Prognostic Significance of BMI1 Gene in Chronic Myeloid Leukemia Patients

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Abstract

BMI1 is a polycomb group (PcG) proteins which maintain self-renewal of stem cells, and is overexpressed in leukemia. This study was aimed to investigate the expression of BMI1 chronic myeloid leukemia (CML) and its clinical significance. Expression levels of BMI1 in 45 CML patients and 10 healthy controls were measured by real time quantitative polymerase chain reaction (RQ-PCR). The results showed that the expression of BMI1 was significantly higher in advanced phase than in chronic phase (p <0.05) and healthy controls (p <0.05). The 3-year survival rate was significantly lower in advance patients than in chronic phase CML patients (95% vs. 50%, p = 0.005). Interstingly, overall survival was longer in low BMI1 expression patients than in high BMI1 expression patients (p = 0.012). We conclude that detecting BMI1 is helpful for the diagnosis and prognosis by predicting the overall survival and monitoring of patients with CML.

Introduction

Chronic myeloid leukemia is characterized by three clinical phases: the chronic phase (CP) is followed by an accelerated phase and then by blastic crises. The molecular analysis clearly showed that BCR-ABL chimereic protein, which is generated by a t(9;22)(q34;q11) chromosomal translocation, is involved in malignant transformation of hematopoietic stem cells (1). The B-cell-specifics moloney murine Leukemia virus integration site-1 (BMI1 gene) is an important member in the family of polycomb group genes, and it directly participates in the growth and regulation of cell proliferation (2). The polycomb group gene BMI1 fulfills essential roles in both normal and leukemic stem cells (3) and several published studies have deepened our knowledge of the biology of the PcG in health and disease (4). Many studies have shown that BMI1 expression is frequently upregulated in various types of human cancers, including lung cancer, ovarian cancer, acute myeloid leukemia, nasopharyngeal carcinoma, breast cancer, and neuroblastoma, which indicates that BMI1 might play important roles in cancer initiation and progression (5-10), but the precise mechanism by which it promotes cell growth, have yet to be elucidated (11,12). Mouse models of BMI1 deficiency have also established the importance of BMI1 in the self renewal of neural and hematopoietic stem cells (13,14). In concordance with its role in stem cells, BMI1 has been proposed to maintain cancer stem cell populations in leukemia (15), as well as in breast (16) and lung cancers (17). It is established that CML exhibits marked heterogeneity in prognosis (18) which is reflected even in response to modern therapies (19). As previously established in CML and other malignancies (20-24), BMI1, acting in cooperation with other oncogenes, can induce neoplastic transformation (25,26) and its over expression contributes to disease aggressiveness. Coexpression of BMI1 and other proteins from the PcG, such as EZH2, confers a higher degree of malignancy (27). BMI1 overexpression was described in several types of cancer, including hematologic neoplasms (28-30). This study was designed to investigate BMI1 expression in CML patients and its impact on patients with CML to discover its prognostic significance and whether it might serve as a biomarker to predict disease aggressiveness and progression from CP to more advanced phases.

Material and Methods

This study was carried out on 45 diagnosed chronic myeloid leukemia patients. They were recruited on the basis of standard clinical and hematological criteria for diagnosis of chronic myeloid leukemia. Subjects of this study were selected from the inpatient and outpatient clinics of (OCMU) Oncology Center of Mansoura University. All patients gave informed consent to morphological and molecular examination. Thirty one patients were in the chronic phase of CML and 14 cases were with advanced phase (accelerated and blast phase). They were 24 males and 21 females and their median age were 54 years old. Peripheral-blood mononuclear cells (PBMCs) from 10 healthy donors were also obtained by consent and were used as controls.

All patients underwent a complete physical examination. Treatment was started at dose of 400
mg/day. Before starting imatinib mesylate, complete blood count, serum creatinine and electrolytes were checked. While on therapy, complete blood counts were monitored weekly for the first month and then fortnightly thereafter till patient achieved hematological response and then monthly. Treatment was held if absolute neutrophils count dropped below 500/cumm and platelets less than 50,000/cumm. On recovery, therapy was resumed at the full initial dose. Hematological response was evaluated after 8 weeks of commencement of therapy. Special investigations for detection of BMI1 was done by gene expression quantification of BMI1 gene in patients wit CML by RQ-PCR.

Gene Expression Quantification assay of BMI1 using quantitative real time PCR (Applied Biosystem, USA):

**Total RNA extraction**

Between 2 and 3 ml of EDTA-anticoagulated peripheral blood was collected and Total RNA was extracted with QIA amp RNA blood mini kits and RNA integrity was checked by gel electrophoresis and assuring its purity by the measurement of RNA concentration which was accepted if absorbance readings is more than 0.15.

**cDNA synthesis:**

A reaction mixture of 50 micron in each tube containing 25 micron of RNA sample, 5 micron of RT buffer, 4 micron of dNTP’s Mix, 5 micron of random hexamers, 2.5 micron of RT enzyme and 8.5 micron of water (free nuclease). These tubes were placed in the thermal cycle of gene Amp 7700 with program which was 10 min at 25 ºC, 1 hr at 42 ºC and 5 min at 95ºC

**cDNA amplification**

AmpliTaq Gold DNA polymerase amplifies cDNA using the TaqMan pre-Developed assay Reagents (PDARs) and the TaqMan universal PCR master mix (Applied Biosystem, USA). The TaqMan PDARs, composed of two primers and TaqMan fluorescent probe designed for the detection and quantitation of specific genetic sequence of BMI1. The real time PCR assay was performed by using a mixture of 50 micron in each tube containing 25 µl Universal Master Mix, 1 µl Forward Primer, 1 µl Reverse Primer, 0.5 µl Probe, 2.5 µl GAPDH (Primer – Probe Mix), 10 µl Water and 10 µl Sample. These were placed in the thermal cycle of gene Amp 7700 program which was 2 min at 50ºC, 10 min at 95ºC, 15 sec at 95ºC and 1 min at 60ºC. The comparative expression levels were determined as a ratio between the BMI1 and the housekeeping gene (GAPDH) to correct for variation in the amounts of mRNA.

**Statistical analysis**

Patient data were tabulated and processed using SPSS (Statistical Package for Social Science) for Windows and Excel program. Using fisher’s exact test for parametric data and Mann-Whitney U test for non parametric data. Kaplan Meier survival curves test for survival analysis. A log-rank test was used to compare the differences among survival curves, where p < 0.05 had statistical significance.

**Results**

BMI1 expression level was different among patients with chronic phase and advance phase (accelerated and blast phases). In 45 patients with diagnosed CML either in chronic or advance phase, BMI1 transcript levels were assessed by quantitative real time PCR. BMI1 expression in CML patients at diagnosis in CP was significantly lower compared with patients in more advanced disease stage. Of note, BMI1 expression from healthy donors was significantly lower Compared with CML patients either in chronic or advanced phases (p <0.05) (Table 1).

Clinical follow up was made in 45 patients. The Comparative expression levels were determined as a ratio between the BMI1 and GAPDH to correct for variation in the amounts of mRNA. The BMI1 expression level was unaffected by age or other pretreatment factors. Based on the detection of BMI1
median expression level (2.17), patients were divided into the low-expression group (BMI1 expression level < 2.17) and the high-expression group (BMI1 expression level > 2.17) (Table 2).

The 3 year survival rate was different among CML patients. Patients displaying a low BMI1 expression level at diagnosis had significantly longer survival than patients in advanced phase (P =0.012) (figure 1).

There was significant statistical difference in the overall survival between groups according to the phase of disease. It was 95% for chronic phase versus 50% for advanced phase (P = 0.005) (figure 2).

Factors, such as HB, WBCs, peripheral blood blast count, basophil count, BMI1 expression, which could possibly affect a patient’s prognosis, were introduced into multivariate analysis. The results of a multi-factorial prognostic analysis showed that high BMI1 expression (R= 0.751, P= 0.000), was a highly significant risk factor for influencing prognostic outcome of patient with CML (Table 3). No apparent clinical characteristics were significantly different for the patients with high BMI1 expression when compared with those of low expression BMI1 expression level (Table 3).

Discussion

Although tyrosine kinase inhibitors (TKIs) are now the frontline treatment for CML, a significant proportion of CP patients does not achieve optimal responses and require alternative therapy (31). Furthermore, even patients who obtain a good response to TKIs remain at risk of drug resistance, and disease progression to advanced phase of CML as leukemic stem cells are not adequately targeted by TKIs (32, 33).

Several lines of evidence imply that BMI1 plays an important role in the regulation of cell proliferation and senescence and is required for maintenance of adult hematopoietic and neural stem cells (34-37). Induction of BMI1 would change the composition of the PcG complex to favor proliferation over cell-cycle arrest, because the relative amounts of BMI1 in the complex determine its biochemical and biologic functions (38).

Many reports confirmed that BMI1 was expressed in many kinds of human tumors, such as lymphoma, leukemia, myelodysplastic syndrome (MDS), breast cancer, etc (39,40). BMI1, an essential gene for the self-renewal of normal as well as cancer stem cells (41-43), plays an important role in CML pathophysiology and prognosis in the non-transplantation setting (44). As previously established in CML and other malignancies (45,46), BMI1, acting in cooperation with other oncogenes, can induce neoplastic transformation (47), and its overexpression contributes to disease aggressiveness. BMI1 expression level was significantly higher in the advanced phase patients than in the chronic phase and was lower in healthy subjects than in CML patients, which indicates that BMI1 has a diagnostic value among CML patients. Our data were in agreement with the findings of other publication. They Interestingly found that the level of BMI1 expression was significantly higher in CP than in controls and was further increased during the course of the disease progression and usefulness of BMI1 expression as a molecular marker for monitoring patients with CML(31,44).

The present study indicates that BMI1 gene expression affect the outcome in CML patients. BMI1 gene expression appeared to be associated with significant difference for overall survival in CML patients. Patients displaying a low BMI1 expression level at diagnosis had significantly longer survival than other patients. This finding has been confirmed by others who showed significantly shorter survival in patients who had high BMI1 expression (44).

Despite their great success, it is still unclear whether tyrosine kinase inhibitors can cure CML. Therefore, the prospective screening for BMI1 expression in combination with other molecular markers (48), can help refine CML disease staging and prognosis toward optimizing therapeutic interventions, including perhaps BMI1-targeted inhibitors.

So, we believe that further investigations on larger series of CML patients, including clinical follow-up and other molecular markers, are needed to confirm whether BMI1 can be used for accurate prediction of its prognostic role in CML patients and its potential chemosensitivity to current treatment needs further study.

References


Chinese Journal of Cancer 2008, 27:12, 574-577


Illustrations

Illustration 1

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Chronic phase</th>
<th>advanced phase</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>33</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Median</td>
<td>2.04</td>
<td>4.84</td>
<td>0.15</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

P: chronic and advanced phases groups versus control group, P1: advanced phase group versus chronic phase group.
Table (2): BMI1 expression and clinical characteristics in CML cases

<table>
<thead>
<tr>
<th></th>
<th>Patients with high expression level ( &gt; 2.17 )</th>
<th>Patients with low expression level ( &lt; 2.17 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Median 35-58 (range 47)</td>
<td>Median 49 (range 42-61)</td>
<td>0.42</td>
</tr>
<tr>
<td>WBCs</td>
<td>349.50 (range 63.00-470.0)</td>
<td>325.00 (range 56.0-462.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>HB</td>
<td>10.90 (range 6.90-13.20)</td>
<td>12.00 (range 6.50-13.90)</td>
<td>0.06</td>
</tr>
<tr>
<td>PLT</td>
<td>322.00 (range 59.00-460.00)</td>
<td>342.00 (range 50.0-463.0)</td>
<td>0.54</td>
</tr>
<tr>
<td>P.B Blast</td>
<td>4.00 (range 1.00-29.00)</td>
<td>4.00 (range 1.00-32.00)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Table 3: Multivariate of predictors for the failure to achieve hematological response in CML patients

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs</td>
<td>0.064</td>
<td>0.623</td>
</tr>
<tr>
<td>HB</td>
<td>-0.079</td>
<td>0.557</td>
</tr>
<tr>
<td>B.M blast</td>
<td>1.455</td>
<td>0.094</td>
</tr>
<tr>
<td>P.B blast</td>
<td>1.357</td>
<td>0.083</td>
</tr>
<tr>
<td>Esinophils</td>
<td>0.074</td>
<td>0.541</td>
</tr>
<tr>
<td>Basophils</td>
<td>-0.086</td>
<td>0.600</td>
</tr>
<tr>
<td>Low BMI1 expression</td>
<td>0.167</td>
<td>0.488</td>
</tr>
<tr>
<td>High BMI1 expression</td>
<td>-0.698</td>
<td>0.001</td>
</tr>
<tr>
<td>R=0.751</td>
<td></td>
<td>P=0.000</td>
</tr>
</tbody>
</table>
Illustration 4

Figure 1

Illustration 5

Figure 2
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