Targeting mitochondrial metabolism in Pancreatic Ductal Adenocarcinoma via blockade of the APE1/Ref-1 signaling cascade: using single-cell RNA sequencing to identify novel targets for combination drug therapies

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related mortality in the United States, and is accompanied by a fibrotic phenotype that contributes to chemotherapeutic resistance. Combination therapies including Gemcitabine (Gemzar®) and sustained release, nab-paclitaxel (Abraxane®) and FOLFIRINOX (5-FU/leucovorin/irinotecan/oxaliplatin) offer modest improvement in survival, albeit at an increase in side effects.


Results & Summary

We previously used single cell RNA sequencing to identify 1950 differentially expressed genes (DEGs) between the scrambled control and siAPE1 PDAC cells in normoxia (Shah et al 2017). Under hypoxic conditions, 2113 DEGs were identified between the scrambled and siAPE1 cells. Ingenuity Pathway Analysis (IPA) identified over 100 pathways affected by APE1 knockdown in co-culture. Metabolic pathways such as oxidative phosphorylation and mitochondrial function were among the top 5 pathways identified. In this study we analyze the role of APE1 in mitochondrial metabolism. We also show how the combination of targeting APE1 redox function along with mitochondrial metabolism enhances tumor efficacy.

Materials and Methods

Methods: We stained single cell RNA sequencing data of APE1 knockdown and untreated patient cells from the scrambled control and siAPE1 samples to extract RNA for sequencing. Cells were cultured in normoxia (1% CO2) or hypoxia (0.1% CO2). The knockdown and control samples were cultured in an ex vivo 3-Dimensional tumor-infiltrated mouse model using patient-derived tumor cells along with patient-derived cancer-associated fibroblasts (CAFs).

Cell culture

Patient derived pancreatic cancer cells (PDAC) (kindly provided by Arshad Malik) were cultured in normoxia (1% CO2). Cancer-associated Fibroblasts (CAF19) (kindly provided by Arshad Malik) were cultured in hypoxia (0.1% CO2). APE1 knockdown and control samples were cultured in a 3-Dimensional tumor-infiltrated mouse model using patient-derived tumor cells along with patient-derived cancer-associated fibroblasts (CAFs).

Cell lines used

A549: Lung adenocarcinoma (kindly provided by Thomas F. Arbeit)

SiHa: Cervical carcinoma cells (kindly provided by Dr. Robert C. Gillies)

HCT116: Human colorectal carcinoma cells (kindly provided by Dr. Carol Giaccia)

U251: Human glioblastoma multiform cells (kindly provided by Dr. Robert C. Gillies)

CA9: Human chondrosarcoma cells (kindly provided by Dr. Robert C. Gillies)

MCF-7: Human breast adenocarcinoma cells (kindly provided by Dr. Carol Giaccia)

MCF-10A: Human mammary epithelial cells (kindly provided by Dr. Carol Giaccia)

HeLa: Human cervical carcinoma cells (kindly provided by Dr. Robert C. Gillies)

SMARTpool: A combination of shRNA sequences targeting APE1

Materials

- American Type Culture Collection (ATCC)
- Qiagen® Real Time Detections system
- Enzymatic Protein Assay Kit

Acknowledgment

- Biotech S-1 Assay plates

References


Figure 1: APE1 expression both in tumor cells and the stroma of patient tissue

Figure 2: APE1 siRNA knockdown results in a heterogeneous population

Figure 3: Single Cell RNA Sequencing Analysis of PDAC cells following APE1 knockdown in Normoxia and Hypoxia

Figure 4A: Different Gene Expression patterns in Cancer-related Pathways between Normoxia and Hypoxia

Figure 4B: Different Gene Expression Patterns in Cancer-related Pathways between Normoxia and Hypoxia

Figure 5: Validation of Hypoxia Single Cell RNA Sequencing by qRT-PCR

Figure 6: Functional effects of APE1 knockdown on tumor cell growth and metabolism