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 nation for 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 in previous studies. 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 added to opti-mem and Dharmafect-1 were added to opti-mem. Non-small cell cancer cell lines expressing CAS9 were transfected with gRNAs to knock out the expression of the 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. gRNA 2 and 3 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 gRNA 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 the 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.
- 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. Optimum and Dharmafect-1 was added to optimum 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.

Results

- gRNA 1 effectively knocked out CIR-1 in A549, H1437, and H2009 cell lines. gRNA 2 and 3 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 gRNA 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.
- PDSS-2 was validated as a synthetic lethal target to KRAS mutant cell lines.
- 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.

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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