The Regulation of Apoptosis by Cooperative Src and MAPK Signaling

Introduction: Thyroid cancer is the most common endocrine malignancy with poor survival rates for patients with advanced and aneuploidic thyroid cancer due to lack of effective therapy. While genetic alterations in the MAPK pathway account for the majority of driver mutations expressed in thyroid cancer (BRAF, RAS, RAF, ERBB2), there has been mixed success in targeting this pathway in clinical practice. Here we demonstrate that combined Src and MAPK inhibition results in synergistic inhibition of growth in vitro and in vivo, and increased apoptosis in BRAF- and RAS-mutant cells, while PI3K/Akt/mTOR are resistant. Here we have further dismantled the mechanism(s) of apoptosis regulation by dual Src and MAPK inhibition.

Methods/Case Presentation: Reverse Phase Protein Array (RPPA) was performed on a panel of thyroid cancer cell lines treated with a Src inhibitor and/or MEK1/2 inhibitor. Western blotting was performed using Odyssey Imaging, growth assays were performed using Sulfotitration B (DPI) or CellTiter-Glo 2.0 and apoptosis assays were performed using Caspase-Glo 3/7. IC50 values from CellTiter-Glo were calculated in GraphPad Prism 9 using the nonlinear regression analysis with a variable slope.

Results/Discussion: RPPA identified the pro-apoptotic protein BIM as a key regulator of the apoptotic response. Western blotting showed a 4-fold reduction of BIM in BRAF- and RAS-mutant cells that are sensitive to combined Src and MEK1/2 inhibition when treated with the combination, and only a 1.5- to 3-fold induction of BIM in cells that are resistant. Ecotopic expression of inducible BIM sensitizes a PI3K/Akt/mTOR resistant cell to combined Src and MEK1/2 inhibition. The efficacy of combined Src and MEK1/2 inhibition can be increased through the addition of a BIM mimetic targeting Bcl-2.

Conclusion: In summary, BIM is a key pro-apoptotic protein cooperatively regulated by the Src and the MAPK pathways and is sufficient to induce sensitization to combined Src and MEK1/2 inhibition in a resistant cell. The efficacy of combined Src and MEK1/2 inhibition can be increased through the addition of a BIM mimetic targeting Bcl-2.