ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer with high mortality and limited efficacious therapeutic options. PDAC cells undergo metabolic alterations to survive within a nutrient-depleted tumor microenvironment. One critical metabolic shift in PDAC cells occurs through altered isoform expression of the glycolytic enzyme, pyruvate kinase (PK). Pancreatic cancer cells preferentially switch from the constitutively active pyruvate kinase muscle 1 isoform (PKM1) to the allosterically regulated pyruvate kinase muscle isoform 2 isoform (PKM2). Overexpression of PKM2 in PDAC produces a profound reprogramming of many metabolic pathways including glucose and glutamine metabolism, but little is known about the impact on cysteine metabolism. Cysteine metabolism is critical for supporting survival through its role in defense against ferroptosis, a non-apoptotic iron-dependent form of cell death characterized by unchecked lipid peroxidation. Exploiting this cell death mechanism has enormous potential for treating PDAC cells that are vulnerable to cystine starvation.

To improve our understanding of the metabolic adaptations that cancer cells depend on for survival and proliferation, we generated PKM2 knockout (KO) human PDAC cells. We evaluated PKM2KO cell tolerance of low cystine environments, sensitivity to compounds known to induce ferroptosis, and expression of ferroptosis related proteins. Fascinatingly, PKM2KO cells demonstrate a remarkable resistance to cystine starvation mediated ferroptosis. This response to cystine starvation was found to be caused by decreased PK activity, rather than an isoform specific effect. We further utilized stable isotope tracing to evaluate the impact of glucose and glutamine reprogramming in PKM2KO cells. PKM2KO cells demonstrate a dependence on

glutamine metabolism to support antioxidant defenses against lipid peroxidation. This is attributed primarily to increased glutamine flux through the malate aspartate shuttle and utilization of ME1 to produce NADPH. Lastly, we found that ferroptosis could be synergistically induced by the combination of PKM2 activation with TEPP-46 and cystine starvation with imidazole ketone erastin (IKE) *in vitro*. Preliminary investigations *in vivo* show strong potential for this drug combination as a novel and effective therapy for PDAC.