The polycomb group BMI-1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia*

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Abstract

Because the polycomb group gene BMI-1 regulates the proliferation of both normal and leukemic stem cells, we examined whether BMI-1 expression was associated with disease progression in chronic myeloid leukemia (CML). Levels of BMI-1 RNA were significantly higher in patients with advanced phase than in patients with chronic phase CML in both CD34+ cells (P=0.006) and total peripheral blood mononuclear cells (P<0.0001). E2F-1, a transcription factor regulating BMI-1, was upregulated in CML as compared to controls (P=0.001). In a cohort of 64 CML patients, the level of BMI-1 at diagnosis correlated with time to transformation to blast crisis, and the combination of low BMI-1 and high proteinase-3 expression was associated in multivariate analysis with an improved overall survival (P=0.001). We conclude that BMI-1 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.

Introduction

Despite a consistent molecular abnormality, the BCR-ABL oncogene, chronic myeloid leukemia (CML) exhibits marked heterogeneity in prognosis, and various attempts have been made to determine prognosis for individual patients at the time of diagnosis in chronic phase (CP). For example, the Sokal and Hasford prognostic scores derived from study of patients treated predominantly with busulfan and interferon-α, respectively, have proved moderately useful in predicting the duration of survival for individual patients, and recent clinical experience suggests that this heterogeneity is still reflected in response to therapy with imatinib.

The polycomb group (PcG) gene BMI-1 plays an essential role in regulating the proliferative activity of both normal and leukemic stem cells. BMI-1 is a transcriptional repressor likely restricted to stem cells and progenitors. Coexpression of BMI-1 and other proteins from the PcG, such as EZH2, confers a higher degree of malignancy. BMI-1 overexpression was described in several types of cancer, including hematological neoplasms. We therefore measured BMI-1 expression in CML to discover whether it might be involved in pathogenesis, and might serve as a biomarker to predict disease aggressiveness and progression from CP to more advanced phases.

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); 2) patients with cryopreserved cells collected at CP or blast crisis (BC). Informed consent for the use of these cells for research was obtained with approval from the Hammersmith and Queen Charlotte’s & 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. Peripheral blood mononuclear cells (PBMC) from healthy donors, granulocyte-colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC) from non-CML patients, and bone marrow from healthy donors (StemCell, London, UK) were also obtained by informed consent and were used as controls.
Quantitative real-time reverse transcription and polymerase chain reaction (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, UK), treated with DNase I (Invitrogen, Paisley, UK) to eliminate genomic DNA, and reverse-transcribed into cDNA according to standard methods. Expression of BMI-1, E2F-1 and GAPDH was assessed by Q-RT/PCR using the Applied Biosystems 7300/7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA). 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 BMI-1, E2F-1, and GAPDH were utilized according to the manufacturer’s instructions.

Statistical methods

Groups were compared using the Mann-Whitney test for continuous data and Fisher’s exact test for categorical data. Survival curves were calculated using the Kaplan-Meier method, and groups 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 0.20 (P<0.20) 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 two-sided tests with values <0.05 considered significant.

Results and Discussion

BMI-1 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=0.006; Figure 1A). The same significant difference held also true when BMI-1 expression was compared in unfractionated CML-derived PBMCs (P<0.0001; Figure 1B). In 8 patients for whom both CD34+ cells and PBMCs were available, a paired comparison disclosed a trend towards a significant correlation between BMI-1 expression in the two cell populations (P=0.05; Pearson correlation R=0.7). Of note, BMI-1 expression in bone marrow-derived CD34+ stem cells from healthy donors was significantly lower as compared to CML patients (P=0.003; Figure 1A). In order to gain insights into the mechanisms underlying BMI-1 upregulation in CML, we also assessed the expression of E2F-1, a transcription factor that controls various genetic programs including cell cycle progression and apoptosis,12 and that was shown to directly regulate BMI-1 activity.13 We found that PBMCs from CML patients (all disease stages) displayed significantly higher levels of E2F-1 as compared to healthy controls (P=0.001; Figure 1C).

We have previously shown that the combination of CD7, proteinase-3 (PR-3) and elastase-2 (ELA-2) 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 >7 years after diagnosis.11 We therefore measured BMI-1 expression in CD34+ cells from a cohort of 64 CP CML patients (for details see supplementary Table 1. We found a significant difference in BMI-1 levels between patients with an “indolent” or an “intermediate” (patients surviving between 3 and 7 years without developing BC) clinical pattern as compared to those who had an “aggressive”
clinical evolution ($P=0.01$ for comparing the 3 groups, Kruskal-Wallis test; Figure 2A). Patients displaying a low $BMI-1$ expression level at diagnosis had significantly longer survival than other patients. ($P=0.005$; Figure 2B). When $BMI-1$ was included in a Cox multivariate survival analysis model (together with the previously established prognostic markers, $CD7$, $ELA-2$, $PR-3$, and other relevant demographic and clinical parameters; Supplementary Table 1), the combination of low $BMI-1$ and high $PR-3$ expression levels was found to be a strong independent marker associated with significantly longer overall survival ($P=0.001$; RR=0.20, 95%CI; 0.08-0.54; Figure 2C).

Our observations suggest an important role for $BMI-1$ in CML pathophysiology and prognosis. $BMI-1$ is essential for the self-renewal of both hematopoietic and neuronal stem cells, as well as cancer stem cells.\textsuperscript{5,14,15} It has also been shown to cooperate with $MYC$ in the generation of lymphomas in double transgenic mice.\textsuperscript{16} Furthermore, $BMI-1$ blocks senescence and immortalizes mouse embryo fibroblasts and, in combination with an activated $H-RAS$ gene, leads to neoplastic transformation.\textsuperscript{17} These oncogenic functions depend in part on the ability of $BMI-1$ to repress the $INK4A$ locus, which encodes the tumor suppressor proteins p16\textsuperscript{Ink4a} and p14\textsuperscript{Arf}.\textsuperscript{18} All these pathways are known to be involved in the proliferation of $BCR-ABL$ positive cells,\textsuperscript{19} suggesting that overexpression of $BMI-1$ 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.

Though our data do not provide a complete picture of the mechanisms involved in $BMI-1$ upregulation in CML, they show that these likely involve the $E2F-1$ gene, which we also found to be overexpressed in CML. Thus, $E2F-1$ (i) directly regulates $BMI-1$,\textsuperscript{13} (ii) has its activity controlled by the retinoblastoma-cyclin pathway\textsuperscript{20} and, (iii) via this pathway, defines a route from Bcr-Abl to $MYC$ transcription, which is required for Bcr-Abl transformation\textsuperscript{21}. This implies that genetic alterations impairing $E2F-1$, $BMI-1$ and their downstream targets may render hematopoietic cells refractory to the induction of differentiation, as previously demonstrated in myeloid cell line models,\textsuperscript{22} and are thereby likely to play a major role in the progression and aggressiveness of CML. Moreover, induction of $BMI-1$ would change the composition of the PcG complex to favor proliferation over cell cycle arrest, since the relative amounts of $BMI-1$ in the complex determine its biochemical and biological functions.\textsuperscript{23} The identification of $BMI-1$-cooperative factors in CML will surely help defining it as a \textit{bona fide} cancer stem cell inducer.

From the clinical standpoint, our findings demonstrate that $BMI-1$ 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 $PR-3$.\textsuperscript{11,24} An interesting and useful aspect of our study, from a practical point of view, was the indication that the expression of $BMI-1$ in CD34+ cells tends to parallel that found in total PBMCs, as these provide a more easily obtainable and less expensive biological 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 $BMI-1$ expression in combination with other molecular markers,\textsuperscript{25} can help refining CML disease staging and prognosis towards optimizing therapeutic interventions, including perhaps $BMI-1$-targeted inhibitors.

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Szydlo: performed the statistical analysis and helped to write the report; J. F. Apperley: provided clinical care and recorded clinical data; J. V. Melo: conceived and designed the study, supervised its execution and helped writing and revising 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 1. BMI-1 expression in CML as assessed by Q-RT/PCR. (A) BMI-1 expression in CD34+ immunomagnetically selected hematopoietic progenitors from CML patients at diagnosis in chronic phase (CP) as compared to patients in more advanced disease stage (acceleration phase and blast crisis). The definition of CP and advanced phases (acceleration phase and blast crisis) was based on previously established criteria. CP <10% blasts, accelerated phase 10–30% blasts or <10% blasts with clonal evolution, and blast crisis >30% blasts. Bone marrow (BM)-derived CD34+ cells from healthy donors, and G-CSF-mobilized CD34+ stem cells (PBSC) from non-CML donors were used as controls. As previously described, G-CSF-mobilized CD34+ PBSCs express high levels of BMI-1 as compared to non-stimulated normal cells. (B) BMI-1 expression in total unfractionated PBMCs from CML patients at diagnosis in CP as compared to patients in more advanced disease stage (accelerated phase and blast crisis). Total PBMCs from healthy donors were used as controls. (C) E2F-1 expression in total unfractionated PBMCs from CML patients at diagnosis in CP as compared to patients in more advanced disease stage (accelerated phase and blast crisis). Total PBMCs from healthy donors were used as controls. Horizontal bars denote the medians. Values of genes represent the Q-RT/PCR expression as a ratio of the gene of interest to the GAPDH control gene. For establishment of the Q-RT/PCR assay, the Jurkat and HeLa cell lines were used as a positive controls for BMI-1 and E2F-1 expression, respectively, with a standard curve being generated for the amplification of logarithmic dilutions (10^{-1} to 10^{-5}) of their cDNAs. An average of the duplicates of each datapoint was taken and plotted against the cycle threshold (Ct). The technical variability between duplicate samples in our RT/PCR assays has been established for a number of different genes as < 1.3-fold at the 95% level of confidence (data not shown).
Figure 2. BMI-1 expression and probabilities of overall survival. (A) BMI-1 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 over 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 BMI-1 expression among the 3 groups (P=0.01 when comparing all three groups), between patients with “intermediate” and “aggressive” disease (P=0.01) and between “indolent” and “aggressive” disease (P=0.01), but not between “indolent” versus “intermediate” disease, P=NS). The median age at diagnosis of the selected patients was 45.7 years (range 17.6-68.3). 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 BMI-1 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 BMI-1” group (BMI-1 expression<median) and a “high BMI-1” group (BMI-1 expression>median). (C) Cox multivariate analysis yielded a model with the combination of low BMI-1 and high proteinase-3 (PR-3) expression as predictive of significantly improved survival. The median gene expression levels were used to segregate the patients into a “low BMI-1 - high PR-3” group (BMI-1 expression<median and PR-3>median; n=21) and a “high BMI-1 - low PR-3” group (BMI-1 expression>median and PR-3<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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