BTK participates in MYD88 signaling in malignant cells expressing the L265P mutation in Waldenstrom’s Macroglobulinemia, and shows robust tumor cell killing with the BTK-inhibitor PCI-32765 in combination with MYD88 pathway inhibitors


Bing Center for Waldenstrom’s Macroglobulinemia, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA

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 (LPCs). Bruton’s tyrosine kinase (BTK) promotes B-cell receptor signaling along with B-cell expansion and survival through NF-κB and MAPK. MYD88 L265P is a widely expressed somatic mutation in tumor cells from WM patients. MYD88 L265P promotes enhanced tumor cell survival through IRAK 1/4 mediated NF-κB and MAPK signaling. We therefore sought to clarify the role of BTK signaling in MYD88 L265P expressing WM cells, and the impact of BTK and MYD88/IRAK inhibition in WM cell signaling and survival.

Results

BTK was highly expressed and phosphorylated in MYD88-L265P expressing WM cells and PCI-32765 significantly blocked the BTK activation.

Increased phosphorylation of BTK was confirmed by western blotting with phospho-specific antibody in Waldenstrom’s Macroglobulinemia (WM) cell lines, BCWM.1 and MWCL-1, compared to multiple myeloma cell lines, ANBLL and INAH. Antibody against total BTK was used as loading control. PCI-32765 significantly blocked the BTK phosphorylation in WM cells.

PCI32765 significantly reduced downstream NF-κB, MAPK and STAT3 signaling in WM cells.

Knockdown of MYD88 by lentiviral transduction, and/or use of a MYD88 inhibitor leads to decreased BTK phosphorylation.

Increased phosphorylation of BTK was confirmed by western blotting with phospho-specific antibody in Waldenstrom’s Macroglobulinemia (WM) cell lines, BCWM.1 and MWCL-1, compared to multiple myeloma cell lines, ANBLL and INAH. Antibody against total BTK was used as loading control. PCI-32765 significantly blocked the BTK phosphorylation in WM cells.

Patients and Methods

Western blot analysis was performed using total and phospho-specific antibodies in MYD88 L265P expressing WM cells, BCWM.1 and MCWL-1 following MYD88 knockdown by lentiviral transduction, and/or use of MYD88 or IRAK signal inhibitors. Cells were also treated with the BTK inhibitor PCI-32765, in the presence or absence of MYD88 homodimerization or IRAK1/4 inhibitors. Annexin V / PI staining was used to assess cell survival, and synergism assessed with CalcuSyn software.

Conclusion

BTK activation is facilitated by MYD88 pathway signaling in MYD88 L265P expressing WM cells, and participates in MYD88 downstream signaling. Inhibition of BTK by PCI-32765 leads to robust tumor killing of MYD88 L265P expressing WM cells, which is potentiated by MYD88 pathway inhibitors. These studies provide the framework for the investigation of BTK inhibitors in WM, as single agents and in combination with MYD88 pathway inhibitors.

PCI-32765 shows synergistic tumor cell killing in combination with an IRAK 1/4 kinase inhibitor.

BTK activation is facilitated by MYD88 pathway signaling in MYD88 L265P expressing WM cells, and participates in MYD88 downstream signaling. Inhibition of BTK by PCI-32765 leads to robust tumor killing of MYD88 L265P expressing WM cells, which is potentiated by MYD88 pathway inhibitors. These studies provide the framework for the investigation of BTK inhibitors in WM, as single agents and in combination with MYD88 pathway inhibitors.