Background

Waldenstrom’s macroglobulinemia (WM) is a distinct B-cell lymphoma resulting from the accumulation, predominantly in the bone marrow, of clonally related IgM secreting lymphoplasmacytic cells (LCs). MYD88 L265P is a somatic mutation present in more than 90% of Waldenstrom’s Macroglobulinemia (WM) patients. The MYD88 L265P mutant was reported to promote malignant cells growth and survival in ABC type Diffuse Large B-cell Lymphoma (DLBCL) (Ngo et al, Nature 2011) and WM (Yang et al, Blood 2013). The MYD88 L265P mutant assembled a signaling complex that simultaneously triggers IRAK1 and BTK, leading to downstream NF-κB activation in supporting WM cells growth and survival (Treon et al, NEJM 2012; Yang et al, Blood 2013). In addition to IRAK1 and BTK, the downstream signaling pathways remain to be fully clarified.

Results

Modulation of MYD88 affected downstream signaling proteins that involved in canonical BCR, PI3K / AKT and ERK / MAPK signaling in MYD88-L265P expressing WM cells.

Arrays were scanned by Axon GenePix Microarray Scanner and data analyzed by Ingenuity Pathway Analysis. Western blot analysis was performed using total and phospho-specific antibodies in WM cells. CellTiter-Glo® Luminiscant cell viability assay (Promega) was used to assess cell survival following treatment with the PI3K-delta inhibitor, CAL-101 (Idelalisib, GS-1101) (Selleck Chemicals).

Methods

To further clarify the downstream signaling associated with MYD88 L265P in WM cells, we employed Phospho Explorer Antibody Arrays in MYD88 L265P expressing BCWM.1 and MCWL-1 WM cells following lentiviral mediated knockdown of MYD88, or over-expression of MYD88 L265P; or the use of MYD88 homodimerization inhibitor that block MYD88 signaling.

Conclusion

In addition to activation of NF-κB through IRAK and BTK signaling, MYD88 L265P also promotes enhanced survival of WM cells also by activation of PI3K/AKT signaling. Inhibition of PI3K is associated with robust killing of WM cells. The combination of PI3Kδ inhibitor, CAL-101, with the BTK inhibitor, Ibrutinib resulted in synergistic WM tumor cell killing. These studies provide the framework for the investigation of PI3Kδ inhibitors, alone and in combination with Ibrutinib in WM.