Short Communication

The Prevalence of Epstein-Barr Virus Infection in Different Types and Sites of Lymphomas

Yang Zhang, Jinyun Peng, Yunlian Tang, Jie He, Juan Peng, Qiang Zhao1, Rongfang He1, Xiaoli Xie, Xueqin Peng, and Runliang Gan*

Cancer Research Institute, School of Medicine, University of South China, Hengyang; and* Department of Pathology, The First Affiliated Hospital, University of South China, Hengyang, China

(Received September 14, 2009. Accepted January 28, 2010)

SUMMARY: To analyze the association of several types of malignant lymphomas in different anatomical sites with the Epstein-Barr virus (EBV) infection status, 127 cases of formalin-fixed paraffin-embedded samples of malignant lymphomas were investigated with in situ hybridization detecting EBV-encoded small RNA (EBER) in tumor cells. Forty-six out of 108 non-Hodgkin lymphoma (NHL) cases were positive for EBER (42.6%). The EBER-positivity rate of NHL in the nasal cavity and nasopharynx (35/60 cases, 58.3%) was higher than that of NHL in stomach (9/30 cases, 30%) and in the superficial lymph nodes (2/18 cases, 11.1%) (P < 0.05). The EBER-positivity rate of Hodgkin lymphoma in the superficial lymph nodes was 26.3% (5/19 cases). These findings suggest that the EBV-positivity rate in lymphomas is related to their histological types and locations.

Lymphoma is a group of malignant neoplasms in the immune system that originate from the lymph nodes and extranodal lymphatic tissues. The cause of malignant lymphoma remains unclear, but it is related to immunodeficiency, viral infection, gene mutation, ionization radiation, and so on (1,2). In recent years, the viral cause of lymphoma has received more and more attention. Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus that infects more than 90% of the worldwide adult population (3). EBV has been linked to the development of a variety of human malignancies, including Burkitt’s lymphoma, Hodgkin’s disease (HD), nasopharyngeal carcinoma, some T-cell lymphomas, and post-transplant lymphoproliferative disease (4,5). Our former experimental results in vivo confirmed that EBV can induce normal human lymphocytes in the body of hu-PBL/SCID chimeras to form B-cell lymphomas derived from human B-lymphocytes. In addition, the induced tumors in SCID mice were found to be nodular solid tumors, while their histopathology was mainly diffuse large B-cell lymphoma, thus demonstrating that the EBV has oncogenic properties in relation to human normal lymphocytes (6–8). According to the literature, there is a difference in the positivity rate of EBV in malignant lymphomas according to the area, race, sex, age, and type of lymphoma (9,10), and the prognosis also can differ between EBV-positive/or -negative lymphoma patients (11,12).

One hundred and twenty-seven cases of human lymphomas occurring in recent years were collected from three hospitals (First Affiliated Hospital of University of South China [1993–2007], Xiang-ya Hospital of Central South University [2001–2006], and Cancer Hospital of Hunan Province [2000–2007]). The tumor locations were as follows: the nasal cavity and nasopharyngeal lymphomas, 60 cases; primary stomach lymphomas, 30 cases; lymphomas in superficial lymph nodes, 37 cases (32 neck lymphomas, 4 inguinal lymphomas, and 1 armpit lymphoma). According to the histopathological analysis, the diagnosis was Hodgkin lymphoma (HL)/HD in 19 cases, including 15 males and 4 females, with a male:female ratio of 3.75:1. HD patients were 5–65 years old, and the median age was 24. A total of 108 cases with non-Hodgkin lymphoma (NHL) were enrolled, including 66 males and 42 females, and the male:female ratio was 1.57:1. NHL patients were 11–76 years old, and the median age was 50 years. For the immunohistochemical examination, formalin-fixed paraffin-embedded specimens were dewaxed and stained using the streptavidin-biotin peroxidase-labeled complex method. One hundred eight cases of NHL were divided into three kinds of immunity phenotypes: CD20/CD79a positive and CD45RO, CD3/CD56 negative were ascribed to B-cell lymphoma; CD45RO/CD3 positive and CD20, CD79a/CD56 negative were ascribed to T-cell lymphoma; CD45RO/CD3 positive, CD20/CD79a negative, and CD56 positive/negative in the nasal cavity and nasopharyngeal lymphoma were ascribed to NK/T-cell lymphoma. Normal lymph node tissues were used as positive control samples, and PBS instead of the primary antibody was used as a negative control. The operation steps of the immunohistochemical stain were carried out according to the kit instructions.

At present it is thought that the EBV-encoded small RNA (EBER) detection is a reliable index for EBV infection in tumor tissues (13–15). In situ hybridization of EBER was detected with a kit from Taipu Co. (Fujian, China) by the method given in the manufacturer’s manual. Mouse tissues were used as a negative control for EBER in situ hybridization, because mice cannot be infected with EBV (6). Additionally, PBS treatment instead of hybridization solution was used for the negative controls, and EBER-positive lymphoma specimens were used for the positive controls. Categorical variables among groups of EBER detection data were compared by the χ2 test. P values of <0.05 were considered statistically significant.

In this study, we gathered 60 cases of nasal and nasopharyngeal NHLs, including 35 cases of EBER-positive, and the EBER-positivity rate was 58.3% (35/60). Among these samples, there were 29 cases of NK/T-cell lymphoma, includ-
ing 19 cases of EBER-positive, and the EBER-positivity rate was 65.5% (19/29); there were 31 cases of B-cell lymphoma including 16 cases of EBER-positive, and the EBER-positivity rate was 51.6% (16/31). These results indicate that the difference in EBER-positivity rates between NK/T-cell lymphoma and B-cell lymphoma in the nasal cavity and nasopharynx is not significant (P > 0.05). Primary gastric NHLs are commonly B-cell lymphomas, and most of them are lymphomas of mucosa-associated lymphoid tissue (MALT) and diffuse large B-cell lymphomas but rarely T-cell lymphoma. In the present study, the EBER-positivity rate of 30 cases of primary gastric lymphomas was 30% (9/30). Among these samples, there were 28 cases of B-cell lymphoma, including 9 cases of EBER-positive (Figs. 1A, B), and the EBER-positivity rate was 32.1% (9/28); 2 cases of T-cell lymphomas in the stomach were EBER-negativity. The EBER-positivity rate of 18 cases of superficial lymph node NHLs (including neck, inguen, and armpit) was 11.1% (2/18). Among them, there were 8 cases of T-cell lymphomas, including 1 case of EBER-positive, and 10 cases of B-cell lymphomas, including 1 case of EBER-positive.

The tumor tissues of the 108 NHLs in this data were from the nasal cavity/nasopharynx, stomach, and superficial lymph node. The results show that the EBV-positivity rate of NHL in the nasal cavity/nasopharynx (58.3%) is higher than that in the stomach (30.0%), with the difference between them being significant (P < 0.05) (Table 1). The EBV-positivity rate of NHL in the nasal cavity and nasopharynx was much higher than that in the superficial lymph node (11.1%), and the difference was highly significant (P < 0.001). The difference in the EBV-positivity rate between the stomach and superficial lymph node NHL was non-significant (P > 0.05).

There were 5 cases of EBER-positive in 19 cases of HLs, and the positivity rate was 26.3% (5/19). Among EBER-positive cases, there were 2 cases of lymphocyte-predominant HL and 3 of mixed cell-type HL (Figs. 1C, D). EBER hybridization was negative for 1 case of nodular lymphocyte-predominant lymphoma and 4 cases of nodular sclerosis lymphomas.

These data show that the EBV-positivity rate of NHL was slightly higher than that of HL (Table 2), although the difference was non-significant (P > 0.05).
In the 66 cases of NHL male patients, there were 28 cases of EBER-positive, accounting for 42.4% of male patients. In the 42 cases of NHL female patients, there were 18 cases of EBER-positive, accounting for 42.9% of female patients. The difference in the EBER-positivity rate between male and female NHL was non-significant ($P = 0.847$). In this group of 108 cases of NHL, the EBER-positivity rate increased along with the age elevation before 35 years of age, but assumed a drop tendency after 35 years. In HDs, there were 4 cases of EBER-positive in 15 cases of male patients, accounting for 26.7% of all cases; there was 1 case of EBER-positive in 4 cases of female patients, accounting for 25% of all cases. The difference in the EBER infection rate between male and female HL was non-significant ($P = 0.728$).

This group of materials showed an EBER-positivity rate of NHL in the nasal cavity and nasopharynx of 58.3%. The EBV infection percentage of NHLs in the nasal cavity and nasopharynx was higher than that in the stomach and the superficial lymph node, suggesting that EBV infection is position-dependent, which may be correlated with the characteristics of respiratory or salivary spread of EBV in people. Primary stomach lymphoma is the most common type of gastrointestinal lymphoma. The cases of stomach lymphoma in these data are mainly B-cell lymphomas, and the positivity rate of EBER was 30%. The superficial lymph nodes (including cervical regions, inguinal, and axilla) are the predilection site of NHL, but here they presented a lower positivity rate of EBV infection (11.1%).

The nasal cavity and nasopharynx NHL is a group of more common extra-nodal malignant lymphomas. The nasal cavity/ nasopharynx is in a position susceptible to EBV infection, and it has obvious relevance between EBV and NK/T-cell lymphoma, for which the positivity rate of EBV infection may reach 80–90% without racial difference (16,17). In this study, we examined 60 cases of NHL from the nasal cavity and the nasopharynx lymphoma and found that the EBER-positivity rate of NK/T-cell lymphoma was 65.5%; the EBER-positivity rate of B-cell lymphoma was 51.6%. The positivity rate of EBER between NK/T cell lymphoma and B-cell lymphoma was non-significant, suggesting an intimate correlation between these two kinds of immunophenotyping lymphomas and EBV infection in our local area of south China. The reports regarding EBV infection and its relation to the clinical prognosis of lymphoma patients are inconsistent. For example, Lee et al. (18) found that EBV infection has no effect on the prognosis of angioimmunoblastic T-cell lymphoma (AILT), but others have reported that the survival rates of EBER-positive NK/T-cell lymphoma patients are lower than those of EBER-negative patients (11). Previous studies have found that the target cell of EBV infection is the human B lymphocyte (19), but in recent years more evidence has shown that human T-cell lymphomas also exist with EBV infection (20,21). In an in vitro experiment it was discovered that immortalized cell lines will be difficultly transformed from normal T lymphocyte with EBV. However, the clinical data showed that the EBV detection rate in some types of human T-NHLs is higher than that in B-NHLs according to the situation of lymphoma morbidity, suggesting that T-NHL is more relevant with EBV infection. All these contradictory phenomena indicate that there may be different mechanisms between the T-cell lymphoma and B-cell lymphoma infected with EBV, and this is the focus of T-NHL etiologic and pathogenetic study.

In classical HLs approximately 40% of Hodgkin and Reed-Stampenberg (HRS) cells are infected with EBV (22), accompanied by immune globulin gene mutation and/or loss of expression of B-cell receptor. EBV infection is a risk factor for HL. In classical HL, there was a great difference in the EBV detection rates in different geographic regions and the EBER-positivity rate was generally 20–50% (23). In this group of materials, we examined 19 cases of HL and found that the EBER-positivity rate was 26.3% (5/19 cases), consisting 2 cases of lymphocyte-predominant HL and 3 of mixed cell type; however 1 case of nodular lymphocyte-predominant lymphoma and 4 of nodular sclerosis lymphoma were found to be negative for EBER. The fact of EBV detection in all subtypes of HL here is consistent with the results reported from developed countries.

In the group of cases we examined, the EBV infection positivity rate of NHL was 42.6%, and that of HL was 26.3%. There is a range of infection positivity rates for the EBV in variant positions and different types of human lymphomas. Further study is needed regarding the effects of EBV on the pathogenesis of lymphoma and its molecular mechanism.

ACKNOWLEDGMENTS
This work was supported by the grants: National Natural Science Foundation of China (30801335), the Construct Program of the Key Discipline in Hunan Province, and the Opening Foundation of Key Lab of Hunan Province (09K075).

REFERENCES