· Original article ·

The expression and meaning of Skp2, p27 proteins in Mucosa-associated lymphoid tissue lymphoma of ocular adnexal

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Foundation item: Supported by Public Domain of Science and Technology Project of Qingdao (No. 2012-1-3-2-14-nsh)
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Received: 2014-03-07 Accepted: 2015-05-19

Skp2,p27 在眼附属器黏膜相关淋巴组织结外边缘区 B 细胞淋巴瘤中的表达和意义

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基金项目:青岛市公共领域科技支撑计划项目(No. 2012-1-3-2-14-nsh)

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摘要

目的:探讨眼附属器黏膜相关淋巴组织结外边缘区 B 细胞淋巴瘤(mucosa-associated lymphoid tissue lymphoma of ocular adnexal,MALT lymphoma of ocular adnexal)中 Skp2 和 p27 的表达及联系。

方法:收集 1995~2011 年青岛大学附属医院眼科切除的眼附属器 MALT 淋巴瘤患者及眼部反应性淋巴组织增生患者的石蜡包埋标本,用免疫组化法分别检测两组标本中 Skp2 和 p27 的表达。

结果:眼附属器 MALT 淋巴瘤中 Skp2 表达率与眼部反应性淋巴组织增生相比显著增高($P<0.05$)。p27 表达率与反应性淋巴组织增生相比显著降低($P<0.05$)。Skp2 和 p27 的表达与患者 Ann Arbor 病理分级无关。在眼附属器 MALT 淋巴瘤中 Skp2 与 p27 成负相关($r=-0.129$,$\chi^2=15.39$,$P<0.05$)。

结论:综合分析眼附属器 MALT 淋巴瘤中 P27 及 SKP2 的表达对本病的预后有一定意义。两者的表达彼此相关。

关键词:粘膜相关淋巴组织淋巴瘤;眼附属器;SKP2;p27

引用:林红,胡丽婷,赵桂秋,杨文毅,张静静. Skp2,p27 在眼附属器黏膜相关淋巴组织结外边缘区 B 细胞淋巴瘤中的表达和意义. 国际眼科杂志 2015;15(9):1489-1493

Abstract

• AIM: To investigate the expression and relationship of S-phase kinase-associated protein 2 (Skp2) and p27 in mucosa-associated lymphoid tissue (MALT) lymphoma of ocular adnexal.

• METHODS: The expression of Skp2, p27 were detected on resected specimens from patients suffering from MALT lymphoma and lymphadenosis of ocular adnexal in Ophthalmology Department, Affiliated Hospital of Medical College Qingdao University from 1995 to 2011 by immunohistochemical analysis.

• RESULTS: The expression of Skp2 in MALT lymphoma was higher than that in lymphadenosis ($P<0.05$). The expression of p27 in MALT lymphoma was lower than that in lymphadenosis ($P<0.05$). The expression of Skp2 and p27 do not related with the Ann Arbor clinical stage. There was a negative correlation between p27 and Skp2 in MALT lymphoma ($r=-0.129$, $\chi^2=15.39$, $P<0.05$).

• CONCLUSION: Comprehensive analysis of the expression of p27 and Skp2 protein may have certain guiding significance on the prognosis evaluation of the disease. The expression of two substances may correlate with each other.

• KEYWORDS: mucosa axsociated lymphoid tissue lymphoma; ocular adnexal; Skp2; p27
DOI:10.3980/j.issn.1672-5123.2015.9.01

Citation: Lin H, Hu LT, Zhao GQ, Yang WY, Zhang JJ. The expression and meaning of Skp2, p27 proteins in Mucosa-associated lymphoid tissue lymphoma of ocular adnexal. *Guoji Yanke Zazhi* (*Int Eye Sci*) 2015;15(9):1489-1493

INTRODUCTION

Mucosa-associated lymphoid tissue (MALT) lymphoma, namely extranodal marginal zone B-cell lymphoma of MALT, is the most common type of ocular adnexal lymphoma and accounts for 10%-15% in the orbital solid tumors[1]. And the incidence has increased in recent years[2]. Most studies suggest that the majority of MALT lymphoma is low-grade malignant B-cell lymphoma which rarely appears to be apt to metastasize and seriously deteriorate[3]. But at present, some foreign experts believe that extranodal lymphomas occurring in ocular adnexal is more aggressive[4]. Studies of the disease have asattracted many domestic and foreign scholars. The cell cycle inhibitory protein p27 may combine

with cyclin directly to inhibit biological activity of cyclin-CDK complex, and high expression in G_0 phase to prevent cell shift from G1 to S phase, also inhibits cell cycle progress. Skp2 which is part of ubiquitin ligase complex can specifically recognize negative cell cycle regulatory factors, of which p27 is an important representative, can promote the progress of the cell cycle from G1 to S phase, and promotes cell proliferation. In recent years, the relationship between Skp2 and p27 increasingly attracts the attention of the researchers, but domestic research on both in the pathological changes of eye lymphoid tissue is not much. High expression of Skp2 and low expression of p27 may play a role in the occurrence and development of ocular lymphoma, and is associated with prognosis. This study detected the expression of Skp2 and p27 in MALT lymphoma by immunohistochemistry and combined with clinical data and pathological classification to discuss the effect of the two proteins on the prognosis of eye adnexal MALT lymphoma and explore their roles in the development of the diseases.

SUBJECTS AND METHODS

Subjects

Specimens collection The study included 20 specimens of ocular adnexal MALT lymphoma (which from 20 patients, 8 from eyelid, 10 from orbit and 2 from lacrimal gland) at the Pathological Lab of Ophthalmology Department, Affiliated Hospital of Medical College Qingdao University from 1995 to 2011. The ages of patients arranged from 52 to 88, with an average of 70.2±5.9. According to Ann Arbor clinical stage, the numbers of specimens from Ⅰ to Ⅳ phase were 16, 2, 1 and 1 respectively. 10 cases of reactive hyperplastic lymphadenopathy were used as positive control. For the use of these clinical materials for research purposes, the patient's consent and approval from the Institutional Research Ethics Committee were obtained.

HE and immumohistochemical staining The tissue from various stages was made to paraffin section and hematoxylin-eosin (HE) staining, and the tissue structure was observed under common microscope. 3-μm-thick paraffin sections were cut on a microtome (CM1900; Leica Microsystems, Deerfield, IL, USA) and then mounted onto glass slides with 100 g/L polylysine. Immunohistochemical staining was performed according to the manufacturer's instructions [SP kit (Biosynthesis Biotechnology)]. In brief, after deparaffinising in xylene and dehydrating in ethanol, the sections were immersed in a citrate buffer and heated in a microwave oven for 15min to retrieve antigens, then incubated with the mouse nonspecific immune serum (Liquid A) for 20min at room temperature. After that, the sections were incubated with either mouse anti-Skp2 polyclonal antibody (1:100) or mouse anti-p27 polyclonal antibody (1:40) (Biosynthesis Biotechnology) overnight at 4℃, and then incubated with horseradish peroxidase labeled goat anti-mouse polymer (Liquid B) for 30min at 37℃. After washing three times with PBS, diaminobenzidine was used as a chromogen, and haematoxylin was used as a counterstain. All specimens were sealed by neutral balsam for microscopy observation. As a negative control, the primary antibody was replaced with PBS. Specificity of primary antibody was validated by positive tests using reactive lymphoid hyperplasia. The positive expression is located in nucleus and sometimes in cytoplasm, which appears as diffuse brown yellow particles.

To avoid staining underestimation due to considerable regional variations, five representative fields were examined, and a total of 1000 cells (200 for each field) were counted under the microscope with a high power (×400) objective. Briefly, based on the scale of Xu *et al*[5], the immunostaining grade was as follows: specimens in which ≤25%, >25%-<75%, ≥75% of cells showed positive staining were defined as (-), (+), (++) respectively.

Statistical Analysis Statistical analysis was performed by SPSS 17.0 software (SPSS, Chicago, IL, USA). The statistical differences of Skp2 and p27 expressions among the groups were determined by χ^2 tests, and among the stages were determined by Fisher's exact probabilities. The mutual relation between Skp2 and p27 was analyzed by Spearman correlation test. A $P<0.05$ was considered as statistically significant.

RESULTS

HE Staining The samples of reactive lymphoid hyperplasia showed normal follicular structures with mature lymphocytes, whose patterns were various. It could be seen sporadic plasmocytes, histocytes and immunoblasts (Figure 1A). The samples of MALT lymphoma showed that high-differentiated and moderately-differentiated lymphoma was composed of diffuse, similar and small lymphocytes which appeared as circular nucleus, deep staining, little cytoplasm and extracellular matrix (Figure 1B).

Immumohistochemical Staining

Expression of Skp2 in mucosa-associated lymphoid tissue lymphoma There were no statistical differences in the expression rate of Skp2 between the groups with different Ann Arbor clinical stages, age, sex, occurrence site ($P>0.05$, Table 1). The positive staining of Skp2 in MALT lymphoma and reactive lymphoid hyperplasia were mainly in nucleus, while there was no positive staining in the control groups. Moreover, the expression rate of Skp2 in MALT lymphoma (14/20, 70.0%) (Figure 2A) was higher than that in reactive lymphoid hyperplasia (0/10) (Figure 2B) significantly ($P<0.05$, Table 2).

Expression of p27 in mucosa-associated lymphoid tissue lymphoma There were no statistical differences in the expression rate of p27 between the groups with different Ann Arbor clinical stages, age, sex, occurrence site ($P>0.05$, Table 1). The positive staining of p27 was mainly in nucleus. In reactive lymphoid hyperplasia, the positive staining of p27 was mainly located in small, mature and resting lymphocyte outside of germinal center (Figure 3A). The expression rate of p27 was low in tumor cells, while there were positive staining in normal small lymphocytes, mucosal epithelial cells and vascular endothelial cells in MALT lymphoma (Figure 3B). There was no positive staining in the control groups.

Table 1 Relationship between the expressions of Skp2/p27 and the clinical features of ocular adnexal MALT lymphoma *n*(%)

Features	*n*	Positive expressions of Skp2	*P*	Positive expressions of p27	*P*
Age					
<60	10	6 (60.0)	0.244	2 (20.0)	0.244
≥60	10	8 (80.0)		4 (40.0)	
Gender					
M	11	6 (54.5)	0.315	5 (45.5)	0.315
F	9	4 (44.4)		4 (44.4)	
Occurrence site					
Eyelid	8	4 (50.0)		2 (25.0)	
Orbit	10	7 (70.0)	0.964	4 (40.0)	0.49
Lacrimal gland	2	2 (100.0)		0 (0.0)	
Ann Arbor clinical stagea					
Ⅰ	16	7 (43.8)		10 (62.5)	
Ⅱ	2	2 (100.0)	0.326	0 (0.0)	0.087
Ⅲ	1	1 (100.0)		0 (0.0)	
Ⅳ	1	1 (100.0)		0 (0.0)	

The expression rate of Skp2 and p27 in different Ann Arbor clinical stages had significant difference ($^aP<0.05$).

Table 2 The expression of Skp2 and p27 in MALT lymphoma and reactive lymphoid hyperplasia *n*(%)

Groups	*n*	Positive expressions of Skp2	Positive expressions of P27
Reactive lymphoid hyperplasia	10	0 (0.0)	10 (100)
MALT lymphoma	20	14 (70.0)	6 (30.0)

The expression rate of Skp2 in MALT lymphoma was higher than that in reactive lymphoid hyperplasia significantly ($P<0.05$). While the expression rate of p27 in MALT lymphoma was lower than that in reactive lymphoid hyperplasia significantly ($P<0.05$).

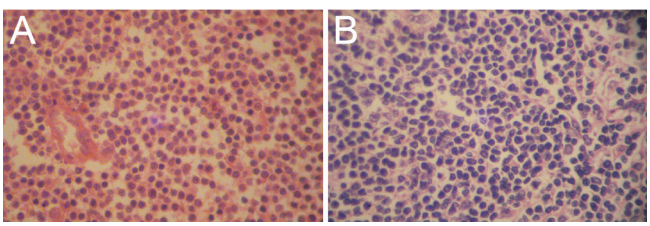

Figure 1 HE Staining of reactive lymphoid hyperplasia and MALT lymphoma A: The samples of reactive lymphoid hyperplasia showed various cell patterns, sporadic plasmocytes, histocytes and immunoblasts. B: The samples of MALT lymphoma showed that high-differentiated and moderately-differentiated lymphoma was composed of diffuse, similar and small lymphocytes which appeared as circular nucleus, deep staining, little cytoplasm and extracellular matrix (HE×400).

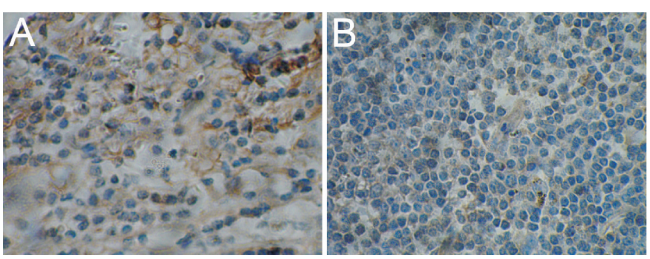

Figure 2 Immumohistochemical Staining of Skp2 in MALT lymphoma and reactive lymphoid hyperplasia A: The expression rate of Skp2 in MALT lymphoma was high. The positive staining of Skp2 was mainly in nucleus. B: The expression rate of Skp2 in reactive lymphoid hyperplasia was low. The positive staining of Skp2 was mainly in nucleus (IH×400).

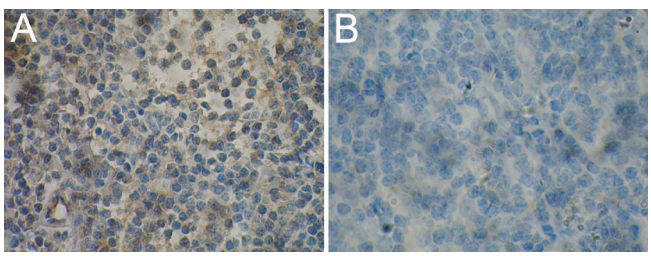

Figure 3 Immumohistochemical Staining of p27 in reactive lymphoid hyperplasia and MALT lymphoma A: The expression rate of p27 in reactive lymphoid hyperplasia was high. The positive staining of p27 was mainly in nucleus. B: The expression rate of p27 in MALT lymphoma was low. The positive staining of p27 was mainly in nucleus (IH×400).

The expression rate of p27 in MALT lymphoma (6/20, 30.0%) was lower than that in reactive lymphoid hyperplasia (10/10, 100%) significantly ($P<0.05$, Table 2).

Mutual relation between Skp2 and p27 in mucosa-associated lymphoid tissue lymphoma The results showed a negative relation between the expression of Skp2 and that of p27 in MALT lymphoma ($r=-0.129$, $\chi^2=15.39$, $P<0.05$; Table 3).

DISCUSSION

Cell cycle regulation imbalance is considered to be a common feature of cell tumor. Cell cycle is regulated by multiple factors, of which the G1/S and G2/M are the two major turning points and respectively control cells into S and M phase. G1/S limit points are the key and rate-limiting stage to control the growth of the cells and determine their fate. In

Table 3 The relation between Skp2 and p27 in MALT lymphoma

p27	Skp2			
	n	−	+	++
	14	1	5	8
+	5	3	2	0
++	1	1	0	0

The results showed a negative relation between the expression of Skp2 and that of p27 in MALT lymphoma ($r=-0.129$, $\chi^2=15.39$, $P<0.05$).

recent years, with the development of molecular biology, much attention is attracted to the researches on cell cycle regulatory protein. While the cell cycle regulatory protein is combined with the detection of the molecular markers of Skp2 andp27, it can provide important index and basis for the clinic and scientifically predict the patient's disease type and offer help to select the appropriate clinical treatment plan[6]. Skp2, found in recent years, is a kind of F-box protein. It functions as a recognition subunit to the substrates of SCF complex. It can specifically recognize 187th threonine phosphorylated p27 and mediate its poly-ubiquitination and degradation. Skp2 must compound with other subunits, such as Cull, Rbxl, Skpl to form SCF-Skp2 compounds to play a role, mediate poly-ubiquitination reaction. Abnormity of this process directly affects the absolute content of intracellular p27 protein, promotes the progress of the cell cycle from G1 to S phase, and promotes cell proliferation[7,8]. Skp2 appears in G0 advanced stage and acts on S phase. If Skp2 expression increased, p27 expression would reduce, these would allow the tumor cells to constantly pass through the Go/S regulation points to enter the cell cycle and lead to overgrowth and malignant proliferation of the cells and tumor formation[9]. Previous studies had shown that Skp2 overexpresses in many human cancer tissues and high expressions are often associated with the occurrence of tumor[10-12]. In this study, we detected that the positive rate of Skp2 in MALT lymphoma was 70.0% (14/20), which is similar to the result of Shigemasa *et al*[13]. But there was no expression of Skp2 in reactive lymphoid hyperplasia, which suggests that high expression of Skp2 is associated with the occurrence of MALT lymphoma. Skp2 protein was highly expressed in ocular adnexal MALT lymphoma, suggesting that such indicator has obvious heterogeneity and capable to be used as important basis of diagnosis and differential diagnosis for ocular adnexal MALT lymphoma. Meanwhile, the study showed that the expression of Skp2 protein was not related with patients' Ann Arbor staging, which was different from the point of White *et al*[14]. And there was not obvious correlation with age, gender and occurrence site.

p27 is a member of the family of cKip/ciP, which is the first to inhibit cyclin E/CDK 2 and cyclin A/CDK 2 complex activity. Cell cycle protein E-CDK2 is the key to the cells to through G1/S limit point, P27 stagnates cells in G1 phase by inhibiting cell cycle protein E-CDK2. As Gl period level checkpoint related gene, p27, in the normal cell cycle, expresses as a curve, and in G0 and G1 phase to achieve highest, once the cells come into the cell cycle, lower levels of p27 may maintain the stability of the phase of the cell in G0. Therefore deficiency of p27 protein will inevitably lead to cell cycle process accelerated, have close relationship with the occurrence of tumor development[15,16].

The expression of p27 in MALT lymphoma was analyzed. It showed that the average expression level of p27 protein in lymphoid tumor cells was significantly lower than that in reactive lymphoid hyperplasia (outside of germinal centers). Compared with reactive lymphoid tissue hyperplasia, the positive rate of p27 protein declined, which suggested that low expression of p27 might be associated with the occurrence of MALT lymphoma and it was in agreement with the study of M Kiviniemi[17]. P27 protein of Ocular adnexal MALT lymphoma was in low expression. Meanwhile, the study showed that the expression of p27 protein was not significantly related with patients' Ann Arbor staging, and not with age, gender and occurrence site.

It is generally believed that Skp2 expression is negatively correlated with p27[18]. F-box protein Skp2 is the recognition subunits of SCFSkp2 composite substrate[19]. It can play a role to mediate ubiquitination reaction only if Skp2 and other subunits combine to form complexes. Thrl87 phosphorylation is the necessary condition for Skp2 to identify the ubiquitin degradation of p27 and it can directly weaken the degradation pathway mediated by Skp2 and lead to cell cycle arrest if the phosphorylation of the site is disturbed[20]. This study found that in MALT the expression of Skp2 and p27 was negatively correlated, which supports the point of Lahav-Baratz *et al*[21].

The pathogenic mechanism of ocular adnexal MALT lymphoma is the joint action of multiple factors. This experiment suggests that decrease in p27 expression and increase in Skp2 expression may play a certain role in the development of MALT lymphoma. It was reported that high expression of Skp2 and lower levels of p27 give us a selection for drug therapeutic development by targeting these molecules. Continued investigations into the molecular mechanisms of MALT lymphoma may provide to establish novel biomarkers for this disease and generate therapeutic targets for improved treatment[22]. Comprehensive analysis of the expression of p27 and Skp2 protein may have certain guiding significance on the prognosis evaluation of the disease. The exact mechanism of how the expression of Skp2 and pP27 protein affects the prognosis is still required to be studied further, while the in-depth discussion on the action mechanism of both will also provide a new idea for the treatment of ocular adnexal MALT lymphoma.

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