Original Article

Clinical Impact of t(14;18) in Diffuse Large B-cell Lymphoma

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ABSTRACT

Objective: Recent studies have suggested that t(14;18) is present in a significant proportion of diffuse large B-cell lymphomas (DLBCLs). However, the prognostic significance of this translocation and its relationship with BCL-2 protein expression remains controversial. Our study aimed to investigate the predictive power of t(14;18) and BCL-2 protein expression in the prognosis of DLBCLs.

Methods: Biopsy specimens from 106 DLBCLs were analyzed using interphase fluorescence in situ hybridization (FISH). Immunophenotypic analysis of CD20, CD3, CD10, BCL-6, MUM1 and BCL-2 was performed by immunohistochemistry. SPSS 13.0 software was used for statistical analysis.

Results: The t(14;18) was identified in 27 of 106 cases (25.5%). The percentages of tumor cells expressing CD10, BCL-6, MUM1 and BCL-2 were 21.7%, 26.4%, 56.6% and 73.6%, respectively. The presence of this translocation was significantly correlated with the expression of CD10 and immunophenotypic subtype ($p<0.001$). No association was observed between BCL-2 protein expression and the presence of t(14;18). Multivariate analysis confirmed that both t(14;18) and BCL-2 expression were significantly associated with survival. Moreover, patients with t(14;18) had worse prognosis, compared with those with BCL-2 expression (for overall survival: hazard ratio, 4.235; 95%CI, 2.153-8.329, p<0.001 vs. hazard ration, 2.743; 95%CI, 1.262-5.962, p=0.011).

Conclusions: The t(14;18) is a useful prognostic tool for the evaluation of DLBCL immunophenotype and prognosis. The prognosis of GCB (germinal centre-like B cell) DLBCL patients should be made with the consideration of the presence of this translocation, and the detection of t(14;18) should be included as a routine diagnostic test in these cases.

Keywords: Chromosome translocation, t(14;18), FISH, Survival analysis, Diffuse large B-cell lymphoma

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is categorized as a distinct lymphoma entity, according to the World Health Organization (WHO) classification[1]. It is the most common subtype of non-Hodgkin lymphomas (NHLs), representing approximately 40% to 50% adult NHL cases. The incidence of NHL is currently increasing. It is now the fifth most frequent cancer worldwide[2-4].

Evidence suggests that DLBCL is not a single disease but rather a heterogeneous group of tumors with various clinical courses, histology and molecular and cytogenetic characteristics. Currently, the International Prognostic Index (IPI) model is most widely used to predict the outcome of DLBCL[5]. However, even patients within identical IPI categories may exhibit striking variability in prognosis, suggesting the heterogeneity of this malignancy. Molecular and cytogenetic studies of NHLs have shown that chromosomal and genetic abnormalities are associated with the biological and clinical features of these diseases and that they could serve as useful prognostic markers.

The t(14;18)(q32;q21) translocation, reported in approximately 10% to 40% DLBCL cases[6-8], is a characteristic feature of follicular lymphoma and is considered the initiating event of lymphomagenesis[9]. The translocation between these two chromosomes juxtaposes the *Bcl-2* locus (located at 18q21) next to the regulatory regions of the IGH (locus at 14q32), which modifies the regulatory region of the proto-oncogene *Bcl-2* leading to BCL-2 overexpression. BCL-2 is an anti-apoptotic protein whose overexpression opposes mitochondrial apoptotic pathways. The presence of t(14;18) may be important for the pathogenesis of DLBCL with t(14;18). However, the prognostic effect of this translocation and its relationship with BCL-2 protein expression remain controversial[10,11].

In previous reports, polymerase chain reaction (PCR) was often used to detect t(14;18), whereas it has been

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reported that FISH is the gold standard in the study of genetic abnormalities. Therefore, the purpose of this study was to determine the incidence of t(14;18) in DLBCL by FISH and its correlation with BCL-2 protein expression and patient prognosis, providing a scientific foundation for the prognosis of DLBCL patients.

MATERIALS AND METHODS

Case Selection

A total of 106 specimens from DLBCL patients treated at the Shan Xi Tumor Hospital in China were examined in this study. The inclusion criterion was a diagnosis of DLBCL between 2000 and 2007. The t(14;18) translocation was detected by FISH. All cases were confirmed by pathologic review and were classified according to the World Health Organization system[1]. Clinical and follow-up information were obtained from corresponding medical records at the Hematology Department of the same hospital. All patients were newly diagnosed and previously untreated; they received CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) or CHOP-like chemotherapy. Among these patients, nine received rituximab together with combination chemotherapy, and eight received additional radiotherapy. Written informed consent was obtained from all patients, according to the Declaration of Helsinki.

FISH Analysis

Interphase FISH was performed on paraffin-embedded tissue sections to detect t(14;18) as previously described[12]. Commercially available dual-color, dual-fusion IGH/BCL-2 probe sets (05J71-001) were used, according to the manufacturer's instructions (www.Vysis.com). The LSI IGH/BCL-2 dual-color fusion translocation probe is a mixture of a LSI IGH probe labeled with SpectrumGreen and a LSI BCL-2 probe labeled with SpectrumOrange. A translocation was defined by the presence of two yellow fusion signals or adjacent red and green signals on abnormal chromosomes 18 and 14, respectively. At least 200 intact, non-overlapping nuclei were assessed by two pathologists using a Leica TCS SP5 Laser Scanning Confocal Microscope (LSCM). The positive predictive cutoff value used was 3.9% of suspected tumor cells and was determined by examining ten control samples[12].

Immunohistochemistry (IHC)

IHC was performed on archived paraffin-embedded tissue samples by the EnVision method, using the antibodies of CD20 (L26; Maxin Bio, China), CD3 (MAB-0200; Maxin Bio, China), CD10 (clone 56C6; Maxin Bio, China), BCL-6 (GI191E/A8; Santa Cruz, China), MUM1 (clone MUM1p; Santa Cruz, China), and BCL-2 (MAB-0012; Santa Cruz, China). For BCL-6 and MUM1, the immunohistochemical results were considered positive if at least 30% of the tumor cells showed nuclear immunoreactivity. For CD10 and BCL-2, membranous reactivity in more than 30% of cells was considered positive[13]. According to Hans's method, immunohistochemical detection of CD10, BCL-6 and MUM1 was used to classify DLBCL into GCB and non-GCB groups[13]. The GCB subgroup included all tumors with the $CD10^+$ or $CD10^-/BCL\text{-}6^+/MUM1^-$ immunophenotype. Other cases were classified into the non-GCB subgroup, including $MUM1^+$ tumors, regardless of their BCL-6 status ($CD10^-/BCL\text{-}6^+/MUM1^+$ or $CD10^-/BCL\text{-}6^-/MUM1^+$).

Statistical Analysis

Overall survival (OS) was defined as the time interval between the date of diagnosis and the date of death or the last follow-up. Deaths from other causes during lymphoma remission were censored. Progression-free survival (PFS) was measured as the time from diagnosis until progression, relapse after response, or death from lymphoma. Pearson's χ^2 test or Fisher's exact test was used to determine the differences between the variables examined. The Kaplan-Meier method with the log rank test was performed to estimate the OS rate and to compare survival differences between groups. *P*-value <0.05 was considered statistically significant, and all *P* values were two-sided. Survival differences between groups were analyzed using the Cox proportional hazard model. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical Characteristics of Patients

The follow-up period of the 106 patients ranged from 0.5 to 104 months, and their median survival time was 23.4 months. In total, 73 patients were diagnosed with primary nodal presentation, and 33 patients were diagnosed with extranodal presentation, with a median age of 56.5 years (range, 15 to 92). In all, 44 patients presented high ECOG performance status (≥2), and 39 patients presented a high IPI score (≥3). There were 56 cases of Ann Arbor stage I-II, and the remaining cases were at III-IV stage. Elevated levels of serum lactate dehydrogenase (LDH) were observed in 55 patients, ranging from 242 to 1651 IU/L (normal: ≤240 IU/L). All patients, except two cases, received intensive combination chemotherapy after admission, and one patient underwent radiation therapy.

t(14;18) Chromosomal Translocation

The t(14;18) translocation was observed in 25.5% (27 of 106) of our cases with DLBCL (Figures 1A and 1B). The primary sites of t(14;18) were variable: lymph node (n=16), eye (n=1), thyroid gland (n=2), cervical (n=1), gastrointestinal tract (n=4), skin (n=1) and breast (n=2). There was no significant difference in the incidence of t(14;18), according to the primary site. The relationships between t(14;18) and the CD10 immunophenotype were observed. Of 23 CD10-positive cases, 14 (60.9%) were t(14;18)-positive (P<0.001). Of 26 GCB DLBCL cases, 14 (53.8%) were t(14;18)-positive (P<0.001). However, no significant correlation was observed between BCL-2 protein expression and the presence of t(14;18) (Table 1).