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RESEARCH

The Expression of High Mobility Group Box 1 is Associated with Lymph Node Metastasis and Poor Prognosis in Esophageal Squamous Cell Carcinoma

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Abstract The objective is to explore the expression of high mobility group box 1 (HMGB1) in esophageal squamous cell carcinoma (ESCC) and its relationship with lymph node metastasis and the prognosis of patients as well as possible mechanism. The expression of HMGB1, vascular endothelial growth factor C (VEGF-C) and lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) in ESCC tissues, which were obtained from 72 patients who underwent radical esophagectomy, was detected through immunohistochemistry, firstly. The correlations between HMGB1 and VEGF-C, and micro-lymphatic vessel density (MLD), and lymph node metastasis, and the prognosis of patients, were analyzed by statistic analysis. The plasmid of small interference RNA (siRNA) targeting HMGB1, giving siHMGB1, was transfected into exponentially growing KYSE150 human esophageal squamous cancer cells and the expression of HMGB1 mRNA and protein was observed by Real-time PCR and Western Blot and the expression of VEGF-C was examined by ELISA. HMGB1 expressed highly in the nuclei and cytoplasm of carcinoma cells as well as the extracellular space in ESCC and was associated with lymph node metastasis, MLD, the expression of VEGF-C, TNM stage and the prognosis of patients ($P<0.05$ or $P<0.01$). *In vitro*, siHMGB1 inhibited the expression of HMGB1 mRNA and protein and the secretion of VEGF-C in KYSE150 cells. In ESCC, HMGB1 expresses highly and affects the prognosis of patients through regulating the expression of VEGF-C to promote lymphangiogenesis and lymph node metastasis, and HMGB1 might serve as the marker of progression and potential target for anti-lymphangiogenesis therapy.

Keywords High mobility group box 1 (HMGB1) · Esophageal squamous cell carcinoma (ESCC) · Vascular endothelial growth factor C (VEGF-C) · Lymphangiogenesis

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Introduction

Esophageal squamous cell carcinoma (ESCC) has a high incidence in China, especially in Henan province, Iran, Iceland, India, Japan and the United Kingdom and is a highly malignant digestive tumor with a very poor prognosis. The overall five-year survival rate for ESCC is less than 15 % and lymph node metastasis is an important prognostic factor because esophageal cancer easily metastasizes to regional or distant lymph nodes [1]. Evidence has showed that vascular endothelial growth factor C (VEGF-C) is a specific lymphatic vessel growth factor and can induce the proliferation and migration of lymph vessel endothelial cells, promote lymphangiogenesis and lymph node metastasis [2], because VEGF-C can enhance the lymph vessel permeability of tumors, which facilitates entry of cancer cells into the lymph circulation to metastasize to distant sites [3]. Recently, it has proved that VEGF-C expresses highly in ESCC and plays a key role in lymph node metastasis and the prognosis of patients [4]. However, it is unknown what mechanism can promote VEGF-C over-expression in ESCC.

High mobility group box 1 (HMGB1), as an important member of the high mobility group protein superfamily, has been implicated in a variety of biologically important processes, including transcription, DNA repair, extracellular signaling [5]. Recently, HMGB1 over-expression has been described in many kinds of tumors, such as colorectal cancer [6], breast cancer [7], prostate cancer [8], and so forth. Interestingly,

over-expression of HMGB1 is shown to be related to the proliferation, metastasis, infiltration of tumor cells and the angiogenesis of tumor and the prognosis of patients [9, 10]. Many studies have indicated that HMGB1 can be actively released from tumor cells into the extra-cellular space or serum [11]. Kang HJ et al [12] found that HMGB1 is phosphorylated firstly, then transported to the cytoplasm, subsequently secreted from the cells, and plays a key role in tumor progression. It was reported that HMGB1 may bind to its receptor, such as the receptor for advanced glycosylation (RAGE), and activate signal pathway, such as Ras/MAKP, PI3K/Akt, NF-kB [13], which leads to some genes over-expression and the change of tumor cell biological behavior.

However, there is no report about the expression of HMGB1 as well as HMGB1 related to lymph node metastasis and the prognosis of patients in ESCC. At the same time, it is unclear whether HMGB1 may have positively effect on VEGF-C expression. In this study, we investigated the expression of HMGB1 in ESCC through immunohistochemistry and explored its relationship with lymph node metastasis by statistic analysis as well as further studied its impact on VEGF-C expression *in vitro*, and, finally, investigated possible mechanisms.

Materials and Methods

Patient Characteristic and Clinical Data

72 ESCC patients, male 52 cases and female 20, ranging in age from 43–77 years with a mean age of 61 years, who has no diabetes, nervous system diseases, cardiovascular diseases, were received radical Ivor-Lewis esophagectomy without preoperative chemotherapy or radiotherapy during 2001–2003 in this retrospective study. At the same time, 72 reserved paraffin-embedded, formalin-fixed specimens of ESCC with detail clinical, pathological and follow-up data were obtained from Tianjin Medical University Cancer Institute and Hospital, Tianjin, China. This study was approved by the Tianjin Medical University Ethics Committee. Clinical TNM stage was performed according to post-operation data, using the criteria of International Union against Cancer (UICC) and the American Joint Committee on Cancer (AJCC) in 2002. Of 72 cases, three were classified as stage I, 34 as stage IIA, four as stage IIB and 31 as stage III. Moreover, 33 cases had lymph node metastasis and 39 cases did not. The median follow-up time for overall survival for 5 years survival was 42.5 months for patients still alive at the time of analysis.

Immunohistochemical Staining

Anti-HMGB1 rabbit polyclonal antibody and anti-LYVE1 rabbit polyclonal antibody were purchased from Abcam (Cambridge, UK). Anti-VEGF-C rabbit polyclonal antibody was purchased from Santa Cruz (CA, USA). Streptavidin-peroxidase (SP) assay kit (Invitrogen, CA, USA) was used. Slides were deparaffinized, and the endogenous peroxidase activity was quenched with 3 % H_2O_2. Nonspecific binding sites were blocked with goat serum after recovering antigens. HMGB1, VEGF-C, and LYVE1 antibodies were pipetted onto slides and kept overnight at 4 °C. Slides were rinsed with phosphate buffered saline (PBS); horseradish peroxidase-conjugated goat anti-rabbit IgG was dropped and incubated for 15 min. Slides was washed with PBS, added with 3, 3-daiminobenzidine tetrahydrochloride (DAB), and counterstained with hematoxylin.

Immunohistochemical Scoring

The expression of HMGB1 and VEGF-C was ranked according to the percentage of positive cells and the intensity of coloration. The percentage of positive staining cells: 0 score, <10 %; 1 score, 10–25 %; 2 score, 25–50 %; 3 score, >50 %. As to the intensity of coloration: 0 score, negative; 1 score, buff; 2 score, yellow; 3 score, brown. The total score was calculated by multiplying the quantity and staining intensity scores. An immunohistochemical score ≥3 was considered as positive expression. Immunohistochemical scoring was performed independently by two observers and the inter-observer variability was <3 %.

Determination of Micro-lymphatic Vessel Density (MLD)

Immunostaining for lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) was to count MLD as suggested by Weidner et al [14]. The area of tissue with the greatest number of distinctly highlighted micro-lymphatic vessels ("hot spots") was selected at low magnification (40×), firstly. Then, MLD was determined by counting all immunostained lymphatic vessels at a total magnification of 200×. Of course, determination of the staining reaction was strictly confined to the "hot spots". MLD was considered evident if at least one tumor cell cluster was clearly visible inside the LYVE1-stained lymphatic vessel space. MLD counts were done by two independent observers, too.

The Construction of Vector and Grouping and Administration In Vitro

According to GenBank No. NM_002128, the sequence of targeting HMGB1 small interference RNA (siHMGB1) and the negative control sequence were designed using computer software. Target sequence, 5′-CCATCA CAGTGT TGTTAA TGT-3′, was selected and it is a most effective sequence of our designed several sequences to inhibit the expression of HMGB1 in our preliminary experiment. At

the same time, a sequence of negative control, 5′-GCTCTG GAGCAG TTCCGA TAT-3′, was selected, too. The shRNA was synthesized and subcloned into the pAAV-H1-hrGFP Vector, giving pAAV-H1-shHMGB1-hrGFP [15].

Three experimental groups were used, namely the blank control group (Wt), the negative control group (Nc) and small interference RNA group (Si). Wt group consisted of non-transfected wild-type human esophageal squamous cancer cell line, KYSE150 cells (DSMZ, Germany), Nc group was KYSE150 cells transfected with negative sequence small interfering RNA vector and Si group was KYSE150 cells transfected with trageting HMGB1 small interfering RNA vector. All groups were cultured in Dulbecco's modified Eagle's medium (DMEM) including 10 % fetal calf serum (FCS) in flasks incubated at 37 °C in 95 % air and 5 % CO_2. When the cells were 90 % confluent, the negative control plasmid and RNA interference plasmid were transfected into the KYSE150 cells using a lipofectamine 2000 kit (Invitrogen, CA,USA), respectively. We tested the expression of HMGB1 mRNA and protein and the secretion of VEGF-C after 24 h and 48 h. For each experiment, five identical replicates would be performed.

Real-time PCR to Detect the Expression of HMGB1 mRNA

The total RNA was extracted from the KYSE150 cells and cDNA was synthesized by AMV reverse transcriptase at 42 °C for 10 min and 95 °C for 2 min. A SYBR ExScript™ RT-PCR Kit (TaKaRa, Tokyo, Japan) and ABI (CA, USA) Prism 7900HT sequence detection system were used. The reagents were subjected to 95 °C for 30 sec and were then cycled 40 times of 95 °C for 5 sec and 60 °C for 15 sec and 72 °C for 30 sec. The primers of HMGB1 were 5′-ATATGG CAAAAG CGGACA AG-3′ as forward primer and 5′-GCAACA TCACCA ATGGAC AG-3′ as reverse primer. The primers of β-actin were 5′-TGGCAC CCAGCA CAATGA A-3′ as forward primer and 5′-CTAAGT CATAGT CCGCCT AGAAGC A-3′ as reverse primer. The results of relative quantification were analyzed by an ABI 7900HT software system.

Western Blot to Detect the Expression of HMGB1 Protein

HMGB1 polyclonal antibody was purchased from Abcam (Cambridge, UK) and β-actin polyclonal antibody was purchased from Santa Cruz (CA, USA). The total proteins were extracted from the KYSE150 cells and the concentrations were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA) and by Biophotometer (Eppendorf, Hamburg, Germany). The total proteins were separated on 12 % SDS-PAGE gel for HMGB1 and β-actin, transferred to polyvinylidene difluoride membranes by MINI TRANS-BLOT (BIO-RAD, CA, USA). The blot membrane was then incubated with primary and secondary antibodies and treated with enhanced chemiluminescence detection reagents (Amersham, Buckinghamshire, UK). The specific blotting band was recorded on film. The results were analyzed by ImageJ software (available from the NIH at http://rsb.info.nih.gov/ij/).

ELISA to Detect the Secretion of VEGF-C

KYSE150 cells of three groups were seeded in 96-well culture plate (10^4 cells/well) and five different wells were used for each experimental condition. Then, culture medium was assayed for VEGF-C in three groups using the human VEGF-C ELISA kit (BOSTER, China) according to the manufacturers' instructions. Absorbance in every well was measured in spectrophotometer at a wavelength of 450 nm.

Statistical Analysis

All data were processed with the statistic software SPSS 11.5. For categorical data, Pearson's $\chi 2$ test was used. Means were compared using the Student t-test. Survival analysis was carried out using the Kaplan-Meier method with log rank Mantel Cox comparison. Spearman's rank correlation analysis was performed to assess the correlations between HMGB1 and clinicopathologic characteristics. The significance of variables for survival was analysed by the Cox proportional hazards model in a multivariate analysis. Results were considered significant at $p<0.05$.

Results

Immunohistochemical Analysis and Clinicopathological Significance

HMGB1 protein was mainly localized in the nuclei and cytoplasm of carcinoma cells. At the same time, HMGB1 protein also presented in extracellular space (Fig. 1b). HMGB1 staining was obviously weaker in normal adjacent esophageal mucosa epithelial cells than in ESCC tissues (Fig. 1a). Overexpression of HMGB1 staining was found in 50 of 72 (69.4%) ESCC tissues. Moreover, of 33 ESCC tissues with lymph node metastasis, 27 were found to shown HMGB1 overexpression (81.82%). VEGF-C expression was observed in the cytoplasm of cancer cells and there was no obvious staining in normal esophageal mucosa tissues (Fig. 1c). Among 33 specimens with lymph node metastasis, 25 (75.76 %) had VEGF-C expression. Moreover, we found that LYVE1 expressed richly in surrounding tissues of carcinoma and weakly in carcinoma. At the same time, MLD was high in surrounding tissues of carcinoma, too (Fig. 2). In the Table 1, we summarized the relationship between HMGB1 expression and clinicopathological

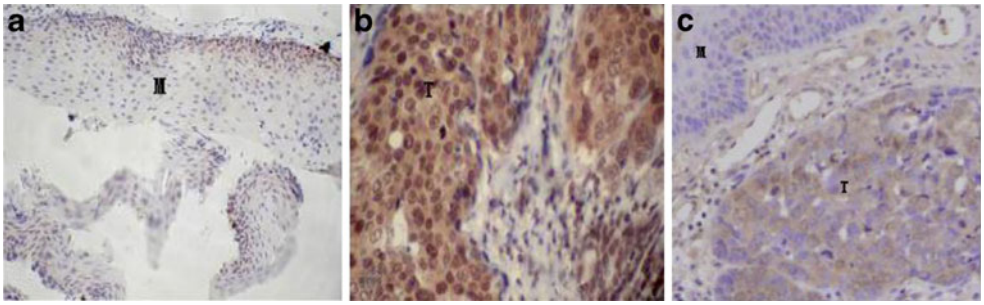

Fig. 1 The lower expression of HMGB1 in normal esophageal mucosa (**a**) (SP×100). The high expression of HMGB1 (**b**) and VEGF-C (**c**) in ESCC (SP×200). The negative expression of HMGB1 in normal esophageal mucosa (**c**) (SP×200). M=mucosa, T=tumor

features in ESCC. There was no significant association between HMGB1 expression and age, gender, tumor size, tumor site, and tumor differentiation grade in ESCC. However, HMGB1 expression was positively correlated with the expression of VEGF-C, lymph node metastasis, MLD, and TNM stage (see Tab. 1).

The Association Between HMGB1 Expression and Survival

Using Kaplan-Meier analysis method, we found that the expression of HMGB1 in ESCC was significantly correlated with overall survival (Fig. 3). In fact, of 22 patients without HMGB1 expression, 10 survived over 5 years (45.45 %). However, of 50 patients with HMGB1 expression in tumor, only 12 survived more than 5 years (24 %). The survival time of patients was significantly difference between groups with positive and negative expressions of HMGB1 (P= 0.0475), indicating that the high level of HMGB1 was correlated with a shorter survival times. In multivariate Cox's regression analysis, we analyzed whether HMGB1 expression was an independent prognostic factor. Multivariate survival analysis including lymph node metastasis, TNM stage, tumor differentiation grade and HMGB1 expression was done. Results showed that HMGB1 expression had a significant correlation with prognosis and could be as an independent prognostic factor of survival (see Tab. 2).

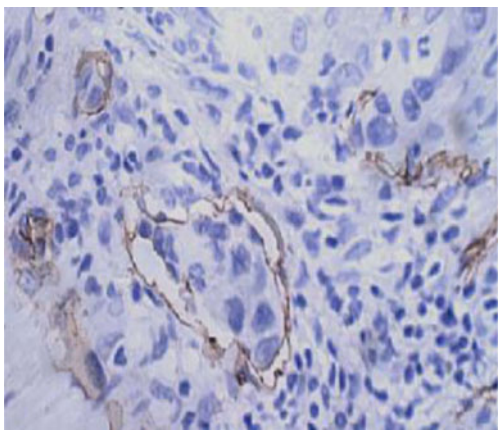

Fig. 2 The expression of LYVE1 in ESCC (SP×200)

The Effect of HMGB1 on VEGF-C Expression In Vitro

After transfection of HMGB1 shRNA into KYSE150 cells, Real-time PCR showed that HMGB1 shRNA inhibited the expression of HMGB1 mRNA in the KYSE150 cells after 24 h and after 48 h the expression decreased nearly 90 % compared to Wt group or Nc group (see Fig. 4a). Western blot showed that HMGB1 shRNA suppressed the expression of HMGB1 protein after 24 h and after 48 h the protein expression declined over 90 % compared to Wt group or Nc group (see Fig. 4b, c and d). From ELISA, HMGB1 knockdown

Table 1 Relationship between HMGB1 and clinicopathological variables

	n	HMGB1 Expression		P
		+	−	
Gender				
Male	52	35	17	0.562
Female	20	15	5	
Age				
≥60	41	28	13	0.807
<60	31	22	9	
Tumor Size				
>5 cm	30	21	9	0.931
≤5 cm	42	29	13	
Tumor Site				
Upper	12	8	4	0.824
Middle	43	30	13	
lower	17	12	5	
Differentiation Grade				
I,I~II,II	61	42	19	0.797
II~III, III	11	8	3	
Tumor Stage				
I	3	1	2	0.043
IIA, IIB	38	23	15	
III	31	26	5	
Lymph Node Metastasis				
+	33	27	6	0.036
−	39	23	16	
MLD	72	12.24±2.64	6.74±2.25	0.000
VEGF-C				
+	54	42	12	0.008
−	18	8	10	

Table 2 Summary of survival analyses by multivariate Cox regression analysis

Parameter	Overall survival		
	P	HR	95%CI
Gender			
Male vs Female	0.514	0.824	0.517–1.330
Age			
≥60 vs <60	0.685	1.058	0.801–1.387
Tumor Size			
>5 cm vs ≤5 cm	0.720	0.912	0.602–1.420
Differentiation Grade			
I,I~II,II vs II~III, III	0.712	1.062	0.792–1.412
Lymph Node Metastasis			
+ vs −	0.006	1.984	1.201–2.952
Tumor Stage			
I, IIA,IIB vs III	0.013	3.080	1.284–7.094
HMGB1 expression			
+ vs −	0.001	2.280	1.431–3.643

cells exhibited significantly reduced VEGF-C secretion compared with cells treated with non-specific siRNA (see Fig. 5). Therefore, disruption of endogenous HMGB1 expression may result in inhibition of VEGF-C expression in ESCC.

Discussion

Metastasis is a most important biological characteristic for tumor cells. Invasion of tumor cells into blood and lymphatic vessels is one of the critical steps for the establishment of metastasis. In fact, tumor cells exposed to an increased number of micro-lymphatic vessels are more likely to spread to lymph nodes and to distant sites. The VEGF-C/VEGFR3 pathway is primarily required for lymphangiogenesis and lymphatic metastasis [16], because VEGF-C could bind to its receptor (VEGFR-3) on the lymphatic endothelial cells, stimulate lymphangiogenesis, enhance MLD and result in lymphatic node metastasis. Thus, MLD is a key factor and important feature for tumor progression and dissemination. In the past, it was very difficult to study MLD due to the absence of lymphatic endothelial marker. Nowadays, LYVE1 has been considered as a specific lymphatic vessel marker, making it easier to count MLD.

HMGB1 is a versatile protein with intranuclear and extracellular functions, which is involved in numerous biological and pathological processes. Studies have shown that extracellular HMGB1 binds to RAGE in tumors and induces cancer cell growth, mobility, invasion and metastasis, and blockade of the HMGB1-RAGE interaction suppresses tumor growth and metastasis [17, 18]. However, it is unclear whether HMGB1 may stimulate VEGF-C expression and lymphangiogenesis as well as enhance MLD in ESCC.

In this study, we found that HMGB1 expressed highly not only in the nuclei and cytoplasm but also in extracellular space in ESCC, which indicated that HMGB1 can play an important role in the nuclei and cytoplasm as well as in extracellular space. For example, Schlueter et al [13] reported that extracellular HMGB1 has positively effect on signal transduction in tumor cells. Moreover, we found that HMGB1 over-expression was significantly associated with TNM stage, lymph node metastasis, MLD, VEGF-C, but not with patient gender, age, tumor site, tumor size and tumor differentiation, which indicated that HMGB1 plays a key role in ESCC and has importance influence on biological behavior of esophageal cancer cells, lymphangiogenesis and lymph node metastasis. However, using univariate and multivariate analysis for overall survival, HMGB1expression had a significant correlation with prognosis and was an independent prognostic factor of survival, which indicated that high expression of HMGB1 was a significant predictor of prognosis for ESCC patients. In fact, HMGB1 has been known as a metastasis associated gene in colorectal cancer [6]. In this study, we found that VEGF-C was only expressed in the cytoplasm of esophageal cancer cells and had no expression in normal esophageal mucosa and was closely related to lymph node metastasis. Interestingly, HMGB1 was significantly associated with VEGF-C expression in ESCC, while VEGF-C was correlated to lymph node metastasis and poor prognosis [4]. So, we speculated that HMGB1 might have important effect on VEGF-C expression in ESCC. To further study whether HMGB1 plays a key role in the expression of VEGF-C, which is a specific lymphangiogenesis factor [16], a RNA interference technique was used to knockdown endogenous

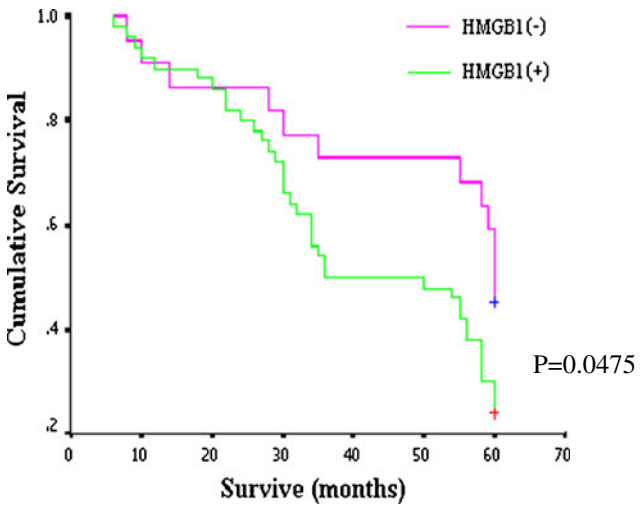

Fig. 3 Survival curves for ESCC patients with regard to HMGB1 expression

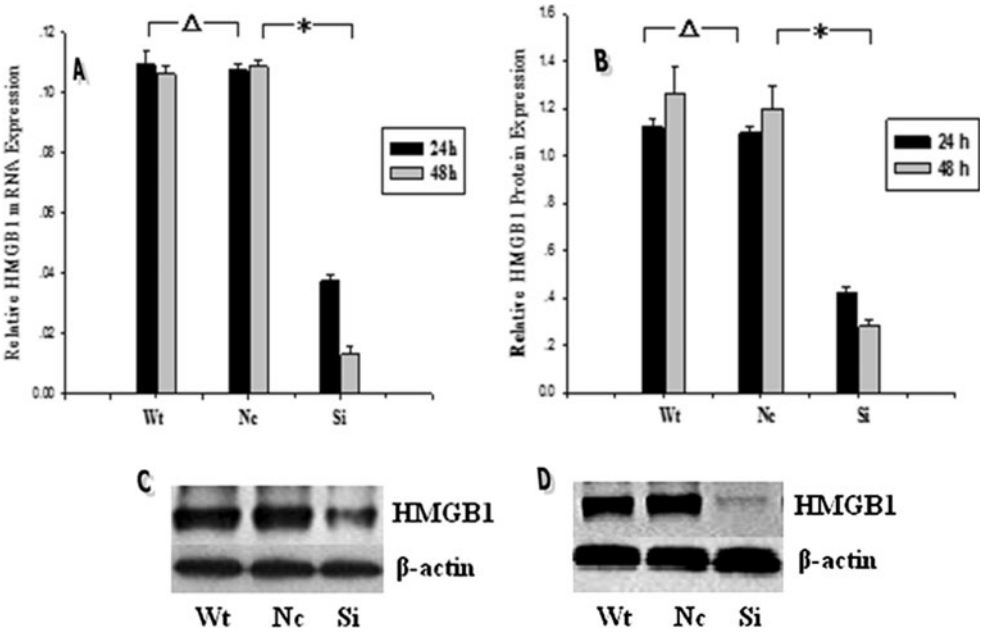

Fig. 4 Expression of HMGB1 and protein after 24 h and 48 h. **a** Expression of HMGB1 mRNA determined by real-time PCR. **b** Expression of HMGB1 protein determined by western blot. Western blot of HMGB1 protein expression **c** after 24 h and **d** after 48 h. Wt: non-transfected wild-type KYSE150 cells; Nc: KYSE150 cells transfected with non-specific siHMGB1; Si: KYSE150 cells transfected with siHMGB1. *$p<0.01$ and $^{\triangle}p>0.05$

HMGB1 and to observe the effect on VEGF-C expression *in vitro*. As a result, siRNA targeting HMGB1 suppressed the expression of VEGF-C in KYSE150 cells while non-specific siRNA did not, which indicated that HMGB1 can stimulate VEGF-C expression in ESCC. The mechanism might be that the interaction of HMGB1 with its receptors activates several intracellular signal transduction pathways such as Ras/MAPK, NF-kB, Rac, and Cdc42, which activate the expression of VEGF-C. Furthermore, HMGB1 expression was inversely correlated with overall survival, that is to say, patients without HMGB1 expression in tumors will have a better overall survival than patients with HMGB1 expression. The reason might be that HMGB1 can activate VEGF-C expression, while VEGF-C is a key factor of lymphangiogenisis and lymph node metastasis, which finally affects the survival and prognosis of ESCC patients. Thus, HMGB1 over-expression could be an important prognostic factor of survival in ESCC.

In conclusion, HMGB1 expressed highly in ESCC and was significantly associated with VEGF-C, MLD, metastatic lymph nodes, TNM stage and poorer clinical survival. At the same time, inhibition of HMGB1 expression led to the decrease of VEGF-C expression *in vitro*. So, HMGB1 may exert positively influence on the prognosis of patients through stimulating VEGF-C expression to promote lymph node metastasis, and serve as the potential target for anti-lymphangiogenesis therapy.

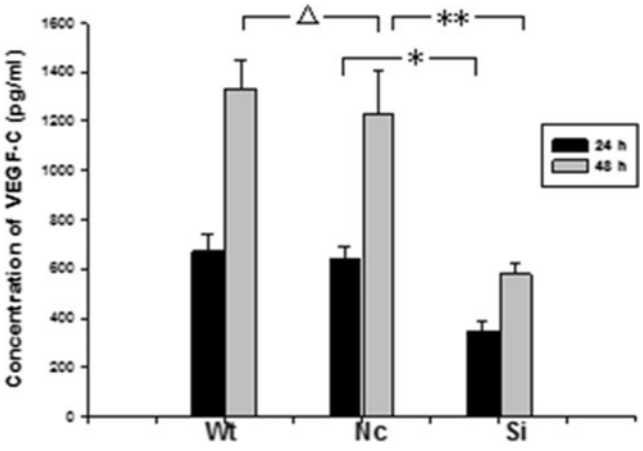

Fig. 5 Secretion of VEGF-C was determined by ELISA in three groups after 24 and 48 h. Wt: non-transfected wild-type KYSE150 cells; Nc: KYSE150 cells transfected with non-specific siHMGB1; Si: KYSE150 cells transfected with siHMGB1. *$p<0.05$, **$p<0.01$, and $^{\triangle}p>0.05$

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References

1. Duff SE, Li C, Jeziorska M, Kumar S, Saunders MP, Sherlock D, O'Dwyer ST, Jayson GC (2003) Vascular endothelial growth factors C and D and lymphangiogenesis in gastrointestinal tract malignancy. Br J Cancer 89:426–30
2. Aurello P, Rossi Del Monte S, D'Angelo F, Cicchini C, Ciardi A, Bellagamba R, Ravaioli M, Ramacciato G (2009) Vascular endothelial growth factor C and microvessel density in gastric carcinoma: correlation with clinicopathological factors. Our experience and review of the literature. Oncol Res 17:405–11

3. Shimoda H, Takahashi Y, Kajiwara T, Kato S (2003) Demonstration of the rat lymphatic vessels using immunohistochemistry and in situ hybridization for 5′- nucleotidase. Biomed Res 24:51–7
4. Liu P, Chen W, Zhu H, Liu B, Song S, Shen W, Wang F, Tucker S, Zhong B, Wang D (2009) Expression of VEGF-C correlates with a poor prognosis based on analysis of prognostic factors in 73 patients with esophageal squamous cell carcinomas. Jpn J Clin Oncol 39:644–50
5. Czura CJ, Wang H, Tracey KJ (2001) Dual roles for HMGB1: DNA binding and cytokine. J Endotoxin Res 7:315–21
6. Yao X, Zhao G, Yang H, Hong X, Bie L, Liu G (2010) Overexpression of high-mobility group box 1 correlates with tumor progression and poor prognosis in human colorectal carcinoma. J Cancer Res Clin Oncol 136:677–84
7. Palumbo R, Sampaolesi M, De Marchis F, Tonlorenzi R, Colombetti S, Mondino A, Cossu G, Bianchi ME (2004) Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. J Cell Biol 164:441–49
8. Gnanasekar M, Thirugnanam S, Ramaswamy K (2009) 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 34:425–431
9. Tang D, Kang R, Zeh HJ 3rd, Lotze MT (2010) High-mobility group box 1 and cancer. Biochim Biophys Acta 1799:131–40
10. Kusume A, Sasahira T, Luo Y, Isobe M, Nakagawa N, Tatsumoto N, Fujii K, Ohmori H, Kuniyasu H (2009) Suppression of dendritic cells by HMGB1 is associated with lymph node metastasis of human colon cancer. Pathobiology 76:155–62
11. Moriwaka Y, Luo Y, Ohmori H, Fujii K, Tatsumoto N, Sasahira T, Kuniyasu H (2010) HMGB1 attenuates anti-metastatic defense of the lymph nodes in colorectal cancer. Pathobiology 77:17–23
12. Kang HJ, Lee H, Choi HJ, Youn JH, Shin JS, Ahn YH, Yoo JS, Paik YK, Kim H (2009) Non-histone nuclear factor HMGB1 is phosphorylated and secreted in colon cancers. Lab Invest 89:948–59
13. Schlueter C, Weber H, Meyer B, Rogalla P, Röser K, Hauke S, Bullerdiek J (2005) Angiogenetic signaling through hypoxia HMGB1: an angiogenetic switch molecule. Am J Path 166:1259–63
14. Weidner N, Semple JP, Welch WR, Folkman J (1991) Tumor angiogenesis and metastasis –correlation in invasive breast carcinoma. N Engl J Med 324:1–8
15. Chen C, Sun J, Liu G, Chen J (2009) Effect of small interference RNA targeting HIF-1α mediated by rAAV combined L-ascorbate on pancreatic tumors in athymic mice. Pathol Oncol Res 15:109–14
16. Tuomela J, Valta M, Seppänen J, Tarkkonen K, Väänänen HK, Härkönen P (2009) Overexpression of vascular endothelial growth factor C increases growth and alters the metastatic pattern of orthotopic PC-3 prostate tumors. BMC Cancer 9:362
17. Zeh HJ 3rd, Lotze MT (2005) Addicted to death: invasive cancer and the immune response to unscheduled cell death. J Immunother 28:1–9
18. Ito N, DeMarco RA, Mailliard RB, Han J, Rabinowich H, Kalinski P, Stolz DB, Zeh HJ 3rd, Lotze MT (2007) Cytolytic cells induce HMGB1 release from melanoma cell lines. J Leukoc Biol 81:75–83