Biochemistry and Biophysics Reports 15 (2018) 69–75

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

BCL2 inhibitor ABT-199 and JNK inhibitor SP600125 exhibit synergistic cytotoxicity against imatinib-resistant Ph+ ALL cells

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ARTICLE INFO

Keywords:
Ph+ ALL
Imatinib-resistance
JNK inhibitor
BCL2 inhibitor
Synergistic action

ABSTRACT

Imatinib (IMT), a specific tyrosine kinase inhibitor (TKI), has drastically changed the treatment strategy for Ph+ ALL (Philadelphia chromosome-positive acute lymphoblastic leukemia). However, TKI resistance remains a serious problem for patient prognosis. Here, a Ph+ ALL cell line NphA2 and the IMT-resistant subline NphA2/STIR were analyzed to identify a potential novel treatment strategy. We also examined other Ph+ ALL cells, MR87 and its IMT-resistant subline, MR87/STIR. IMT induced apoptosis of NphA2 and MR87 but had no effect on resistant sublines. Increased phosphorylated ERK and BCL2, but not BCL-XL, were observed in NphA2/STIR compared with NphA2. NphA2/STIR but not NphA2 was moderately sensitive to U0126, an ERK inhibitor. Interestingly, SP600125, a JNK inhibitor, was potent in cell growth inhibition and apoptosis induction of both parental and IMT-resistant NphA2 and MR87 cells. Moreover, NphA2 and MR87 and their IMT-resistant sublines were sensitive to ABT-199, a specific BCL2 inhibitor. The combination of SP600125 and ABT-199 synergistically suppressed both parental and IMT-resistant cells, including one with T315I mutation, suggesting that Ph+ ALL exhibits high sensitivity to ABT-199 and SP600125 regardless of TKI resistance. This combination might be a possible therapeutic strategy for Ph+ ALL in the future.

1. Introduction

The Philadelphia chromosome (Ph) cytogenic abnormality results in the *BCR-ABL* fusion gene and comprises at least 25% of adult acute lymphoblastic leukemia (ALL) cases and up to 50% in the older population [1]. These leukemia cells are mostly sensitive to conventional cytotoxic drugs, but complete remission is not durable [2,3]. Before the era of molecularly targeted therapy, less than 30% of these patients achieved stable remission, even with aggressive chemotherapy [4]. Although allo-stem cell transplantation represents an effective form of treatment, the long-term overall survival is still not satisfactory.

The *BCR/ABL* fusion gene encodes a tyrosine kinase that plays a critical oncogenic function in Ph+ ALL and chronic myeloid leukemia (CML). The development of BCR/ABL-specific tyrosine kinase inhibitors (TKIs) has drastically changed the treatment of these diseases [5,6]. Treatment with the first TKI, imatinib (IMT), was tolerable with no therapy-related death, and induced complete hematological remission as well as cytogenetic response. TKIs thus represent the first-line treatment of these diseases.

Despite the implementation of TKIs, this disease still shows relapse [7]. One reason for relapse is the acquired resistance against IMT due to point mutation (including T315I) or compound mutations of BCR/ABL. More potent TKIs, including second, third, and new generation TKIs, have been or will be introduced to the clinic; however, resistance against third generation TKIs has already been reported [8]. In addition to resistance issues, some TKIs also produce serious vascular effects [9].

In this study, we examined IMT resistance using NphA2 [10], a Ph+ ALL cell line, as well as MR87 (Ph+ bi-phenotypic AL) [11], along with the IMT-resistant sublines, NphA2/STIR and MR87/STIR. NphA2/STIR cells do not have mutation in the kinase domain of BCR/ABL and do not

Abbreviations: ABL, Abelson kinase; ALL, acute lymphoblastic leukemia; BCR, break clustering region; CML, chronic myeloid leukemia; DST, dasatinib; IMT, imatinib; NRT, nirotinib; Ph+, Philadelphia chromosome positive; TKI, tyrosine kinase inhibitor

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https://doi.org/10.1016/j.bbrep.2018.07.001
Received 26 March 2018; Received in revised form 28 June 2018; Accepted 2 July 2018
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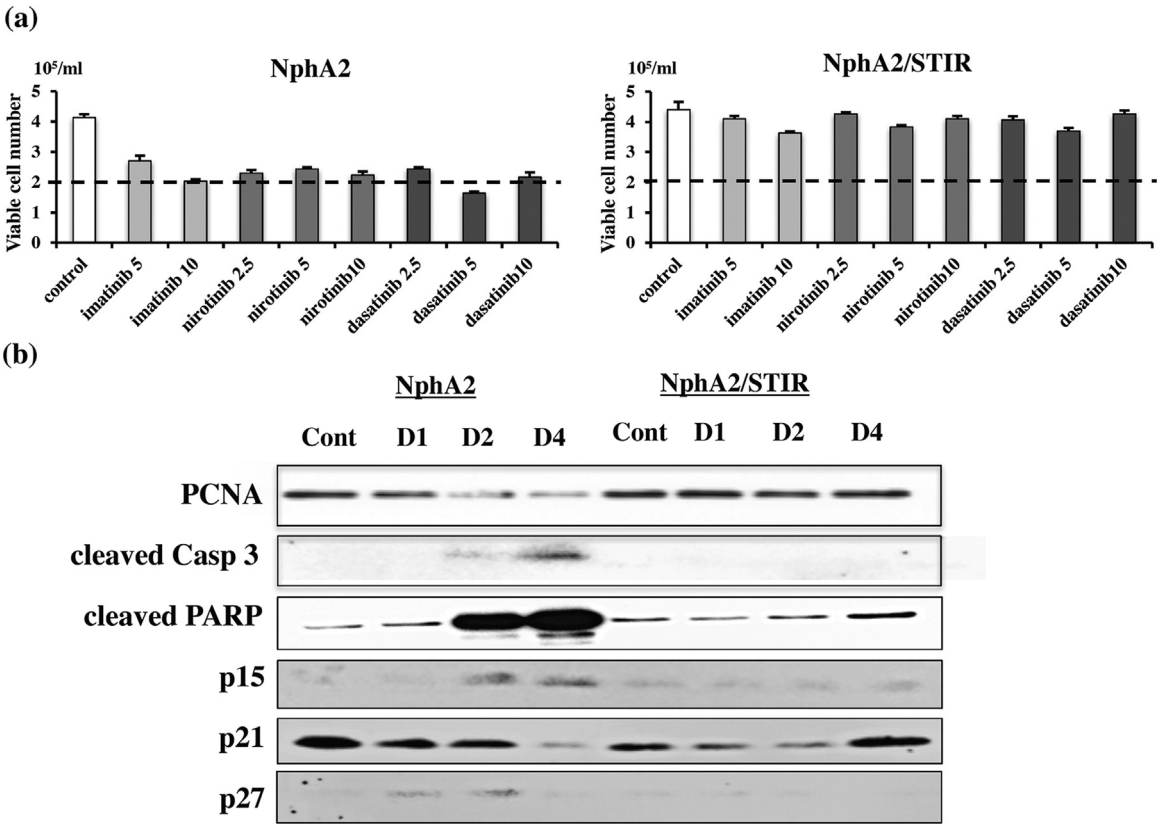

Fig. 1. Characterization of NphA2/STIR cells. (a) Viabilities of NphA2 and NphA2/STIR cells treated with indicated concentrations of IMT, NRT and DST (in μM) for 2 days. Dotted line indicates initial concentration. Cell culture experiments were performed in triplicate. Data are shown as mean ± SD. (b) NphA2 and NphA2/STIR cells were cultured with 10 μM of IMT and collected on day 1, day 2 and day 4 for western blot analysis.

show increased phosphorylated ABL [10]. We used these cells to analyze the BCR/ABL-independent IMT-resistance mechanism and focused on several key signaling and apoptosis pathways.

2. Materials and methods

2.1. Cell lines

NphA2 and its IMT-resistant subline NphA2/STIR were previously reported [10]. K562 cells and the doxorubicin-resistant K562/DNR cells were described before [12]. The MR87 cell line has been reported, and the IMT-resistant subline, MR87/STIR, was established by gradually increasing IMT concentration up to 10 μM during cell culture. TCCY/sr is a Ph+ ALL cell line with T315I mutation [13]. NALM16, Jurkat and U937 cells were reported previously [14]. CML-derived MEGA2 cells were described before [15].

2.2. Reagents

IMT, NRT (nirotinib), and DST (dasatinib) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Cell signaling pathway inhibitors U0126, LY294002, SB203580, and SP600125 were obtained from Calbiochem (San Diego, CA, USA). The BCL2-specific inhibitor ABT-199 (venetoclax), pan-BCL2 family inhibitor ABT-263 (navitoclax), and MCL1 inhibitor A-1210477 were from Abcam (Cambridge, UK). The MCL1 inhibitor marinopyrrole A (moritoclax) was purchased from ChemScene (Monmouth Junction, NJ, USA). The JNK inhibitor JNK-IN-8 was obtained from Sigma (St. Louis, MO, USA).

2.3. Cell viability assays

Cells were plated in triplicate at 2×10^5/ml (in most cases) in 24-well plates and treated with drugs at various concentrations as indicated in the experiments. Cells were cultured for the indicated number of days and then viable cells were counted by trypan blue dye exclusion. Assays were performed in triplicate.

2.4. Western blotting

Western blotting was performed as previously described [12] using the antibodies shown in Supplementary Table 1.

2.5. Isobologram analysis

The isobologram method was performed according to the previous report [14].

2.6. Statistical analysis

Statistical significance was analyzed by Student's *t*-test or one-way ANOVA with Tukey's test for multiple comparisons. All analyses were performed using Prism 6 software (GraphPad; La Jolla, CA, USA).

3. Results

3.1. Characterization of NphA2/STIR cells

A previous study showed that NphA2/STIR exhibits BCR-ABL-independent and RAS/MAPK pathway-dependent IMT resistance [10]. We tested whether NphA2/STIR cells show resistance to other BCR/ABL kinase inhibitors, NRT and DST. Cell viability assays showed that the BCR/ABL-specific TKIs (IMT, NRT, and DST) all effectively suppressed proliferation of parental NphA2 cells (Fig. 1a). However, NphA2/STIR cells were resistant to IMT, as well as NRT and DST. NphA2 cells

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