Introduction

- Pancreatic ductal adenocarcinoma (PDA) is the third leading cause of cancer-related death in the US.
- PDA is resistant to conventional chemotherapy; however, mechanisms that contribute to this chemoresistance are not well-described.
- The tumor microenvironment in PDA has a dense stromal reaction, which is thought to result in low oxygen and low nutrient conditions (Feig, C., et al. 2012).
- Isocitrate Dehydrogenase 1 (IDH1) has been identified as an enzyme that plays an important role in chemoresistance in PDA (Zarei, M., et al. In progress).
- We sought to establish an IDH1 knockout cell line to further study its role in PDA using the CRISPR-Cas9 targeted genome editing system.

Methods

- The Hs 766T cell line, which is known to harbor common genetic mutations found in PDAs, such as KRAS, p53, and SMAD4 was used.
- Individual clones were validated with Sanger Sequencing, mRNA expression levels, and protein expression was determined with immunoblotting.

Results

- Fifteen clones were screened: 3 heterozygous clones and 11 homozygous knockout clones.

Conclusion

- CRISPR-Cas9 is an ideal way to reliably cause targeted genomic mutations resulting in altered mRNA and protein expression of a target gene.
- RNAi, shRNA inducible knockdowns, or siRNA transient transfections are possible, but provide an incomplete knockdown of gene expression and lend themselves to yielding inconsistent results.
- CRISPR-Cas9 knockout of IDH1 is a novel way to investigate the role of this protein in chemoresistance in PDA.
- In the future, we hope to further describe the phenotypic differences in clones with varying levels of IDH1 expression (i.e., wild type, heterozygous, and homozygous clones) through in vivo and in vitro assays.

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