Sky Foundation Research Update
Identification of novel signaling nodes for autophagy inhibition
Kirsten L. Bryant, PhD

We proposed to validate PIKFYVE inhibitors as potent and selective inhibitors of autophagy in PDAC and to identify nodes within the autophagic pathway that enhance ERKi-mediated cell death. Thus far, despite capacity constraints due to the COVID-19 pandemic, we have made significant, promising progress on our Aim 1 studies focused on PIKFYVE inhibition and have begun the initial steps of our CRISPR screening proposed in Aim 2.

PIKFYVE is a lipid kinase that converts phosphatidylinositol 3-phosphate to phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$) in the endocytic pathway (Sbrissa et al., 1999). Loss of PIKFYVE results in enlargement of late endosomal/lysosomal vesicles (Ikonomov et al., 2001; Nicot et al., 2006), potentially through the disruption of the balanced process of lysosome fusion and fission (Choy et al., 2018). Indeed, PIKFYVE activity is necessary for the recovery of engulfed nutrients (Krishna et al., 2016) and recently, PIKFYVE inhibitors have been proposed to have therapeutic potential in autophagy-dependent cancer cells (Sharma et al., 2019). Accordingly, we hypothesized that PIKFYVE inhibition would result in a reduction of autophagic flux in KRAS-mutant PDAC cell lines.

We have addressed this question using two complementary methods. Using the PIKFYVE inhibitor apilimod, we treated PDAC cell lines with a dose response of this drug and used immunoblotting to assess levels of well-validated autophagy-related proteins p62 and LC3B. p62 is an autophagosomal cargo protein, and hence, when autophagy is inhibited p62 accumulates. LC3B exists in two proteoforms in the cell, a cytosolic-localized LC3B-1 form and a lapidated, autophagosomal-associated LC3B-II form. The ratio of the abundance of LC3B II / I can be calculated and is an indicator of autophagic flux. When cells were treated with a dose response of apilimod we observed an accumulation of both p62 and LC3B-II, both of which are indicative of autophagy inhibition. As a complementary method to assess autophagic flux we utilized cells expressing the autophagosomal reporter construct mCherry-EGFP-LC3B. When this construct is associated with autophagosomes, both EGFP and mCherry fluorescence is visible. When the autophagosome fuses with a lysosome the EGFP fluorescence is quenched and just mCherry fluorescence is visible. One can quantify the ratio of mCherry to EGFP fluorescence as an indicator of flux. Using this methond, we observed that treatment with a PIKFYVE inhibitor is capable of significantly reducing ERKi-induced autophagic flux in a panel of PDAC cell lines.

Previously, we observed synergistic growth inhibition when PDAC cells were treated with MEKi (binimetinib) or ERKi (SCH772984) in combination with chloroquine (CQ), an indirect inhibitor of autophagy, as evaluated by a number of different 2D growth assays (Bryant et al., 2019). Additionally, we also demonstrated synergistic increased apoptosis when ERKi and CQ are used in combination, whereas each drug causes little to no apoptosis when used as monotherapy (Bryant et al., 2019). We hypothesized that the autophagy pathway can be targeted multiple different ways and CQ could potentially be replaced with a different agent, such as a PIKFYVE inhibitor. Indeed, when we treated a panel of PDAC cell lines with a combination of either a MEKi or ERKi with the PIKFYVE inhibitor apilimod, we observed synergistic growth suppression. Further, when we performed apoptosis assays assessing this combination, we observed that PIKFYVE inhibition substantially increases MEKi-induced apoptosis.

These studies have nicely validated our screening results that indicated that inhibition of PIKFYVE would decrease PDAC cell growth. Our druggable genome library includes sgRNA targeting only 72 autophagy-related genes. Of those genes, 27 (37% hit rate) were depleted in the vehicle treated cells. This result confirms that autophagy is an essential process for PDAC and one which should be studied in more detail. Thus, we are eager to begin studies with our much more comprehensive autophagy-focused CRISPR library. Thus far we have chosen the 570 autophagy-related genes to be included in the library, as well as

100 positive / negative control genes for normalization of our final results. After assembling and packaging this library we will stably express it in our panel of eight PDAC cell lines, each biological replicate will be separated into four technical replicates and treated with vehicle control or a GI_{20} dose of ERKi, and CQ, collecting samples at two and four weeks. Because progress on these large scale, extremely labor intensive studies has been slowed due to occupancy restrictions due to the COVID-19 pandemic, it is unclear whether we will have these studies completed by 5/1/2021; however, I think that it is very feasible to have results by the end of the year.

References

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