The effects of EGFR knockout via CRISPR-Cas9 system

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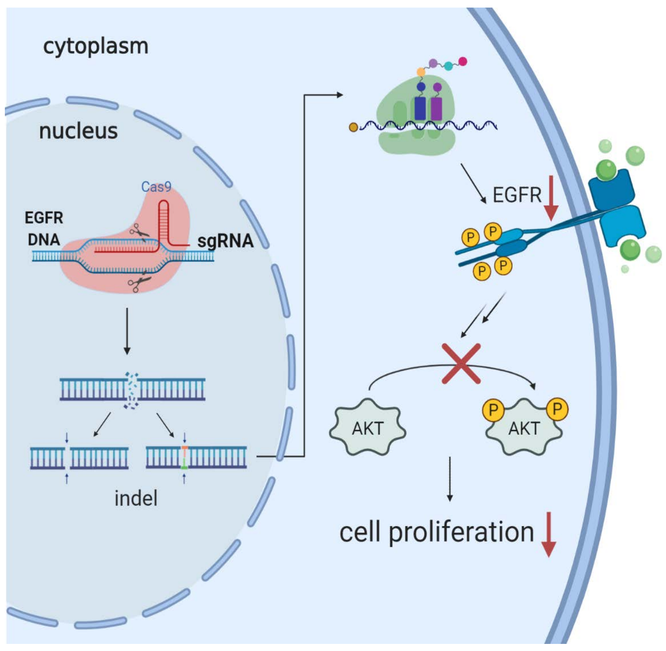
ABSTRACT

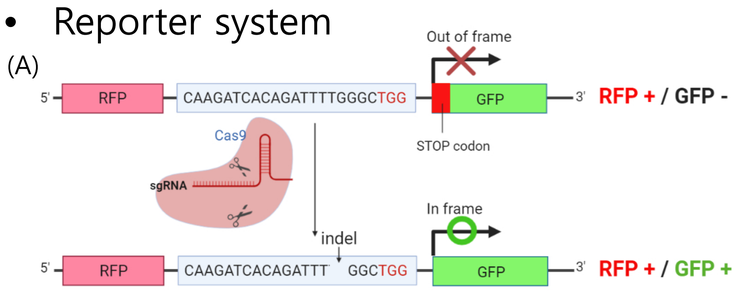


EGFR, or Epithelial Growth Factor Receptor, is one of the frequently mutated genes found in NSCLC patients. (Approximately 30%) As a member of the tyrosine kinase family, activation of EGFR leads to autophosphorylation of cytoplasmic region and subsequently increases phosphorylation of proteins in downstream signal cascade which leads to increased cell proliferation. This normal signaling cascade is manipulated by the cancer cells by making the cell produce excessive amounts of EGFR or by making the EGFR protein to be constitutively active through gene mutation. In any way, downstream signals of EGFR increase greatly, thus causing the cancer cell to have an uncontrollable proliferative ability.

CRISPR-Cas9 system has an ability to create a double-strand break at a specific site. As such, we assessed the possibility of CRISPR-Cas9 system being used for lung cancer patients with abnormal expression of EGFR by observing the effect of CRISPR-Cas9 induced knockout of the EGFR gene on HEK 293 cell line. To see this effect, we checked the ability of Cas 9 and sgRNA to introduce insertion/deletion mutation into the EGFR gene of both exogenous reporter plasmid and endogenous genomic DNA. mRNA and protein level of cellular growth-related gene (AKT) was also observed, to show the effect of EGFR knockout mutation. Lastly, cell growth assay was carried out to show the connection between EGFR knockout and cell proliferation. By these experiments, we could show that targeting the EGFR gene using CRISPR-Cas9 system might be a way of treating lung cancer. Though the decrease in cell growth was not extreme, in combination with already existing EGFR inhibitors, CRISPR-Cas9 therapy might open the way for new era of cancer treatment.

Scheme

- Reporter system



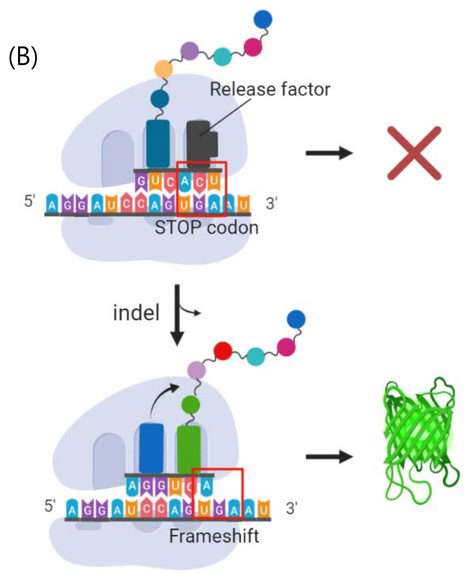
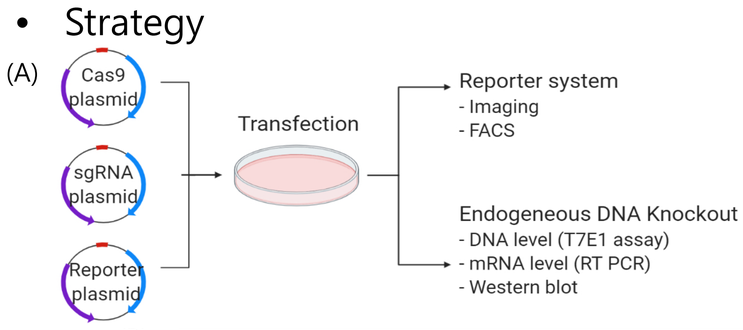


Fig1. (A) Reporter system (B) Frameshift principle

- Strategy



(B)

	control1	control2	control3	Experimental group
Reporter plasmid	+	+	+	+
sgRNA plasmid	-	+	-	+
Cas 9 plasmid	-	-	+	+

Fig2. (A) Experimental plan (B) Experimental setting

Results

- Fluorescence microscope

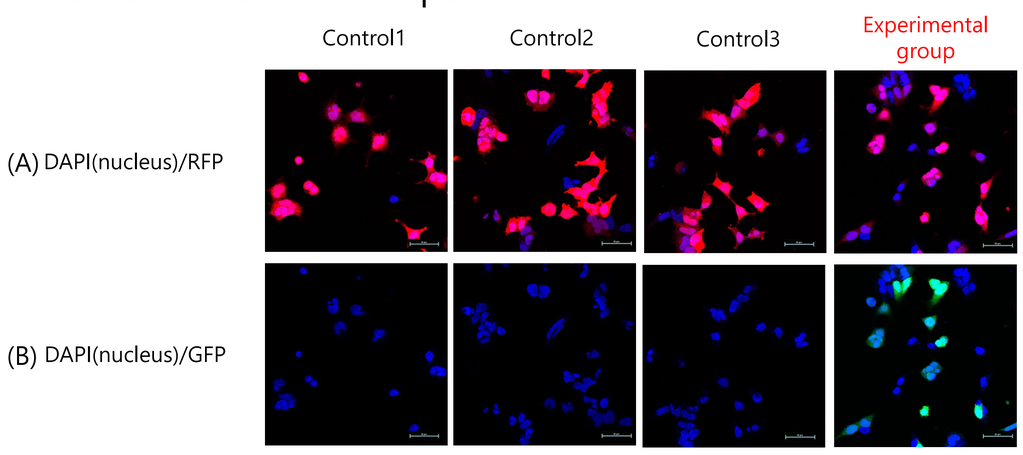
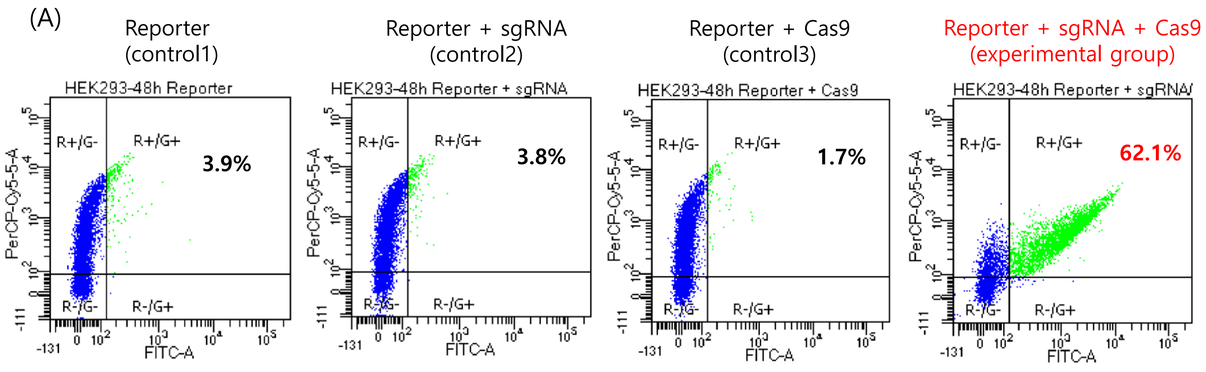


Fig3. Transfected HEK 293 imaged by confocal fluorescence microscope. Nucleus dyed with DAPI(blue) , red indicates RFP and green indicates GFP. (A) DAPI/RFP merge (B)DAPI/GFP merge

- FACS



(B)
Reporter (control1)
Reporter + sgRNA (control2)
Reporter + Cas9 (control3)
Reporter + sgRNA + Cas9 (experimental group)

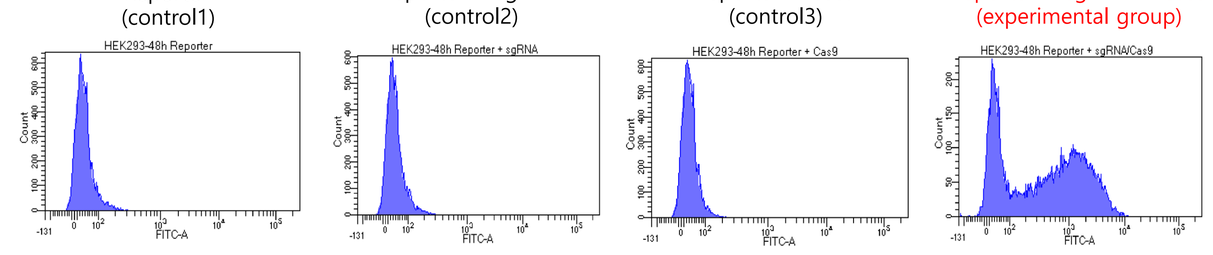
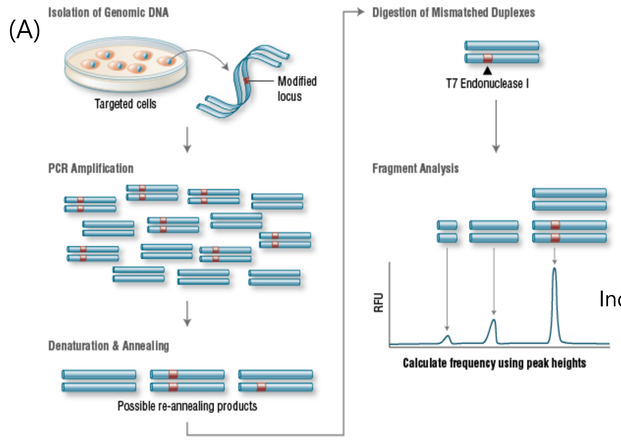


FIg4. FACS data of transfected HEK293 cells. (A) Dot plot graph. One dot represents one cell. X axis shows GFP intensity. Y axis shows RFP intensity. (B) Histogram. X axis shows GFP intensity. Y axis shows cell count.

- T7E1 assay



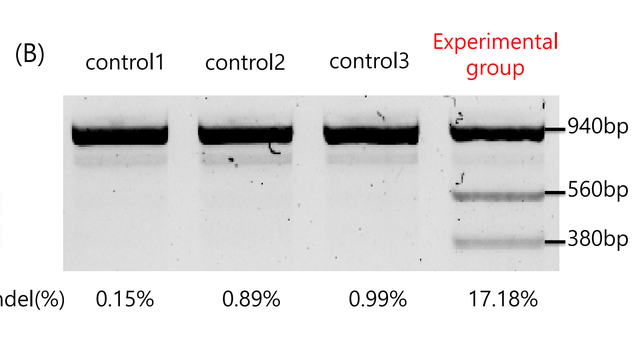


FIg5. (A)T7E1 assay principle (B)T7E1 assay electrophoresis result.

- Western blot & RT-PCR

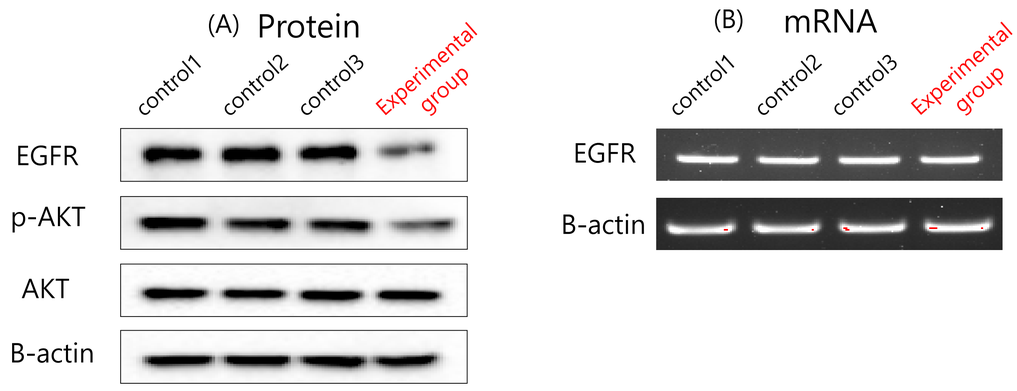
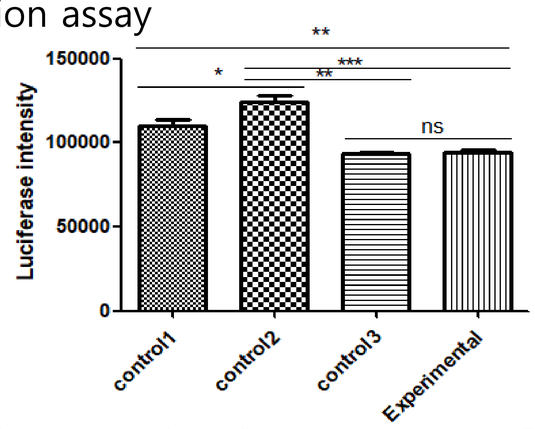


Fig 6. (A) Western blot result. Band size shows target protein amount. (B) mRNA RT-PCR electrophoresis result. Band size shows target mRNA amount.

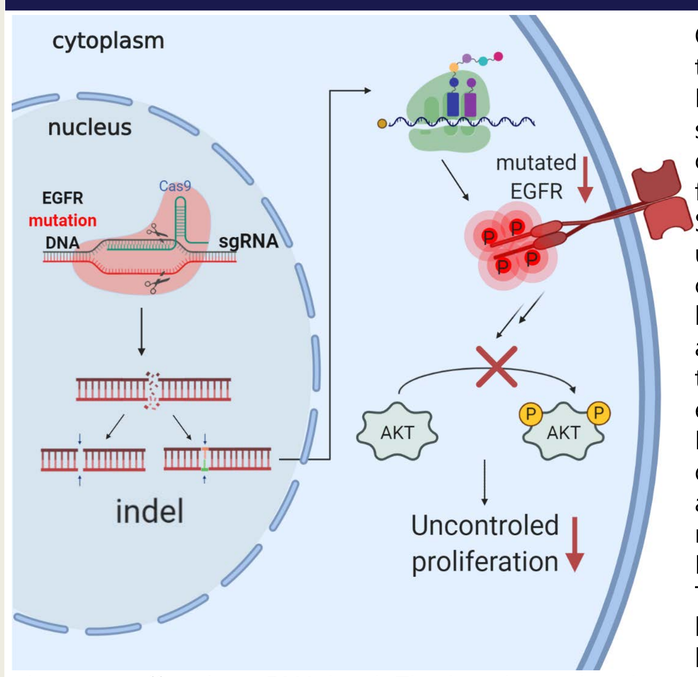
- Cell proliferation assay



* means p<0.05
** means p<0.01
*** means p<0.001
ns means non significance

Fig 7. Transfected HEK293 was grown for 4 days. On second day, treated with EGF.

Conclusion



Our team conducted experiments to confirm that CRISPR-Cas9 system can knockout the EGFR gene in human cells. We used reporter system and confirmed that GFP is expressed only in the experimental group by using a fluorescence microscope and FACS machine. So, we concluded that CRISPR-Cas9 system we used can target the EGFR gene. And we checked the effect of endogenous EGFR gene knockout in human cells at the DNA, mRNA, and protein level. By T7E1 assay, we concluded that CRISPR-Cas9 system cleaves the exact endogenous target sequence. Next, performing RT-PCR, we could confirm that there was no difference at the mRNA level between control and experimental groups. The western blot result showing protein level revealed lower EGFR expression in the experimental group. Through it all, we could conclude that EGFR knockout using CRISPR-Cas9 system certainly leads to a decrease in EGFR level, though it does not affect its mRNA level. The knockout also decreased the level of phospho-AKT, the downstream target of EGFR and activator protein of cell proliferation. Following this line of thought, we conducted a cell proliferation assay to figure out the effect of EGFR gene knockout on cell proliferation, but we couldn't get a meaningful result due to cell concentration inconsistency when seeding the transfected cells. The effectiveness of using reporter system before checking the endogenous level that we performed can be expected to be used in further experiments that use CRISPR-Cas9 system. Also, we checked EGFR knockout and its effect in wild type human cells. EGFR mutation is one of the common factors causing cancers. So, if CRISPR-Cas9 system is studied more and is set targeting for EGFR mutation, it can be used for treating cancer caused by EGFR mutation, such as lowering the condition of cancer.

References

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