

The Regulation of Apoptosis by Cooperative Src and MAPK Signaling M Rose, (Ph.D., GS), LA Pike, VL Espinoza, and RE Schweppe, Department of Endocrinology, University of Colorado Anschutz Medical Campus

Advanced thyroid cancer (TC) patients have poor survival rates due to lack of effective therapies. Genetic alterations in the MAPK pathway account for most driver mutations expressed in TC, but clinically there has been mixed success targeting this pathway. We've shown that combined Src (Srci) and MAPK inhibition (MEKi) results in synergistic inhibition of growth *in vitro* and *in vivo*, and increased apoptosis in *BRAF*- and *RAS*-mutant cells, while *PIK3CA*-mutants are resistant. Here we further delineated the mechanism(s) of apoptotic regulation by dual Srci and MEKi. Reverse Phase Protein Array (RPPA) was performed on a panel of TC cell lines treated with Srci and/or MEKi. Western blotting was performed using Odyssey Imaging, growth and apoptosis assays were performed using Sulforhodamine B or CellTiter-Glo, and Caspase-Glo 3/7 assays. All stats were calculated in GraphPad Prism 9. RPPA identified the pro-apoptotic protein BIM as a key regulator of response. Western blots showed a 6-fold induction of BIM in cells that are sensitive to combined Srci and MEKi, and a  $\leq 3$ -fold induction of BIM in resistant cells. Overexpression of BIM in resistance cells promoted sensitivity to combined Srci and MEKi. A previous study (Sale et al.) showed that low Bcl-xL expression in melanoma compared to high expression of Bcl-xL in pancreatic cancer (PC) predicted sensitivity to an MCL1 or Bcl-xL inhibitor, respectively. We showed that TC cells align with PC, expressing high levels of Bcl-xL and so are sensitive to a Bcl-xL inhibitor. In summary, BIM is a key protein regulated by the Src and the MAPK pathways and is sufficient to induce sensitivity to combined Srci and MEKi in a resistant cell. The efficacy of combined Srci and MEKi can be increased through the addition of a Bcl-xL inhibitor.