Mantle cell lymphoma (MCL) is a rare subtype of aggressive B-cell non-Hodgkin lymphoma that is incurable with standard therapy. Overexpression of B-cell receptor signaling through Bruton’s tyrosine kinase (BTK) is a hallmark of MCL (Pal Singh et al., 2018). Inactivation of BTK signaling with the small molecule inhibitor ibrutinib is currently the most broadly used treatment for B-cell lymphoma. However, ibrutinib only induces a minimal degree of B-cell apoptosis in vitro at clinically achievable concentrations. Frequently, primary and acquired resistance to ibrutinib is observed (Chiron et al., 2014; Wang et al., 2013). One of the molecular mechanisms of acquired resistance is the development of BTK mutations (Martin et al., 2016). In addition, the tumor microenvironment (TME), in which mesenchymal stroma cells (MSC) and vascular endothelial cells (ECs) are specialized components, has increasingly been recognized as a central determinant of drug resistance, subclonal evolution, and late progression/ transformation of B-cell lymphomas (Balsal et al., 2017; Weiss and Chereshe, 2011). Although the pro-tumoral ecosystem that supports MCL is still poorly understood, it has been reported that MCL cells express high levels of functional CXCR4 and CXCR5 chemokine receptors, and VLA-4 adhesion molecules (Kurtova et al., 2009). Lymphoma cells also display high levels of CD44, one of the E-selectin ligands, in co-culture with ECs (Cao et al., 2014). These findings strongly suggest an association between acquired BTK mutations and the TME-mediated resistance in BTK-targeted therapy of MCL. Therefore, we hypothesized that the disruption of crosstalk between MCL cells and the TME (i.e., by blocking CXCR4/CXCL12 or E-selectin/CD44) could enhance BTK-targeted therapy against MCL.

Materials and Methods

Drugs & Cell Lines: Multi-kinase inhibitor CG-10 µM was provided by Apteose Sciences. E-selectin antagonist was provided by GlycoMimetics Inc.; BTK inhibitor (ibrutinib), U101 kinase inhibitor SB-0206965 and putative autophagy inhibitor Chloroquine (CQ) were purchased from Selleckchem. Mantle cell lymphoma cell lines Jeko-1, JVM2, and HUVEC endothelial cells were from ATCC. MSC were derived from normal bone marrow donors. IC50 and EC50: The 50% inhibitory concentration (IC50) for cell growth inhibition (using Trypan blue dye exclusion method) and the 50% effective concentration (EC50) for apoptosis induction (using FACS for measuring annexin V positivity) were calculated using CalcuSyn (Biosoft, Cambridge, UK). Immunoblot Assays: Cells were treated with indicated concentrations of drugs and collected for immunoblot analysis. Flow cytometry: MCL cells were exposed in CG-806 for 24 h and E-selectin and CXCR4 levels were measured with FACS Calibrus using the Cell Quest program.

Abstract:

MCL targets BTK by activating the downstream STAT and PI3K-Akt signaling pathways, which provide an important mechanism of resistance to anti-MCL drugs (CG-10 µM). The study shows that CQ blockade of autophagy with CQ sensitizes MCL cells to CG-806-induced apoptosis. Overall, these findings suggest that blockade of autophagy with CQ could improve the efficacy of anti-MCL therapy.

Conclusions

- CG-806 exerts potent cell growth inhibitory effects in ibrutinib-resistant MCL cells.
- CG-806 increases autophagy in MCL cells, which may be associated with resistance to CG-806-mediated apoptosis. Inhibition of autophagy re-sensitizes MCL cells to CG-806-induced apoptosis.
- CG-806 treatment upregulates CXCR4/E-selectin levels in MCL cells.
- The TME (i.e., MSC and HUVEC cells) protects MCL cells from CG-806-induced apoptosis, which is partially abrogated by CXCR4/E-selectin antagonists to enhance CG-806-induced MCL cell killing.