Chemical Activation of the SHIP1 Inositol Lipid Phosphatase: A Novel Therapeutic Strategy to Suppress B-Cell Receptor Signaling and CXCR4 Expression in Malignant Human B Cells

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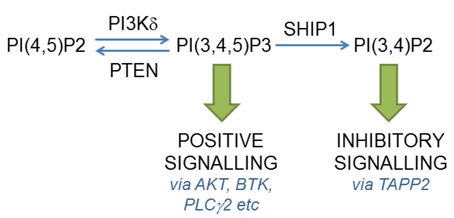

Figure 1: The role of SHIP1 in inositol lipid metabolism. PI3 kinase (PI3K) catalyzes the conversion of PI(4,5)P2 to PI(3,4,5)P3 to promote 'positive' signaling via the plasma membrane recruitment of intermediates such as AKT, BTK and PLCγ2. SHIP1 reduces this positive signaling by decreasing levels of PIP3. SHIP1 also drives accumulation of PI(3,4)P2 which can act as an inhibitory second messenger in B cells via adaptors such as TAPP2.[1]

In this work we investigated the potential therapeutic effects of AQX-C5, a novel pelorol-based chemical activator of SHIP1,[3] focusing on chronic lymphocytic leukemia (CLL) where activation of PI3Kδ downstream of the cell surface B-cell receptor (BCR) plays a central role in malignant cell proliferation and survival.[2] We investigated effects of SHIP1 activation on PIP3-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.[4]

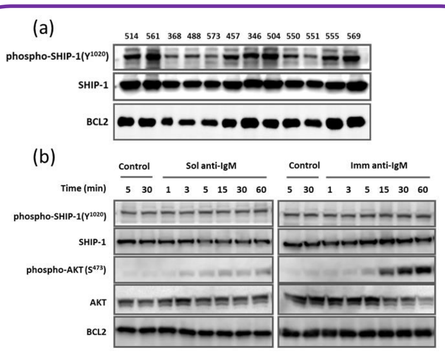

Figure 2. SHIP1 is constitutively expressed and phosphorylated in primary CLL blood samples. Immunoblot analysis of total and phosphorylated (Y1020) SHIP1 in (a) unstimulated CLL blood samples (n=12) and (b) following *in vitro* stimulation using soluble (Sol) or immobilised (Imm) anti-IgM to induce weak or strong BCR signal responses, respectively (n=13; representative sample shown).

References

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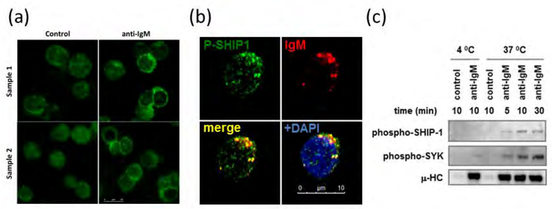

Figure 3. Anti-IgM induces membrane relocalisation of phospho-SHIP1 and its association with surface IgM. (a) Immunofluorescent imaging of phospho-SHIP1 in 2 representative CLL samples with or without soluble anti-IgM (30 minutes). (b) Confocal microscopy demonstrating co-localisation of IgM and phospho-SHIP1 in anti-IgM treated CLL cells. (c) Induced association between phospho-SHIP-1 and sIgM was confirmed using co-immunoprecipitation. Here, cells were treated with immobilised anti-IgM, or control immobilised antibody and incubated at 4°C (control) or at 37°C (to initiate signalling) for the indicated times. Beads were collected and bound heavy-chain (μ-HC) and associated phospho-SHIP1 and phospho-SYK were analyzed by immunoblotting. Representative of two experiments.

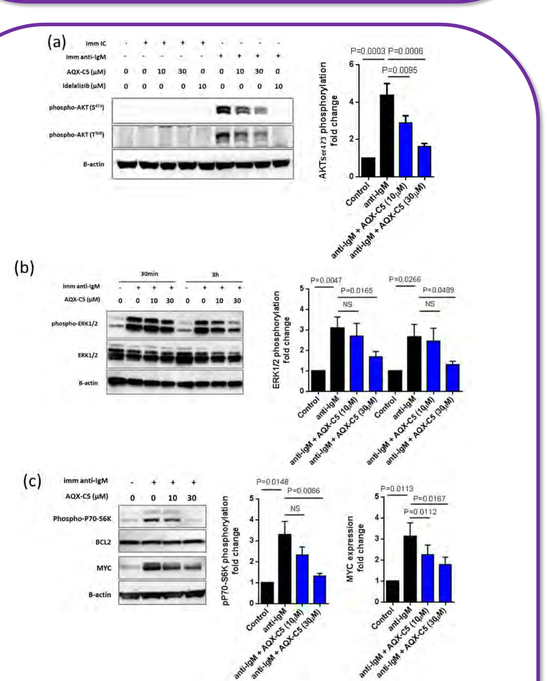

Figure 4. The SHIP1 activator AQX-C5 effectively inhibits anti-IgM-induced, PIP3-mediated signalling responses in CLL samples. CLL samples were pre-treated with the indicated concentrations of the small molecule SHIP1 activator AQX-C5 and then stimulated with immobilised anti-IgM. Data show effect on (a) AKT phosphorylation at 30 min, (b) ERK1/2 phosphorylation (at 30 and 180 min), and (c) phospho-p70-S6K and MYC expression (both at 180 min). Results show representative immunoblots and quantitation from multiple samples (n=9-11). Effects of the PI3Kδ inhibitor idelalisib are shown in (a) as a comparator. Statistical significance of differences are shown (Student's t-test).

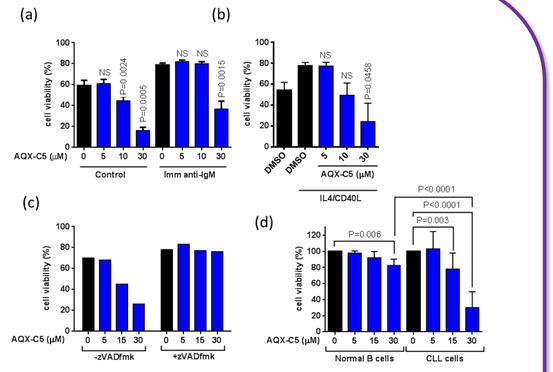

Figure 5. AQX-C5 induces caspase-dependent apoptosis in CLL cells and overcomes pro-survival promoting effects of BCR signalling and microenvironmental stimuli. (a) CLL samples were pre-treated with AQX-C5 for 1 hour and then stimulated with immobilized anti-IgM or a control antibody (n=12). (b) CLL samples were pre-treated with CD40L + IL4 (to mimic tissue-based supporting factors) for 6 hours and then treated with AQX-C5 or DMSO as a control for an additional 16 hours (n=4). (c) Representative sample showing that the caspase inhibitor ZVADfmk (100 μM) effectively suppresses AQX-C5-induced apoptosis (24 hours). (d) Comparison of effects of treatment with AQX-C5 for 24 hours on viability of CLL cells (n=12) and non-malignant blood CD19+ B cells from healthy donors (n=5). In all experiments, viability of CD19+CD5+ CLL cells was determined by annexin V/PI staining and flow cytometry. Statistical significance of differences are shown (Student's t-test). In (d) mean viability of control cells was set to 100%.

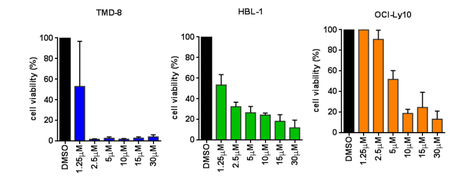

Figure 6. AQX-C5 induces apoptosis of ABC-DLBCL cell lines whose growth *in vitro* is dependent on chronic BCR signalling.[5] Effect of AQX-C5 treatment for 72 hours on viability (annexin V/PI staining) of HBL-1, OCI-Ly-10 and TMD-8 cells (n=3 repeat experiments for each cell line). Viability of control cultures at the end of the experiment was set to 100%.

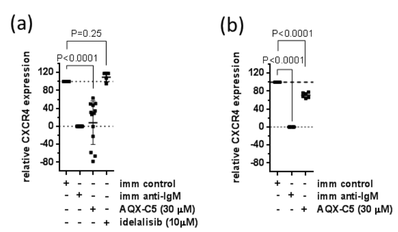

Figure 7. AQX-C5 triggers down-modulation of CXCR4. (a) CLL samples (n=13) were treated with immobilised anti-IgM, AQX-C5 or idelalisib. After 6 hours CXCR4 expression on CD19+CD5+ CLL cells was quantified by flow cytometry. (b) Similar experiments were performed to quantify effects of AQX-C5 on CXCR4 expression on normal CD19+ blood cells from healthy donors (n=6). Statistical differences between groups are shown (Student's t-test).

CONCLUSIONS

- SHIP1 is constitutively expressed and phosphorylated in primary CLL cells and is recruited to surface IgM complexes following B-cell receptor activation
- Chemical activation of SHIP1 is sufficient to effectively suppress PIP3-mediated signalling responses, including induction of the MYC oncoprotein
- SHIP1 activation promotes apoptosis of CLL and ABC-DLBCL cells
- SHIP1 activation overcomes protective effects of BCR stimulation and microenvironmental stimuli in CLL cells
- In contrast to the direct PI3Kδ inhibitor idelalisib, SHIP1 activation results in CXCR4 down-modulation on CLL cells, especially compared to normal B cells. We speculate that CXCR4 down-modulation may we a consequence of SHIP1-mediated PI(3,4)P2 accumulation rather than decreased PI(3,4,5)P3 *per se*

Chemical SHIP1 activation is an attractive therapeutic approach for CLL and other BCR driven cancers via inhibition of PIP3-mediated signalling. In addition, SHIP1 activation results in downmodulation of the key tissue homing receptor CXCR4, not observed with direct PI3Kδ inhibition.

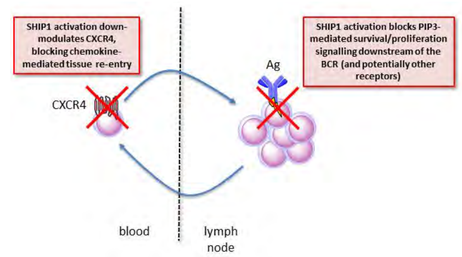

Figure 8. Potential dual-edged therapeutic effects of SHIP1 activators against CLL

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Disclosures

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