Validation of Novel KRAS-mutant Synthetic Lethal Target in Non-Small Cell Lung Cancer

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Abstract

Introduction: Lung cancer is the leading cause of cancer related deaths in both men and women. Kentucky is ranked highest in the country in incidence of lung cancer. The KRAS mutation is one of the most common mutations found in non-small cell lung cancers (NSCLC). The KRAS protein is very important in regular cell functioning, but when mutated, the cells proliferate uncontrollably. There are currently no targeted treatments for KRAS mutant NSCLCs. Studies have been performed to target KRAS, however they have not been very successful. This is possibly because the tumor microenvironment has not been taken into account. The tumor microenvironment is inherently hypoxic, around 5% oxygen. We hypothesize that KPI-2 and CIR-1 are synthetic lethal targets to KRAS mutant NSCLCs.

Objective: To validate KPI-2 and CIR-1 as synthetic lethal targets to KRAS mutant NSCLC.

Methods: KRAS mutant and Non-KRAS mutant cell lines expressing CAS9 were transfected with gRNAs to knock out the expression of target gene. The cells were monitored for growth in both 5% oxygen conditions and 20% oxygen conditions. Protein expression was measured by western blot.

Results: gRNA 1 effectively knocked out CIR-1 in A549, H1437, and H2009 cell lines. gRNAs 1 and 2 effectively knocked out KPI-2 in the H2009 cell line. From these results, the gRNAS were pooled (1:1 ratio) and it was found that gRNAs 1 and 2 pooled together knocked out KPI-2 in cell line H2009. A colony formation assay confirmed previous studies that showed validation of PDSS-2 as a synthetic lethal to KRAS mutant cell lines.

Conclusions: PDSS-2 was validated as a synthetic lethal to KRAS mutants as confirmed in a previous study. The knock out of CIR-1 and KPI-2 was effective using their respective gRNAs. The clinical implications are that these genes could be potential synthetic lethal targets for KRAS mutant NSCLC with further validation.

Background

The state of Kentucky is ranked highest in the nation for lung cancer incidences. KRAS is one of the most common mutations in non-small cell lung cancer. Non-small cell lung cancer accounts for about 85% of lung cancer cases, with adenocarcinoma as the main type. Currently, there are no targeted therapies for KRAS mutant lung cancers. The tumor microenvironment has not been taken into account in previous studies. Normally cells in the lab are cultured in 20% oxygen, but in the body, oxygen is at a much lower concentration. In this project, data was validated from a whole genome wide screen in which virtually every gene in the genome was knocked out in KRAS mutant and non-KRAS mutant cell lines using CRISPR/CAS9 and then grown in both normoxic (20% oxygen) and hypoxic (5% oxygen) conditions. The goal of the project is to find a target that, when knocked out, is synthetically lethal to the KRAS mutant cancer cells.

Methods

- Non-small cell cancer cell lines A549, H2009, and H1437 were obtained from ATCC. They were cultured in RPMI-1640 +10% FBS and Pen-strep. The cell lines were grown in 37℃ at 5% CO^2.
- Non-small cell cancer cell lines expressing CAS9 were transfected with gRNAs to knock out the expression of the target gene.
  - The gRNAs were obtained from Synthego
- Transfection: Opti-mem and Dharmafect-1 were added to opti-mem and gRNA. 3gRNA/gene, 2 genes used were KPI-2 and CIR-1. 3 cell lines tested.
- Growth was maintained in 20% oxygen conditions.
- Protein expression was measured by western blot using SuperSignal West Femto which was obtained from Thermo Scientific.
- Colony Formation of PDSS-2 KO cells: 300 cells/well were plated. Growth was monitored over about a 14 day period. Each well was stained and counted.

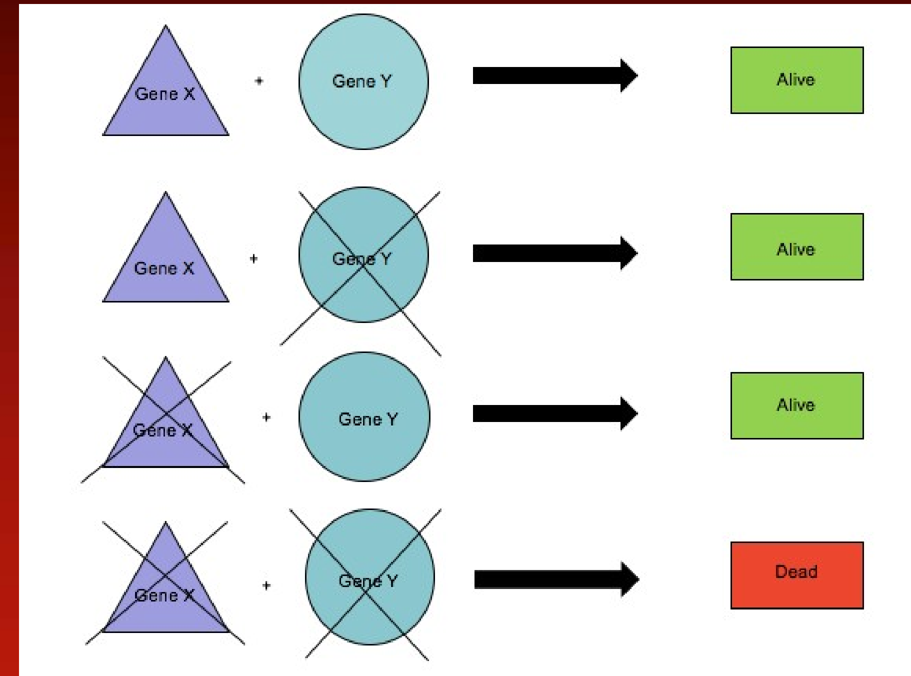


Figure 1. Schematic explaining synthetic lethality.

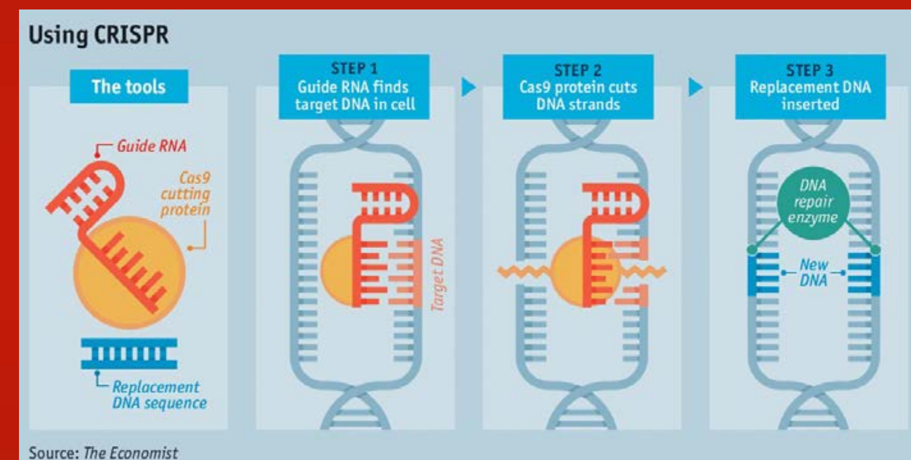


Figure 2. CRISPR/CAS9 knock out system.

Current Estimate	20,000-25,000 genes in human genome
Genes targeted in Gecko library	19,050 genes targeted in this library
# of gRNAs/gene	4-6 guides
Total gRNAs	123,411
Multiplicity of infection	300 cells infected with each gRNA
Total # of gRNA infection	37 million
Infection ratio	0.3
Total 3 of cells used for each infection	123 million
Selection of infected cells	Puromycin selection for 2-days to kill uninfected

Table 1. Gecko Screen Information

- KPI-2: Kinase/phosphatase/inhibitor 2. Protein coding gene.
- CIR-1: Corepressor interacting with RBPJ. Protein coding gene.
- PDSS-2: Decaprenyl diphosphate synthase subunit 2. Protein coding gene.
- GAPDH: Glyceraldehyde-3 phosphate dehydrogenase. Protein coding gene.

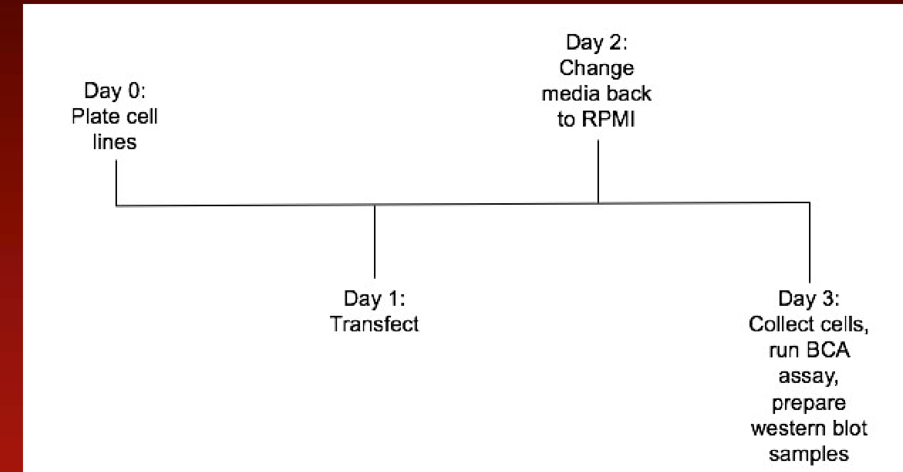


Figure 3. Schematic depicting the experimental design and time line.

Results

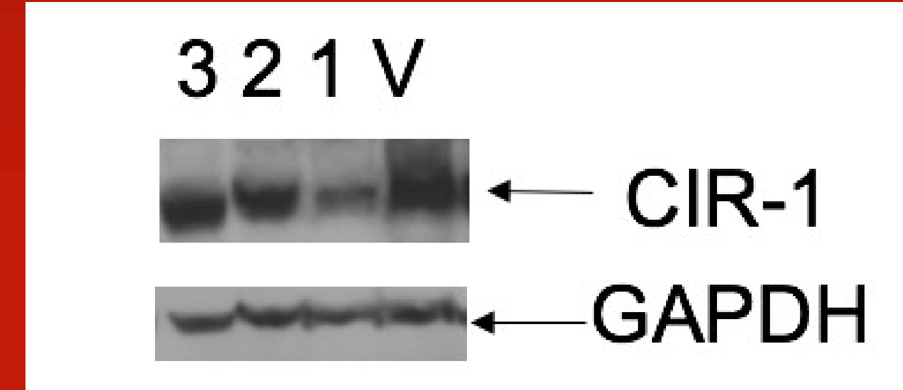


Figure 4. Western Blot analysis of H1437 cell line. 1, 2, and 3 represent the number of the gRNA used and V represents the vehicle. gRNAs were used to knock out CIR-1. gRNA 1 was shown to knock out CIR-1.

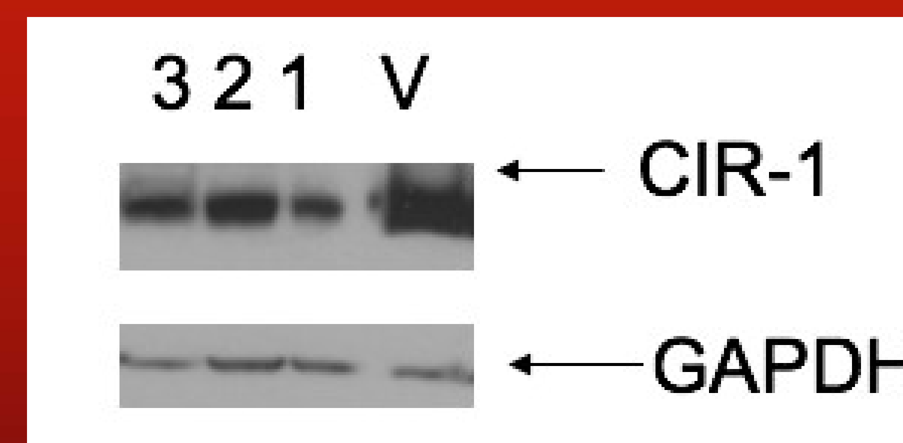
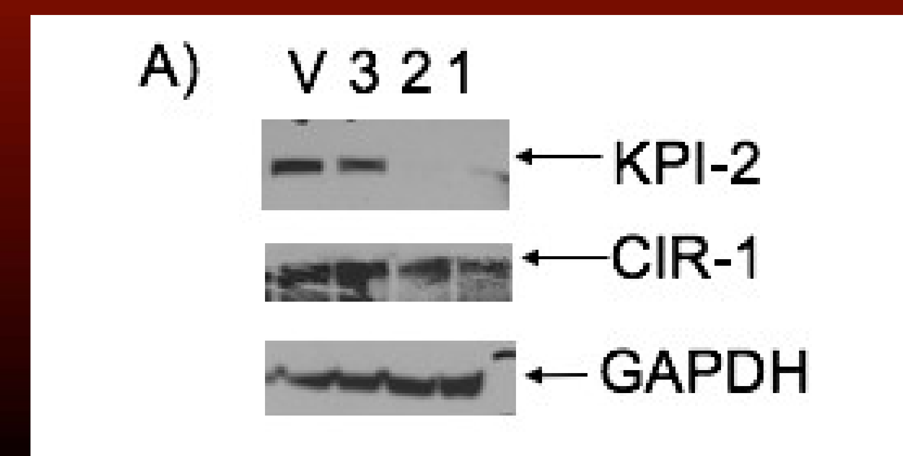


Figure 5. Western Blot analysis of A549 cell line. 1, 2, and 3 represent the number of the gRNA used, V represents the vehicle. gRNAs were used to knock out CIR-1. gRNA 1 knocked out CIR-1.



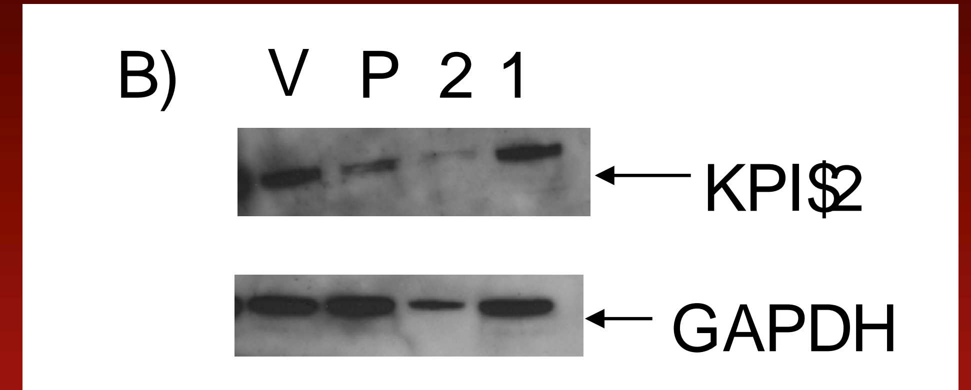


Figure 6. A) Western Blot analysis of H2009 cell line. 1, 2, and 3 represent the number of the gRNA used and V represents the vehicle. gRNAs were used to knock out KPI-2 and CIR-1. For KPI-2, gRNAs 1 and 2 knocked out the protein and for CIR-1 gRNA 1 knocked out the protein.
B) Western Blot analysis of H2009 cell line. 1 and 2 represent the number of the gRNA used, P represents pooled gRNAs, and V represents vehicle. From the results of figure 6a, the corresponding gRNAs were pooled. For KPI-2, gRNAs 1 and 2 were tested individually and pooled. Knock out is once again seen in gRNAs 1 and 2 and now is also seen in the pooled 1 and 2.

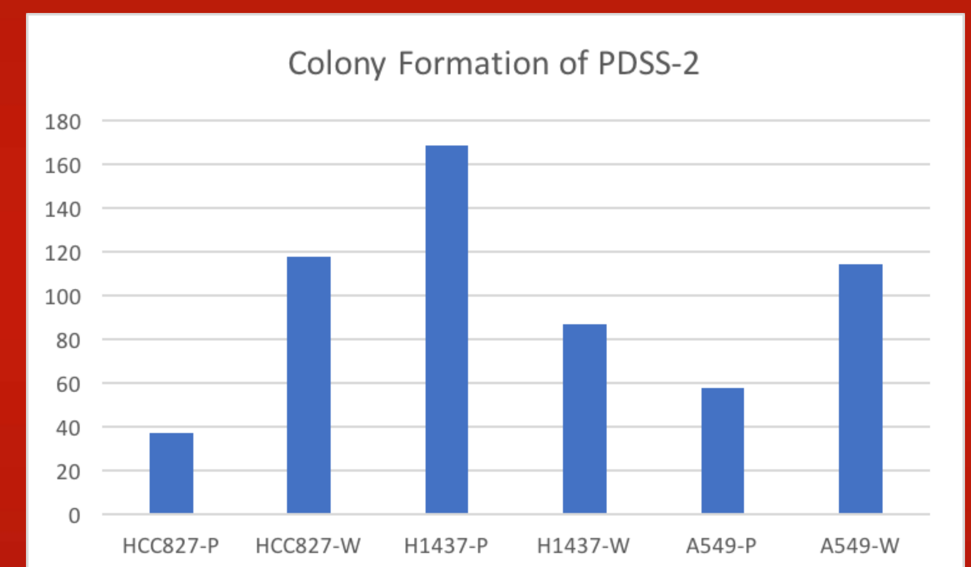


Figure 7. Colony formation of PDSS-2. P represents the gRNAs that were pooled and W represents the wildtype. Colonies were counted and averaged.

Conclusions and Clinical Implications

- PDSS-2 was validated as a synthetic lethal target to KRAS mutant cell lines and confirmed previous studies through analyzing colony formation.
- Knock out of KPI-2 was done using gRNAs 1 and 2 in cell line H2009, a KRAS mutant cell line.
- Knock out of CIR-1 was done using gRNA 1 in H1437 and A549 cell lines.
- Clinical implications: CIR-1, KPI-2, and PDSS-2 are possible synthetic lethal targets for KRAS mutant non-small cell lung cancer and with future studies and testing, are potential targeted therapies for KRAS mutant non-small cell lung cancer.

Future Aims

- Validate CIR-1 and KPI-2 for more KRAS and Non-KRAS cell lines.
- Determine mechanistic pathways as to why the targets are synthetic lethal in KRAS mutant cell lines.

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