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BCR-ABL tyrosine kinase inhibition induces metabolic vulnerability by preventing the integrated stress response in K562 cells

Yu Kato [a, b], Kazuhiro Kunimasa [a], Yoshikazu Sugimoto [b], Akihiro Tomida [a, *]

[a] *Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-8-31, Ariake, Koto-ku, Tokyo, 135-8550, Japan*
[b] *Division of Chemotherapy, Faculty of Pharmacy, Keio University, 1-5-30, Shibakoen, Minato-ku, Tokyo, 105-8512, Japan*

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The integrated stress response (ISR) is a cellular process that is characterized by activation of eukaryotic initiation factor (eIF)2α kinases and subsequent induction of activating transcription factor (ATF)4. The ISR plays an important role in protecting cells from tumor-related metabolic stresses, such as nutrient deprivation and perturbed proteostasis. Here, we showed that disruption of the ISR, together with increased cellular stress vulnerability, was produced by pharmacological inhibition of BCR-ABL, the oncogenic driver in chronic myeloid leukemia (CML). Treatment of CML-derived K562 cells with BCR-ABL tyrosine kinase inhibitors, including imatinib, dasatinib, nilotinib and ponatinib, prevented activation of eIF2α kinases, protein kinase-like endoplasmic reticulum kinase (PERK) and general control non-derepressible 2, and downstream ATF4 induction during metabolic stress. Prevention of ATF4 induction likely occurred as a result of the combinatorial suppression of the eIF2α kinase and phosphoinositide 3-kinase/mammalian target of rapamycin signaling pathways. In addition, we found that pharmacological inhibition of PERK mimicked BCR-ABL inhibition to enhance apoptosis induction under stress conditions. These findings indicate that the ISR is under the control of BCR-ABL and may foster adaptation to tumorigenic stresses in CML cells.

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1. Introduction

The integrated stress response (ISR) is a cellular adaptation mechanism to various tumor-related metabolic stresses, such as endoplasmic reticulum (ER) stress or amino acid deprivation, and plays important roles in cell proliferation and survival [1–3]. Under distinct stress conditions, ISR is activated by four different eukaryotic initiation factor (eIF)2α kinases, including general control nonderepressible (GCN)2 [2], protein kinase-like endoplasmic reticulum kinase (PERK) [3], double-stranded RNA-dependent protein kinase [4] and heme-regulated inhibitor [5]. These eIF2α kinases commonly phosphorylate eIF2α at Ser51, reducing general protein synthesis and subsequently promoting translation of activating transcription factor (ATF)4 mRNA [6,7]. ATF4 increases transcription of genes for stress adaptation such as protein folding, amino acid metabolism and redox metabolism [6,7].

The ISR can be triggered by oncogene activation that alters nutrient demands and translation control in cancer cells [8,9]. For example, *PIK3CA*-mutated colon cancer cells show high glutamine dependency, and mutated *PIK3CA* contributes to the glutamine metabolism though ISR activation [10]. Transformation and tumor growth induced by c-Myc overexpression can be promoted by PERK-initiated ISR activation to cope with perturbed proteostasis [11]. These previous findings have revealed a link between ISR activation and adaptation to oncogenic signal-associated stress. However, it is not known whether activated oncogenes are always involved in the control of ISR activation.

In this study, we focused on the *BCR-ABL* fusion gene, the product of the Philadelphia chromosome, which is expressed in most chronic myeloid leukemia (CML) patients [12]. The BCR-ABL protein possesses constitutively activated tyrosine kinase activity [13] that transmits oncogenic signals to various downstream effectors, such as Ras [14,15], phosphoinositide 3-kinase (PI3K) [16,17] and signal transducer and activator of transcription (STAT)5 [18,19]. Here, we showed that BCR-ABL was involved in regulation of the ISR through its tyrosine kinase activity. Indeed, BCR-ABL tyrosine kinase inhibitors suppressed activation of ISR, resulting in enhanced cell death under metabolic stress conditions.

* Corresponding author.
E-mail address: akihiro.tomida@jfcr.or.jp (A. Tomida).

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2. Materials and methods

2.1. Cell culture and reagents

Human CML cell line K562 was obtained from the American Type Culture Collection (Manassas, VA, USA). K562 cells were maintained in RPMI 1640 (Wako Pure Chemical Industries, Osaka, Japan) with 5% fetal bovine serum (Merck, Burlington, MA, USA), as described previously [20]. Imatinib, dasatinib, nilotinib, ponatinib, BKM120, MK2206, AZD6244, Rapamycin, TG101348, PP242 and BEZ235 (Selleck Chemicals, Houston, TX, USA), GSK2656157 (Merck, Burlington, MA, USA), tunicamycin (Nacalai Tesque, Kyoto, Japan) and thapsigargin (Wako) were dissolved in DMSO (<0.5% final concentration) as a stock solution and were added to culture medium. Histidinol (Merck) was dissolved in sterilized distilled water.

2.2. Immunoblot analysis

Immunoblot analysis was performed as described previously [20]. Cell lysates were prepared using SDS lysis buffer (62.5 mM Tris−HCl [pH 6.8], 2% SDS, 50 mM dithiothreitol, 10% glycerol). Protein concentrations were determined using the Bio-Rad Protein Assay (Hercules, CA, USA). Protein samples were subjected to SDS-PAGE and subsequently transferred to nitrocellulose membranes. Membranes were incubated with the following primary antibodies: phospho-GCN2 (Thr899) and PERK (Abcam, Cambridge, UK), ATF6 (Proteintech, Rosemont, IL, USA), GCN2, ATF4, c-ABL, phospho-c-ABL (Y412), X-box binding protein (XBP)1s, inositol-requiring enzyme (IRE)1, phospho-Akt (Ser473), Akt, phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204), p44/42 MAPK (ERK1/2), phospho-STAT5 (Tyr694), STAT5, phospho-4EBP1 (Ser65), 4EBP1, phospho-S6 (Ser235/236), S6, cleaved-poly (ADP-ribose) polymerase (PARP) and ribosomal protein S3 (Cell Signaling Technology, Danvers, MA, USA). The specific signals were detected using Western Lighting plus ECL (Perkin Elmer, Waltham, MA, USA).

2.3. Detection of apoptotic cells

Cells were seeded at 2×10^4/well in 96-well plates and treated with the indicated concentration of reagents. After 18 h, the nuclei were stained with 10 μg/mL Hoechst 33342 (Thermo Fisher Scientific) for 10 min. Fluorescent images were acquired by IN Cell Analyzer 6000 (GE Healthcare, Little Chalfont, UK). The percentage of apoptotic cells was calculated by dividing the number of apoptotic cells by the total number of cell. Results are shown by boxplot diagram (12 fields of view in total under identical conditions, 3 fields of view/well). The boxplot shows the median and dispersion of each group. The box represent distributions of data from 25% to 75% and the whiskers show the range of 1.5-fold of the box.

2.4. Cell proliferation assay

Cells were seeded at 1.5×10^4/well in 96-well plates and treated with the indicated concentrations of reagents. After 48 or 72 h, cell viability was measured by the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA). The cell viability is shown as the percentage of the controls.

3. Results

3.1. BCR-ABL inhibition prevents activation of PERK and GCN2

To investigate the effects of BCR-ABL inhibition on the PERK signaling pathway, we treated human CML K562 cells with BCR-ABL inhibitors imatinib (1 or 10 μM), dasatinib (10 or 100 nM) or ponatinib (10 or 100 nM) for 18 h under ER stress conditions of glucose withdrawal (Fig. 1A) as well as tunicamycin (10 μg/mL), an N-linked glycosylation inhibitor [21] (Fig. 1B). Activation of the PERK signaling pathway by glucose withdrawal and tunicamycin treatment was demonstrated by phosphorylation of PERK, which was seen as a bandshift from lower (underphosphorylated) to higher (phosphorylated), and by induction of ATF4. PERK

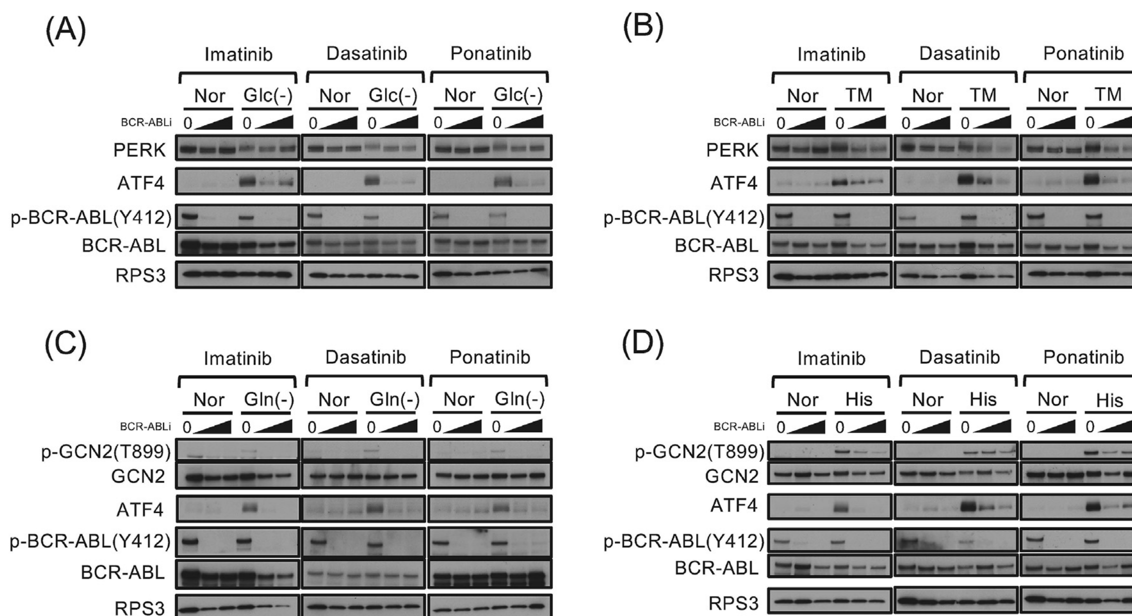

Fig. 1. BCR-ABL inhibitors prevent eukaryotic initiation factor 2α kinases activation and activating transcription factor 4 (ATF4) induction. K562 cells were treated with imatinib (1 or 10 μM), dasatinib (10 or 100 nM) or ponatinib (10 or 100 nM) for 18 h under normal (Nor) or the following stress conditions: (A) glucose (Glc) withdrawal; (B) 10 μg/mL tunicamycin (TM) addition; (C) glutamine (Gln) withdrawal; and (D) 2 mM histidinol (His) addition. The cell lysates were subjected to immunoblot analysis with specific antibodies, as indicated. GCN2, general control nonderepressible 2; PERK, protein kinase-like endoplasmic reticulum kinase; RPS3, ribosomal protein S3.

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