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Molecular effects of the phosphatidylinositol-3-kinase inhibitor NVP-BKM120 on T and B-cell acute lymphoblastic leukaemia

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Abstract ***Background:*** Constitutive activation of the PI3K pathway in T cell acute lymphoblastic leukaemia (T-ALL) has been reported and in a mouse model, PI3K activation, together with MYC, cooperates in Burkitt lymphoma (BL) pathogenesis. We investigated the effects of NVP-BKM120, a potent pan-class I PI3K inhibitor, in lymphoblastic leukaemia cell lines.
Methods: Effects of NVP-BKM120 on cell viability, clonogenicity, apoptosis, cell cycle, cell signalling and autophagy were assessed *in vitro* on T-ALL (Jurkat and MOLT-4) and BL (Daudi and NAMALWA) cell lines.
Results: NVP-BKM120 treatment decreased cell viability and clonogenic growth in all tested cells. Moreover, the drug arrested cell cycling in association with a decrease in Cyclin B1 protein levels, and increased apoptosis. Immunoblotting analysis of cells treated with the drug revealed decreased phosphorylation, in a dose-dependent manner, of AKT, mTOR, P70S6K and 4EBP1, with stable total protein levels. Additionally, we observed a dose-dependent decrease in BAD phosphorylation, in association with augmented

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BAX:BCL2 ratio. Quantification of autophagy showed a dose-dependent increase in acidic vesicular organelles in all cells tested.

Conclusion: In summary, our present study establishes that NVP-BKM120 presents an effective antitumour activity against T-ALL and BL cell lines.

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1. Introduction

PI3K/AKT/mTOR pathway controls most hallmarks of cancer, including cell growth, survival and metabolism [1,2]. In haematologic malignancies, constitutive PI3K activation is associated with oncogenesis and disease progression [3,4]. In particular, in T-cell acute lymphoblastic leukaemia (T-ALL), PI3K/AKT/mTOR pathway is frequently activated, which can contribute to cell proliferation, survival and drug resistance [5–7]. Despite recent advances in chemotherapeutic protocols, both adult and paediatric patients with relapsed or refractory disease have a very poor prognosis [8,9]. Therefore, new therapeutic approaches are necessary for these patients.

Burkitt lymphoma (BL) is an aggressive form of non-Hodgkin lymphoma and the translocation of the oncogene MYC is the hallmark of this disease [10–12]. However, PI3K signalling has emerged as an essential pro-survival mechanism in BL [13,14]. Although intensive chemotherapeutic regimens can cure many patients with BL, less toxic yet effective therapies are desired especially for elderly patients and those in developing countries [10,15].

NVP-BKM120 is an orally bioavailable 2,6-dimorpholino pyrimidine derivative, and is considered to be a highly selective pan-class I PI3K inhibitor [16]. In preclinical studies, it has been shown to suppress cell growth and induces apoptosis in a variety of malignancies. It is currently being investigated in phase I/II/III clinical trials, mainly in advanced solid tumours (clinicaltrials.gov). Herein, we investigated the anti-leukaemic activity and molecular mechanisms of NVP-BKM120 on T-ALL and BL cells.

2. Materials and methods

2.1. Cell culture and chemical reagents

T-ALL cells lines, Jurkat and MOLT-4, and BL cell lines, NAMALWA and Daudi, were obtained from ATCC (Philadelphia, PA, United States of America (USA)). Cells were grown in RPMI 1640 medium supplemented with 10% FBS, penicillin/streptomycin. NVP-BKM120 was kindly provided by Novartis (Basel, Switzerland), and was prepared as a 10 mM stock solution in dimethyl sulfoxide (Me_2SO_4; DMSO). Different concentrations of the drug were used as indicated, where cells treated only with DMSO served as a control.

2.2. Cell viability

Cell viability was measured by methylthiazoletetrazolium (MTT) assay. After 16 h of serum starvation, cells were stimulated to reenter the cell cycle and to proliferate using RPMI supplemented with 10% FBS [17]. A total of 5×10^4 cells per well were plated in 96-well plates in RPMI 10% FBS at different concentrations of NVP-BKM120 (1, 10 and 50 μM) for 48 h. In brief, 10 μL of a 5 mg/mL solution of MTT was added to the wells and incubated at 37 °C for 4 h. The reaction was stopped by using 100 μL of 0.1 N HCl in anhydrous isopropanol. Cell growth was evaluated by measuring the absorbance at 570 nm, using an automated plate reader. All conditions were tested in five replicates.

2.3. Colony formation

Cells were submitted to a colony formation assay in the presence or absence of NVP-BKM120 (1, 10 and 50 μM). Colony formation was observed in semisolid methyl cellulose medium (3×10^3 cell/mL; MethoCut 4230; StemCell Technologies Inc). Colonies were detected after 8 days of culture by adding 1 mg/mL of MTT reagent and scored by Image J quantification software (U.S. National Institutes of Health, Bethesda, MD, USA). All conditions were tested in triplicate.

2.4. Annexin V-APC/propidium iodide (PI) staining

Cells were seeded on 24-well plates and treated with different concentrations of NVP-BKM120 (1, 10 and 50 μM) for 6 h. Cells were then washed twice with ice cold PBS and resuspended in binding buffer containing 1 μg/mL APC labelled Annexin-V and 1 μg/mL PI. All specimens were analysed by flow cytometry utilising a FACSCalibur (Becton–Dickinson, CA, USA), after incubation for 15 min at room temperature in a light-protected area. Ten thousand events were acquired for each sample.

2.5. Caspase activity

Caspase activity was measured as recommended by the manufacturer (Calbiochem, CA, USA). Cells were

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