Brief report

The polycomb group BMI1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia

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Because the polycomb group gene BMI1 regulates the proliferation of both normal and leukemic stem cells, we examined whether BMI1 expression was associated with disease progression in chronic myeloid leukemia (CML). Levels of BMI1 RNA were significantly higher in patients with advanced-phase than in patients with chronic-phase CML in both CD34+ cells (P = .006) and total peripheral-blood mononuclear cells (P < .001). E2F1, a transcription factor regulating BMI1, was upregulated in CML compared with controls (P = .001). In a cohort of 64 CML patients, the level of BMI1 at diagnosis correlated with time to transformation to blast crisis, and the combination of low BMI1 and high proteinase-3 expression was associated in multivariate analysis with an improved overall survival (P = .001). We conclude that BMI1 may be a biomarker for the intrinsic heterogeneity of CML, and its measurement at diagnosis can help predict overall survival and thus contribute to better therapeutic decisions. (Blood. 2007;110:380-383)

Q-RT/PCR amplification

PBMCs from cryopreserved material were isolated by density gradient centrifugation (Lymphoprep; Nycomed, Oslo, Norway). CD34+ cells were selected by binding to immunomagnetic beads (MiniMACS; Miltenyi Biotech, Bergisch-Gerbach, Germany). Total RNA was extracted using the Qiagen RNeasy kit (Qiagen, Crawley, United Kingdom), treated with DNase I (Invitrogen, Paisley, United Kingdom) to eliminate genomic DNA, and reverse transcribed into cDNA according to standard methods. Expression of BMI1, E2F1, and GAPDH was assessed by quantitative real-time reverse-transcription and polymerase chain reaction (Q-RT/PCR) using the Applied Biosystems 7300/7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). All Q-RT/PCR reactions were performed in 25-μL volume.11 GAPDH expression was used as the endogenous cDNA quality control. The ABI Assays-on-demand TaqMan probe-and-primer reagents for BMI1, E2F1, and GAPDH were used according to the manufacturer’s instructions.

Patients, materials, and methods

Patients and controls

Two independent cohorts of CML patients were studied: (1) patients in CP whose nucleated cells were collected by leukapheresis and cryopreserved within 3 months of diagnosis (before start of treatment) and for whom complete follow-up was available (n = 64)11; (2) patients with cryopreserved cells collected at CP or blast crisis (BC). Informed consent for the use of these cells for research was obtained in accordance with the Declaration of Helsinki and with approval from the Hammersmith and Queen Charlotte’s and Chelsea Research Ethics Committee Institutional Review Board. Diagnosis of CML and disease staging was based on clinical parameters and morphology of blood and bone marrow,11 Peripheral-blood mononuclear cells (PBMCs) from healthy donors, granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral-blood stem cells (PBSCs) from non-CML patients, and bone marrow from healthy donors (StemCell, London, United Kingdom) were also obtained by informed consent and were used as controls.

Statistical methods

Groups were compared using the Mann-Whitney test for continuous data and Fisher exact test for categoric data. Survival curves were calculated using the Kaplan-Meier method, and groups were compared using the
log-rank test. Patients were divided into groups using Q-RT/PCR values delineated by the median. Genes or parameters identified from the univariate analysis with P values of less than .20 (P < .20) were entered into a Cox regression analysis, and a forward and backward stepping procedure was used to find the best model to predict survival. All quoted P values are from 2-sided tests with values less than .05 considered significant.

Results and discussion

**BMI1** expression levels in CD34+ cells were significantly lower in CP (n = 13) than in more advanced stages (accelerated phase and BC, n = 17) of CML (P = .006; Figure 1A). The same significant difference also held true when **BMI1** expression was identified in un_fractionated CML-derived PBMCs (P < .001; Figure 1B). In 8 patients for whom both CD34+ cells and PBMCs were available, a paired comparison disclosed a trend toward a significant correlation between **BMI1** expression in the 2 cell populations (P = .05; Pearson correlation R = 0.7). Of note, **BMI1** expression in bone marrow-derived CD34+ stem cells from healthy donors was significantly lower compared with CML patients (P = .003; Figure 1A). In order to gain insights into the mechanisms underlying **BMI1** up-regulation in CML, we also assessed the expression of **E2F1**, a transcription factor that controls various genetic programs including cell-cycle progression and apoptosis13 and that was shown to directly regulate **BMI1** activity.14 We found that PBMCs from CML patients (all disease stages) displayed significantly higher levels of **E2F1** compared with healthy controls (P = .001; Figure 1C).

We have previously shown that the combination of **CD7**, protease-3 (**PR3**), and elastase-2 (**ELA2**) expression levels at diagnosis can reflect the intrinsic molecular heterogeneity of CML in CP, especially duration of CP. This was observed in patients with an “aggressive disease” who develop BC early after diagnosis (<3 years), as opposed to patients with an “indolent disease” whose BC occurs more than 7 years after diagnosis.11 We therefore measured **BMI1** expression in CD34+ cells from a cohort of 64 CP CML patients (for details see Table S1, available on the Blood website; see the Supplemental Table link at the top of the online article). We found a significant difference in **BMI1** levels between patients with an “indolent” or an “intermediate” (patients surviving between 3 and 7 years without developing BC) clinical pattern compared with those who had an “aggressive” clinical evolution (P = .01 for comparing the 3 groups, Kruskal-Wallis test; Figure 2A). Patients displaying a low **BMI1** expression level at diagnosis had significantly longer survival than other patients (P = .005; Figure 2B). When **BMI1** was included in a Cox multivariate survival analysis model (together with the previously established prognostic markers **CD7**, **ELA2**, and **PR3** and other relevant demographic and clinical parameters; Table S1), the combination of low **BMI1** and high **PR3** expression levels was found to be a strong independent marker associated with significantly longer overall survival (P = .001; RR = 0.20, 95% confidence interval [CI]; 0.08-0.54; Figure 2C).

Our observations suggest an important role for **BMI1** in CML pathophysiology and prognosis. **BMI1** is essential for the self-renewal of both hematopoietic and neuronal stem cells, as well as cancer stem cells.5,15,16 It has also been shown to cooperate with MYC in the generation of lymphomas in double-transgenic mice.17 Furthermore, **BMI1** blocks senescence and immortalizes mouse embryo fibroblasts and, in combination with an activated H-RAS gene, leads to neoplastic transformation.18 These oncogenic functions depend in part on the ability of **BMI1** to repress the INK4A locus, which encodes the tumor suppressor proteins p16Ink4a and p14Arf.19 All of these pathways are known to be involved in the proliferation of **BCR-ABL**-positive cells,20 suggesting that overexpression of **BMI1** acts in conjunction with its related partner genes in the genesis and transformation of CML in a manner analogous to its role in other malignancies.

Although our data do not provide a complete picture of the mechanisms involved in **BMI1** up-regulation in CML, they show that these probably involve the **E2F1** gene, which we also found to be overexpressed in CML. Thus, **E2F1** (1) directly regulates **BMI1**,14 (2) has its activity controlled by the retinoblastoma-cyclin pathway,21 and, (3) via this pathway, defines a route from Bcr-Abl to **MYC** transcription, which is required for Bcr-Abl transformation.22 This implies that genetic alterations impairing **E2F1**, **BMI1**, and their downstream targets may render hematopoietic cells
immune-related proteins such as CML, particularly in conjunction with the expression level of cancer stem cell inducer. Cooperative factors in CML will surely help define it as a bona fide "low BMI1" group (BMI1 expression < median) and a "high BMI1" group (BMI1 expression > median). (C) Cox multivariate analysis yielded a model with the combination of low BMI1 and high proteinase-3 (PR3) expression as predictive of significantly improved survival. The median gene expression levels were used to segregate the patients into a "low BMI1-high PR3" group (BMI1 expression < median and PR3 > median; n = 21) and a "high BMI1-low PR3" group (BMI1 expression > median and PR3 < median; n = 43). Values of genes represent the Q-RT/PCR expression as a ratio of the gene of interest to the GAPDH control gene.

From the clinical standpoint, our findings demonstrate that BMI1 can serve as a novel molecular marker to predict prognosis in CML, particularly in conjunction with the expression level of immune-related proteins such as PR3. An interesting and useful aspect of our study, from a practical point of view, was the indication that the expression of BMI1 in CD34+ cells tends to parallel that found in total PBMCs, as these provide a more easily obtainable and less expensive biologic material in which a rapid Q-RT/PCR prognostication test can be done at diagnosis of the disease. 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 can help refine CML disease staging and prognosis toward optimizing therapeutic interventions, including perhaps BMI1-targeted inhibitors.

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Authorship

Contributions: M.M. conceived and designed the study, collected patient data, processed samples, performed experiments, analyzed data, performed bibliographic search, and wrote and revised the report; A.S.M.Y. conceived and designed the study, processed samples, and performed experiments; R.M.S. performed the statistical analysis and helped to write the report; J.F.A. provided clinical care and recorded clinical data; and J.V.M. conceived and designed the study, supervised its execution, and helped write and revise the report. The corresponding author (J.V.M.) had full access to all the data in the study and had final responsibility for the decision to submit it for publication.

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Figure 2. BMI1 expression and probabilities of overall survival. (A) BMI1 expression in CD34+ immunomagnetically selected hematopoietic progenitors from diagnosis in a cohort of 64 CP CML patients, showing different disease evolution patterns: patients who developed blast crisis (BC) within 3 years of diagnosis were defined as having “aggressive disease” (n = 17), whereas those who survived for longer than 7 years prior to the onset of BC were defined as having “indolent disease” (n = 23). Patients who survived between 3 and 7 years without developing BC were categorized as having “intermediate disease” (n = 24). There was a significant difference in BMI1 expression among the 3 groups (P = .01 when comparing all 3 groups), between patients with “intermediate” and “aggressive” disease (P = .01), and between “indolent” and “aggressive” disease (P = .01) but not between “indolent” versus “intermediate” disease (P = not significant [NS]). The median age at diagnosis of the selected patients was 45.7 years (range, 17.6-68.3 years). The male-female ratio was 1.8:1 (41 males, 23 females). The majority of patients were diagnosed in the pre-imatinib era. (B) Overall survival according to BMI1 expression as assessed by Q-RT/PCR in the whole cohort of the above 64 patients. The median gene expression level is used to segregate the patients into a "low BMI1" group (BMI1 expression < median) and a "high BMI1" group (BMI1 expression > median). (C) Cox multivariate analysis yielded a model with the combination of low BMI1 and high proteinase-3 (PR3) expression as predictive of significantly improved survival. The median gene expression levels were used to segregate the patients into a "low BMI1-high PR3" group (BMI1 expression < median and PR3 > median; n = 21) and a "high BMI1-low PR3" group (BMI1 expression > median and PR3 < median; n = 43). Values of genes represent the Q-RT/PCR expression as a ratio of the gene of interest to the GAPDH control gene.


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