The Ref-1/APE1 signaling node as a target for precision cancer therapy: Integrating preclinical and clinical bioinformatic data

Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related mortality in the US. Most patients present with advanced disease and ~93% die within five years, with most surviving less than six months. Combination therapies including Gemcitabine (Gemzar™) and sustained release, nab-paclitaxel (Abraxane™) and FOLFIRINOX (5-FU/leucovorin/irinotecan/oxaliplatin) offer modest improvement in survival. Therefore, new therapeutic options are highly needed. Here we investigate a unique target, Apurinic/apyrimidinic endonuclease/redox factor-1 (APE1/Ref-1 or APE1) and the effects of redox-specific APE1 inhibitor, APX3330 on tumor cell growth.

APE1 is a multifunctional protein involved in repairing DNA damage via endonuclease activity and in redox signaling regulation of transcription factors such as HIF-1α, NFκB and STAT3. In PDAC, both tumor and stroma express APE1 with the tumor having significantly elevated levels of APE1 protein. Because APE1 is essential for cell viability, generation of APE1 knockout cell lines and determining a comprehensive list of genes regulated by APE1 has been difficult. To circumvent this, we performed single cell RNA-Sequencing on PDAC cells following APE1 knockdown under normoxia and hypoxia to identify differentially expressed genes and further explore APE1’s effects on its target transcription factors under both conditions. Proteomic analysis on PDAC cells following APE1 knockdown in normoxia and hypoxia revealed changes in signaling downstream of APE1, complementing the transcriptomic data and providing a more complete understanding of pathways affected by APE1.

Ingenuity Pathway Analysis (IPA) identified over 100 pathways affected by APE1 knockdown under both conditions. Interestingly, metabolic pathways such as oxidative phosphorylation and mitochondrial function were among the top 10 pathways affected. The effect of APE1 knockdown on gene expression associated with mitochondrial Complexes I-IV was significantly greater in hypoxia. We validated the scRNA-seq results via qPCR, and further confirmed the effect of APE1 knockdown on mitochondrial health and identified the complexes affected using different biochemical assays. We used the APE1 redox inhibitor, APX3330 to illustrate that disruption of the redox function of APE1 was responsible for mitochondrial dysregulation.

Furthermore, we combined APX3330 and next generation analogs with different mitochondrial inhibitors to test the effect on tumor cell growth. These combinations were tested using an ex vivo 3D tumor-stroma model system using patient-derived tumor cells as well as cancer-associated fibroblasts. We identified synergy with agents such as CPI-613, a dehydrogenase inhibitor targeting the mitochondrial tricarboxylic acid (TCA) cycle. With the phase I clinical trial for APX3330 finishing (NCT03375086), this study highlights the potential for combining APE1 targeted therapy with mitochondrial metabolic inhibitors in order to enhance tumor efficacy.

Additional data will be discussed on identified combinations of APE1 and other inhibitors based on bioinformatic data analysis.