The side population of T-cell acute lymphoblastic leukemia cells is sensitive to modulators of PI3K/AKT/mTOR signaling

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The side population (SP), which overexpresses ABC-family membrane transporters including ABCG2 (also referred to as Breast Cancer Resistance Protein or BRCP), is thought to be enriched in cancer stem cells (CSC). SP cells are characterized by the capacity of extruding the Hoechst 33342 dye. The difficulty in eradicating tumors might be due to the fact that conventional treatments target the bulk of the tumor cells leaving behind the CSCs. Since CSCs may be responsible for aggressive behaviour of certain tumors as well as their sustained growth, strategies that target these cells will have significant clinical implications. So far the SP of T-cell acute lymphoblastic leukemia (T-ALL) has not been identified. Here, we have investigated the possible existence of SP cells in T-ALL cell lines and patients. In addition, we have studied if SP cells were sensitive to modulators of the PI3K/Akt/mTOR pathway. SP cells were analyzed using a Cell Lab Quanta SC flow cytometer equipped with an UV lamp and a 488 solid state laser. The Hoechst 33342 dye was excited at 366 nm. SP cells were gated on the FL1/FL3 histogram, while ABCG2 staining was evaluated on the FL2 channel. Hoechst staining specificity was demonstrated by the use of ABC-family transporter blocking agents (verapamil, fumitremorgin C, Ko143). The analysis documented the existence of cells displaying SP features (2.7-7.5%). Flow cytometric analysis demonstrated that the T-ALL SP expresses high levels of ABCG2, whereas the expression of two other ABC-family member transporters, P-gp or MRP1, was not detected. SP cells disappeared in samples treated with BEZ-235 (a dual inhibitor of PI3K/mTOR) or rapamycin (an mTORC1 inhibitor). Our findings indicate that SP cells exist also in T-ALL and are sensitive to PI3K/Akt/mTOR inhibition. Taken together, our preclinical findings strongly suggest that modulators of PI3K/AKT/mTOR could be valuable compounds for treating T-ALL because of their capability of targeting a population enriched in CSCs.

Key words
PI3K/AKT/mTOR signaling pathway, side population, T-ALL