Figure 1: The role of SHIP1 in inositol lipid metabolism. PI3 kinase (PISK) catalyzes the conversion of PI(4,5)P2 to PI(3,4,5)P3 to promote signal relay via the plasma membrane recruitment of intermediates such as AKT, BTK and PLCγ2. SHIP1 reduces the plasma membrane by dephosphorylating levels of PI3, SHIP1 also drives accumulation of PI(3,4,5)P3 which can act as an inhibitory second messenger in B cells via adaptors such as TAPP2.

In this work we investigated the potential therapeutic effects of AK2-CS, a novel PI3 phosphatase-directed chemical inhibitor of SHIP1.1 focusing on chronic lymphocytic leukemia (CLL) where activation of PI3Ks downstream of the cell surface B-cell receptor (BCR) plays a central role in malignant cell proliferation and survival.3 We investigated effects of SHIP1 inhibition on PI3-mediated signaling and survival, and also on expression of the chemokine receptor CXCR4 which plays an important role in homing of CLL cells to supportive tissue microenvironments.

Figure 2: SHIP1 is constitutively expressed and phosphorylated in primary CLL blood samples. Immunoblot analysis of total and phosphorylated (Y1020) SHIP1 in (n) unstimulated CLL blood samples (n=12) following in vitro stimulation using soluble (Sol) or immobilized (Imm) anti-IgM to induce weak or strong BCR signal responses, respectively (n=12; representative sample shown).

Figure 3. Anti-IgM induces membrane relocalisation of phospho-SHIP1 and its association with surface IgM. (a) Immunofluorescence imaging of phospho-SHIP1 in 2 representative CLL samples with or without soluble anti-IgM (30 minutes). (b) Control microscopy demonstrating co-localisation of IgM and phospho-SHIP1 in anti-IgM treated CLL cells. (c) Reduced association between phospho-SHIP1 and IgM was confirmed using co-immunoprecipitation. Here, cells were treated with immobilised anti-IgM or control (immobilized antibody and incubated at 4°C or 37°C (to see signal) for the indicated hours. Effects were followed and bound heavy-chain (μHC) and associated phospho-SHIP1 and phospho-SYK were analysed by immunoblotting. Representative of two experiments.

Figure 4. The SHIP1 activator AK2-CS effectively inhibits anti-IgM-induced, PI3P-mediated signalling responses in CLL samples. CLL samples were pre-treated with the indicated concentrations of AK2-CS and respective SHIP1 activator AK2-CS (0 μM) and then stimulated with immobilised anti-IgM. Data show effect on (a) AKT phosphorylation at 30 min, (b) ERK1/2 phosphorylation at 30 and 180 min), and (c) phospho-p70S6K and MYC expression (both at 180 min). Results show representative immunoblots and quantification from multiple samples (n=9-11). Effects of the PI3K inhibitor ICG-001 are shown in (a) as a comparator. Statistical significance of differences are shown (Student’s t-test).

Figure 5. AK2-CS induces caspase-dependent apoptosis in CLL cells and overcomes pre-existing inhibitory effects of BCR signalling and microenvironmental stimuli. (a) CLL samples were pre-treated with AK2-CS for 1 hour and then stimulated with immobilized anti-IgM or a control antibody (n=12) (b) CLL samples were pre-treated with CD40L, 40 μM (to mimic tissue-based supporting factors) for 1 hour and then treated with AK2-CS or DMSO as a control for an additional 16 hours (n=10). (c) Representative sample showing that the caspase inhibitor Z-DEVD-FMK (10 μM) effectively suppresses AK2-CS-induced apoptosis (24 hours). (d) Comparison of effects of treatment with AK2-CS for 24 hours on viability of AK2-CS resistant and nonresistant blood CD19+ B cells from healthy donors (n=8). In all experiments, viability of CD19+CD21+ CLL cells was determined by annexin V/PI staining and flow cytometry. Statistical significance of differences are shown (Student’s t-test). (d) mean viability of control cells was set to 100.

Figure 6. AK2-CS induces apoptosis of ABC-OLBCL cell lines whose growth in vitro is dependent on chronic BCR signalling. 4 Effect of AK2-CS treatment for 72 hours on viability (annexin V/PI staining) of RPMI-8226, Daudi-10 and RPMI-8226 (n=4) repeat experiments for each cell line. Viability of control cultures at the end of the experiment was set to 100.

Figure 7. AK2-CS inhibits chemotaxis of CLL cells. (a) CLL samples (n=12) were treated with immobilized anti-IgM, AK2-CS or solvent. After 6 hours CHROM expression on 6292/CD38+ CLL cells was quantified by flow cytometry. (b) Similar experiments were performed to quantify effects of AK2-CS on CHROM expression on normal CD19+ blood cells from healthy donors (n=4). Statistical differences between groups are shown (Student’s t-test).

References

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