Investigating the effect of KRAS gene in human lung cancer using CRISPR-Cas9 system

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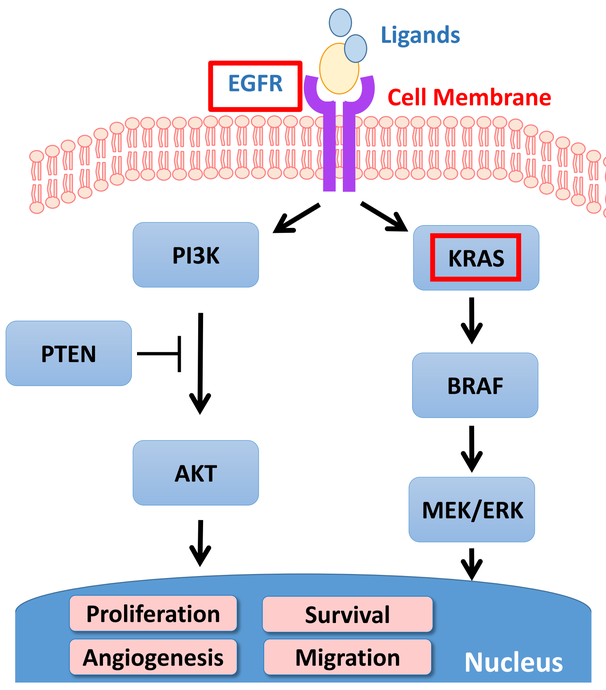
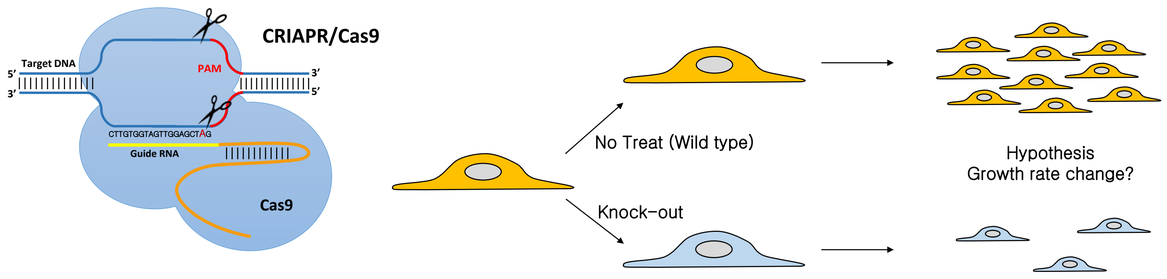
ABSTRACT



Lung cancer, also known as lung carcinoma, is a malignant lung tumor characterized by uncontrolled cell growth in tissues of the lung. It is known to be difficult to detect on early stages, and commonly spreads to other tissues.
Currently available treatments usually targets the EGF(Epidermal growth factor) receptor. However, recent studies show that the EGF receptor targeting molecules have low efficiency - which might be due to the mutations on downstream signaling molecules.
In this study, we knocked out important signaling kinase, KRAS, and identified its role on cell growth rate. When we knocked out the KRAS gene, a significant decrease on cellular growth rate was observed, regardless of the existence of mutations on the KRAS gene.
Our data might provide a new stepping stone into the development of new lung cancer drug targeting KRAS in the future, as it could indicate a potentially more efficient method in decreasing the growth level of tumor cells in lung tissues.

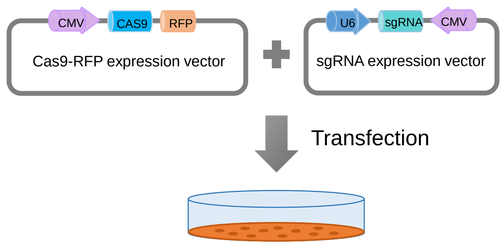
SCHEME

Key Concept

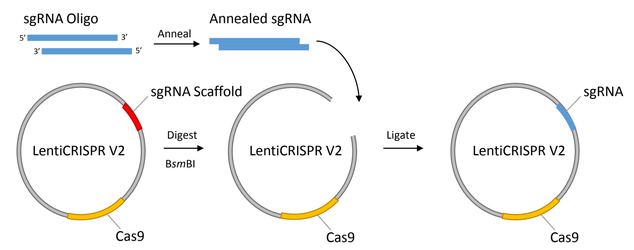


Strategy

I. ToolGen Knock-out



II. lentiCRSIPRv2 Cloning



RESULTS

ToolGen Knock-out

A

	A	B	C	D
1	sgRNA Sequence	Combined Rank	On-Target Rank	Off-Target Rank
2	ACATCAGCAAAGACAAGACA	1	3	4
3	AAGAGGAGTACAGTGCAATG	2	2	7
4	CTTGTGGTAGTTGGAGCTAG	3	10	1
5	GTAGTTGGAGCTAGTGGCGT	4	11	5
6	TCTCGACACAGCAGGTCAAG	5	7	12
7	CTGAATTAGCTGTATCGTCA	6	9	10
8	CAACAATAGAGGATTCCTAC	7	15	6
9	CAGTACATGAGGACTGGGGA	8	4	22
10	CCAGTACATGAGGACTGGGG	9	6	20
11	CAATGAGGGACCAGTACATG	10	5	21
12	AGAGGAGTACAGTGCAATGA	11	12	14
13	TTGGATATTCTCGACACAGC	12	13	17
14	CCTCCCCAGTCCTCATGTAC	13	25	11
15	GATGTACCTATGGTCCTAGT	14	22	15
16	AAAGAAAACATGAGCAAAGA	15	16	23
17	TTTTAATTTGTTCTCTATAA	16	37	2
18	GGACCAGTACATGAGGACTG	17	1	38
19	CTGTAGGAATCCTCTATTGT	18	36	3
20	GGACTCTGAAGATGTACCTA	19	23	18
21	AGAACAAATAAAAGAGTTA	20	32	9
22	GAATATAAACTTGTGGTAGT	21	34	8
23	AACATCAGCAAAGACAAGAC	22	8	36
24	TTATTTCCTACTAGGACCAT	23	18	27

	A	B	C	D
1	sgRNA Sequence	Combined Rank	On-Target Rank	Off-Target Rank
2	ACATCAGCAAAGACAAGACA	1	3	3
3	AAGAGGAGTACAGTGCAATG	2	2	5
4	TCTCGACACAGCAGGTCAAG	3	8	10
5	CTGAATTAGCTGTATCGTCA	4	10	8
6	CAACAATAGAGGATTCCTAC	5	15	4
7	CAGTACATGAGGACTGGGGA	6	4	20
8	CCAGTACATGAGGACTGGGG	7	6	18
9	CAATGAGGGACCAGTACATG	8	5	19
10	AGAGGAGTACAGTGCAATGA	9	12	12
11	TTGGATATTCTCGACACAGC	10	13	15
12	GTAGTTGGAGCTGGTGGCGT	11	11	21
13	GATGTACCTATGGTCCTAGT	12	22	13
14	CCTCCCCAGTCCTCATGTAC	13	26	9
15	AAAGAAAAGATGAGCAAAGA	14	16	22
16	GGACTCTGAAGATGTACCTA	15	23	16
17	TTTTAATTTGTTCTCTATAA	16	38	1
18	CTGTAGGAATCCTCTATTGT	17	37	2
19	AGAACAAATAAAAGAGTTA	18	33	7
20	GGACCAGTACATGAGGACTG	19	1	39
21	GAATATAAACTTGTGGTAGT	20	35	6
22	CTTGTGGTAGTTGGAGCTGG	21	7	35
23	TTATTTCCTACTAGGACCAT	22	18	26
24	CAGGACTTAGCAAGAAGTTA	23	34	11

B

	KRAS guide RNA seq
A549 (Human)	CTTGTGGTAGTTGGAGCT**A**G (TGG–PAM) MUTANT (G12S)
H1299 (Human)	CTTGTGGTAGTTGGAGCT**G**G (TGG–PAM) WILD TYPE

Fig. 1. Guide-RNA sequence design. (A) Using guide-RNA evaluation tool from Harvard medical school, lists of potent candidates for guide-RNA are shown. (B) Guide RNAs are selected based on paper and score.

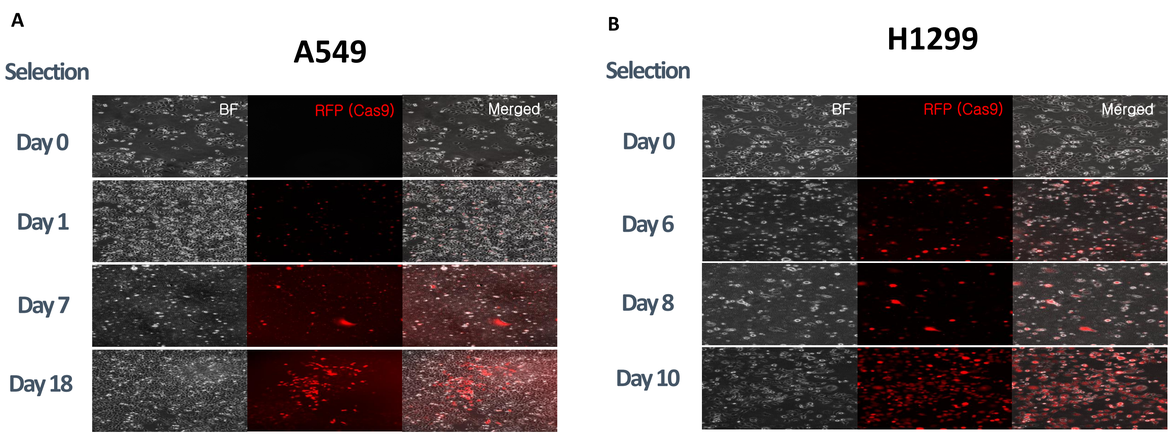


Fig. 2. Cas9 transfection and selection. Cas9-RFP vector was transfected into each cell line (A549–A, H1299-B) and grown under the condition of Puromycin.

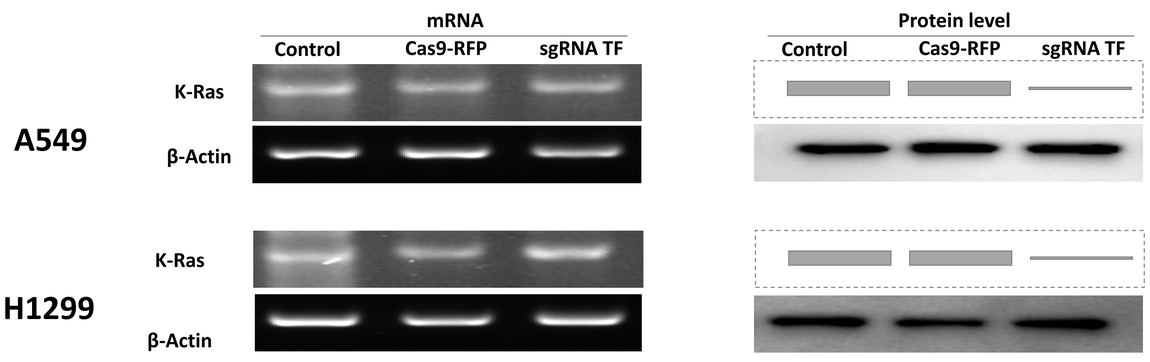
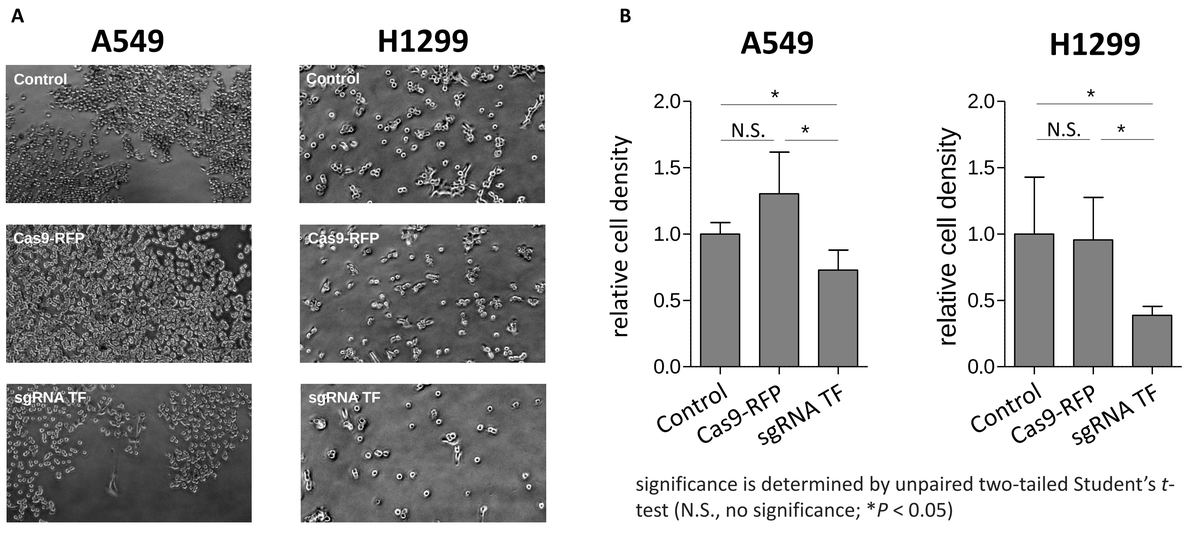


Fig. 3. Comparison of mRNA and protein level. RT-PCR and PCR were done to compare mRNA level. Western blot analysis was used to check the protein level. K-Ras bands were replaced by a drawing since antibody for K-Ras shows low specificity.



significance is determined by unpaired two-tailed Student's *t*-test (N.S., no significance; $^*P < 0.05$)

Fig. 4. Cell growth rate analysis. (A) Images of cells were obtained using brightfield microscopy. (B) sgRNA transfected cells had decreased cell growth rate.

lentiCRISPRv2 Cloning

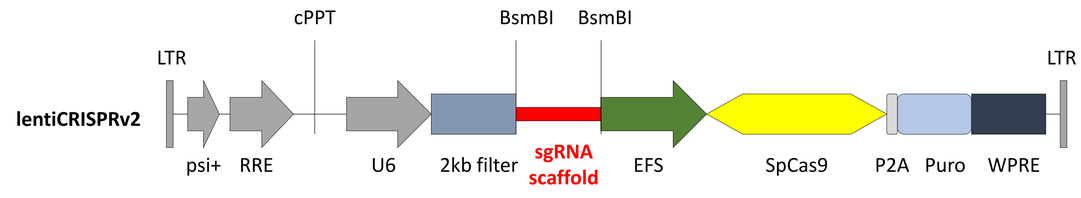


Fig. 5. lentiCRISPRv2 vector design.

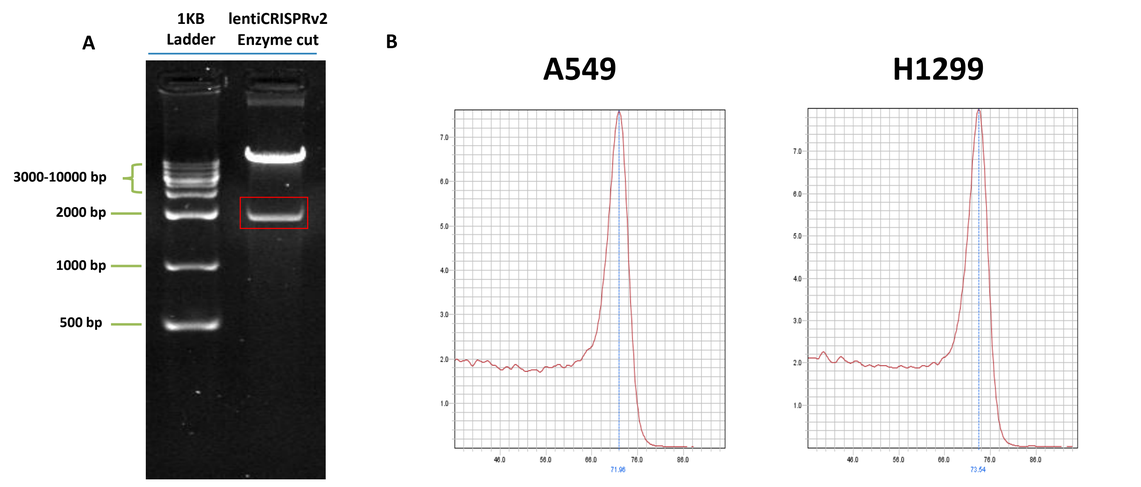


Fig. 6. lentiCRISPRv2 Cloning. (A) lentiCRISPRv2 vector was cut using BsmBI restriction enzyme. sgRNAs were annealed (B) and ligated with lentiCRISPRv2 vector.

CONCLUSION & FURTHER STUDY

Our team conducted a total of three experiments to confirm successful knock out using CRISPR-Cas9. Because of the low efficiency of antibodies and property of KRAS gene itself, experiments that directly check the amount of mRNA and protein did not produce satisfactory results. However, in the process of counting the number of cells and confirming their growth rate, a significant difference was observed. Later, a quantitative experiment will be conducted to confirm the decrease in growth rate. Also, by knocking in the KRAS gene again and seeing whether the growth rate is restored, we could solidify our results.
Cloned lentiCRISPRv2 that we made for this study might use for knock-out experiment for further studies. This might increase the chance to make efficient KRAS knocked out human lung cancer cell line library.
If knocking out the KRAS gene yields a significant decrease in tumor cell growth rate, further research into developing drugs targeting the KRAS gene might be possible.

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