Study of ARG (ABL Related Gene-ABL2) Protein, a Tyrosine Kinase Protein, in Burkitt's Lymphoma

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Abstract

There are some evidences on involvement of ABL2 in a number of cancers, especially ABL2 overexpression was observed in Raji, a Burkitt's lymphoma (BL) cell line. Therefore ABL2 overexpression may be involved in pathogenesis of Burkitt's lymphoma. The aim of this study was to evaluate ABL2 significance in BL. For this purpose 20 formaldehyde fixed paraffin embedded blocks – 16BL and 4 normal lymph nodes – were used. Sections were prepared from the paraffin blocks and treat with primary and then secondary antibodies. Two proteins, ABL2 (the test protein) and GAPDH (a control house keeping protein) were studied, ABL2 was labeled green and GAPDH red. After capturing the pictures using a CCD camera, the intensity of green and red colours were measured and ratio between green/red, that demonstrate changes in ABL2 expression, were calculated.

The expression of ABL2 protein in BL and normal lymph node was considerably different and BL had higher expression of ABL2 protein compared to normal lymph nodes. The mean ratio of ABL2/GAPDH in BL and normal lymph node was 0.68 and 0.65, respectively.

Like other malignancies, finding about gene expression patterns in BL seems to be helpful in future board therapeutic guidelines. Then specific inhibitors of ABL2 would be considered as new chemotherapy agents in BL treatment.

Keywords: Burkitt's lymphoma; c-abl; ABL2 protein; IHC (Immunohistochemistry)

Introduction

Burkitt's lymphoma (BL) is an aggressive, undifferentiated and rapidly growing malignant non-Hodgkin's lymphoma with low long-term survival rates. BL also is a germinal centre derived B-cell malignancy that commonly presents in the abdomen in sporadic BL, while endemic BL manifested as a large osteolytic
ABL2 like c-abl is a proto-oncogene and has important role in pathogenesis of some cancers [3]. In some studies down regulation of ABL2 were reported such as; human gastric carcinoma [15], hepatoma cell lines HLE [16] and BEL-7402 [17]. However, controversial results were reported in many studies for example, overexpression of ABL2 was reported in pancreatic adenocarcinoma [18], breast cancer [19] and bladder cancers [20] among others. In human adenocarcinoma, it is shown that there is close association between ABL2 expression and vessels invasion. This report indicated a potential involvement of ABL2 in gastric cancer invasion and progression [21].

As it is mentioned above the highest level of ABL2 expression is observed in mature B cells. These results came from a study on Raji cell line, a cell line derived from Burkitt lymphoma [13]. Here we propose that ABL2 overexpression may be involved in pathogenesis of Burkitt's lymphoma, the same as other cancers that are mentioned above. The aim of this study was to evaluate ABL2 significance in BL.

**Materials and Method**

To measure changes in ABL2 protein between BL and normal lymph node we developed a semi-quantitative method. We measured intensity of the signals derived from ABL2 and at the same time intensity of the signals from a house keeping protein, GAPDH. The expression of GAPDH protein supposes to be constant in both set of studied samples (test and control). Any alteration in the ratio of ABL2/GAPDH demonstrates changes in the ABL2 expression. To do this we used 2 antibodies against ABL2 and GAPDH [22].

The study groups included 16 available paraffin-embedded tissues from BL patients and 4 normal lymph nodes that had undergone surgery in Isfahan Medical University Hospital, Sayed Al-Shohada (PBH) and AL-Zahra between 1999 and 2005.

The samples were already processed for paraffin wax histology using standard method. Histological section (4µm) were cut using a Jung rotary microtome, floated out on distilled water at 50°C, and mounted on glass microscope slides, previously coated with 3-aminopropyltriethoxysilane prior to over night storage at 37°C.

ABL2 and GAPDH protein expression were measured by Immunohistochemistry (IHC) assay. ABL2 primary antibody (sc-6356) was a Goat polyclonal antibody mapping at C-terminal of human ABL2 and GAPDH primary antibody was Rabbit polyclonal IgG.
antibody specific for human GAPDH (sc-25778). Fluorochrome-conjugated secondary antibodies against ABL2 and GAPDH were Mouse anti-goat IgG FITC conjugated (sc-2356) and Donkey anti Rabbit IgG Texas Red conjugated (sc-2095) respectively. All antibodies were purchased from Santa Cruz biotechnology company (USA).

Slide preparation involves transferring sections to slides, removal of paraffin, re-hydration and antigen retrieval.

Sections were deparaffinized and gradually rehydrated in the following manner: Xylene (2×10 min); absolute alcohol (2×5 min); 95% alcohol (2×5 min); 80% alcohol (5 min); deionised water (5 min); phosphate buffered saline (2×5 min).

After re-hydration, slides were placed in HIER solution (Citrate, 0.01 M; pH 6.0), in microwave oven and heated until boiling (3 min 900 W, 10 slides in 100 ml HIER solution). Then slides were allowed to reach room temperature and rinsed in distilled water. 150 µl blocking peptide solution (1/200 dilution in BSA) was added to the slides and incubated for 1 h at 37°C in a humidified chamber before rinsing in PBS. Primary antibodies (ABL2 and GAPDH) were diluted to their optimal dilution (1/400) in diluents. 150 µl primary antibodies were added to the slides and incubated for 1 h at 37°C and rinsed in PBS (2×10 min).

The secondary conjugated antibodies were diluted to 1/200 in PBS. 150 µl anti ABL2 and anti GAPDH antibodies were applied to each slide, and covered with coverslip and sealed with clear nail polish. The slides were then incubated for 1 h at 37°C, before washing them in PBS (2× 10 min). The secondary antibody step needed to be done in dark.

Slides were then examined using a LEICA fluorescence microscope (BZ00) with filter sets for FITCand Texas red. Two pictures were taken from each microscope field, one red and one green. Images were captured using a Cooled charge Coupled Device (CCD) camera (LEICA; DC 350F) interfaced with a PC computer, the pictures were transferred to computer monitor and saved.

The saved images were processed using image analysis software [IP Lab Spectrum (Signal Analytics, Tucson, USA), modified by Digital Scientific UK and supplied by Vysis, USA]. The intensity of green (ABL2 protein) and red (GAPDH protein) colors were measured on each individual cells of microscope field, ratio between green to red colors were obtained and average of these ratio were calculated for all of the normal lymph nodes and BL samples. Because expression of GAPDH is the same in normal lymph nodes and neoplastic lymphoid tissues [23], differences between this ratio in BL and normal lymph node samples indicated difference in the expression of ABL2 between samples (BL and normal lymph nodes). One-way analysis of variance was applied to study significance of the observed differences between each group.

**Result**

We examined ABL2 protein expression in 16 patients with BL diagnosis and also 4 normal lymph nodes.

ABL2 expression was analyzed in paraffin sections of lymphoid tissue from BL patients and patients with normal lymph nodes. A multi-color fluorescence immunohistochemistry method that was developed to enable more than one gene to be investigated at the same time within the same cell was used in this study.

Although because of the different variables in each reaction, it is not possible to accurately estimate amounts of proteins, it is possible to obtain a relative quantitation by using the digital imaging capabilities of the CCD camera to determine the relative fluorescence signal intensities from two proteins. The mentioned method is well established in quantifying protein expression in Western blotting. To do this, the fluorescence signals produced by a control (housekeeping) gene are compared to those produced by gene under the study. In our study GAPDH was used as the housekeeping control gene, as it has fixed level of expression in lymphoma or normal lymphoid tissue [23].

Accurate data of alternation in the levels of protein expression within each individual cell was provided by analyzing 100 cells in sections of BL samples and normal lymph nodes.

We calculated changes in the expression of ABL2 protein in our samples based on decrease or increase in ABL2/GAPDH ratio. It is shown that GAPDH expression is constant in cancers, therefore any changes in the ratio of green to red indicates changes in the expression of ABL2. Figure 1 is a representative of ABL2 and GAPDH protein expression in both Burkitt's lymphoma and normal lymph node samples.

In BL samples, the mean ratio of ABL-2/GAPDH expression was 0.68 and in normal lymph nodes this mean ratio was 0.66. This difference was highly significant (p<0.001). These results are demonstrated in Diagram 1 and summarized in Table 1.

**Discussion**

Burkitt's lymphoma is an aggressive malignancy and patients will have a very short survival without
chemotherapy. Understanding the pathogenesis of BL, the same as other cancers could play an important role in treatment. Therefore search for similarities and differences between BL and normal B cells can provide strategies for BL treatment. In our study expression of ABL2 as a proto-oncogene was compared in BL patients and normal lymph nodes. Our data showed that expression of ABL2 protein in BL and normal lymph node was considerably different.

Firstly ABL2 was identified as a homolog of c-abl through hybridization with v-abl to human placenta DNA [24]. Although some evidences showed that these two proteins have different functions [5], but other studies suggested that they may have similar function in cell mechanism. This difference between studies may be related to uncertain function of ABL2 [3]. Although the exact role of ABL2 in cancers is not yet clear, the c-abl role is definitely recognized in hematological malignancies and other cancers [3].

Our results demonstrated overexpression of ABL2 in Burkitt's lymphoma. Recently a number of studies were performed on alteration of ABL2 in different cancers. The results of these studies would help to understand not only function of ABL2, but also mechanism of carcinogenesis.

So far there are only three reports of down regulation of ABL2 in cancers, including human gastric carcinoma and two hepatoma cell lines of HLE [14] and BEL-7402 [15]. However there are a great number of reports on overexpression of this protein in cancers including melanoma [26], pancreatic adenocarcinoma [18], breast cancer [19], bladder cancer [20], thymoma [27], gastric adenoid carcinoma [21] and AML-M3 cell line [25]. So overexpression of ABL-2 as a proto-oncogene in cancer can propose that ABL-2 may play an important role in BL pathogenesis. The role of ABL2 could be through interfering with cell growth and proliferation, cytoskeleton reorganization [28] and apoptosis pathway [6], cell migration [29] and angiogenesis [21]. Since functions of ABL2 are not well established, more investigations seem to be necessary.

It was shown that ABL2 expression was higher in mature than immature B cell lines. Our results showed that ABL2 is over expressed in Burkitt lymphoma which is a mature B cells lymphoma.

Based on our results as well as the cell line study results [13], it can be suggested that ABL2 may play a potential role in differentiation of B cells and transformation of normal B cells to neoplastic cells. But this needs to be reconfirmed by further studies.

Analysis of gene expression patterns in BL like other cancers may also lead to new guides with respect to the identification of additional therapeutic targets.

**Figure 1.** Representative of GAPDH expression detected by Texas red labeled antibodies and ABL-2 expression detected by FITC in BL (A and B) and normal lymph node (C and D).


Table 1. Comparison between mean, maximum, and minimum of ABL2/GAPDH ratio. A minimum of 100 cells were studied for each group

<table>
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<tr>
<th>Group</th>
<th>Mean Ratio</th>
<th>Min</th>
<th>Max</th>
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<tbody>
<tr>
<td>Normal lymph node</td>
<td>0.66</td>
<td>0.62</td>
<td>0.67</td>
</tr>
<tr>
<td>BL</td>
<td>0.68</td>
<td>0.65</td>
<td>0.70</td>
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Because ABL2 has overexpression in BL, so the specific inhibitor of this protein might be useful in BL chemotherapy. At the moment there is one FDA approved drug available that inhibits ABL-2, c-abl, c-kit, PDGFR-α and PDGFR-β [27]. This drug is taught to be effective on BL however it needs to be examined in a clinical trial.

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References