

RESEARCH ARTICLE

Expression of HMGB1 and its Clinical Significance in T-cell Lymphoma

Xing-Jiang Mao^{1*}, Geng-Fu Wang², Zhi-Jun Chen¹, Li-Na Wang³, Jun-Biao Zhang⁴, Hui-Ling Wang⁵

Abstract

Objectives: To evaluate the clinical significance of HMGB1 expression in T-cell lymphoma. **Methods:** Immunohistochemical staining for HMGB1 and survivin was performed with specimens from 120 cases of T-cell lymphoma and 40 cases of reactive lymphoid hyperplasia with antibodies against human HMGB1 and survivin. **Results:** The expression of HMGB1 and survivin was significantly higher in tissues of T-cell lymphoma than in reactive lymphoid hyperplasia. Positive expression of HMGB1 and survivin was observed in 63.7% (65/102) and 61.8% (63/102) of T-cell lymphoma cases, respectively. While was associated with gender, age, and tumor location, significant correlations with malignancy and clinical stage were observed. Spearman rank correlation analysis revealed that the expression of HMGB1 and survivin was positively correlated in T-cell lymphomas ($P < 0.01$). **Conclusions:** Expression of HMGB1 and survivin in T-cell lymphomas is significantly associated with malignancy and clinical stage, but not with gender, age and tumor location. Elevated expression of HMGB1 may be an important biomarker for the development and progression of T-cell lymphoma.

Keywords: HMGB1 - survivin - T-cell lymphoma - prognostic significance

Asian Pacific J Cancer Prev, **13** (11), 5569-5571

Introduction

T-cell lymphoma is a heterogeneous tumor in which T lymphocyte cells become cancerous. It is a common cancer in China and its incidence rate increases steadily in recent years. High mobility group box-1 (HMGB1) protein is a highly conserved nuclear protein which binds to DNA and regulates gene transcription. Recent studies show that HMGB1 plays important roles in the development and progression of tumors. It is highly expressed in many malignant tumors including liver cancer, breast cancer, colon cancer, and ovarian cancer. Survivin is a newly described member of the inhibitor of apoptosis (IAP) family. Its expression is undetectable in normal adult tissues but significantly upregulated in transformed cell lines and several malignant tumors. It may protect cells against apoptosis and promote tumor growth and invasion (Lu et al., 2007; Reis et al., 2011). Wang et al showed that HMGB1 may inhibit apoptosis of hepatoma cells and promote their proliferation via Survivin (Wang et al., 2004). However, in T cell lymphoma the expression of HMGB1 and its relationship with Survivin have not been reported. In this study, we investigated the expression of HMGB1 and Survivin in tissues from patients with T cell lymphoma and its correlation with clinicopathology.

Our results may shed light on the role of HMGB1 in the development and progression of T-cell lymphoma.

Materials and Methods

Materials

Tissue samples from 102 patients diagnosed with T-cell lymphoma by histopathology and immunohistochemistry from Jan 2002 to Dec 2011 (60 males, 42 females, age range 14-81 years, mean age 56 ± 4.5 years) were included in this study. The grading of pathology and malignancy of T-cell lymphoma were determined according to "WHO Classification of Tumours of Haematopoietic and Lymphoid, Fourth Edition, 2008". Pathological stages of T-cell lymphoma were classified according to Ann Arbor staging system. Tissues from 40 cases of reactive lymphoid hyperplasia were obtained from biopsy specimens in our hospital.

Immunohistochemical analysis

Samples were fixed in 4% formaldehyde solution, embedded in paraffin, and sectioned (4 μ m). The sections were dewaxed in xylene, dehydrated in alcohol, and subjected to antigen retrieval by microwave irradiation. Then, sections were incubated with rabbit

¹Department of General Surgery, ²Department of Anesthesiology, ³Operating Room, ⁴Department of Cardiology, ⁵Department of Obstetrics and Gynecology, The First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan, China *For correspondence: xingjiangmaocn@163.com

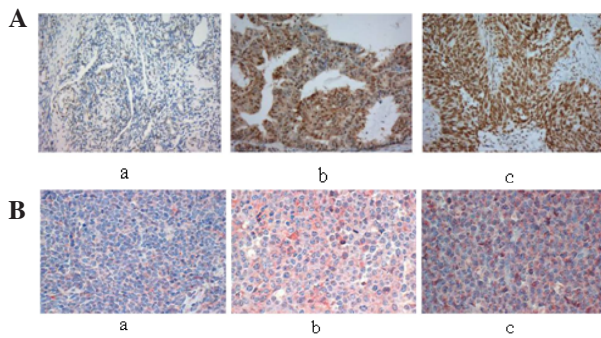


Figure 1. A. Expression of HMGB1 in Different Tissues. a: normal tissues; b: tumor lymphoid tissues; c: reactive lymphoid hyperplasia tissues. **B. Expression of Survivin in Different Tissues.** a: normal tissues; b: tumor lymphoid tissues; c: reactive lymphoid hyperplasia tissues

Table 1. Expression of HMGB1 and Survivin in T-cell Lymphoma and its Relationship with Clinicopathologic Features

Group	Case	Survivin			HMGB1		
		+	-	P value	+	-	P value
	102						
Gender							
Male	60	39	21	0.535	36	24	0.3035
Female	42	24	18		29	13	
Age(years)							
<45	29	18	11	1	20	9	0.6485
≥45	73	45	28		45	28	
Location							
Intranodal	31	20	11	0.8256	23	8	0.1819
Extranodal	71	43	28		42	29	
Malignancy							
I	31	5	26	<0.001	10	21	<0.001
A	56	44	12		40	16	
HA	15	14	1		15	0	
Stage							
I/II	71	34	37	<0.001	35	36	<0.001
III/IV	31	29	2		30	1	

I = inert; A = aggressive; HA = highly aggressive; “+” = positive; “-” = negative

anti-human polyclonal antibodies against HMGB1 and Survivin (1:200 dilution, Santa Cruz, USA), followed by incubation with peroxidase-conjugated secondary antibodies. The peroxidase reactivity was visualized using a 3, 3'-diaminobenzidine (DAB) according to the manufacturer's instruction of the DAB detection kit. As a negative control, a parallel experiment in which the primary antibodies were substituted by phosphate buffered saline (PBS) was performed.

Evaluation of immunohistochemistry

Antigen expression was evaluated using light microscopy. The immunoreactivity of HMGB1 was localized to the cytosol of tumor cells, while the immunoreactivity of Survivin was in nucleus. The dark brown staining indicates positive expression of HMGB1 and Survivin. Using a semiquantitative scoring system (Kawasaki et al., 1998), we evaluated the intensity and extent of HMGB1 and Survivin expression. The percentage of cells positive for HMGB1 and Survivin was determined and graded as follows: 0 = 0%-5%, 1 =

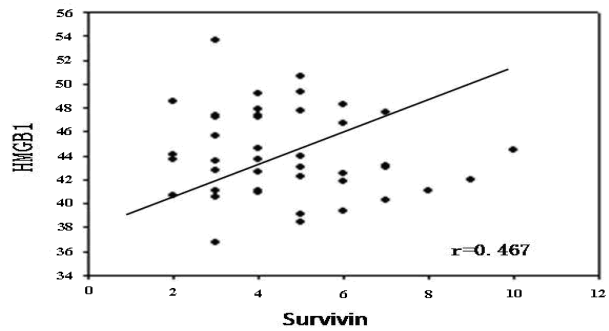


Figure 2. The Correlation Between HMGB1 and Survivin Expression

Table 2. The Relationship Between HMGB1 and Survivin Expression in T-cell Lymphoma

Survivin	HMGB1		Total	r value	P value
	+	-			
+	57	6	63	0.467	0.001
-	8	31	39		
Total	65	37	102		

“+” = positive; “-” = negative

6%-25%, 2 = 26%-50%, 3 = 51%-75%, and 4 = 76%-100%. The intensity of HMGB1 and Survivin staining was graded as follows: 0 = none, 1 = weak, 2 = moderate, and 3 = intense. An immunoreactive score was calculated by adding the grade of percentage of positive cells to the grade of intensity of staining. Scores of 0 and 1 were judged as HMGB1 or Survivin negative, and scores of 2 and 2+ were judged as positive.

Statistical analysis

All data were analyzed with the SPSS 17.0 statistical software. Chi-square test was used to determine the relationship between the expression of HMGB1/Survivin and clinicopathologic features. Spearman rank correlation analysis was used to analyze the correlation between HMGB1 expression and Survivin expression. Statistically significance was defined as P < 0.05.

Results

Expression of HMGB1 and Survivin in samples of T-cell lymphoma and reactive lymphoid hyperplasia and its relationship with clinicopathologic features

The positive immunoreactivity of HMGB1 was observed in the cytosol of cells. As shown in Figure 1A, specimens from 65 of 102 (72%) cases of T-cell lymphoma were HMGB1 positive, which is much higher than that of reactive lymphoid hyperplasia (16 of 40, 40%, P<0.01). The immunoreactivity of Survivin was localized to the nucleus. The percentage of Survivin-positive cases was also significantly higher in T-cell lymphoma than that in reactive lymphoid hyperplasia (61.8% [63/102] vs 45% [18/40], P < 0.05) (Figure 1B).

As shown in Table 1, there was no correlation between the expression of HMGB1 or Survivin and the gender, age, or tumor location in patients with T-cell lymphoma (P>0.05). In inert, aggressive, and highly aggressive lymphoma, the positive expression rate was 32.3%, 71.4%,

and 100.0% respectively for HMGB1, and 16.1%, 78.6%, and 93.3% respectively for Survivin. Overexpression of HMGB1 and Survivin was associated with tumor aggressiveness ($P < 0.001$). The positive expression rate in stage I/II lymphoma and stage III/IV lymphoma was 49.3% and 96.8% respectively for HMGB1 and 47.9% and 93.5% respectively for Survivin. Quantitative analysis showed that the expression of HMGB1 and Survivin were associated with the tumor stage ($P < 0.001$).

The correlation between HMGB1 expression and Survivin expression in T-cell lymphoma

In patients with T-cell lymphoma, 65 cases were positive for HMGB1. Of them, 57 cases (87.7%) were also positive for Survivin. However, of 37 HMGB1-negative cases, only 6 cases (16.2%) were positive for Survivin. Statistical analysis revealed a significant correlation between the expression of the two proteins ($r = 0.467$, $p = 0.001$) (Figure 2 and Table 2).

Discussion

HMGB1, originally identified as a chromatin protein in 1973, is an important regulator of cell cycles (Wang and Chen, 2009). It is expressed in tumor tissues of several cancers including liver cancer, breast cancer, colon cancer, and ovarian cancer, and plays critical roles in gene regulation and tumor immunology (Jube et al., 2012). It has been shown that HMGB1 can be secreted from cells and its level in serum is significantly upregulated in cancer patients compared to that in normal controls (Cheng et al., 2008). We found that HMGB1 was expressed in T-cell lymphoma as well as reactive lymphoid hyperplasia and normal lymphoid. However, its expression was significantly elevated in T-cell lymphoma compared to that in reactive lymphoid hyperplasia and normal lymphoid. Survivin is an endogenous inhibitor of apoptosis and participates in early event of tumorigenesis. It is highly expressed in many malignant tumors and its expression is correlated with the malignancy, stage, recurrence, and transfer of tumors (Wang et al., 2004; Lu et al., 2007; Reis et al., 2011). Our results showed that the expression of Survivin was upregulated in T-cell lymphoma compared to that in reactive lymphoid hyperplasia. Consistent with previous reports (Kuniyasu et al., 2002; Cheng et al., 2008; Chung et al., 2009), the expression of HMGB1 and Survivin was correlated with the aggressiveness and stage of tumors. Therefore, HMGB1 and Survivin may be involved in the development and progression of T-cell lymphoma and could be new targets for targeted molecular therapy.

Both HMGB1 and Survivin function in the G2/M phase to regulate cell cycles. They also promote tumorigenesis by inhibiting apoptosis (Tang et al., 2010). Wang et al. (2004) found that HMGB1 may promote tumor proliferation by upregulating Survivin expression. We evaluated the correlation between HMGB1 expression and Survivin expression, and found that they were positively correlated in T-cell lymphoma, indicating that Survivin may be a downstream target of HMGB1 in T-cell lymphoma and that HMGB1 may promote proliferation by upregulating

Survivin expression.

Taken together, our findings suggest that HMGB1 may play important roles in the development and progression of T-cell lymphoma. Examination of the expression of both HMGB1 and Survivin will help us to determine the malignancy and stage of T-cell lymphoma.

Acknowledgements

The author(s) declare that they have no competing interests.

References

- Cheng BQ, Jia CQ, Liu CT, et al (2008). Serum high mobility group box chromosomal protein 1 is associated with clinicopathologic features in patients with hepatocellular carcinoma. *Dig Liver Dis*, **40**, 446-52.
- Chung HW, Lee SG, Kim H, et al (2009). Serum high mobility group box-1 (HMGB1) is closely associated with the clinical and pathologic features of gastric cancer. *J Transl Med*, **7**, 38.
- Jube S, Rivera ZS, Bianchi ME, et al (2012). Cancer Cell Secretion of the DAMP Protein HMGB1 Supports Progression in Malignant Mesothelioma. *Cancer Res*, **72**, 3290-301.
- Kawasaki H, Altieri DC, Lu CD, et al (1998). Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. *Cancer Res*, **58**, 5071-4.
- Kuniyasu H, Oue N, Wakikawa A, et al (2002). 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.
- Lu YH, Luo XG, Tao X, et al (2007). Survivin gene RNA interference induces apoptosis in human HL60 leukemia cell lines. *Cancer Biother Radio Pharm*, **22**, 819-25.
- Reis FR, Vasconcelos FC, Pereira DL, et al (2011). Survivin and P-glycoprotein are associated and highly expressed in late phase chronic myeloid leukemia. *Oncol Rep*, **26**, 471-8.
- Tang D, Kang R, Cheh CW, et al (2010). HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. *Oncogene*, **29**, 5299-310.
- Wang SY, Chen ZY (2009). High mobility group box-1 protein and cancer. *Shanghai Medical Journal*, **32**, 757-60.
- Wang H, Yang H, Tracey KJ, et al (2004). Extracellular role of HMGB1 in inflammation and sepsis. *J Intern Med*, **255**, 320-31.