Evaluation of ATF-2 expression and its clinical significance in DLBCL

Xun-Xiu Ji, Xin-Bao Hao
The First Affiliated Hospital of Hainan Medical University

Objective: Detection of Activating Transcription Factor-2 (ATF-2) expression in Diffuse Large B-cell Lymphoma (DLBCL) and its relationship with clinicopathological significance.

Method: Pathological diagnosis and clinical data were collected in DLBCL. Immunohistochemical (IHC) was applied for ATF-2 expression in DLBCL.

Result: Positive rate of ATF2 expression in DLBCL was 81% (64/79). We found ATF2 expression was not related to gender, age, clinical staging, immunological phenotype, and EBV infection, Ki-67, CyclinD1 and Bcl-2. The positive rate of both ATF-2, Bcl-6 was 62.0% (49/79), ATF-2 was associated with Bcl-6; the higher expression of ATF-2 is correlated with the poor survival time in DLBCL.

Conclusion: High expression of ATF-2 expression is associated with poor prognosis in DLBCL, suggesting that ATF-2 may be an independent prognostic factor for diffuse large B cell lymphoma.

1. Introduction

DLBCL is the most common type of Non-Hodgkin’s Lymphoma (NHL), constituting 30%–40% of NHL in the middle and old age[1]. DLBCL is a malignant tumor with high malignancy and also with obvious heterogeneity, and its clinical manifestation, morphological, cytogenetic and immunophenotypic features are different in different people. The pathogenesis of DLBCL is unclear. In recent years, research found that ATF-2 protein plays a role in carcinogenesis and tumor inhibition in breast cancer and melanoma cells, respectively, associated with their position in the nucleus and cytoplasm. The high expression of ATF-2 had been detected in DLBCL cell lines BJAB, SUDHL-4, HBL-1 and OCI-LY3; after blocking ATF-2, cell growth was inhibited[2]. However, the expression of ATF-2 in DLBCL and its relationship with clinicopathological features have not been reported. In this study, the expression of ATF-2 in 79 cases of DLBCL was explored by IHC, and the relationship with clinicopathological features was analyzed.

2. Materials and methods

2.1 Material

A total of 79 patients with DLBCL and follow-up information in the First Affiliated Hospital of Hainan Medical University from January 2009 to March 2016 were corrected. All diagnoses of DLBCL conformed to the Han’s Classification. 50 men, 29 women, age from 23 to 92, the average age (61±16). DLBCL were divided into germinal center B cell source (GCB 29 cases) and non-germinal center B cell (non-GCB) in 50 cases. All specimens were fixed in 10% neutral formalin liquid, conventional dehydration, paraffin section and HE staining, determined by the pathological diagnosis. All patients were not treated with radiotherapy and chemotherapy before operation.

2.2 Reagents

ATF-2 rabbit mAb antibody was purchased from American Cell Signaling Technology; Bcl-2, CyclinD1 mouse mAb, enzyme
labeled Goat anti mouse/rabbit IgG complexes and DAB staining were purchased from Fuzhou Maixin Biotechnology Development Co., Ltd.

2.3 Methods

2.3.1 IHC

According to reagent instruction, with known positive control specimens of endometrial carcinoma were be the ATF-2 positive control, tonsil tissue was the Bcl-2 positive control, breast invasive carcinoma was the CyclinD1 positive control, PBS replace the first antibody as a negative control.

2.3.2 Microscope

5 visual fields were randomly selected under microscope × 40. ATF-2 was positive in nucleus/cytoplasm staining, Bcl-2 was positive in cell membrane/cytoplasm staining, and CyclinD1 was positive in nucleus staining. With histological grading (ΣPi) integral calculation, i represents staining degree (0 points: no color or color is not clear; 1 points: light yellow; 2 points: brown; 3 points: dark brown) 4; P represents the percentage of positive cells: 0 point: <5%, 1 point: 5%-20%, 2 points: 20%-50%, 3 points: 50%-75%, 4 points: ≥75%. The finally score divided into 3 grade: ≤ 4: weak positive (+), 5-8 were moderately positive (++), 9 - 12: strong positive (+++).

2.4 Statistical analysis

SPSS 19.0 was used for data processing, Pearson test and Spearman test to carry on the correlation analysis, the Chi-Square test was used to analyze the significance, Kaplan-Meier analysis plays for survival analysis. there was statistical significance of P<0.05.

3. Results

3.1. Expression of ATF-2 and its relationship with clinicopathological features

In 79 cases of DLBCL, the positive rates of ATF-2 and Bcl-6 were 81% (64/79) and (56/72).respectively. ATF-2/Bcl-6 co-expression rate was 62.0% (49/79). Figure 1. No correlation between the expression of ATF-2 with Bcl-2 and CyclinD1 (P > 0.05), and ATF-2 was positively correlated with Bcl-6 expression (P<0.05), Table 1; had no significant difference to gender, age, clinical stage, immunophenotype, EBV infection, Ki-67 (P>0.05); survival analysis of Kaplan-Meier showed that the higher expression of ATF-2 is correlated with the poor survival time in DLBCL (P<0.05), Figure 2. ATF-2 /Bcl-6 co-expression group had no relation with survival time (P>0.05).

Figure 1. Expression of ATF-2, Bcl-2 and DLBCL in CyclinD1 (IHC, 40). A: HE revealed a large number of specific lymph nodes in the lymphoid cell diffuse hyperplasia, lymph node structure disappeared, nuclear fission was visible; B: ATF-2 expressed in nucleus; C: Bcl-2 expressed strongly in membrane; D: CyclinD1 showed moderate positive expression in nucleus.

Table 1.
The relationship between the expression of ATF-2 and CycinD1, Bcl-2, Bcl-6.

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<th>CycinD1</th>
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<td>Number</td>
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<td>72</td>
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Figure 2. The higher the expression of ATF-2, the shorter survival time of patients (P<0.05).

4. Discussion

ATF2 is a member of AP-1 family which consists of 505aa, located in chromosome 2q31, characterized by a basic structural region which belongs to N-terminal and c-Terminal has the bZIP basic Leucine zipper domain belonging to the DNA-binding domain[5-7]. ATF-2 bZIP DNA-binding domain loops onto the amino-terminal region form a heterodimerization, resulting in intramolecular
inhibition. Therefore, transcriptionsal activities[8]. When subjected the stimulation of hypoxia and inflammatory cytokines, pathogen infection, radiation, DNA damage, both Thr69 and Thr71 can be phosphorylated by c-JUN SAPK, MAPK, N-terminal kinase (JNK) and p38, PKC. After that the p-ATF-2 formed as an heterodimer by binding to CRE element belongs to c-fos or c-jun, and then activated the transcription, finally Mediated downstream gene such as CyclinB1 and CyclinD1, Snail, Vimentin, Bcl-2 expression which regulated cell proliferation, differentiation, apoptosis and other biological process[9,10].

Carcinogenic effects have been reported in breast cancer, melanoma, liver cancer, esophageal cancer, lung cancer and renal cell carcinoma. High expression of ATF-2 protein was associated with poor prognosis. The results showed that ATF-2 was highly expressed in DLBCL cell line, which could promote the growth of DLBCL cell line[2]. ATF-2 can accelerate the progression of renal cell carcinoma cells to increase the proportion of S phase cells and accelerate the growth of cells. When blocking the expression of ATF-2, G0/G1 cells increased, the proportion of S cells decreased, cell growth was inhibited[11], ATF-2 carcinogenic effect mainly depends on its nuclear localization and regulated by PKC protein, the expression of PKC protein increased in the nucleus associate with the expression of ATF-2 in nucleus and cell growth increased, blocking ATF-2 resulted in apoptosis of tumor cells; in a word. High expression of ATF-2 expression is associated with poor prognosis and survival time[8,11-14].

Bcl-6 is located in the human chromosome 3q27, belongs to the family of anti-apoptosis, the N side of the POZ encoding protein is the basis of protein-protein interaction; C-terminal has the zinc finger structure, belonging to the DNA binding region. Bcl-6 is mainly expressed in germinal center B cells, which is one of the markers of germinal center B cells, and has the functions of regulating lymphocyte differentiation, immune regulation, DNA damage repair, cell cycle regulation and so on .Relationship between Bcl-6 and cell cycle is through By inhibiting the activity of transcription factors such as competitive suppress STAT6, CD23 and IgE, and then inhibit cell apoptosis[15,16]. Bcl-6 expression and its relation with prognosis of DLBCL are still controversial.

ATF-2 can promote the expression of downstream proteins Bcl-2 and CyclinD1, by inhibiting the apoptosis and cell cycle progression, and promote the growth of tumor cells. In this study, we found that ATF-2 was not correlated with Bcl-2 and CyclinD1 expression, suggesting that ATF-2 may not be involved in the regulation of Bcl-2 and CyclinD1 up regulation of tumor cell proliferation in DLBCL. The survival time of patients with high expression of ATF-2 protein was shorter. In addition, we also found that the expression of ATF-2 was positively correlated with the expression of Bcl-6. Bcl-2 and Bcl-6 belong to the anti-apoptotic family members, and the nucleotide sequence of the gene BANK shows that both Bcl-6 and Bcl-2 have a base sequence that binds to the ATF-2 promoter transcription activator binding site CRE element. Whether ATF-2 and Bcl-6 play an important role in regulating cell cycle needs further study. In conclusion, ATF-2 may be involved in the development of DLBCL, and the relationship with Bcl-6 may provide a new direction for the pathogenesis, diagnosis, treatment and prognosis of DLBCL.

References