Serum high mobility group box protein 1 as a clinical marker for ovarian cancer

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The aim of the study was to evaluate the clinical value of serum high mobility group box chromosomal protein 1 (HMGB1) in ovarian cancer and analyze the correlation between HMGB1 and ovarian cancer clinicopathologic outcomes.

A total of 105 patients with diagnosed epithelial ovarian cancer, 46 patients with ovarian benign disease and 33 healthy volunteers were enrolled from January 2011 through January 2013. Serum HMGB1 levels were analyzed by enzyme-linked immunosorbent assay.

The mean value of serum HMGB1 levels in ovarian cancer patients (78.18±54.87ng/ml) was significantly higher than those in benign patients (33.98±9.97ng/ml) and healthy control (26.71±7.99ng/ml, p < 0.0001), respectively. The serum HMGB1 levels were 40.33±6.50ng/ml, 61.16±20.15ng/ml, 81.81±51.15ng/ml and 119.48±84.28ng/ml in patients with TNM stage I, II, III, and IV, respectively (p < 0.0001). There were 81 of the 105 ovarian cancer patients obtained complete remission, the serum HMGB1 levels before treatment(71.99±42.49ng/ml) were much higher than that at remission stage(42.10±15.48ng/ml) (p < 0.0001). During our investigating period, 28 ovarian cancer patients underwent recurrence, the serum HMGB1 levels were 75.54±39.50ng/ml in these recurrent ovarian cancer patients compared to 42.04±10.68ng/ml in non-recurrent ovarian cancer (p < 0.0001). None of the remission or recurrent patients came from benign ovarian tumor group.

Our study suggests that HMGB1 may be a useful clinical marker for evaluating progression and predicting prognosis of ovarian carcinoma. Targeting HMGB1 production or release might have potential approaches for ovarian carcinoma treatment.

Key words: high mobility group box chromosomal protein 1, ovarian cancer, prognosis

Ovarian cancer is one of the most lethal gynecologic malignancies with rapid progression and poor survival. Primarily due to asymptomatic presentation and lacking of highly sensitive biomarkers for early detection, women continue to be diagnosed with advanced-stage disease. Despite substantial advances in ovarian cancer research and improvements in treatments, survival to incidence ratio is still poor and overall cure rate remains very low [1]. For further insights into the treatment of ovarian cancer, we need to find new tools for early detection and to study the molecular biology that characterises ovarian cancer cells.

HMGB1 is a non-histone, chromatin-binding nuclear protein which has a highly conserved sequence among various species with 98% identity between rodent, bovine and human proteins[2]. HMGB1 appears to have two distinct functions in cellular systems. In nucleus, HMGB1 can bind chromatin, stabilize nucleosome and regulate transcription[3], while secreted outside cells, it acts as an extracellular signaling molecule[4]. Some recent studies indicated that HMGB1 regulates the transcription of many cancer genes, such as E-selectin, TNF-α, insulin receptor, and BRCA1[5-7], suggested an important relationship between HMGB1 and cancer. By present, many researches confirmed the facilitating effect of HMGB1 on tumor, for example, HMGB1 has been reported to be increased in colorectal cancer[8], nasopharyngeal cancer[9], lung cancer[10], gastric cancer[11] and so on, indicating HMGB1 as an important mediator for cancer transformation, proliferation and invasion.

By present, there have been some achievements demonstrated the facilitating effects of HMGB1 on ovarian cancer[12]. However, the relationship between serum HMGB1 and ovarian cancer disease progression have not been evaluated. In this study,

we measured the serum levels of HMGB1 by ELISA, analyzed relationship between HMGB1 and ovarian cancer patients' clinic pathological parameters, therapy efficacy, and recrudescence.

Patients and methods

Patients. A total of 105 patients with diagnosed epithelial ovarian cancer, 46 patients with diagnosed benign ovarian tumor and 30 healthy volunteers were enrolled from January 2011 through January 2013 at department of Gynecological Cancer, Tianjin medical university cancer institute and hospital. The present study and the experiments were approved by the ethics committee of the Tianjin medical university cancer institute and hospital, and all the volunteers and patients gave written informed consent prior to participation. All of the ovarian cancer patients were clinically staged according to the FIGO staging system. The control subjects are free from significant disease, prior tumor illness, trauma/fracture, inflammatory systemic disease or infection, rheumatologic disease, stroke, vascular or other internal medical condition. All cancer patients were diagnosed for the first time during the enrollment period and their blood samples were collected before they received any treatment such as surgery, chemotherapy or radiotherapy. Among all of the malignant ovarian tumor patients enrolled in our investigation, 81 patients obtained disease remission whose serum HMGB1 levels were measured before treatment and at remission. Furthermore, there were 28 ovarian cancer patients underwent recurrence during the study period, and their serum HMGB1 levels were measured after recurrence. The clinic pathologic characteristics of patients were in Table 1.

HMGB1 measurements. Approximately 10 ml of whole blood was collected in non-heparinized tubes from each fasting

subject and allowed to clot at room temperature for half an hour, then centrifuged at 1,000×g for 15 min, and serum collected for storage at −80°C in microfuge tubes until assayed. HMGB1 was measured by the commercially available HMGB1 ELISA Kit II (SHINO-TEST Corporations, Kanagawa, Japan). Briefly, 100μl of sample diluent was added to each well and then 10μl of standard, sample or control was added to the well. Then the microtiter plates were incubated for 20–24 h at 37°C. After 5 washes, 100μl/well of anti-human HMGB1 peroxidase-conjugated monoclonal anti-body was added and incubated at room temperature for 2 h. After 5 washes, the chromogen 3,3',5,5'-tetra-methylbenzidine was added to each well. After reacting for 30 min at room temperature, the chromogenic substrate reaction was stopped by the addition of stop solution and the absorbance was read at 450 nm. The results were calculated using a calibration curve prepared from standards.

Statistical analysis. The results were expressed as the mean± standard deviation. One-way analysis of variance (ANOVA) with post-hoc Bon-ferroni adjustment for pairwise comparison was used to compare the means of HMGB1 among groups. Pearson correlation analysis was performed to assess the correlations between HMGB1 and the continuous variables, and Spearman correlation was performed to assess the correlations between HMGB1 and the non-continuous variables. Statistical Package for Social Sciences software (SPSS, Chicago, Illinois, USA version 17.0) was used for data support and analysis and p-values < 0.05 were considered as statistically significant differences.

Results

Serum HMGB1 levels in ovarian cancer patients, ovarian benign tumor patients and healthy control. The mean value of serum HMGB1 levels in 105 patients with ovarian cancer was 78.18±54.87ng/ml and was significantly higher than those in 46 ovarian benign tumor patients (33.98±9.97 ng/ml), and 30 healthy control (26.71±7.99ng/ml, p < 0.0001, respectively, Fig. 1). The serum HMGB1 levels in ovarian benign tumor patients were higher than those in healthy, but there was no statistical significance between them.

The serum HMGB1 levels in patients with different tumor stage of ovarian cancer. The tumor stage was defined by the FIGO staging (TNM stage) for ovarian cancer to evaluate whether HMGB1 was associated with ovarian cancer metastases to lymph nodes, distant organs, vascular invasion and tumor characteristics. The serum HMGB1 levels were 40.33±6.50ng/ml, 61.16±20.15ng/ml, 81.81±51.15ng/ml and 119.48±84.28ng/ml in patients with TNM stage I, II, III, and IV. There were significant differences among four groups and between two groups (Fig. 2).

The serum HMGB1 levels in patients with ovarian cancer before treatment and at remission stage. To determine whether the serum HMGB1 level was associated with clinical treatment, we measured the serum HMGB1 levels of the same patients before treatment and after complete remission.

Table 1. Baseline characteristics of the ovarian cancer patients.

Variables	N(%)	HMGB1 (ng/ml) mean±SD	P
Age			0.504
<50	36 (34.3)	76.19±25.02	
>50	69 (65.7)	79.22±59.89	
Pathology type			0.728
Serous cystadenocarcinoma	48 (45.7)	74.67±43.95	
Endometrioid carcinoma	39 (37.1)	79.81±66.05	
Mucinous cystadenocarcinoma	18 (17.1)	84.59±57.07	
Tumor stage			<0.001
stage I	12(11.4)	40.33±6.50	
stage II	29(27.6)/	61.16±20.15	
stage III	45(42.9)	81.81±51.15	
stage IV	19(18.1)	119.48±84.28	
Distance metastasis			0.002
Non-metastasis	86(81.9)	68.97±41.40	
Metastasis	19(18.1)	119.89±84.13	

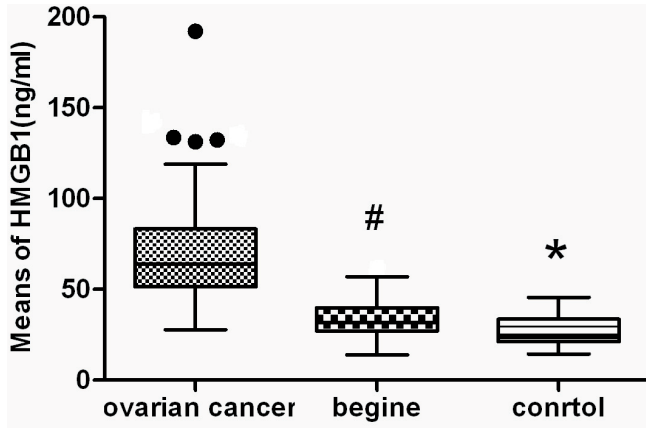**Figure 1. Serum levels of HMGB1 levels in patients with ovarian cancer**
*, # Significantly lower versus ovarian cancer (p < 0.0001, respectively).
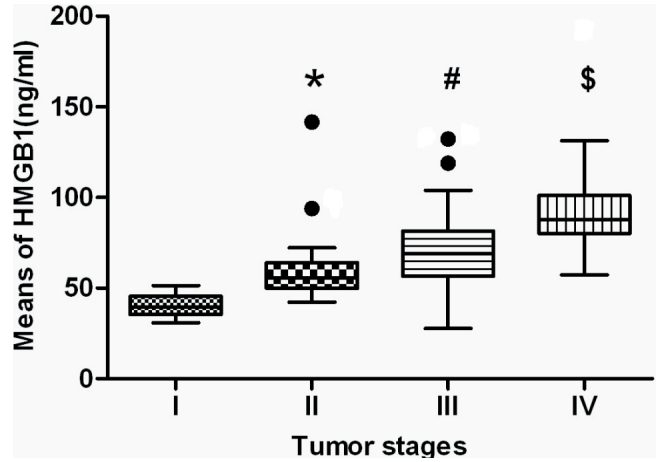

Figure 2. Serum levels of HMGB1 in patients with tumors of different stage
Value among four groups was significantly different (p < 0.0001); *versus stage I group, p < 0.01; # versus stage II group, p < 0.01; $ versus stage III group, p < 0.01.

The serum HMGB1 levels were 71.99±42.49ng/ml in patients before therapy, and 42.10±15.48ng/ml after remission (p < 0.0001) (Fig. 3).

The serum HMGB1 levels in patients with recurrent ovarian cancer and non-recurrent ovarian cancer. To determine whether the serum HMGB1 level was associated with ovarian cancer progression and recurrence, we compared the serum HMGB1 levels in recurrent ovarian cancer patients during the study period with the non-recurrent patients. Serum HMGB1 levels in patients with recurrent ovarian cancer (75.54±39.50 ng/ml) were significantly higher than in patients with non-recurrent disease (42.04±10.68 ng/ml) (Fig. 4).

Discussion

In this study, we evaluated serum HMGB1 concentrations in 105 patients with ovarian carcinoma and compared them to the concentrations in benign ovarian tumors and healthy control subjects. Analysis of serum HMGB1 levels revealed that HMGB1 was overexpressed in malignant ovarian tumors and associated with high stage and distant metastasis. These results indicated that HMGB1 may play a pivotal role in the development of ovarian cancer. However, HMGB1 was unassociated with patient ages and pathology types of ovarian carcinoma, suggested that HMGB1 was not affected by tissue histologic type. These results are consistent with those of Jie Chen et al. in which HMGB1 was evaluated in ovarian cancer tissue[13]. Numerous reports indicated that the serum HMGB1 level was elevated in patients with various types of cancer. Hanna Lee et al. showed that the serum HMGB1 level was increased by 1.5-fold in patients with colorectal carcinoma compared to those
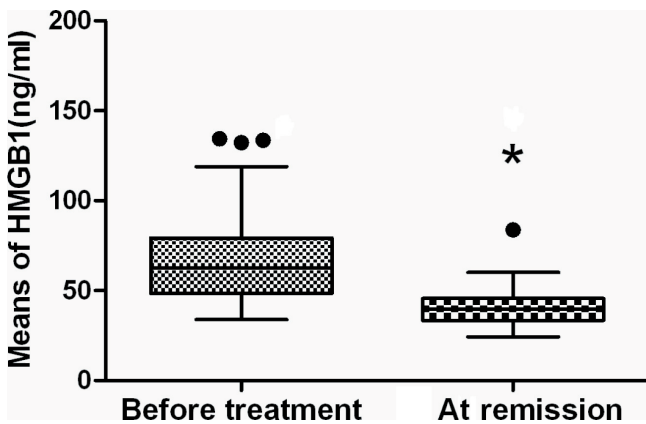

Figure 3. Serum levels of HMGB1 in patients with ovarian cancer before treatment and after remission
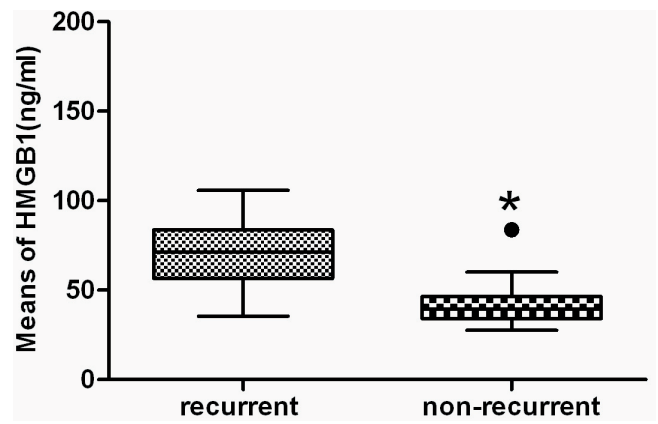*Significantly lower versus before treatment (p < 0.0001).

Figure 4. Serum levels of HMGB1 in patients with recurrent ovarian cancer and non-recurrent ovarian cancer
*Significantly lower versus recurrent ovarian cancer (p < 0.0001).

in healthy control[14]. HMGB1 is also closely associated with the clinical and pathologic features of gastric cancer[11]

To our knowledge, this report demonstrated for the first time that ovarian cancer patients' serum HMGB1 level dropped significantly after the developing remission of the disease. Moreover, the patients who underwent recrudescence had a higher serum HMGB1 level compared with the non-recurrent patients. This relatively new discovery might provide insight that HMGB1 may play an important role not only in development of ovarian cancer but also in poor outcome of ovarian cancer patients. Similar results have been indicated by Xiugui Sheng and his colleagues that serum HMGB1 level could be a useful marker for evaluating the disease recurrence and predicting prognosis in patients with cervical squamous cell carcinomas[15].

Several factors may contribute to elevation in serum HMGB1 levels in the patients with ovarian cancer. One is that HMGB1 can be passively released from dying ovarian tumor cells. Tumor cells proliferation may outpace the rate of angiogenesis, resulting in tissue severe ischeamic hypoxia[16] . It is well known that HMGB1 can be passively released by necrotic and ischeamic cells[17, 18]. Considered these results together, HMGB1 release through tumor cell death might contribute to the elevation of serum HMGB1 levels. On the other hand; it was well known that HMGB1 can be actively released from immune cells into the extra-cellular space or serum[19]. HMGB1 was known to be important in malignant cell transformation. As reported by Poser I and his colleagues, HMGB1 leads to malignant transformation and melanoma development [20]. Recent researches have shown that HMGB1 overexpression can improve proliferation[21] and decrease drug sensitivity of cancer cell[22]. In addition, HMGB1 functions as an anti-apoptotic oncoprotein by activating NF-κB and the apoptosis inhibitor c-IAP[23]. Moreover, a variety of agents such as cancer chemotherapeutic agents, oncolytic viruses, ultraviolet or gamma irradiation, cytolytic T and nature killer cells that leading to tumor cell death can indicate classic apoptosis markers that were associated with HMGB1 release[24]. These reports suggest that HMGB1 could be an oncoprotein for contribution to the tumor development and formation.

Post-translational modification has also significant effects on HMGB1. Compared to the nonmodified proteins, acetylated HMGB-1 exhibited both stronger binding to linker DNA-containing nucleosomes and a higher co-remodeling activity[25]. The acetylated protein was 3-fold more effective in inducing ligase-mediated circularization of a 111-bp DNA fragment[26]. Researchers has also found that acetylation is critical for active HMGB1 release[27]. In addition, acetylation affects HMGB1 to promote inflammation and determine its role in inflammation and immunity[28]. It is well known that chronic inflammation is an important tumor promoter. Considering these together, acetylation of HMGB1 may contribute to the development of malignancies. However, there has been no direct evidence about the relationship between the HMGB1 acetylation and cancer progression.

Extracellular HMGB1 transduse cellular signals by interacting with at least three receptors: RAGE, TLR2 and TLR4. Researchers showed that malignant cells and immature cells express high levels of HMGB1 and RAGE[29]. Furthermore, RAGE was found in 19%, 81% and 100% of Dukes' B, C and D cases, respectively, correlating with invasiveness and poor prognosis[30]. HMGB1/RAGE signaling pathways activate nuclear factor-κB (NF-κB) pathway, mitogen activated protein kinase (MAPK) and type IV collagenase (MMP-2/MMP-9), cause extracellular matrix degradation and tumor cells metastasis.[31] Recent research indicated that knockdown of HMGB1 in gastric cancer can inhibit cell migration and invasion, induce cell apoptosis and decrease MMP-9 expression[32].

In solid tumors, necrosis is commonly found in the core region in response of oxygen and glucose deletion because rapid growth of tumors may outpace the rate of angiogenesis, resulting in tissue severe ischemic hypoxia. HMGB1 released from necrotic tumor cells will function as a proinflammatory cytokine to create a microenvironment which is similar to chronic inflammation and in return contribute to the development of epithelial malignancies[33].

Conclusion

The results of our study showed that HMGB1 is overexpressed in ovarian cancer and related with clinical treatment and disease outcome, indicating that blocking of HMGB1 production or release, or preventing its interaction with its receptor(s) might provide an important opportunity for the prevention or treatment of ovarian cancer. Therefore, further studies are needed to reach on a deeper understanding of the biology of HMGB1 in ovarian cancer and to evaluate its therapeutic usefulness.

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