Generation of CRISPR knockout of *IDH1* in pancreatic ductal adenocarcinoma cell line: An optimal model to study pancreatic cancer metabolic reprogramming

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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

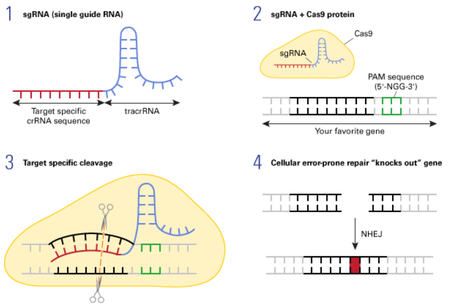
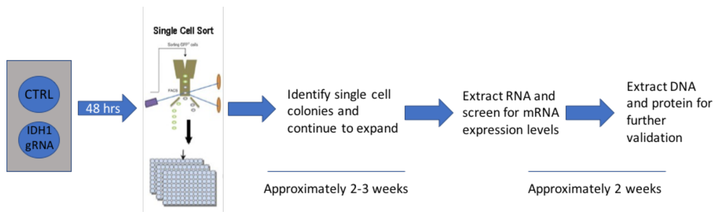


Figure: Takara, Inc.

- Plasmids containing Cas9 protein fused with GFP and a guide RNA (gRNA) were designed to target exon 3 of *IDH1*.
- 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.

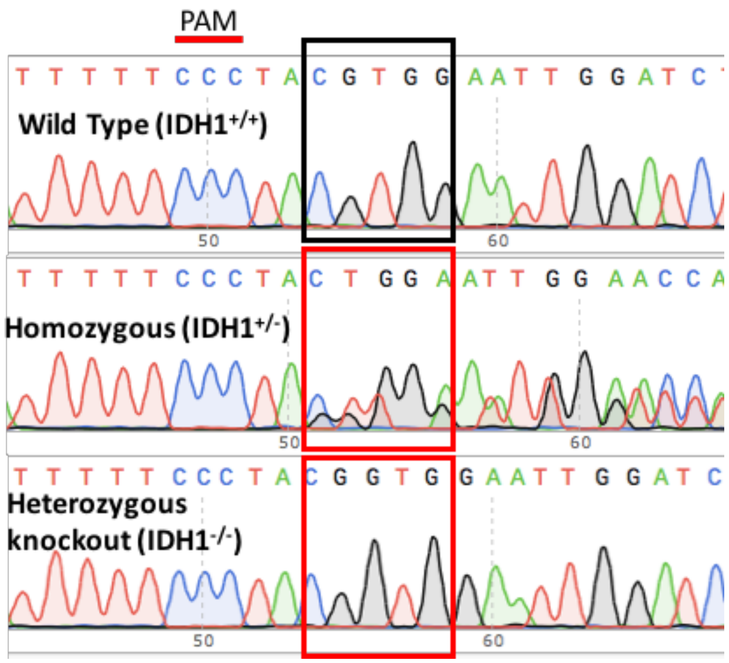


Figure A: Sanger sequencing of wild type clone, heterozygous clone, and a homozygous knock-out clone.

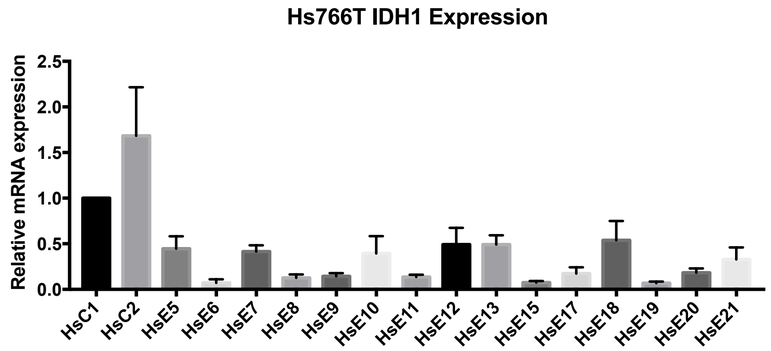


Figure B: RT-PCR showing significantly decreased mRNA expression in all of the screened clones.

Results

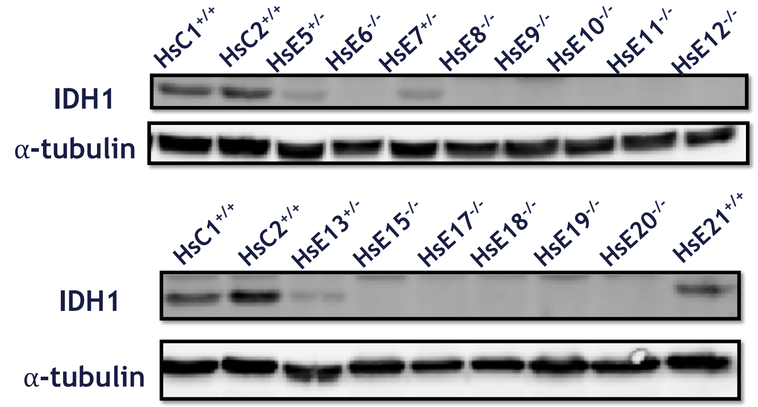


Figure C: Immunoblotting demonstrating decreased IDH1 expression in heterozygous clones (HsE5, HsE7, and HsE13). There was no IDH1 expression in the homozygous knock-out clones. Sanger sequencing of clone HsE21 demonstrated wild-type sequence and preserved IDH1 protein expression, despite decreased mRNA levels.

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.

Acknowledgements & Support

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