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Comparative effect of two pan-class I PI3K inhibitors used as anticancer drugs on human T cell function

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ABSTRACT

The phosphatidylinositol 3-kinase (PI3K) pathway is commonly deregulated in cancer and, thus, PI3K has been recognized as an attractive molecular target for novel anti-cancer therapies. However, the effect of PI3K inhibitors on T-cell function, a key component of antitumor immunity, has been scantly explored. The objective of this study was to investigate the effect on human T-cell activation of two PI3K inhibitors currently being tested in clinical trials: PX-866 and BKM120. Their activity against a leukemic T cell line was also assessed. For that purpose, Jurkat cells or anti-CD3/anti-CD28 stimulated human peripheral blood mononuclear cells were cultured in the presence of different concentrations of PX-866 or BKM120 and their effect on T-cell proliferation, apoptosis, expression of activation markers and cytokine secretion was analyzed by flow cytometry. In addition, Akt and Erk phosphorylation was analyzed by Western blotting. Both PX-866 and BKM120 decreased viability of Jurkat cells and blocked cell cycle progression. Regarding primary T cells, both compounds similarly inhibited expression of activation markers and cytokine secretion, although they did not induce apoptosis of stimulated T cells. Interestingly, we found differences in their ability to block T-cell proliferation and IL-2 secretion, exerting BKM120 a more potent inhibition. These disparate effects could be related to differences observed in PI3K/Akt and RAS/MEK/ERK signaling between PX-866 and BKM120 treated cells. Our results suggest that, when selecting a PI3K inhibitor for cancer therapy, immunosuppressive characteristics should be taken into account in order to minimize detrimental effects on immune function.

1. Introduction

Phosphatidylinositol-3-kinases (PI3K) are a family of enzymes that act downstream of cell surface receptors, leading to activation of multiple signaling pathways, including the Akt–mTOR axis, that regulate cell growth, proliferation, differentiation, metabolism, motility and survival.

The PI3K family comprises eight catalytic isoforms subdivided into three classes. The class I subset includes four p110 catalytic subunits, which have been further subdivided into class IA (p110α, p110β, and p110δ) and IB (p110γ), depending on whether they associate with a p85/p55/p50 (class IA) or with a p101 or p84/p87 (class IB) regulatory subunit. In mammals, the four class I PI3K isoforms are present in all cell types, with p110δ and p110γ highly enriched in leukocytes [1].

Deregulation of PI3K signaling is frequently involved in cancer. PIK3CA, the gene encoding p110α, is mutated in multiple tumor types [2–8]. In addition, loss of the tumor suppressor PTEN (phosphatase and tensin homologue), which negatively regulates PI3K signaling, is also found in different neoplastic diseases [9–13]. Mutations of the p85 regulatory subunit are also oncogenic [2,14]. Finally, upstream growth factor receptors with increased activity in some cancers, such as EGF receptor, activate downstream PI3K signaling [15]. Thus, PI3K represents an attractive target for therapeutic intervention [16] and antitumor activity of several PI3K inhibitors is being evaluated in clinical trials. However, the central role of PI3K signaling in diverse biologic processes raises concerns about its use in therapeutics and efforts must be made to define the effect of PI3K inhibitors in different cell types, in order to achieve the optimal response minimizing off-target effects. In particular, it is essential to gain knowledge about their effect on immune cells, as it would be desirable to preserve patient's immunity and immunosurveillance. In this regard, very few studies have analyzed the impact of PI3K inhibitors on human T cells and the scant published data have been mainly obtained using the non PI3K-selective inhibitors wortmannin and LY294002 [17] or p110δ-selective inhibitors [18,19]. For that reason, the aim of this study was to analyze the effect on human primary T cells of two pan-class I PI3K selective inhibitors that are currently being evaluated in clinical trials: the irreversible inhibitor PX-866 [20] and the reversible inhibitor BKM120 [21,22]. Both ATP-competitive inhibitors of PI3K have shown antitumor efficacy *in vitro*

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and in *in vivo* models [23–32] and their safety in completed clinical trials [33,34]. In the present study we have assessed the anti-tumor activity of PX-866 and BKM120 on a human leukemic T cell line and evaluated their effect on normal primary T cell activation, proliferation and survival.

2. Material and methods

2.1. Materials

The pan class I PI3K inhibitor PX-866 was purchased from Active Biochemicals (Hong Kong, China) and the pan class I inhibitor BKM120 was purchased from Selleck Chemicals (Houston, TX). Table 1 shows IC50 of these drugs for p110 isoforms. Both compounds were dissolved in DMSO and stored at −80 °C until use.

2.2. Cells and cell culture

Jurkat human T-ALL cell line was grown in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (all from GIBCO, Grand Island, NY).

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of volunteer healthy donors by density gradient centrifugation using Ficoll-Paque solution (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Buffy coats were provided by the Centro de Hemodonación de Castilla y León (Chemcyl). For Western blot analysis, the cells were allowed to adhere to the tissue culture dish (Becton Dickinson, Franklin Lakes, NJ) O/N at 37 °C. Non adherent cells, referred to as T-cell enriched PBMCs, were collected, washed and re-suspended in culture medium. For the cell cycle, apoptosis and cytokine assays, T cells were purified with the Automacs™ Separator, using the Pan T Cell Isolation Kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's instructions. The purity of isolated populations was routinely >95%.

PBMCs or isolated T cells were cultured at a density of 10^6/ml in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (GIBCO) and 10% human AB serum (Sigma, St. Louis, MO).

For T lymphocyte stimulation, plate-bound anti-CD3 (5 μg/ml) and soluble anti-CD28 (2.5 μg/ml) (both from BD Biosciences) were added to cultures.

2.3. Jurkat proliferation/viability assays

The effect of PI3K inhibitors on proliferation/viability of Jurkat cells was assessed by measuring the 3-(4.5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye absorbance of the cells. For that purpose, 2.5×10^4 cells/100 μl were plated in triplicate into 96-well tissue culture dishes in culture medium with increasing doses of the drugs (from 1 nM to 20 μM). After 24 h, 0.5 mg/ml MTT was added and the cells were incubated for 2 h at 37 °C. Then, 100 μl of 10% SDS (Merck Millipore, Darmstadt, Alemania) — 0.01 N HCl (Panreac, Barcelona, Spain) were added. The plate was shaken for 24 h in the dark and absorbance at 570 nM was measured in a Tecan Ultra Evolution detection platform (TECAN, Männedorff, Switzerland).

Table 1
Reported IC50 of PX-866 (Hong DS, Clin Cancer Res 2012) and BKM120 (www.selleckchem.com) for the different p110 isoforms.

	p110α	p110β	P110δ	p110γ
PX-866	39 nM	88 nM	124 nM	183 nM
BKM-120	52 nM	166 nM	116 nM	262 nM

2.4. Primary T cell proliferation assays

T cell proliferation was analyzed by flow cytometry. Briefly, PBMCs were stained with PKH-67 green fluorescent dye (SIGMA). 2×10^5 unstimulated or anti-CD3/anti-CD28 stimulated PKH-stained cells were seeded in 96-well plates with different concentrations of the drugs (1, 2.5, 5, 10 and 20 μM). After five days the cells were collected, stained with CD25-PE, 7-amino-actinomycin D (7AAD) and anti-CD3 APC (all from BD Biosciences, San Jose, CA) and acquired on a FACSCalibur flow cytometer (BD Biosciences). Percentage of proliferating T cells (PKH^{low}) and $CD25^+$ cells was calculated using the Infinicyt software (Cytognos, Salamanca, Spain).

2.5. Cell cycle analysis

Jurkat cells or isolated primary T cells were cultured for 24 h or four days, respectively, in the presence of different concentrations of the compounds (0 to 20 μM). Then, the cells were stained with propidium iodide, using the kit Cycletest (BD Biosciences). Samples were acquired on a FACSCalibur flow cytometer. The distribution of the cells along the cell-cycle phases was analyzed using Infinicyt software program (Cytognos, Spain).

2.6. Apoptosis assessment

After 24 h (Jurkat cells) or two days (isolated primary T cells) of culture in the presence of different concentrations of the compounds (0 to 20 μM), the cells were stained with Annexin V-PE using the PE Annexin V Apoptosis Detection Kit I (BD Biosciences) following manufacturer's instructions. Additionally, anti-CD25 FITC antibody (BD Biosciences) was added to primary T cell samples to analyze the percentage of $CD25^+$ cells. Samples were acquired on a FACSCalibur flow cytometer. The percentage of annexin V and $CD25^+$ lymphocytes was calculated using the software Infinicyt (Cytognos).

2.7. Immunophenotypic analysis

5×10^5 unstimulated or anti-CD3/anti-CD28 stimulated PBMCs/well were seeded in 48-well plates and different concentrations of the drugs were added (0, 1, 2.5, 5, 10 and 20 μM). After 48 h, brefeldin A (10 μg/ml) was added for the last 4 h prior to acquisition. Then, the cells were stained with anti-CD69 FITC/anti-Granzyme B PE/7AAD/anti-CD3 APC (all from BD Biosciences). For intracellular cytokine staining of Granzyme B, the IntraStain kit (Dako Cytomation, Denmark) was used, following manufacturer's recommendations. For CD45RA expression analysis, cells cultured for five days were stained with anti-CD45RA FITC/7AAD/anti-CD3 APC (all from BD Biosciences). Data acquisition was performed on a FACSCalibur flow cytometer using the CellQuest software program (BD Biosciences) and analyzed using the Infinicyt software (Cytognos). Analysis of CD25 expression has been described in the Primary T cell proliferation assays and Apoptosis assessment sections.

2.8. Cytokine assays

Isolated T cells were cultured for 48 h in the presence of several concentrations of PI3K inhibitors (0, 0.1, 0.5, 1, 2.5, 5, 10 and 20 μM). Then, concentration of different cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ) was measured using the Human Th1/Th2 Cytokine Cytometric Bead Array (CBA) kit (BD Biosciences), according to manufacturer's instructions. Samples were acquired on a FACSCalibur flow cytometer and analyzed using BD CBA software.