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RESEARCH

Aberrant Cytoplasmic Expression of Cyclin B1 Protein and its Correlation with EBV-LMP1, P53 and P16(INK4A) in Classical Hodgkin Lymphoma in China

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Abstract The relationships between the expression of cyclin B1, EBV-LMP1, P53 and P16(INK4A) in Chinese classical Hodgkin lymphoma are unknown and need exploring. Samples of classical Hodgkin lymphoma from 60 Chinese patients were analyzed for the expression of cyclin B1, EBV-LMP1, P53 and P16(INK4A) proteins by immunohistochemistry. Cyclin B1 protein was overexpressed in 90.0% (54/60) of this group of classical Hodgkin lymphoma, staining mainly and strongly in cytoplasm but also sparsely and weakly in nucleus of the Hodgkin and Reed-Sternberg (HRS) cells. EBV-LMP1, P53 and P16 (INK4A) were overexpressed in 85.0%, 96.7% and 71.7% of Hodgkin lymphoma, respectively. EBV-LMP1, P53 and P16(INK4A) were was noted in the nucleus of HRS cells. Microscopically, cyclin B1 and P53 staining distinguished the HRS cells from the complex background of lymphocytes. Cyclin B1 was positively correlated with EBV-LMP1 ($P<0.001$) and P53($P<0.001$), but was inversely related to P16(INK4A) ($P<0.05$). It is suggested that overexpression of cyclin B1 could play an important role in the evolution of classical Hodgkin lymphoma, and cyclin B1 may be considered as a potential adjunct marker to identify HRS cells in diagnosis and be served as Hodgkin lymphoma-associated antigen in the near future.

Keywords Lymphoma · Cyclin B1 · EBV · P53 · p16 · Immunohistochemistry

Introduction

Classical Hodgkin lymphoma is a lymphoid neoplasm composed of monoclonal Hodgkin cells and multinucleated Reed-Sternberg (HRS) cells. HRS cells are currently thought to be derived from mature B cells at the germinal stage of differentiation, and are pathognomonic in Hodgkin lymphoma [1]. However, HRS cells may often be scanty and difficult to identify in sections. The pathogenesis of classical Hodgkin lymphoma remains unknown. Cyclin B1 plays an important role as a mitotic cyclin in G_2/M phase transition during the cell cycle. In the normal cell cycle, it starts to express at the late S phase, increase in G_2 phases and peak at mitosis. Cyclin B1/cdc2 (CDK1) complex has a role as a maturation/mitosis-promoting factor in the G_2–M phase transition during the cell cycle [2]. Thus, dysregulation of the expression of Cyclin B1 may be involved in uncontrolled cell growth and malignant transformation. Indeed, overexpression of Cyclin B1 has been reported in various malignant tumors [3–7] and has been shown to be a poor prognostic factor in some of them. Aberrant expression of cylin B1 has been found in diffuse large B cell lymphoma [8, 9] and MALT-lymphoma [10]. However, there is to date no study in Hodgkin lymphoma in China and the relationship between cyclin B1 and EBV-LMP1, P53 and P16(INK4A) in classical Hodgkin lymphoma also needs exploring. We used the cyclin B1, EBV-LMP1, P53 and P16(INK4A) antibodies to investigate the possible role of the expression of the four proteins in 60 cases of Chinese classical Hodgkin lymphoma. It is hoped that the study will give information on the pathogenesis of this disease.

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Material and Methods

Patients

Sixty patients (36 men, 24 women) with histologically confirmed classical Hodgkin lymphoma were recruited into the study. Ethical approval for this study was not required by our institute as the experiments carried out did not relate to patient's privacy, impairment or treatment.

Immunohistochemistry

A formalin-fixed, paraffin-embedded block was selected from each patient with Hodgkin lymphoma for immunohistochemical study. Paraffin sections, 4 μm in thickness, were cut, dewaxed in xylene and rehydrated in a graded ethanol series. Then sections were immersed in 3% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity. Subsequently antigen retrieval was performed in boiling 0.01 M citrate buffer (pH 6.0) in a pressure cooker, which was then sealed and brought to full pressure for 2 min. The sections were then separately covered the primary antibodies including polyclonal rabbit antibody against human cyclin B1 protein (Santa Cruz, CA, USA), mouse monoclonal antibody to Latent membrane protein 1 of the Epstein-Barr virus (EBV-LMP1) (CS.1-4, DAKO) and mouse monoclonal antibody against human P53 as well as P16(INK4A) protein (Zymed, South San Franciso, CA, USA), all diluted one in 100. After exposure to primary antibody for 1 h, the sections were allowed to react with poly peroxidase-anti-mouse/rabbit IgG for 20 min by the standard PV-6000 Polymer Detection System (Zymed, South San Franciso, CA, USA). Human cervical squamous cell carcinoma tissues were used as a positive control for cyclin B1, P53 and P16(INK4A), and human nasopharyngeal carcinoma was used as a positive control for EBV-LMP1. Negative controls were sections treated as above but with the primary antibody replaced by 0.01M PBS. Expression of cyclin B1, P53, P16(INK4A) and EBV-LMP1 was considered positive when at least 15% of HRS cells were stained. In addition, ten B-cell lymphomas, eight T-cell lymphomas and ten reactive lymph nodes (four lymph nodes sampled during resection of carcinomas and six lymphadenitis) were used for comparison.

Statistical Analysis

The correlation between expressions of the protein was determined by counting the positive cells in at least four medium power fields of serial sections, calculating and presenting as means±SD. The statistical significance of association was assessed by Spearman's correlation coefficient test, with SPSS15.0. A value of P less than 0.05 was accepted as statistically significant.

Results

Expressions of Cyclin B1, EBV-LMP1, P53 and P16 (INK4A) in Classical Hodgkin Lymphoma

Cyclin B1 protein was overexpressed positively in 90.0% (54 out of 60) of the classical Hodgkin lymphomas (Table 1). It was predominantly positive in cytoplasm and also sparsely and weakly in nucleus of the HRS cells

Table 1 Expression of cyclin B1, EBV-LMP1, P53 and P16 (INK4A) in classical Hodgkin lymphoma

Lymphoma	*n*	cyclin B1	EBV-LMP1	P53	P16(INK4A)
Classical HL					
Nodular sclerosis	29	27	24	28	22
Mixed cullularity	11	10	9	11	7
Lymphocyte-rich	16	14	14	15	11
Lymphocyte-depleted	4	3	4	4	3
Non-HL					
DLBCL	5	4	2	5	3
MALT	4	1	0	1	1
Burkitt's	1	1	1	1	1
ALCL	2	2	1	2	1
AILT	2	1	2	1	1
NK/T(nasal type)	2	0	2	2	1
Peripheral TCL	2	1	0	1	1
Non-lymphomatous					
Tonsilla	2	0	0	0	0
Lymph nodes	10	0	0	0	0

HL Hodgkin lymphoma, *DLBCL* Diffuse large B cell lymphoma, *MALT* extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue, *Burkitt's* Burkitt's lymphoma, *ALCL* Anaplastic large cell lymphoma, *AILT* angioimmunoblastic T-cell lymphoma

(Fig. 1), but only minimally or negative in nucleus of reactive B and T lymphocytes in the background of Hodgkin lymphoma. A nuclear staining was found in the mummified Hodgkin cells. It was noted that some HRS cells did not express cyclin B1. In normal lymphoid tissues, cyclin B1 expression was mainly in nucleus of a few activated cells in germinal center. In intratumoral (stromal) lymphoid tissue, cyclin B1 staining could be found in cytoplasm of some mild atypical lymphocytes. Six of ten B-cell lymphomas and four of eight T-cell lymphomas were stained positively for cyclin B1 (Table 1).

EBV-LMP1 protein was expressed in 85.0% (51 out of 60) of this group of classical Hodgkin lymphomas (Table 1). EBV-LMP1 was noted in the HRS cells, highlighting them from the complex background of lymphocytes although positive staining could also be found minimally or occasionally in the surrounding lymphocytes. Staining was mainly nuclear and infrequently cytoplasmic. However, some HRS cells were negative for EBV-LMP1 staining. Three of ten B-cell lymphomas and five of eight T-cell lymphomas, but none of the ten reactive lymph nodes were stained positively for EBV-LMP1 (Table 1).

P53 and P16(INK4A) protein was noted in 96.7%(58 out of 60) and 71.7% (43 out of 60) of the classical Hodgkin lymphomas by immunohistochemistry (Table 1). P53 and P16(INK4A) were overexpressed mainly in the nuclei of Hodgkin and Reed-Sternberg (HRS) cells (Fig. 2). Some Hodgkin cells showed some cytoplasmic staining of P16 (INK4A) protein as well as its nuclear staining. Mummified HRS cells were often negative for P16(INK4A) protein. Many of the HRS cells with a very prominent nucleolus were positive for P16(INK4A). Neither B nor T lymphocytes in the background of the lymphoma or other tissues showed positive staining for P16(INK4A) protein, although histiocytes were weakly positive. There was no P16 (INK4A) staining in the lymphocytes, plasma cells and

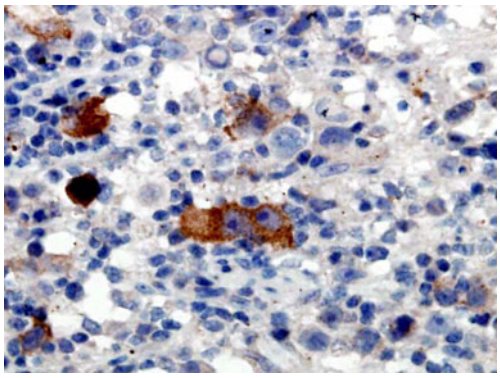

Fig. 1 The results of immunohistochemical staining in classical Hodgkin lymphoma. cyclin B1 was localized in the cytoplasm of Reed-Sternberg cell. [cyclin B1 X 400]

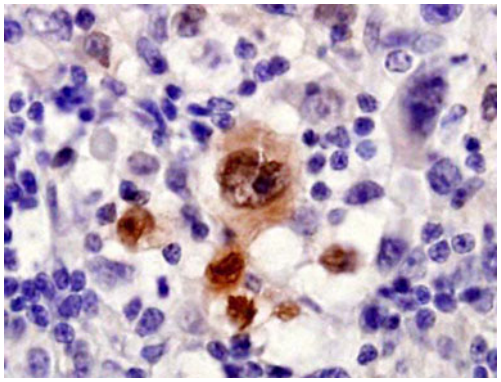

Fig. 2 The results of immunohistochemical staining in classical Hodgkin lymphoma. P16(INKA4A) was positive in the nucleus of HRS cells of classical Hodgkin lymphoma (D). [P16(INKA4A) X 400]

eosinophils. In the control tissues and reactive lymph nodes, histiocytic lineage cells including macrophages, Langerhans cells, and dendritic cells were weakly positive for P16(INK4A). In the non-Hodgkin lymphomas, P53 protein was found in seven out of ten B-cell lymphomas and six out of eight T-cell lymphomas, and P16(INK4A) protein was noted in five out of ten B-cell lymphomas and four out of eight T-cell lymphomas, but none of lymphocytes in the reactive lymph nodes were positive for the two proteins (Table 1).

The Association Between Cyclin B1 and P53, EBV-LMP1 or P16(INK4A)

Under the microscope, cyclin B1 was detected in most EBV-LMP1 and P53 positive HRS cells, and there was a positive correlation between cyclin B1 and the two markers (4.408±2.617 *vs* 4.267±2.842, Rho=0.894, P=0.0001; 4.408±2.617 *vs* 4.700±2.414, Rho=0.959, P=0.0001), respectively. However, cyclin B1 was inversely correlated with P16(INK4A) in this group of Hodgkin lymphomas (4.408±2.617 *vs* 3.5667±2.557, Rho=−0.194, P=0.034). It was also noted that HRS cells positive for cyclin B1 were negative for P16(INK4A) and *vice versa* in the same field on serial sections. Among six cyclin B1 totally negative tumours, five was positive for P16(INK4A), four positive for P53 and also three positive for EBV-LMP1. Of fifty-four cyclin B1 positive cases, sixteen was negative for P16 (INK4A), none negative for P53 and six negative for EBV-LMP1 completely.

Discussion

Cyclin B1, which plays a key role in the control of cell cycle progression from G(2) through M phase, was recently identified as a tumor antigen recognized by human T-cells

[11]. cyclin B1 is not detectable in normal B lymphoid cells but is strongly expressed in 90.0% of HRS cells of classical Hodgkin lymphoma (54/60). Nuclear accumulation is necessary for cyclin B1-dependent apoptosis, in which ectopic expression of cyclin B1-5xE, a protein that preferentially localizes to the nucleus, is sufficient to trigger apoptosis. and conversely, expression of cyclin B1-5xA, a predominantly cytoplasmic protein, fails to induce apoptosis [12]. Yu et al concluded that the cellular localization of cyclin B1 is cytoplasmic rather than nuclear of cancer cells, suggesting its dysregulation of mechanism by non-functional P53 [13]. In this research we found that cyclin B1 was overexpressed in the cytoplasm of vital HRS cells (but in the nucleus of mummified Hodgkin cells), suggesting its mechanism of failing to induce apoptosis in classical Hodgkin lymphoma in accordance with Porter and colleagues [12]. Thus, the cytoplasmic localization of cyclin B1 may be involved in malignant characteristics of HRS cell in classical Hodgkin lymphoma. The cytoplasmic cyclin B1 is found in the vast majority of classical Hodgkin lymphoma (90.0%) so that it may be considered as a potentially useful marker for identifying the HRS cell in classical Hodgkin lymphoma and cyclin B1 in cytoplasm of HRS cells may be served as Hodgkin lymphoma-associated antigen associated with T cell-dependent antibody response in immunotherapy [11, 13–16]. The result that cytoplasmic localization of cyclin B1 was positively related to overexpression of P53 (non-functional P53) in this study also further proved the hypothesis proposed by Yu et al. [13]. The mechanism of nuclear accumulation of cyclin B1 is regulated in part by the CRM1 (exportin 1) protein, which exports cyclin B1 from the nucleus into the cytoplasm [17, 18]. Therefore, the relationship between the overexpression of cyclin B1 and the enhanced function of CRM1 in the tumor may need investigation further. Our result that cyclin B1 was positively correlated with EBV-LMP1 is associated with the previous findings [19, 20], in which EBV-LMP1 regulated by EBNA2 disturbs mitosis control and the function of mitotic checkpoint of cancer cell through up-regulating the expression of P34(cdc2) and cyclin B1 proteins. The result in this study that cyclin B1 correlates inversely with P16(INK4A) in classical Hodgkin lymphoma is also associated with the authors' finding in diffuse large B-cell lymphoma [9]. It is also suggested that the aberrant expression of cyclin B1 in cytoplasm of HRS cells may be served as a potential marker to localize HRS cells and as a Hodgkin lymphoma-associated antigen associated with T cell-dependent antibody response in a subset of patients in the near future.

Declaration of interest The authors report no conflicts of interest. The authors responsible for the content and writing of paper.

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