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AKT1 induces caspase-mediated cleavage of the CDK inhibitor p27Kip1 during cell cycle progression in leukemia cells transformed by FLT3-ITD

Xinping Yang, Suiyang Liu, Surender Kharbanda, Richard M. Stone*

Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, 450 Brookline Avenue, Boston, MA 02115, USA

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ABSTRACT

p27Kip1 cleavage and caspase-3 regulate cell cycle in human myeloma cells and B cells, however regulation of p27Kip1 cleavage during the cell cycle is not known. In BaF3-FLT3-ITD cells, p27Kip1 undergoes C-terminal cleavage. Inhibition of the PI3K/AKT pathway is associated with decreased cleavage of p27Kip1 and G1 phase arrest. A caspase-3 inhibitor reduces p27Kip1 cleavage and inhibits cell proliferation. Knockdown shRNA against AKT1 reduces cleavage of p27Kip1, inhibits caspase-3 activation, and is associated with a delay in cell cycle progression. Taken together, these findings indicate that AKT1 induces caspase-mediated cleavage of p27Kip1, required for G1-S progression in FLT3-ITD cells.

1. Introduction

Leukemogenesis is in part based on deregulation of one or more pathways mediating normal proliferation, apoptosis or self-renewal. The presence of a FLT3 ITD mutation, present in 25% of patients with AML, promotes clonal proliferation and is associated with an adverse outcome in acute myeloid leukemia (AML) patients treated with standard chemotherapy [1,2]. Understanding the downstream effects of FLT3-ITD mediated signals could lead to the development of new therapeutic agents. The PI3K/AKT pathway is constitutively activated by FLT3-ITD mutations [3,4]. AML patients with up-regulated activity of PI3K/AKT pathway have a relatively poor prognosis [5,6]. Pharmacologic inhibition of PI3K by LY294002 results in growth arrest of AML cells [7]. Our previous studies also show that inhibition of the PI3K/AKT pathway leads to cell cycle arrest but only has a minimal effect on apoptosis in FLT3-ITD transduced BaF3 (BaF3/FLT3-ITD) leukemic cells [8].

The AKT1-dependent phosphorylation and cytoplasmic mislocalization of p27Kip1 may account for proliferation mediated by an activated oncogene in cancer cells [9–11]. Previous studies show that the PI3K pathway is crucial in regulating the cyclin-dependent kinase (CDK) inhibitor p27Kip1 during G1/S progression [12]. The CDK inhibitor p27Kip1 forms complexes with cyclin D-CDK4/6 and cyclin E-CDK2, and thus inhibits CDK activity which is required for G1/S transition [13,14]. The amount of p27Kip1 is generally up-regulated in quiescent cells and is down-regulated upon cell cycle entry. Down-regulation of p27Kip1 expression is associated with aggressive tumor behavior and poor clinical outcome in cancers [15]. The down-regulation of p27Kip1 in cell cycle is mainly via decreased translation [16] and increased degradation [14,17]. Proteasome-dependent degradation of nuclear p27Kip1 requires phosphorylation at T187 by CDK2 [18–20]. Phosphorylation-mediated nuclear export of p27Kip1 represents another aspect of p27Kip1 regulation [21–23]; cytoplasmic retention of p27Kip1 is found in cancers [12,24,25]. Cytoplasmic retention of p27Kip1 may involve phosphorylation of S10 by hKIS [22,26], through phosphorylation of T157 and T198 by AKT [9,25–29], and via binding to 14-3-3 in cytoplasm.

Despite the aforementioned convincing evidence that p27Kip1 cleavage is critical for cell cycle regulation in cancer cells, the interaction of this moiety with apoptosis-promoting caspase 3 or caspase 3-like proteases [30,31] remains unclear. Furthermore, the regulation of p27Kip1 cleavage during the cell cycle requires elucidation in leukemia cells. We demonstrate that the PI3K/AKT pathway promotes caspase-3 activation and p27Kip1 cytoplasmic cleavage leading to G1-S progression consequent to the presence of FLT3-ITD. The cleavage of p27Kip1 to p23Kip1 removes the nuclear localization signal (NLS) and thus prevents the protein from entering the nucleus. PI3K/AKT pathway inhibition is associated with inhibition of caspase 3 inhibition limiting p27Kip1 cleavage. Taken together, the AKT-caspase 3-p27Kip1 pathway is involved

* Corresponding author. Tel.: +1 617 632 2214; fax: +1 617 632 2933.
E-mail address: rstone@partners.org (R.M. Stone).

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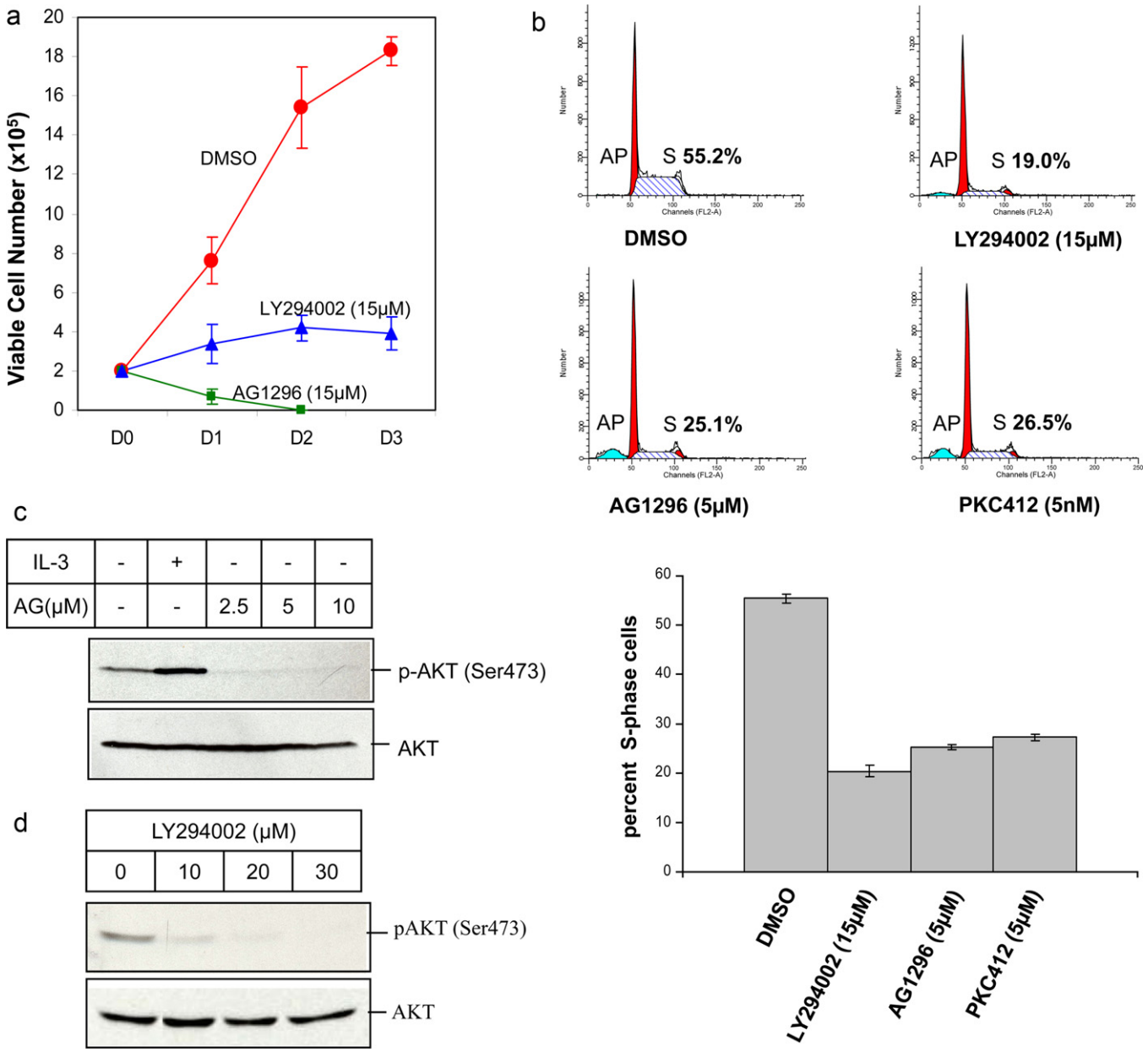

Fig. 1. Arrest of BaF3/FLT3-ITD cells in G1 by inhibiting PI3K/AKT pathway. (a) Cells under indicated treatments were counted on D0 (start time), D1 (1 day after seeding), D2 (2 days after seeding), D3 (3 days after seeding): *X*-axis represents time; *Y*-axis represents cell counts for viable cells. The results are expressed as Mean ± SE from three independent experiments. (b) Cells were collected for cell cycle analysis after indicated treatments for 24 h: the *Y*-axis represents cell counts and the *X*-axis represents DNA amount; the percentages of cells in G1 phase (G1) are shown in heavily shaded, S phase (S) in striped areas and apoptosis (AP) in lightly shaded areas. The experiments were repeated twice. Columns: mean of triplicate data points from one of two independent experiments; bars: SE (bottom panel). (c and d) BaF3/FLT3-ITD cells were starved for at least 8 h in medium containing 1% FBS and then treated with the indicated concentrations of FLT3 inhibitor AG1296 or the PI3K inhibitor LY294002 for 90 min. Western blotting was done to detect phospho-AKT (Ser-473). The growth factor IL-3 was used as a positive control for stimulation of AKT phosphorylation. The membranes were stripped and re-probed with anti-human AKT antibodies to show equal loading.

in FLT3-ITD-mediated cell cycle regulation and could represent a therapeutic target in AML.

2. Materials and methods

2.1. Cell culture, treatments and reagents

FLT3-ITD transduced BaF3 stable cell lines (BaF3/FLT3-ITD) were maintained in RPMI 1640 containing 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin, 100 mg/ml streptomycin, 2 mM L-glutamine and 400 mg/ml G418. The FLT3 inhibitor PKC412 was obtained from Novartis; FLT3 inhibitor AG1296, PI3K inhibitor LY294002 and caspase-3 inhibitor Z-VAD-fms were obtained from Calbiochem-Novabiochem Corp (San Diego, CA). BaF3/FLT3-ITD cells were cultured at a starting density of 2×10^5 cells/ml in RPMI 1640 for 24 h before cells were treated. For drug treatments, the FLT3 inhibitors PKC412 (5 nM) or AG1296 (5 μM), the PI3K inhibitor LY294002 (15 μM) or the caspase-3 inhibiotr Z-VAD-fmk (50 μM) were added to the medium.

2.2. Antibodies

Anti-p27Kip1 rabbit polyclonal antibody and monoclonal antibody, anti-cyclin D1 monoclonal antibody, anti-cyclin D2 rabbit polyclonal antibody, anti-cyclin D3 rabbit polyclonal antibody, anti-β-Tubulin monoclonal antibody, anti-β-actin monoclonal antibody, anti-Lamin B rabbit polyclonal antibody, anti-phospho-pRb rabbit polyclonal antibody and anti-caspase-3 rabbit polyclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Upstate Inc.