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Genomewide Screen for Synthetic Lethal Interactions with Mutant KRAS in Lung Cancer

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14. ABSTRACT Many factors are known to contribute to the development of lung cancer; one of the important contributing ones is genetic mutations. For example, KRAS mutations account for 22% lung cancer cases. Thus, researchers have been trying hard to develop drugs against mutant KRAS. However, to date no effective pharmacological inhibitors targeting mutant RAS have reached the clinic for various reasons. Therefore, the search is still going on. Synthetic lethal approach is a new concept that RAS mutant cancer cells rely on other genes or pathways to survive. Thus, these genes or pathways serve as potential targets for lung cancer therapy. Our proposed work uses the same concept but with a novel approach.					
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Introduction

Lung cancer is treated based on the two major categories, non-small cell lung cancer (NSCLC; 83% cases) and small cell lung cancer (SCLC; 13% cases). Mutations in KRAS (22%) and EGFR (17%) and rearrangements in ALK (7%) are the most frequent drivers in NSCLC adenocarcinoma. Thus, researchers have been trying hard to develop drugs against mutant KRAS. However, to date no effective pharmacological inhibitors targeting mutant RAS have reached the clinic for various reasons. Therefore, the search is still going on. Increasing evidence suggests that expression of mutant RAS function can cause hyperproliferative developmental disorders and cancer. Thus, targeting mutant RAS has been extensively investigated for cancer therapy in past decades. However, a great challenge was the difficulty in targeting mutant RAS. For example, despite of many years of work, to date no effective pharmacological inhibitors targeting RAS have reached the clinic. In this application, we aimed to take an alternative indirect approach, i.e., synthetic lethal approach, which seeks targets that are essential in cells bearing an activated RAS. We used CRISPR/Cas9-based screening approach for this purpose.

Body

The overall objective was to identify synthetic lethal interactions with KRAS in lung cancer with one specific aim and three major tasks as listed below.

Three major tasks were listed in SOW as below

Major Task 1: Generate tet inducible hCas9 construct

Major Task 2: Establish stable cells with an inducible hCas9 at AAVS1 site

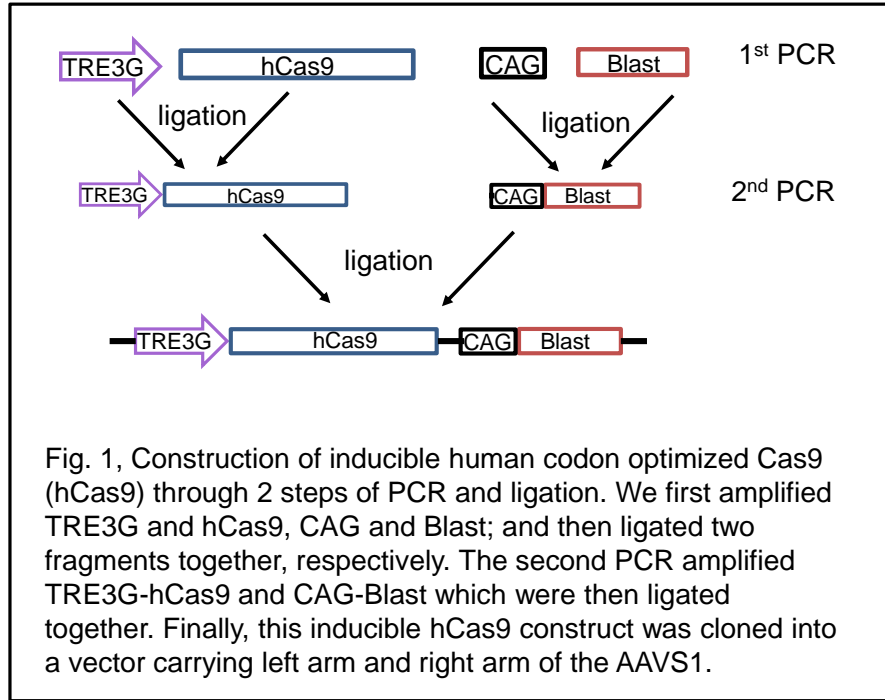
Major Task 3: Selection and validation

Results

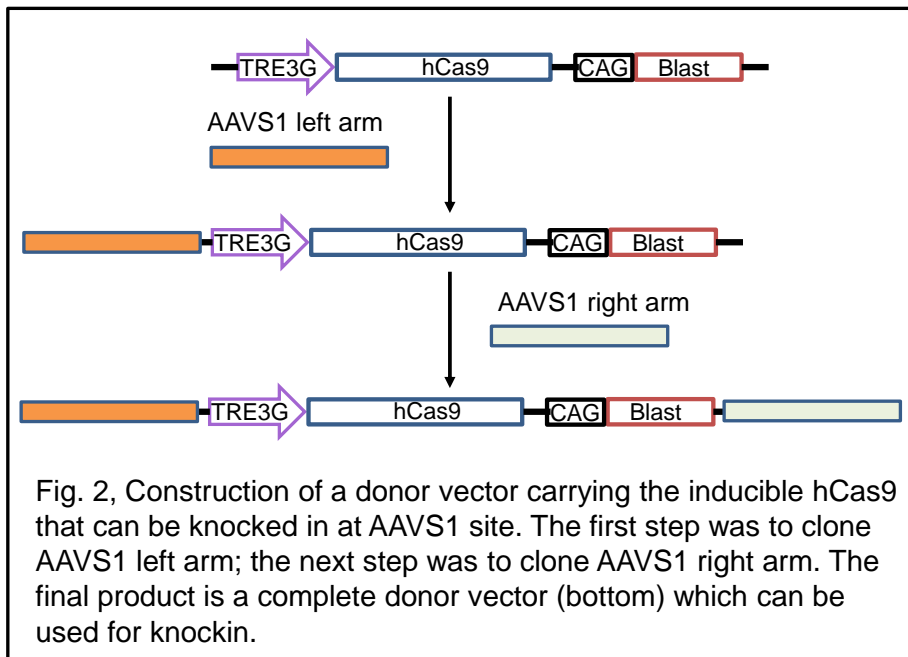
Major Task 1. Generate tet inducible hCas9 construct (complete)

Since the proposed screen platform is based on negative selection, i.e., gRNA clones of our interest are lost after selection, we have to address the technical variability issue. In this regard, we proposed a tetracycline (tet) inducible system for Cas9.

Therefore, we assembled



several components into an expression vector by two steps of PCR reaction. As shown in Fig. 1, the first PCR reaction was to amplify TRE-3G, hCas9, CAG promoter and Blast and then the first two fragments and the second two fragments were ligated together. The second PCR reaction was to amplify TRE-3G-hCas9 and the CAG-Blast fragments and then these two fragments were ligated together.

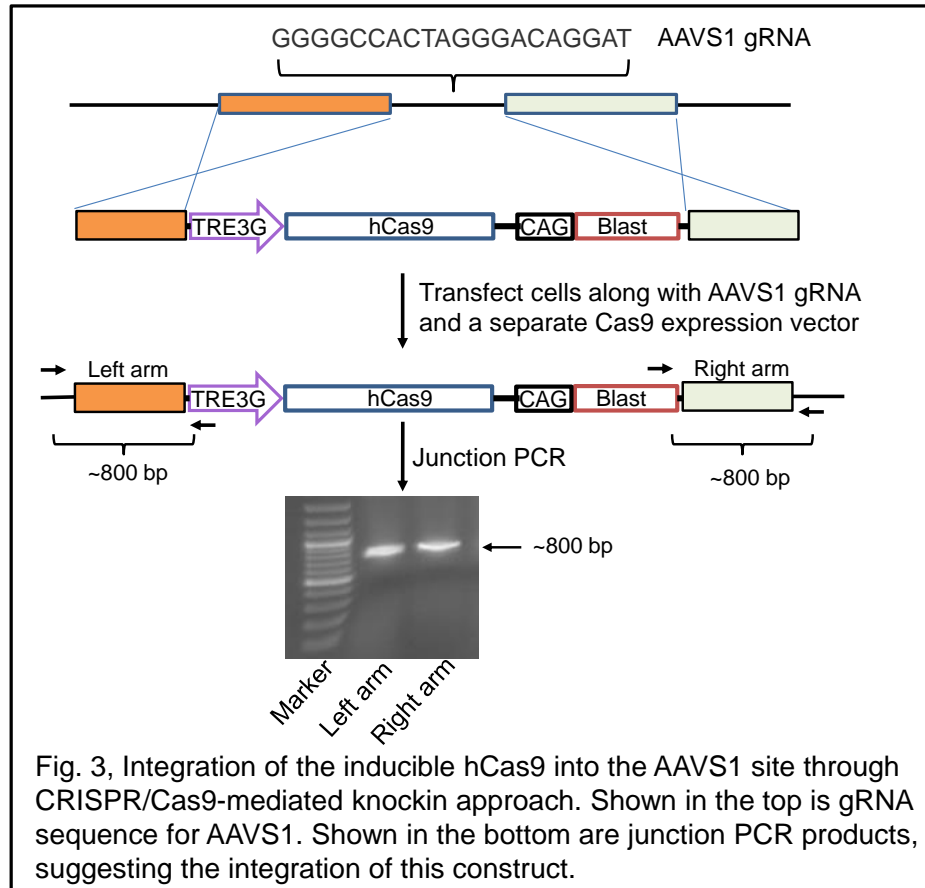


Finally, we cloned left arm and right arm of AAVS1 into this vector sequentially. This resultant construct was used as a donor such that the inducible hCas9 was integrated into the AAVS1 site precisely (Fig. 2).

Major Task 2: Establish stable cells with an inducible hCas9 at AAVS1 site (complete)

We first design and generated AAVS1 specific gRNA (GGGGCCACTAGGGACAGGAT). After synthesis of two complementary oligonucleotides, we cloned this AAVS1 specific gRNA into a U6 driven promoter vector which also carries EF1 driven hCas9. For proof of principle, we first introduced this construct into 293T cells because 293T cells have been widely used for gene manipulation and expression experiments. As shown in Fig. 3, we detected a 800 bp band using specific primers for either left arm or right arm, suggesting that the inducible hCas9 is integrated into the AAVS1 site as expected.

Using the same approach, we introduced the same fragment into the AAVS1 site in lung cancer NCI-H727 cells which carry a mutant KRAS.



Major Task 3: Selection and validation (incomplete)

We co-transfected 293T cells and NCI-H727 cells, respectively, with AAVS1 gRNA expression vector and the inducible hCas9 expression vector (pTRE-3G-hCas9-CAG-Blast). One day after transfection, we added blasticidin for selection and obtained individual colonies 2 weeks after selection. Genomic PCR characterization indicated that the fragment is successfully knocked in (Fig. 3).

To determine whether Cas9 can be induced by doxycycline, we added doxycycline to culture

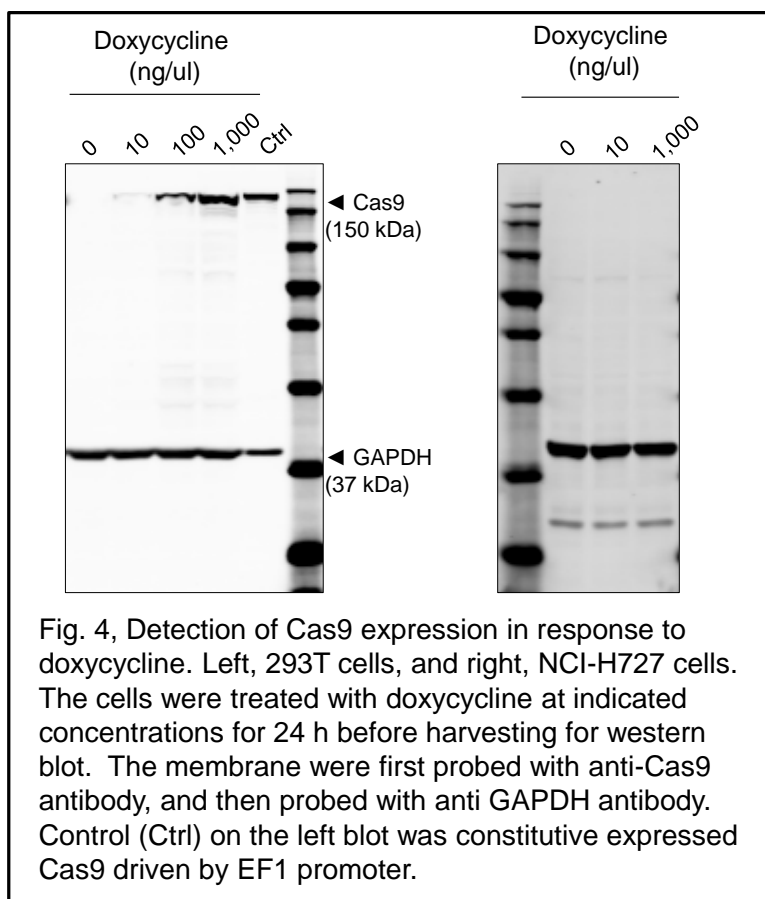


Fig. 4, Detection of Cas9 expression in response to doxycycline. Left, 293T cells, and right, NCI-H727 cells. The cells were treated with doxycycline at indicated concentrations for 24 h before harvesting for western blot. The membrane were first probed with anti-Cas9 antibody, and then probed with anti GAPDH antibody. Control (Ctrl) on the left blot was constitutive expressed Cas9 driven by EF1 promoter.

medium for 24 h before the cells were harvested for Western blot. For 293T cells, we were able to detect a nice induction of Cas9 in response to doxycycline in a dose dependent manner (Fig. 4, left panel).

However, we were not able to detect any induction in NCI-H727 cells (Fig. 4, right panel). In addition, we also tested different time points, but still did not detect Cas9 expression. We then tested another lung cancer cell line (NCI-

H23) and was not able to detect any induction of Cas9 either. It is not clear at this moment why the same construct behavior differently in response to doxycycline in different cell lines.

In any case, we went on producing GeCKO library and vector control in these cells by infection.

After selection by puromycin, these surviving cells are transductant cells that can be used in future for screening synthetic lethal interaction with mutant KRAS.

Key Research Accomplishments

- We successfully generated an inducible hCas9 expression vector and a donor vector for integration of the inducible hCas9 into the AAVS1 site
- We established stable cell lines carrying inducible hCas9 at AAVS1 site
- We were able to detect hCas9 expression in response to doxycycline in a dose dependent manner in 293T cells
- We have established stable NCI-H727 and NCI-H23 cells carrying the GeCKO library and these cells may be used for library screen in future

Reportable Outcomes

Not yet

Conclusions

Although we finished most of tasks proposed in the application, we were not able to finish the last part, i.e., screening for genes that can have synthetic lethal interaction with mutant KRAS in lung cancer, due to lack of Cas9 expression in response to doxycycline induction. Nevertheless, these constructs will be valuable for future screening purpose. While we are not able to figure out why the same construct was able to express tet inducible Cas9 in 293T cells, but not in lung cancer cell lines, we will test more lung cancer lines or take a different approach when we secure further funding.