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Mechanism of action of EBV, Bcl-2, p53, c-Myc and Rb in non-Hodgkin's lymphoma

W. SONG[1], M.-G. LIU[1], J.-B. ZHANG[1], J.-J. ZHANG[2], M.-M. SUN[1], Q.-K. YU[1]

[1]Department of Pathology, Zhengzhou University Affiliated Cancer Hospital (Henan Cancer Hospital), Zhengzhou, China
[2]Department of Cardiology, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou, China

Wei Song and *Mingge Liu* are both the first author

Abstract. – OBJECTIVE: The aim of the present study is to explore the mechanism of action of several proteins, including Epstein-Barr virus (EBV), B-cell lymphoma (Bcl)-2, p53, c-Myc and retinoblastoma (Rb), in Non-Hodgkin's lymphoma (NHL).

PATIENTS AND METHODS: Between July 2010 and July 2015, samples of 142 patients with pathologically confirmed NHL which presented at our institution were included in the observation group. In addition, samples from 55 patients with hyperplastic lymphadenitis presented during the same period were enrolled as control group. The expressions of EBV (+), p53(+), Bcl-2(+), Rb(-) and c-Myc(+) were determined and compared among different subtypes and stages of NHLs of observation group. Besides, the correlation of EBV with p53, Bcl-2, Rb and c-Myc were investigated in NHLs of observation group.

RESULTS: In the observation group, the expression rates of EBV(+), p53(+), Bcl-2(+), Rb(-), and c-Myc(+) were significantly higher than those, respectively, in the control group ($p < 0.05$). No significant correlation was observed between EBV expression and the expressions of p53, Bcl-2, Rb and c-Myc in the observation group ($p > 0.05$). The expression rates of p53(+) and Bcl-2(+) were significantly higher in aggressive and highly-aggressive NHLs than in indolent NHLs of the observation group ($p < 0.05$). The expressions of EBV(+), p53(+), Bcl-2(+), Rb(-), and c-Myc(+) were significantly higher in stage III-IV NHLs than in stage I-II NHLs ($p < 0.05$).

CONCLUSIONS: The expressions of EBV(+), p53(+), Bcl-2(+), Rb(-), and c-Myc(+) are closely associated with NHL pathogenesis. Expressions of these proteins are higher in later stages of NHLs, and expressions of p53(+) and Bcl-2(+) are higher in more aggressive NHLs.

Key Words:
EBV, Bcl-2, p53, c-Myc, Rb, NHL, Mechanism of action.

Introduction

The non-Hodgkin lymphoma (NHL), a heterogeneous group of diseases, is a common malignancy with the higher incidence in China. NHL generally develops in the lymph node, the spleen as well as the thymus. Besides, it can involve the peripheral lymphatic tissue or hematopoietic tissues system[1]. NHL originates from three types of cells, including B cell, T cell and natural killer (NK) cell. Hence, NHL can be classified based on the type of lymphocyte involved. Statistics have shown that the majority of NHLs are B cell lymphomas which is lymphoma affecting B-cell, accounting for 85% of all NHLs. Diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, marginal zone B-cell lymphoma (MZL) or mucosa-associated lymphatic tissue lymphoma (MALT), small lymphocytic lymphoma and mantle cell lymphoma (MCL) are the main common type of NHLs

The Bcl-2 family proteins regulate apoptosis via mitochondrial maintenance. These proteins consist of anti- and pro-apoptotic members, and interactions of them decide whether the mitochondria should initiate the programmed death by releasing pro-apoptotic factors[2]. It has been reported that Epstein-Barr virus (EBV) can induce changes in B-lymphocyte and plays a critical role in the pathogenesis of NHL[3]. At the same time, some study suggested that Bcl-2 and p53 as well as other apoptotic proteins can regulate cell apoptosis of NHL, thereby, promoting the development of the tumor[4]. Therefore, the presence of EBV and the expression of apoptotic proteins are the key to the pathogenesis of NHL. However, few studies have ever been reported regarding the characteristics of EBV and Bcl-2 expression as well as their mechanism of action (MOA). Hence, further work is required. In the present study, the expression of

Corresponding Author: Qingkai Yu, MD; e-mail: qingkaiy@163.com

EBV, Bcl-2, p53, c-Myc and Rb was evaluated, and their MOA were explored, in an effort to advance the understanding of the expression of these pathogenic proteins and improve the treatment of NHL.

Patients and Methods

Patients

Between July 2010 and July 2015, samples of 142 patients with pathologically confirmed NHL presented at our institution were included in the present study. Inclusion criteria: (1) NHL diagnosis satisfies WHO standard[5]. (2) Patients with age > 20 years. (3) Patients with a complete clinical medical record. Exclusion criteria: (1) Patients with other types of tumors. (2) Patients with blood diseases or severe infection. Of 142 patients included, 94 were males and 48 females. The patients were with an age range of 21-65 years and a mean age of 45.24 ± 2.17 years. Among these NHL cases, 132 cases were B-cell lymphomas and 10 T-cell lymphomas (6 peripheral T-cell lymphomas (PTCL), 4 NK/T-cell lymphomas). These cases were subdivided into categories including indolent, aggressive and highly aggressive according to International Working Formulation and were further classified into four clinical stages (Stage I, II, III, IV) according to the Ann Arbor staging system with Cotswolds modifications. In addition, samples from 55 patients (18 males, 37 females, age range 22-64 years, mean age 44.98 ± 1.96 years with hyperplastic lymphadenitis presented during the same period were enrolled as control group. No statistical differences were observed in gender, age or other data between tow groups of patients ($p > 0.05$)[6]. This study protocol was approved by the Ethics Committee of our institution and written informed consent was obtained from all patients.

Methods

Specimens were fixed with 10% of formalin, paraffin-embedded and sectioned, followed by H&E staining and immunohistochemical staining for SP using staining kit (Maixin Biotech, Fuzhou, China) by following manufacturer's instruction. The diagnosis and classification of lymphomas were determined by identifying selected biomarkers, including CD3/20/45RO/15, epithelial membrane antigen (EMA) and leukocyte common antigen (LCA).

Parameters

Expression of protein biomarkers, including EBV(+), p53(+), Bcl-2(+), Rb(-), c-Myc(+) were measured in all patients. The analysis was performed regarding the correlation of NHL with the expression of protein biomarkers, including EBV, p53n, Bcl-2, Rb and c-Myc, and their relationship with pathological subtypes as well as clinical staging of NHL was explored.

Result Assessment[6,7]

The edges of samples were avoided as much as possible when counting the percentage of positive results in an effort to prevent the influence of non-specific staining. Two hundred cells of the area with the highest positivity of each section were selected and the means were obtained. Proportions of tumor cells with positive results were evaluated, with the value < 5% defined as negative and the one > 5% as positive.

Statistical Analysis

Statistical analysis was performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Qualitative data were compared and analyzed using chi-square test and the correlation analysis was performed using Pearson test. $p < 0.05$ was considered statistically significant.

Results

Comparison of Protein Expression Between Two Groups

The expression rates of EBV(+), p53(+), Bcl-2(+), Rb(-) as well asc-Myc(+) of observation group were significantly higher than those, respectively, in the control group ($p < 0.05$) (Table I).

The Correlation of EBV with p53, Bcl-2, Rb and c-Myc in NHL of Observation Group

In NHL patients of observation group, EBV was not significantly correlated with the expression of p53, Bcl-2, Rb or c-Myc ($p > 0.05$) (Table II).

Major Protein Expression in Various Subtypes of NHL in Observation Group

In observation group, expression rates of p53(+) and Bcl-2(+) were significantly higher in aggressive and highly-aggressive NHLs than those in indolent NHLs ($p < 0.05$) (Table III).

Table I. Expressions of major proteins in two groups (n, %).

Groups	N	EBV(+)	p53(+)	Bcl-2(+)	Rb(-)	c-Myc (+)
Observation group	142	27 (19.01)	46 (32.29)	59 (41.55)	40 (28.17)	51 (35.92)
Control group	55	4 (7.27)	2 (3.64)	10 (18.18)	3 (5.45)	11 (20.00)
χ^2	–	4.122	17.791	9.512	11.988	4.656
p	–	0.042	0.000	0.002	0.001	0.031

Table II. Correlation of EBV with p53, Bcl-2, Rb and c-Myc in NHLs of observation group.

		p53		Bcl-2		Rb		c-Myc	
EBV	N	+	-	+	-	+	-	+	-
+	27	7	20	9	18	19	8	12	15
-	115	40	75	51	64	87	28	39	76
r	–	0.095		0.241		0.046		0.106	
p	–	0.501		0.311		0.583		0.227	

Table III. Protein expression in different subtypes of NHLs in observation group (n, %).

NHL subtypes	N	EBV(+)	p53(+)	Bcl-2(+)	Rb(-)	c-Myc(+)
Indolent	31	6 (19.35)	5 (16.13)	8 (25.81)	7 (22.58)	13 (41.94)
Aggressive and highly-aggressive	111	21 (18.92)	41 (36.94)	51 (45.95)	33 (29.73)	38 (34.23)
χ^2	–	0.003	4.791	4.047	0.612	0.624
p	–	0.956	0.029	0.044	0.434	0.429

Major Protein Expression in Various Stages of NHLs

In observation group, the expression rates of EBV (+), p53(+), Bcl-2(+), Rb(-) and c-Myc(+) were significantly higher in stage III-IV NHLs than in stage I-II NHLs ($p < 0.05$) (Table IV).

Discussion

In recent years, the incidence of NHL has been increasing in China, representing the 10th most prevalent malignant tumor and severely threatening human's life. Despite the difficulties remaining in the pathological classifications, clinical staging and individualization, NHLs are still highly curable. Therefore, further researches in the pathogenesis and the treatment of the disease are required to improve the therapeutic outcome[8]. Increasing lines of evidence have shown that the initiation and development of tumors take many stages, which are regulated synergistically by multiple genes[9]. Some studies have demonstrated that part of EBV-encoded protein can regulate and immortalize lymphocytes, which serves the key target for the study of the pathogenesis of NHL pathogenesis[10]. EBV, moreover, may have been associated with the transformation of MCL (Mantle cell lymphoma) to DLBCL (Diffuse large B-cell lymphomas) which can be predicted by c-Myc and Bcl-2[11,12]. p53 overexpression has been as a marker of poor prognosis in mantle cell

Table IV. Protein expression in different stages of NHLs (n, %).

NHL stage	N	EBV(+)	p53(+)	Bcl-2(+)	Rb(-)	c-Myc(+)
Stage I-II	46	4 (8.70)	5 (10.87)	13 (28.26)	7 (15.22)	9 (19.57)
Stage III-IV	96	23 (23.96)	41 (42.71)	46 (47.92)	33 (34.38)	42 (43.75)
χ^2	–	4.705	14.395	4.947	5.641	7.903
p	–	0.030	0.000	0.026	0.018	0.005

lymphomas[13]. In addition, studies have proved that various types of apoptotic proteins can regulate the life as well as the quantity of cells, thereby representing the key to cell proliferation and apoptosis.

According to the present work, in observation group the expression rates of EBV(+), p53(+), Bcl-2(+), Rb(-) and c-Myc(+) were significantly higher than those, respectively in control group, indicating that the expression of EBV(+), p53(+), Bcl-2(+), Rb(-) and c-Myc(+) is closely associated with the pathogenesis of NHL, in agreement with the results of Gandhi et al[14]. The underlying mechanism may be due to the suppression of programmed cell death by Bcl-2, thereby leading to the enhancement of cell survival. EBV can induce the expression of Bcl-2 in B-lymphocytes, which is then involved in the pathogenesis of NHL. p53 gene can directly activate the promoter for the tumor proliferation-related antigens, thereby, increasing cell proliferation and then participating in tumor initiation and development. Rb gene is the first tumor suppressor gene identified by gene cloning. Rb is involved in the regulation of cell cycle, being likely to influence NHL pathogenesis. C-Myc overexpression may induce the malignant transformation and promote the autonomic growth of cells, thereby being closely associated with NHL pathogenesis. In the present study, EBV was not significantly correlated with the expression of p53, Bcl-2, Rb or c-Myc in NHLs of observation group. This finding is consistent with Lombard et al[15,16], which suggested that evaluation of EBV alone cannot reflect accurately the expression of p53, Bcl-2, Rb and c-Myc, but an examination of individual proteins is required. The possible reason is that no correlation exists between EBV and p53, Bcl-2, Rb as well asc-Myc in terms of pathogenic mechanism and change in expression, and hence no regulation of expression can be observed. In addition, the present study showed that, in the observation group, the expression rates of p53(+) and Bcl-2(+) were significantly higher in aggressive and highly-aggressive NHLs than those in indolent NHLs. Besides, the expression rates of EBV(+), p53(+), Bcl-2(+), Rb(-) and c-Myc(+) in stage III-IV NHLs were significantly higher than those in stage I-II NHLs, suggesting that thise protein expression is higher in more advanced stage of NHLs and the expressions of p53 (+) and Bcl-2(+) are higher in more aggressive NHLs, being consistent with the results reported by Karaarslan et al[17]. The underlying mechanism is that high expression of Bcl-2 can regulate and enhance the expression of vascular endothelial growth factor, thereby favoring tumor angiogenesis. Furthermore, a number of works have proven that the expression level of Bcl-2 increases along with the increase in aggressiveness and when approaching more advanced stage of the disease, which is primarily presented by a significant lower Bcl-2 expression in indolent and early stages of NHLs than in aggressive and highly-aggressive as well as in late stages of NHLs. Besides, p53 expression coincides with Bcl-2 expression[18,19]. The pathogenesis and development of NHL are directly correlated with aberrant regulation of cell apoptosis, characterized by aggravation of NHL when cell apoptosis is severely suppressed. No significant differences were observed in the expressions of Rb and c-Myc genes between different stages of NHLs, which may be caused by the fact that the inactivation and expression of these two genes are associated with NHL pathogenesis. These findings also suggest that NHL pathogenesis involves more important and relevant mechanisms, representing a focus for further research.

Conclusions

The expressions of EBV(+), p53(+), Bcl-2 (+), Rb(-), and c-Myc(+) are closely associated with NHL pathogenesis. Higher expression of these proteins is observed in more advanced stages of NHL. Expressions of p53(+) and Bcl-2(+) increase in more aggressive NHLs.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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