RESEARCH ARTICLE

Expression Level of Valosin Containing Protein is Associated with Prognosis of Primary Orbital MALT Lymphoma

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Abstract

Objective: To investigate whether the expression level of valosin-containing protein (VCP) is correlated with the prognosis of primary orbital mucosa-associated lymphoid tissue (MALT) lymphoma. Methods: VCP expression in 58 samples from primary orbital MALT lymphoma patients was determined by immunohistochemisty using monoclonal antibodies. Correlations between VCP expression level and prognosis were clarified by statistical analysis. Results: It was found that the percentage of VCP positive cells in samples of primary orbital MALT lymphoma ranged from 32% to 95%. The samples were divided into two groups (level 1 and level 2) according to the median value (45%) of the percentage of VCP positive cells. It was found that the expression level of VCP was significantly correlated with recurrence (*P*=0.003) and tumor size (*P*=0.008). At the same time, the 5-year disease-free and overall survival rate of patients of level 1 was significantly better than that of level 2 (*P*=0.001; *P*=0.032). There was no observed correlation between the expression level of VCP and other clinical features. Conclusion: VCP could be a useful marker for predicting the prognosis of primary orbital MALT lymphoma.

Keywords: MALT lymphoma - orbit - VCP - recurrence - survival

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Introduction

Non-Hodgkin's lymphoma (NHL) constitutes one half of malignancies arising in the orbit and the ocular adnexae. Mucosa-associated lymphoid tissue (MALT) lymphoma, a kind of B cell lymphoma, is the most common histological category in this anatomic region (Eckardt et al., 2013; Vollmer, 2013). A substantial percentage of NHL arise from tissues other than lymph nodes and even from sites which normally contain no lymphoid tissue (Padhi et al., 2012). In recent years, the incidence of orbital and ocular adnexal MALT lymphoma is increasing worldwide (Sjö et al., 2008; Moslehi et al., 2011). According to the ILSG classification, MALT lymphoma irrespective of origin is thought to be an indolent disease as reflected by the good general prognosis of patients (Smiljanic et al., 2013). However, it has been reported that the recurrence rate of MALT lymphoma is up to 26.1% (Charlotte et al., 2006).

Moreover it is remarkable that as a part of systemic disease, primary orbital MALT lymphoma has the possibility of recurrence in the distant organs after initial therapy (Raderer et al., 2005; Sjö et al., 2009; Goda et al., 2011; Graue et al., 2013). Several reports have revealed that CD5 (Wenzel et al., 2001), CD43 (Nola et al., 2004), and Bcl10 (Franco et al., 2006) are involved in the progression of ocular adnexal lymphoma, but until now, there is no specific marker for recurrence of the primary orbital MALT lymphoma.

Valosin-Containing Protein (VCP), a member of the ATPases associated with various cellular activities (AAA) superfamily, acts as a molecular chaperone in many cellular activities. Dai et al have demonstrated the involvement of VCP in the signaling pathway of IκB degradation in human B cell lines (Dai et al., 1998). As we known, IκB is a specific inhibitor of the NFκB transcription factor (Baeuerle et al., 1988). It has become clear that aberrant deregulated NFκB activation is a hallmark of several lymphoid malignancies, particularly MALT lymphoma (Coupland et al., 2013). Moreover, it has been reported that VCP can inhibit apoptosis and facilitate metastasis of the Osteosarcoma Cell Line (Asai et al., 2002). Several studies have revealed VCP is associated with the prognosis of gastric cancer (Yamamoto et al., 2003), colorectal cancer (Yamamoto et al., 2004), and liver cancer (Yamamoto et al., 2003).

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Table 1. Summary of Clinical Information of 58 Patients with Primary Orbital MALT Lymphoma

Case No.	Age (years)	Sex	Tumour size (mm^3)	LDH (U/L)	ESR	Serum albumin (g/L)	B symptoms	VCP	Recurrence (months after surgery)	Outcome (months after surgery)
1	46	male	≥9	165	normal	43.4	present	level 1	No	alive
2	61	male	<9	173	above normal	29.5	present	level 1	No	alive
3	46	male	≥9	240	normal	32	present	level 1	Yes(40)	dead(46)
4	74	male	≥9	203	normal	36.7	present	level 2	No	alive
5	53	famale	<9	147	normal	35.5	absent	level 1	No	alive
6	70	famale	<9	187	normal	37.4	absent	level 1	No	alive
7	35	male	≥9	344	normal	37.4	absent	level 2	No	alive
8	82	male	≥9	165	normal	36.2	absent	level 2	Yes(50)	alive with disease
9	50	famale	≥9	161	normal	39.7	absent	level 1	No	alive
10	49	male	≥9	267	normal	39.6	absent	level 1	No	alive
11	57	famale	≥9	240	above normal	31.5	absent	level 2	Yes(10)	dead (55)
12	83	famale	≥9	189	above normal	40.6	absent	level 2	Yes(56)	alive with disease
13	55	male	≥9	241	normal	40.1	absent	level 2	No	alive
14	81	famale	≥9	273	above normal	40.6	present	level 1	No	alive
15	85	male	≥9	137	above normal	39.2	present	level 1	No	alive
16	76	male	≥9	180	above normal	41.7	present	level 1	No	alive
17	74	famale	<9	165	normal	30	absent	level 1	No	alive
18	55	male	≥9	170	above normal	39.5	absent	level 2	No	alive
19	41	famale	≥9	106	above normal	36.7	absent	level 2	Yes(55)	alive with disease
20	70	male	≥9	159	above normal	39.6	present	level 1	Yes(47)	dead (52)
21	65	male	≥9	181	above normal	38.5	absent	level 1	No	alive
22	59	male	≥9	180	normal	38.2	absent	level 2	Yes(36)	dead (48)
23	34	male	≥9	153	above normal	39.7	absent	level 1	No	alive
24	81	famale	≥9	160	normal	38.1	absent	level 2	No	dead of other causes(50)
25	58	male	≥9	174	normal	38.4	absent	level 1	No	alive
26	52	male	≥9	161	normal	43.5	absent	level 2	No	alive
27	41	male	<9	106	normal	36.7	absent	level 2	Yes(55)	alive with disease
28	55	male	≥9	241	normal	40.1	absent	level 2	No	alive
29	66	male	<9	187	normal	40	absent	level 1	No	alive
30	76	famale	≥9	157	normal	40.2	absent	level 2	No	dead of other causes(45)
31	46	male	<9	165	normal	43.4	absent	level 2	No	alive
32	71	male	≥9	119	normal	39	absent	level 2	No	alive
33	46	male	<9	240	normal	32	absent	level 1	No	alive
34	74	male	≥9	179	normal	36.7	absent	level 2	Yes(30)	dead (40)
35	53	famale	<9	147	normal	35.5	absent	level 1	No	alive
36	70	famale	<9	187	normal	37.4	absent	level 2	No	alive
37	35	male	≥9	344	normal	37.4	absent	level 2	No	alive
38	82	male	<9	165	normal	36.2	absent	level 1	No	dead of other causes(55)
39	50	famale	<9	161	normal	39.7	absent	level 1	No	alive
40	65	male	<9	181	normal	38.5	absent	level 1	No	alive
41	57	male	<9	240	normal	31.5	absent	level 2	Yes(10)	dead (24)
42	83	male	<9	226	normal	40.6	absent	level 2	Yes(56)	alive with disease
43	76	famale	<9	157	normal	40.2	absent	level 2	Yes(32)	dead (45)
44	81	famale	<9	273	normal	40.6	absent	level 1	No	alive
45	85	male	≥9	137	normal	39.2	absent	level 2	No	alive
46	76	male	<9	180	normal	41.7	absent	level 1	No	alive
47	61	male	<9	173	normal	29.5	absent	level 1	No	alive
48	55	male	≥9	170	normal	39.5	absent	level 2	No	alive
49	70	male	<9	159	normal	39.6	absent	level 1	No	alive
50	74	famale	<9	165	normal	30	absent	level 1	No	alive
51	59	male	≥9	180	normal	38.2	absent	level 2	Yes(36)	dead (48)
52	34	male	<9	153	normal	39.7	absent	level 1	No	alive
53	81	famale	<9	160	normal	38.1	absent	level 2	No	dead of other causes(50)
54	58	male	<9	174	normal	38.4	absent	level 2	No	alive
55	52	male	<9	161	normal	43.5	absent	level 1	No	alive
56	49	famale	<9	267	normal	39.6	absent	level 1	No	alive
57	71	male	≥9	119	normal	39	present	level 2	No	alive
58	66	famale	<9	187	normal	40	absent	level 1	No	alive

Here, we detected the expression level of VCP in 58 samples from primary orbital MALT lymphoma. Combining with the clinical data, the correlation between VCP expression level and the prognosis of the primary orbital MALT lymphoma was analyzed.

Materials and Methods

Patients

A total of 62 patients underwent curative resections for primary orbital MALT lymphoma at the Institute of

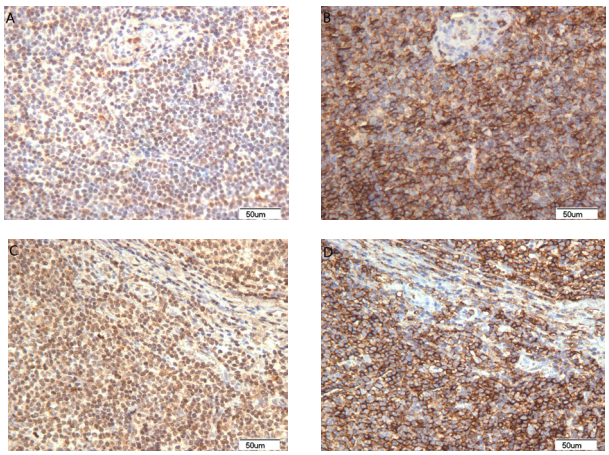

Figure 1. Representative Immunohistochemical Staining for VCP of primary orbital MALT lymphoma samples (×200). A, VCP expression level 1 of primary orbital MALT lymphoma. B, Positive expression of CD 20 in the serial section of (A) section. C, VCP expression level 2 of primary orbital MALT lymphoma. D, Positive expression of CD 20 in the serial section of (C) section

Orbital Disease, General Hospital of Chinese People's Armed Police Forces (Beijing, China) during the period from January 1998 to December 2007. Four of these patients were excluded from the present analysis because of missing clinical information. The remaining 58 patients were selected for this study. There were 39 males and 19 females, with ages ranging from 34 to 85 years (median, 61 years). All of the patients had primary stage IE disease at initial diagnosis. And the patient's IPI scores were zero to two. In the present study, the patients were diagnosed exactly with primary orbital MALT lymphoma by the senior pathologists. Before surgery, the patients received comprehensive examinations including physical examination, computerized tomographic (CT) scans of orbit, thorax, abdomen and pelvic, laboratory examinations and bone marrow biopsy. And there was no tumor metastasis in the distant organ. The surgeon removed the tumor as much as possible intraoperatively. After the incision healed up, all of the patients received the radiation therapy postoperatively. The patients received follow-up visits at 3-month intervals. Through the examination, the recurrence in the orbit and the distant organ could be detected timely. The follow-up period ranged from 24 to 60 months (median, 60 months).

Immunohistochemistry

Samples obtained from the curative resections were fixed in 10% formalin and routinely processed for paraffin embedding. Histologic sections cut at 4 μm were stained immunohistochemically for monoclonal antibody of VCP (abcam, Cambridge, UK). The staining in vascular endothelial cell was used as internal positive control. Stained sections were evaluated in a blinded manner without prior knowledge of the clinicopathologic parameters. The percentage of positive cells was calculated by counting positive cells per 5×100 tumor cells.

Statistical analysis

Time to recurrence was calculated as time to any event related to the same cancer. Overall survival was defined as the time elapsed from the date of surgery to the date of the last follow-up examination or death of any cause. Disease-free survival was defined as the time elapsed from the date of surgery to the date of local or distant recurrence or death of any cause. Statistical analyses were performed using SPSS 17.0 software. The X^2 and Fisher exact test were performed for analyzing the association between VCP expression level and clinicopathologic features of primary orbital MALT lymphoma. Overall survival and disease-free survival curves were calculated by using the Kaplan-Meier method. Different groups were compared with the log-rank test. P values lower than 0.05 were considered statistically significant.

Table 2. Association Between VCP Expression and Clinicopathologic Factors of 58 Orbit MALT Lymphoma Patients

Factors/ Category	Total No. of Patients (n=58)	No. of Patients with VCP level 1		No. of Patients with VCP level 2		*P*
		No.	%	No.	%	
Age, years						
≤60	28	13	46.4%	15	53.6%	0.436
>60	30	17	56.7%	13	43.3%	
Sex						
Male	39	19	48.7%	20	51.3%	0.512
Female	19	11	57.9%	8	42.1%	
Tumor size (mm^3)						
>9cm^3	27	19	70.4%	8	29.6%	0.008
≥9cm^3	31	11	35.5%	20	64.5%	
LDH						
≤240 U/L	46	24	52.2%	22	47.8%	0.893
>240 U/L	12	6	50.0%	6	50.0%	
ESR						
Normal	47	23	48.9%	24	51.1%	0.38
Above normal	11	7	63.6%	4	36.4%	
Serum albumin						
≤35g/L	8	6	75.0%	2	25.0%	0.256
>35g/L	50	24	48.0%	26	52.0%	
B symptoms						
Absent	49	23	46.9%	26	53.1%	0.147
Present	9	7	77.8%	2	22.2%	
Recurrence						
Absent	45	28	62.2%	17	37.8%	0.003
Present	13	2	15.4%	11	84.6%	

Results

Patients outcome

According to the follow-up visit of all patients in this study, we found that 13/58 (22.4%) patients suffered from tumor recurrence. The mean value of recurrence time was 39.5 months (range from 10 to 56 months). 8/58 (13.8%) patients died of tumor, 4/58 (6.9%) patients died of other causes unrelated to the tumor. As shown in Table 1, the 5-year disease-free and overall survival rates were 70.7% and 79.3%, respectively. The mean value of 5-year disease-free survival was 54.7 months (range from 10 to 60 months). The mean value of overall survival in the series was 57.2 months (range from 24 to 60 months).

VCP expression in the tissue samples from patients with primary orbital MALT lymphoma

All the fifty-eight sections of patients that were

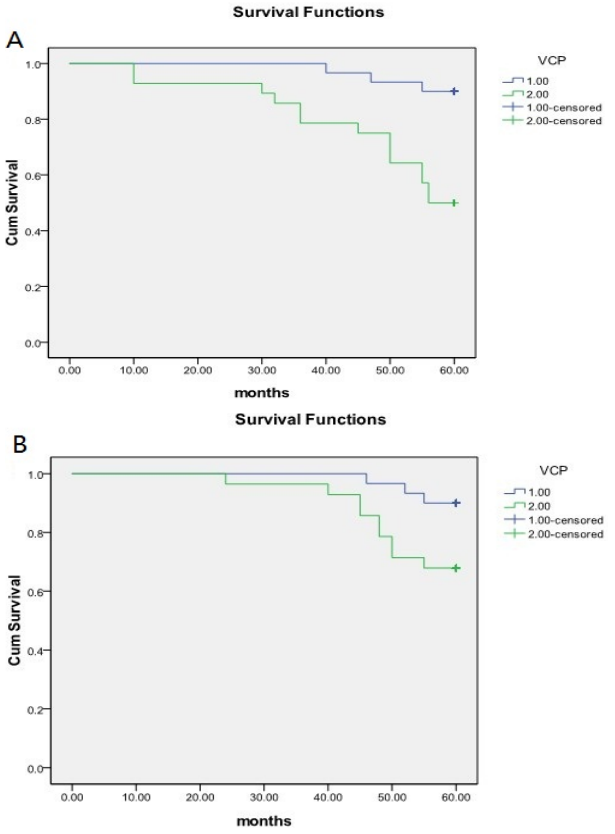

Figure 2. 5-Year Disease-free (A) and Overall (B) Survival of Patients with VCP Level 1 and 2 in Primary Orbital MALT Lymphoma. A significant difference can be observed between the two groups

diagnosed primary orbital MALT lymphoma could be stained by specific monoantibody of VCP. All of the samples were stained by monoclonal antibodies against CD20 (Figure 1B, D). Although the difference of the staining strength was not observed, it was found that the percentage of the VCP stained cells varied from 32% to 95% in these samples. The median value was calculated as 45%. The samples were divided into two groups (level 1 and level 2) according to the median value. The percentage of VCP positive cells lower than 45% was named as level 1, higher than 45% was named as level 2. Thirty sections (51.7%) were regarded as level 1 (Figure 1A). Twenty-eight sections (48.3%) were regarded as level 2 (Figure 1C).

The correlation between VCP level and clinical features of patients with primary orbital MALT lymphoma

We investigated the correlation between VCP expression level and a series of clinical features, including age, sex, tumor size, LDH level, erythrocyte sedimentation rate (ESR) level, serum albumin level, B symptoms, and recurrence (Table 2). Interestingly, it was found that patient's tumor size of level 2 is larger than that of level 1 (P=0.008). Meanwhile, 11/28 (39.3%) patients in level 2 recurred, while, only 2/30 (6.7%) patients in level 1 recurred in the follow-up visit period. The recurrence rate of patients of level 1 was significantly lower than that of level 2 (P=0.003). However, there was no correlation between VCP expression level and the other clinical factors.

Table 3. Univariate Analysis of Clinicopathologic Factors For Disease-free and Overall Survival of 58 Orbit MALT Lymphoma Patients

Factors/ Category	No. of patients	No. of recurrence	5-year Disease-Free Survival Rate (%)	P	No. of Deaths	5-year Overall Survival Rate (%)	P
VCP expression							
Level 1	30	2	90%	0.001	3	90%	0.032
Level 2	28	11	50%		9	67.9%	
Age, years							
≤60	28	7	75%	0.581	5	82.1%	0.628
>60	30	6	66.7%		7	76.7%	
Sex							
Male	39	9	74.4%	0.403	7	82.1%	0.492
Female	19	4	63.2%		5	73.7%	
Tumor size (mm^3)							
<9 cm^3	27	4	77.8%	0.242	4	85.2%	0.315
≥9 cm^3	31	9	64.5%		8	74.2%	
LDH							
≤240 U/L	46	10	69.6%	0.876	9	80.4%	0.655
>240 U/L	12	3	75.0%		3	75.0%	
ESR							
Normal	47	9	72.3%	0.620	10	78.7%	0.737
Above normal	11	4	63.6%		2	81.8%	
Serum albumin							
≤35g/L	8	3	62.5%	0.388	3	62.5%	0.177
>35g/L	50	10	72%		9	82.0%	
B symptom							
Absent	49	11	69.4%	0.652	10	79.6%	0.918
Present	9	2	77.8%		2	77.8%	

Survival analysis

It was shown that the 5-year disease-free and overall survival rates of patients are significantly correlated with VCP expression level. Patients with VCP level 1 had better 5-year survival rates than that with VCP level 2 (5-year disease-free survival, 90.0% v 50.0%, P=0.001; 5-year overall survival, 90.0% v 67.9%, P=0.032; Table 3; Figure 2). However, there was no correlation between the other factors and the survival rates (Table 3).

Discussion

In the present study, it has been demonstrated that the expression of VCP varies in different patients with primary orbital MALT lymphoma. The expression level of VCP is significantly correlated with the tumor size, the recurrence rate and 5-year survival rate, which suggests that VCP may be a potential marker for the prediction of prognosis of patients with primary orbital MALT lymphoma.

VCP might play a crucial role in tumor proliferation and metastasis through the activation of NFκB. So it is reasonable that the expression level of VCP is associated with tumor size, recurrence rate and overall survival rate in these patients.

In our cohort of patients, 22.4% of the patients recurred in 5 years. The recurrence rate was similar to the previous study (Charlotte et al., 2006). Moreover, we found that all of these recurred in the distant organ, which suggests that orbital MALT lymphoma, as a part of a systemic disease, may be easy to recur in other organ besides orbit.

The mechanism of lymphoma recurrence has not been clarified. In our study, we surprisingly found that the percentage of VCP positive cells was associated with the prognosis of patients with primary orbital MALT

lymphoma. These results indicate that VCP may be involved in the development of orbital MALT lymphoma, which will be investigated in the future.

Here we just investigate the correlation between VCP expression level and prognosis of primary orbital MALT lymphoma. Whether VCP is associated with MALT lymphoma in other organs needs to be indentified in the future.

In a word, VCP may serve as a novel prognostic marker for prediction of prognosis of patients with primary orbital MALT lymphoma.

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