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# The sensitivity of chronic myeloid leukemia CD34 cells to Bcr-Abl tyrosine kinase inhibitors is modulated by ceramide levels

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ABSTRACT

Despite BCR-ABL tyrosine kinase inhibitors (TKIs) improved outcome of patients with chronic myeloid leukemia (CML), resistance still develops when progresses to blast phase (BP). The mechanisms underlying resistance to TKIs are not well understood. In this study, we analyzed ceramide levels in CD34 cells derived from BP-CML patients and healthy donor bone marrow (BM) using liquid chromatography mass spectrometry. We found that ceramide level was significantly lower in BP-CML CD34 compared with normal BM counterparts. BP-CML CD34 ceramide$^{low}$ were more resistant to BCR-ABL TKIs compared to BP-CML CD34 ceramide$^{normal}$. Both mRNA and proteins levels of sphingomyelin synthase 1 and 2 are lower in BP-CML CD34 ceramide$^{low}$ compared to normal BM CD34 cells, suggesting that these two ceramide synthesis enzymes maybe the mechanism of how ceramide level is suppressed. Importantly, up-regulation of cellular ceramide level induces apoptosis of multiple CML cell lines and BP-CML CD34 progenitors. Combination of BCR-ABL TKIs with ceramide analog is synergistic in targeting BP-CML 34 progenitors. Collectively, our work provides evidence that down-regulation of ceramide level is involved in the resistance of BP-CML CD34 progenitors to TKIs treatment. Targeting ceramide metabolism together with BCR-ABL inhibition makes it an attractive addition to the armamentarium in BP-CML treatment.


## 1. Introduction

Chronic myeloid leukemia (CML) is a lethal hematological stem cell malignancy due to transformation by oncogene BCR-ABL [1]. BCR-ABL-positive leukemia stem/progenitor cells have a proliferative advantage over normal hematopoietic cells and allow CML to progress from chronic phase (CP) to terminal blast phase (BP) [2]. BCR-ABL tyrosine kinase inhibitors (TKIs), such as imatinib, have improved clinical responses and outcomes significantly. However, the majority of CML patients develop resistance to these agents as the disease progresses to BP which is characterized by the expansion of immature CD34 progenitor cells [3,4]. The mechanisms underlying resistance to TKIs may involve BCR-ABL kinase domain mutations, reduced drug uptake or increased efflux or high levels of BCR-ABL expression [5]. It has been recently reported that activation of Wnt/β-catenin signalling, or polymorphim of a pro-apoptotic gene BIM might also contribute to resistance of CML progenitors to BCR-ABL tyrosine kinase inhibitors treatment [4,6,7].

Sphingolipids are involved in a variety of cellular activities, such as growth, survival and responses to stress stimuli. Ceramide is a central mediator in sphingolipid metabolism and serves as a "tumor suppressor lipid" [8]. Up-regulation of ceramide level using ceramide analogs or ceramide metabolizing enzyme inhibitors have recently become an attractive therapeutic strategy in human leukemia [8–13]. Chemotherapeutic drug etaposide which was shown to increase cellular ceramide levels induces apoptosis of acute myeloid leumia cells [14]. In contrast, inhibition of ceramide metabolism sensitizes human leukemia cells to drug treatment [15]. In addition, ceramide analogs, such as C2-ceramide or C6-ceramide, promote apoptosis in CML-derived K562 cells [16].

In this study, we compared ceramide level in BP-CML CD34 progenitors versus normal bone marrow (BM) counterparts and investigated its impact on resistance of BP-CML primary cells to BCR-ABL TKI treatment. We found that compared to normal BM, BP-CML CD34 progenitors with lower level of ceramide are more resistant to 2nd generation Bcr-Abl TKIs treatment. The lower level of ceramide in BP-CML CD34 cells is controlled by the decreased

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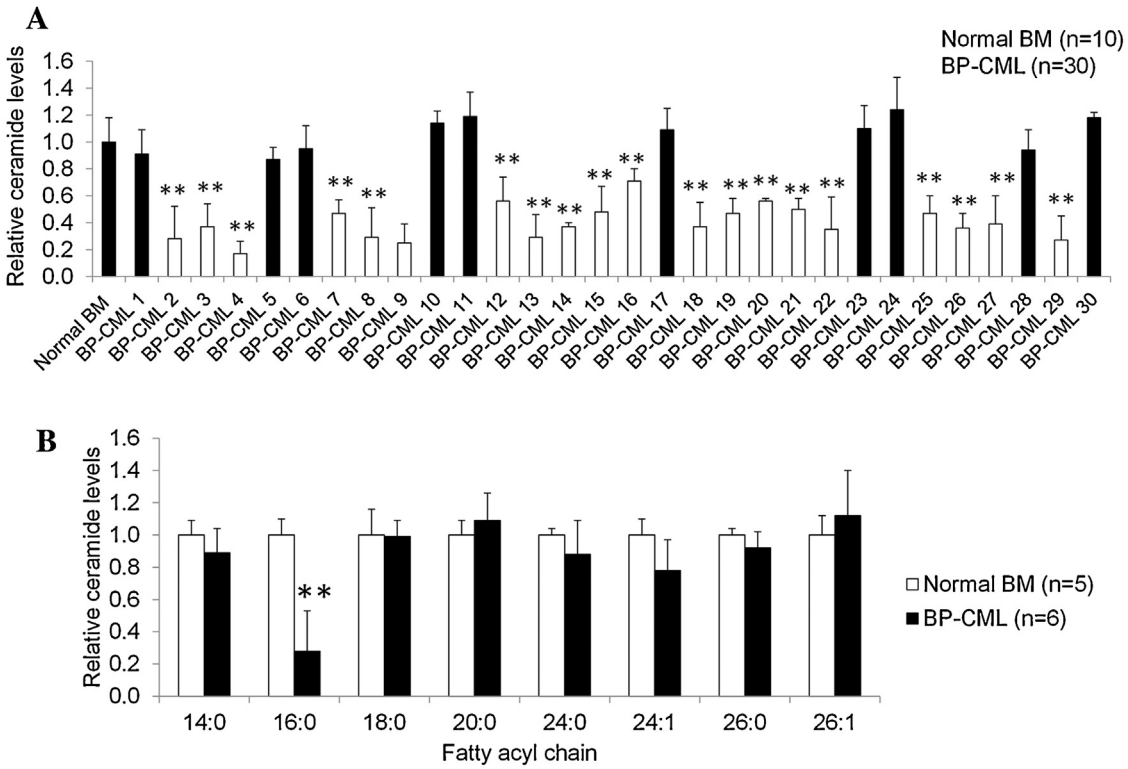


**Fig. 1.** Endogenous cellular ceramide levels in normal BM and BP-CML CD34 progenitors. LC–MS was applied to determine ceramide levels and differentiate distinct fatty acyl subspecies of sphingolipids in CD34 progenitors from 30 patients with BP-CML and 10 healthy donors. CD34 cells at $>10^7$ were isolated from each patient. (A) Ceramide levels were significantly lower in 20 BP-CML and similar in 10 BP-CML CD34 progenitors compared with normal BM CD34 progenitors. The value of normal BM CD34 is the average of 10 samples. Results shown are the ceramide level in BP-CML relative to normal BM. The statistical analysis has been done using a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey honestly significant difference (HSD) statistical analysis. (B) The level of C16:0 but not C14: 0, C18:0, C20:0, C24:0, C24:1, C26:0 and C26: 1 is significantly lower in BP-CML than normal BM CD34 progenitors. **, $p < 0.01$.

**Table 1**
BP-CML patients' information.

| Patient Number | Sex | Age | BCR-ABL mutation | BCR-ABL copies | BCR-ABL/ABL Ratio (IS%) | TKI-resistant | Ceramide Level vs control |
|---|---|---|---|---|---|---|---|
| BP-CML 1 | F | 56 | None | 2500 | 60% | Newly diagnosed | – |
| BP-CML 2 | M | 52 | None | 2987 | 65% | Imatinib | ↓ |
| BP-CML 3 | F | 35 | Y253H | 2873 | 63% | Imatinib, Nilotinib | ↓ |
| BP-CML 4 | F | 23 | None | 4301 | 76% | Imatinib | ↓ |
| BP-CML 5 | F | 67 | None | 3520 | 70% | Imatinib | – |
| BP-CML 6 | M | 65 | E255V | 3091 | 65% | Imatinib Dasatinib | – |
| BP-CML 7 | F | 64 | None | 5826 | 85% | Imatinib Nilotinib | ↓ |
| BP-CML 8 | M | 35 | E287K | 3621 | 67% | Imatinib | ↓ |
| BP-CML 9 | F | 38 | None | 4350 | 70% | Newly diagnosed | ↓ |
| BP-CML 10 | M | 54 | E488K | 2419 | 55% | Newly diagnosed | – |
| BP-CML 11 | F | 47 | None | 7025 | 95% | Imatinib | – |
| BP-CML 12 | M | 49 | E453K | 3985 | 70% | Newly diagnosed | ↓ |
| BP-CML 13 | F | 42 | None | 5690 | 85% | Imatinib | ↓ |
| BP-CML 14 | F | 39 | None | 8466 | 95% | Dasatinib | ↓ |
| BP-CML 15 | F | 26 | None | 8031 | 90% | Imatinib | ↓ |
| BP-CML 16 | M | 29 | None | 8921 | 90% | Imatinib | ↓ |
| BP-CML 17 | F | 58 | E255K | 4896 | 75% | imatinib | – |
| BP-CML 18 | M | 63 | E279K | 4710 | 70% | Imatinib | ↓ |
| BP-CML 19 | F | 71 | E255V | 4865 | 80% | Imatinib | ↓ |
| BP-CML 20 | F | 25 | T315I | 3876 | 65% | Newly diagnosed | ↓ |
| BP-CML 21 | M | 56 | E255V | 3509 | 65% | Imatinib | ↓ |
| BP-CML 22 | M | 52 | T315I | 4826 | 75% | Imatinib, nilotinib | ↓ |
| BP-CML 23 | M | 59 | None | 5210 | 85% | Imatinib | – |
| BP-CML 24 | M | 48 | None | 6712 | 90% | Imatinib, nilotinib | – |
| BP-CML 25 | F | 44 | None | 7099 | 90% | Imatinib | ↓ |
| BP-CML 26 | M | 38 | None | 6302 | 80% | Newly diagnosed | ↓ |
| BP-CML 27 | F | 68 | G250E | 5433 | 70% | Imatinib | ↓ |
| BP-CML 28 | M | 45 | None | 6921 | 80% | Newly diagnosed | – |
| BP-CML 29 | F | 48 | None | 7068 | 85% | Newly diagnosed | ↓ |
| BP-CML 30 | M | 56 | None | 6886 | 80% | Newly diagnosed | – |

*Notes*: –, no change; ↓, decreased; IS, international scale; newly diagnosed indicates that peripheral blood or bone marrow were collected when the patient were naïve to TKI treatment; TKI-resistant indicates that peripheral blood or bone marrow were collected when patients have been treated with one or two TKIs and showed resistance to the treatment. A patient resistant to TKI treatment is diagnosed based on the loss of hematological response (e.g., significantly increased WBC, increased percentage of blast cell, and decreased platelet count) as well as the loss of cytogenetic response (e.g. increased level of BCR-ABL transcripts) after a period time of TKI treatment.