Abstract

Non-Hodgkin's lymphomas (NHL) are a group of heterogenous lymphoproliferative malignancies originating from B/T or NK cells. The deregulation of the phosphatidyl-inositol-3-kinase–protein kinase B/AKT (PI3K-AKT) signaling pathway plays an important role in the pathogenesis, chemoresistance, and progression of B-NHL. The molecular mechanisms leading to the aberrant activation of PI3K-AKT pathway vary in different subtypes of B-NHL.

Mantle cell lymphoma (MCL) is a B cell aggressive non-Hodgkin's lymphoma characterized by the chromosomal translocation (11;14) and the presence of many recurrent molecular and cytogenetic alterations, including gain/amplification of *PIK3CA*, coding for a p110α catalytic subunit of the phosphatidylinositol 3 kinase (PI3K), and loss of phosphatase and tensin homolog (PTEN) function, a negative regulator of PI3K.

The aim of this study was a complex analysis of the effect of the above mentioned *PIK3CA* and *PTEN* gene aberrations on the biology and drug resistance of MCL using (MINO, JEKO-1, UPF1H, UPF19U and Z138) cell lines with transgenic overexpression of *PIK3CA* (*PIK3CA* UP), and knock-out/knock-down of *PTEN* (*PTEN* KO)/ (*PTEN* KD), mimicking total and partial PTEN protein loss. To study the impact of the studied aberrations on the pro-survival signaling from BCR in MCL, cells with an additional knock out of B-cell receptor were derived from *PIK3CA* UP, *PTEN* KO, and *PTEN* KD cell lines. The expression and activity of phosphorylated protein kinase B (AKT) were analyzed using western blotting and Förster resonance energy transfer (FRET). Energy-metabolic pathways were investigated using a Seahorse analyzer and a hypoxic incubator. A panel of different selective PI3K isoform inhibitors, an AKT inhibitor, a Bruton tyrosine kinase (BTK) inhibitor, a glycolysis inhibitor and BH3 mimetics were used to determine sensitivity/resistance of *PIK3CA* UP, *PTEN* KO, and *PTEN* KD cell lines to these treatments. Transgenic over-expression of *PIK3CA* was not associated with appreciable changes in AKT protein phosphorylation or AKT kinase activation. Moreover, the sensitivity of *PIK3CA* UP cell lines to a battery of PI3K inhibitors, or ibrutinib (a BTK inhibitor) remained virtually unchanged. Interestingly, two out of 4 tested cell lines were more resistant to venetoclax (a BCL2 inhibitor), currently used in the treatment of relapsed/refractory (R/R) MCL, and to S63845 (a MCL1 inhibitor). Increased *PIK3CA* expression decreased dependence of the tested MCL cell lines on the pro-survival signaling from BCR, lowered levels of oxidative phosphorylation

(OXPHOS), increased resistance to the inhibitor of glycolysis, 2-deoxy-glucose (2-DG), and increased tumor growth rate in vivo. The precise molecular mechanisms that lay behind these observations remain largely elusive, however, the unchanged activity and phosphorylation status of AKT suggest that *PIK3CA* gain consequences are mediated independently of AKT in MCL. Loss of PTEN was associated with a distinct phenotype with *PTEN* KO cells manifesting hyperphosphorylation and overactivation of AKT protein, increased resistance to the majority of tested PI3K inhibitors, ibrutinib (a BTK inhibitor) and venetoclax (a BCL2 inhibitor), increased sensitivity to BCL-XL inhibitor, increased glycolytic rates associated with increased resistance to 2-DG (a glycolysis inhibitor), increased tumor growth rate in vivo, and remarkably decreased reliance on the pro-survival BCR signaling. Partial PTEN loss had a similar but less pronounced manifestation compared to the total loss of PTEN with *PTEN* KD cells showing overactivation of AKT protein, increased resistance to copanlisib (a pan-PI3K inhibitor), ibrutinib (a BTK inhibitor) and venetoclax (a BCL2 inhibitor), unchanged sensitivity to BCL-XL inhibitor, and increased resistance to 2-DG.

In summary, *PIK3CA* gain and PTEN loss resulted in different phenotypes, however, both attenuate dependence of MCL cells on pro-survival signaling from BCR and increase survival of MCL cells in response to BCL2 inhibition, glycolysis inhibition and under hypoxia.