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Activation of ERK and P38 by the Addition of Arsenic Trioxide in Flt3-ITD Cells

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ABSTRACT

Flt3-internal tandem duplications (*Flt3-ITD*) *is a prevalent mutation in acute myeloid leukemia* (*AML*). *We recently reported arsenic trioxide* (*ATO*) *and Flt3 inhibition synergize to induce apoptosis in Flt3-ITD cells. However, the signaling effect of ATO in these cells has not been elucidated. Here, we demonstrate that the treatment of ATO potently induces the activation of extracellular regulated kinase (ERK)-mitogen activated protein kinase* (*MAPK*), *and modestly activates p38-MAPK in BaF3-Flt3-ITD cells, among other major* (*PI3-kinase-Akt, c-jun N-terminal kinase* [*JNK*]) *signaling pathways examined. In contrast, in BaF3-Flt3-wild type* (*WT*) *cells, slight activation of p38, but none for others, was observed. As MAPK kinase* (*MEK*), *as well as p38 inhibition is reported to enhance ATO-induced apoptosis in AML and various hematological malignancies, our results suggest that Flt3 mutation status is important for the effect of these combinations.*

Keywords*: *Flt3-ITD, ERK, Arsenic Trioxide

1. Introduction

Flt3 is a member of the class III receptor tyrosine kinase family, and approximately one third of AML patients have mutation of this gene. The majority of such mutations are ITD in the juxtamembrane domain of *Flt3*, specifically found in AML, which result in ligand-independent dimerization and tyrosine phosphorylation of the receptor [1,2]. This causes constitutive activation of downstream signaling pathways, including the Ras/MEK/MAPK pathway, leading to aberrant cell growth and differentiation block in leukemia cells [1]. The MEK/MAPK pathway is an important signaling cascade involved in the control of hematopoietic cell proliferation and differentiation [3]. The downmodulation of MEK phosphorylation inhibits proliferation and induces apoptosis of primary AML blasts [4]. ATO has currently widely used in the treatment of the patients with relapsed or refractory APL [5]. It was reported that downmodulation of MEK/ERK phosphorylation significantly enhanced ATO-induced apoptosis in primary APL blasts [6]. Additionally, it was reported that not only in APL blasts, but in AML cases, the combination of ATO with an MEK inhibitor is very efficient [7,8]. We recently reported that ATO and *Flt3* inhibition synergize to induce apoptosis in *Flt3*-ITD cells [8]. In this study, we examined the signaling effect of ATO on *Flt3*-WT and *Flt3* mutated cells, to clarify the mechanisms of the specific effect of ATO on *Flt3*-ITD cells.

2. Materials and Methods

2.1. Cell Culture

BaF3-*Flt3*-ITD and BaF3-*Flt3*-WT cells were recently established in our laboratory [9]. All experiments using BaF3-*Flt3*-ITD cells were performed under IL-3 deprivation because the *Flt3*-ITD signal is redundant in the presence of IL-3 stimulation of the cells [10].

2.2. Westernblot

For assay of phosphorylated proteins, 3×10^6 of BaF3-*Flt3*-WT and BaF3-*Flt3*-ITD cells [9] were washed twice with PBS and resuspended with serum free RPMI and seeded into 10 cm dishes. Then the cells were exposed with FL (100 ng/ml) in the presence or absence of ATO (4 μM). The cells were extracted at indicated time points. Phosphorylated proteins of ERK, p38, JNK and Akt were determined by Westernblot as previously described [11].

3. Results and Discussion

To uncover the signaling difference between *Flt*3-ITD and *Flt*3-WT, we employed BaF3-*Flt*3-ITD and -WT cells those we established in our laboratory [9]. As shown in **Figure 1**, in BaF3-*Flt*3-ITD cells, ERK was potently phosphorylated 30 min after the addition of 4 μM of ATO and its activation was decreased thereafter. There was a modest effect for the phosphorylation of p38-MAPK in BaF3-*Flt*3-ITD cells. In contrast, in BaF3-*Flt*3-WT cells, slight activation of p38-MAPK, but none for others, was observed by the addition of ATO.

It was reported that ATO at clinically achievable concentrations (2 - 7 μmol/l) activated p38-MAPK in several leukemia cell lines or myeloma cells [12,13]. p38-MAPK has been shown to mediate both proapototic/growth inhibitory and antiapoptotic/pro-growth signals in different systems, apparently depending on the stimulus and cell type involved [5,12,14]. Combination of ATO with p38-MAPK inhibition significantly increased the apoptosis and/or growth inhibition induced by ATO treatment in these cells [12,13]. Therefore, current results indicate that combination therapy of p38-MAPK inhibitors with ATO might be effective for both *Flt*3-ITD and WT cells.

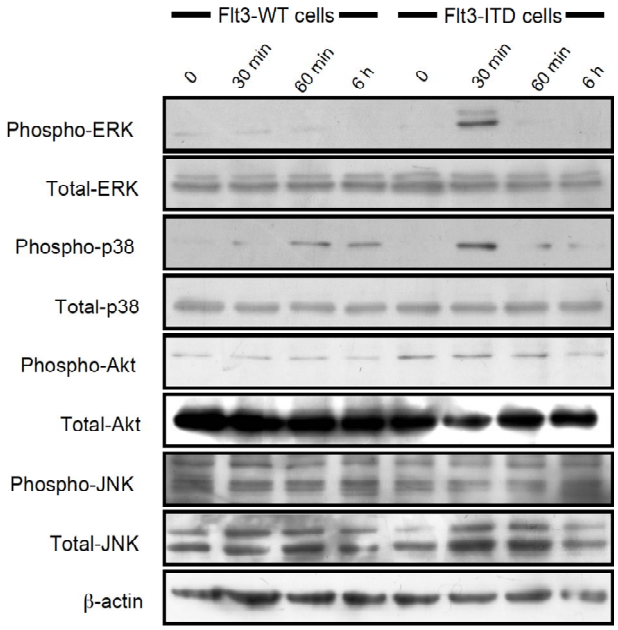

Figure 1. The signaling effect of ATO in BaF3-*Flt*3-WT and BaF3-*Flt*3-ITD cells. 3 × 10^6 of BaF3-*Flt*3-WT and BaF3-*Flt*3-ITD cells were washed twice with PBS and resuspended with serum free RPMI and seeded into 10 cm dishes. Then the cells were exposed with FL (100 ng/ml) in the presence or absence of ATO (4 μM). The cells were extracted at indicated time points. Phosphorylated proteins of ERK, p38, Akt and JNK were determined. β-actin served as a loading control. The results presented are representative of two to three different experiments.

Common chemotherapeutic drugs usually induce a wide range of cytotoxic effects on hematopoietic stem cells or progenitor cells of other tissues. In addition, there are many serious side effects of chemotherapy. In contrast, the therapeutic dose of ATO is associated with an acceptable toxicity level without bone marrow hypoplasia or alopecia [15]. Although QT interval prolongation APL differentiation syndrome are the most serious complications observed in patients with ATO, ATO is well tolerated and toxicities are manageable and reversible. From these points of view, combination therapy with ATO may be advantageous in leukemia therapy. It was noted that ATO is a potent stimulator of ERK and AP-1 transactivational activity and an efficient inducer of c-fos and c-jun gene expression [16,17]. Induction of c-jun and c-fos by ATO is also associated with the activation of JNK [16]. However, a subsequent study indicated that at a therapeutic dose for AML (<5 μM), ATO dominantly induces ERK, but not JNK phosphorylation [18], which is consistent with the current result of our study. As MEK inhibitors are promising agents for the treatment of AML [19-22], our results provide an evidence that the status of *Flt*3 receptor is responsible for the effect of MEK inhibition in combination with ATO.

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