510515-01000072) and Guangdong Provincial Science and Technology Projects of China (2012B-031800302).

Disclosure of conflict of interest

The authors declare no conflict of interest.

Address correspondence to: Shan-Chao Zhao, Department of Urology and Medical Center for Overseas Patients, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong Province, 510515, PR China. E-mail: zhaoshanchaosmu@sina.cn; Da-Yong Zheng, Department of Oncology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong Province, 510515, PR China. E-mail: zhengdayong@hotmail.com

References

- [1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; 61: 69-90.
- [2] Lei T, Mao WM, Yang HJ, Chen XZ, Lei TH, Wang XH, Ying Q, Chen WQ, Zhang SW. Study on cancer incidence through the cancer registry program in 11 cities and counties, China. *Zhonghua Liu Xing Bing Xue Za Zhi* 2009; 30: 1165-70.
- [3] Lei M, Zhang SW, Ma JH, Chen WQ, Na YQ. A comparative study on incidence trends of prostate cancer in part of cities and counties in China. *Chin J Urol* 2009; 30: 568-570.

Co-expression of RAGE and HMGB1 in prostate cancer

- [4] Cuzick J, Fisher G, Kattan MW, Berney D, Oliver T, Foster CS, Møller H, Reuter V, Fearn P, Eastham J, Scardino P; Transatlantic Prostate Group. Long-term outcome among men with conservatively treated localized prostate cancer. *Br J Cancer* 2006; 95: 1186-94.
- [5] Bangma CH, Roemeling S and Schroder FH. Overdiagnosis and overtreatment of early detected prostate cancer. *World J Urol* 2007; 25: 3-9.
- [6] Knudsen KE and Penning TM. Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer. *Trends Endocrinol Metab* 2010; 21: 315-24.
- [7] Schrijvers D, Van Erps P and Cortvriend J. Castration-refractory prostate cancer: New drugs in the pipeline. *Adv Ther* 2010; 27: 285-96.
- [8] Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. *Annu Rev Immunol* 2010; 28: 367-388.
- [9] Sparvero LJ, Asafu-Adjei D, Kang R, Tang D, Amin N, Im J, Rutledge R, Lin B, Amoscato AA, Zeh HJ, Lotze MT. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. *J Transl Med* 2009; 7: 17.
- [10] Logsdon CD, Fuentes MK, Huang EH, Arumugam T. RAGE and RAGE ligands in cancer. *Curr Mol Med* 2007; 7: 777-789.
- [11] Schmidt AM, Yan SD, Yan SF, Stern DM. The biology of the receptor for advanced glycation end products and its ligands. *Biochim Biophys Acta* 2000; 1498: 99-111.
- [12] Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern D, Shaw A. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 1992; 267: 14998-15004.
- [13] Alexiou P, Chatzopoulou M, Pegklidou K, Demopoulos VJ. RAGE: a multi-ligand receptor unveiling novel insights in health and disease. *Curr Med Chem* 2010; 17: 2232-2252.
- [14] 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.
- [15] Kuniyasu H, Chihara Y, Kondo H, Ohmori H, Ukai R. Amphoterin induction in prostatic stromal cells by androgen deprivation is associated with metastatic prostate cancer. *Oncol Rep* 2003; 10: 1863-1868.
- [16] Thomas JO. HMGB1 and 2: architectural DNA-binding proteins. *Biochem Soc Trans* 2001; 29: 395-401.
- [17] Todorova J, Pasheva E. High mobility group B1 protein interacts with its receptor RAGE in tumor cells but not in normal tissues. *Oncol Lett* 2012; 3: 214-218.
- [18] 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.
- [19] 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.
- [20] Sasahira T, Akama Y, Fujii K, Kuniyasu H. Expression of receptor for advanced glycation end products and HMGB1/amphoterin in colorectal adenomas. *Virchows Arch* 2005; 446: 411-415.
- [21] Kuniyasu H, Yano S, Sasaki T, Sasahira T, Sone S, Ohmori H. Colon cancer cell-derived high mobility group 1/amphoterin induces growth inhibition and apoptosis in macrophages. *Am J Pathol* 2005; 166: 751-760.
- [22] Kuniyasu H, Sasaki T, Sasahira T, Ohmori H, Takahashi T. Depletion of tumor-infiltrating macrophages is associated with amphoterin expression in colon cancer. *Pathobiology* 2004; 71: 129-136.
- [23] Sasahira T, Kirita T, Bhawal UK, Ikeda M, Nagasawa A, Yamamoto K, Kuniyasu H. The expression of receptor for advanced glycation end products is associated with angiogenesis in human oral squamous cell carcinoma. *Virchows Arch* 2007; 450: 287-295.
- [24] Kuniyasu H, Oue N, Wakikawa A, Shigeishi H, Matsutani N, Kuraoka K, Ito R, Yokozaki H, Yasui W. 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.
- [25] Gnanasekar M, Thirugnanam S, Ramaswamy K. Short hairpin RNA (shRNA) constructs targeting high mobility group box-1 (HMGB1) expression leads to inhibition of prostate cancer cell survival and apoptosis. *Int J Oncol* 2009; 34: 425-431.
- [26] Li T, Gui Y, Yuan T, Liao G, Bian C, Jiang Q, Huang S, Liu B, Wu D. Overexpression of high mobility group box 1 with poor prognosis in patients after radical prostatectomy. *BJU Int* 2012; 110: E1125-30.
- [27] Gnanasekar M, Kalyanasundaram R, Zheng G, Chen A, Bosland MC, Kajdacsy-Balla A. HMGB1: A Promising Therapeutic Target for Prostate Cancer. *Prostate Cancer* 2013; 2013: 157103.
- [28] Hermani A, Hess J, De Servi B, Medunjanin S, Grobholz R, Trojan L, Angel P, Mayer D. Calcium-binding proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer. *Clin Cancer Res* 2005; 11: 5146-52.

Co-expression of RAGE and HMGB1 in prostate cancer

- [29] Lu B, Song XL, Jia LY, Song FL, Zhao SC, Jiang Y. Differential expressions of the receptor for advanced glycation end products in prostate cancer and normal prostate. *Zhonghua Nan Ke Xue* 2010; 16: 405-409.
- [30] Elangovan I, Thirugnanam S, Chen A, Zheng G, Bosland MC, Kajdacsy-Balla A, Gnanasekar M. Targeting receptor for advanced glycation end products (RAGE) expression induces apoptosis and inhibits prostate tumor growth. *Biochem Biophys Res Commun* 2012; 417: 1133-1138.
- [31] Cuzick J, Swanson GP, Fisher G, Brothman AR, Berney DM, Reid JE, Mesher D, Speights VO, Stankiewicz E, Foster CS, Møller H, Scardino P, Warren JD, Park J, Younus A, Flake DD 2nd, Wagner S, Gutin A, Lanchbury JS, Stone S; Transatlantic Prostate Group. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. *Lancet Oncol* 2011; 12: 245-55.
- [32] Yao X, Zhao G, Yang H, Hong X, Bie L, Liu G. Overexpression of high-mobility group box 1 correlates with tumor progression and poor prognosis in human colorectal carcinoma. *J Cancer Res Clin Oncol* 2010; 136: 677-84
- [33] Wu D, Ding Y, Wang S, Zhang Q, Liu L. Increased expression of high mobility group box 1 (HMGB1) is associated with progression and poor prognosis in human nasopharyngeal carcinoma. *J Pathol* 2008; 216: 167-175.
- [34] He W, Tang B, Yang D, Li Y, Song W, Cheang T, Chen X, Li Y, Chen L, Zhan W, Li W, He Y. Double-positive expression of high-mobility group box 1 and vascular endothelial growth factor C indicates a poorer prognosis in gastric cancer patients. *World J Surg Oncol* 2013; 11: 161.
- [35] Akaike H, Kono K, Sugai H, Takahashi A, Mimura K, Kawaguchi Y, Fujii H. Expression of high mobility group box chromosomal protein-1 (HMGB-1) in gastric cancer. *Anticancer Res* 2007; 27: 449-457.
- [36] Fahmueller YN, Nagel D, Hoffmann RT, Tatsch K, Jakobs T, Stieber P, Holdenrieder S. Immunogenic cell death biomarkers HMGB1, RAGE, and DNase indicate response to radioembolization therapy and prognosis in colorectal cancer patients. *Int J Cancer* 2013; 132: 2349-58.
- [37] Bassi R, Giussani P, Anelli V, Colleoni T, Pedrazzi M, Patrone M, Viani P, Sparatore B, Melloni E, Riboni L. HMGB1 as an autocrine stimulus in human T98G glioblastoma cells: role in cell growth and migration. *J Neurooncol* 2008; 87: 23-33.
- [38] Tateno T, Ueno S, Hiwatashi K, Matsumoto M, Okumura H, Setoyama T, Uchikado Y, Sakoda M, Kubo F, Ishigami S, Shinchi H, Natsugoe S. Expression of receptor for advanced glycation end products (RAGE) is related to prognosis in patients with esophageal squamous cell carcinoma. *Ann Surg Oncol* 2009; 16: 440-6.