Adopting CRISPR-mediated Genomic Editing Technique on the Treatment of Lung Cancer: Using Revolutionary Genomic Editing Technique to Treat Serious Human Disease

Sixian Chen[a]
Beijing Huijia Private School, Beijing China

Keywords: CRISPR, Lung Cancer, EGFR Gene, EML4-ALK Fusion Gene, TSLC1, P107 and P130.

Abstract: CRISPR system was first discovered in E. coli in 1987 by Japanese scientist Yoshizumi Ishino and his team. Since its emergence, it has been widely applied for a variety of medical use, including lung cancer. Traditional treatments of lung cancer gradually lost in their effectiveness by inducing drug resitance. Therefore, more influencing and innovative technologies are needed urgently, and CRISPR-mediated genomic editing technique is one of them. CRISPR system helps scientist to construct tumor model, to identify certain lung cancer related genes as well as deleting or repairing those cancer genes which can be done by targeting specific genes and either inhibit or activate its function. The applications of CRISPR system are now developed in a flying speed that it already moves to the clinical testing stage. Ideally, within a decade, the adoption of CRISPR system on treating lung cancer can be popularized widely. This review summarizes the characteristics of lung cancer and the application of CRISPR system on treating lung cancer. Potential target genes related with lung cancer will be discussed including EFGR, EML4 fusion gene, TSLC1 etc. The application of CRISPR system on deleting various types of cancer gene will be introduced, too. In order to generalize CRISPR technology into human cases, more in-depth investigations of the usage of this system are necessary for future studies.

1 INTRODUCTION

Lung cancer is a common type of cancer happened in lungs that leads to roughly 25% of death among all cancer cases. Its morbidity rate is ranked at the top level among a lot of developing countries including China, Europe and so on. There're mainly two types of lung cancer – non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC takes up approximately 80% - 85% of all lung cancer cases (National Comprehensive Cancer Network). There're a variety of subtypes within NSCLC including adenocarcinoma, large cell carcinoma, squamous cell carcinoma and so on. Although each of them damages different parts of the lungs, they're collectively categorized as non-small cell lung cancer since they possess similar treatment as well as prognoses. On the other hand, SCLC, or oat cell cancer occupies 10% - 15% lung cancer cases. It generally grows in a more rapid rate compared with NSCLC. The metastatic speed is also faster in SCLC patients that by the time the cancer is discovered, it has already diverted to other organs or tissues. It's also possible for tumor cells to be metastasized to the lungs from other parts of the body, but in this case, the tumor will be named after the primary cancer site instead of lung cancer (Niederhuber et al. 2020).

Due to its low surviving rate, the treatment of lung cancer is always the focus of clinical research. The pathology of lung cancer is mostly due to the mutation of certain genes. This mainly includes the transformation of proto-oncogene into oncogene and the inactivation of tumor suppressor gene. For the past decade, people have tried a huge number of therapeutic pathways to treat lung cancer, chemotherapy is one of the most common fashion. However, despite the various types of chemotherapy, many patients developed drug resistance after receiving chemotherapy which lower the efficiency of the treatment significantly. Traditional way of

[a] https://orcid.org/0000-0003-4132-7707

Chen, S.
Adopting CRISPR-mediated Genomic Editing Technique on the Treatment of Lung Cancer: Using Revolutionary Genomic Editing Technique to Treat Serious Human Disease.
DOI: 10.5220/0011228700003444
In *Proceedings of the 2nd Conference on Artificial Intelligence and Healthcare (CAIH 2021)*, pages 168-174
ISBN: 978-989-758-594-4

therapy is already behind the times. To invent new creative way of treatment, it's crucial to understand the causes, mechanisms, and pathologies of lung cancer. Among all possible reasons, genetic mutation is considered as the most essential cause of lung cancer. There're numerous types of gene that might induce lung cancer after being either activated or inhibited. Considering the significant role of mutated genes play in lung cancer, scientists start to investigate cancer gene therapy, a therapy that specifically designed for editing human genome.

Cancer gene therapy is a broad concept that include any treatment related with active cancer genes. In the past few years, using gene editing technologies to treat lung cancer has taken a lot of researchers' attentions. CRISPR system is one of them. CRISPR system is an efficient tool used to edit the genome of cells. The essential components of this system include single-guide RNA (sgRNA) and nuclease in which sgRNA guide the nuclease to cause a DNA cleavage at the targeted site. This enables researchers to edit the removed site, normally by introducing a DNA repair. There are several other types of gene editing technologies including zinc-finger endonucleases (ZFNs), transcription activator-like effector nuclease (TALENs) and so on. Compared with them, CRISPR system is more accurate and straightforward. It also allows researcher to target or edit any type of gene.

There's a huge amount of research aimed to investigate lung cancer and the application of CRISPR system on treating lung cancer. This review will focus on discussing the pathology of lung cancer, the traditional treatment of lung cancer as well as the application of gene editing technology, specifically CRISPR system on treating lung cancer.

2 PATHOLOGY OF LUNG CANCER

The pathogenesis of lung cancer is mostly caused by the mutation of protooncogenes into oncogenes, inactivation of tumor suppressor genes, insensitivity of cancerous treatment as well as dysfunction of immune system (Fig 1) (Jiang, Lin, & Zhao 2019)

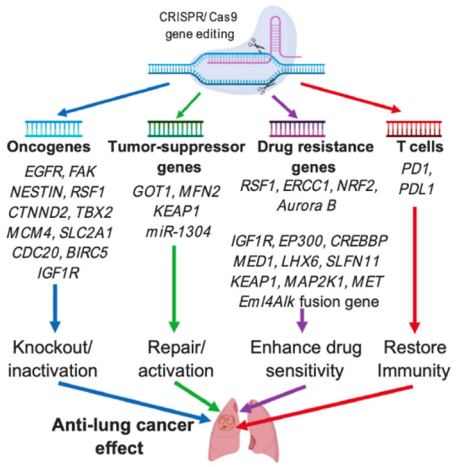

Figure 1: Summary of 4 main pathological causes of lung cancer.

2.1 Mutations of Protooncogene

Cancers are basically caused by mutation of specific genes. Gene that works in its normal state is called protooncogene which is in charge of regulating the progress of cell division. In cancer, protooncogene will mutate into oncogene which will significantly disturb the normal machinery of cell division. This will lead to an uncontrollable proliferation of cancer cells. Below shows several common types of oncogenes related with lung cancer.

2.1.1 Epidermal Growth Factor Receptor (EGFR)

Epidermal growth factor receptor (EGFR) gene mutation is associated with 13% cases of lung cancer. It's commonly detected in non-smoker or Asian patients with NSCLC (Soda et al. 2007). EFGR gene is located at chromosome 7. It is belonged to a class of receptor tyrosine kinases named HER/erbB. The homodimerization or heterodimerization of this class of receptors will induce a tyrosine kinase activity. This process will start a cascade of reactions which will eventually couple the receptor to the pathway of downstream signaling. This signaling will cause various cancerous phenomenon like decreased apoptosis (Koivunen et al. 2008). The expression of EGFR gene is the main inducing factor for rapid cell proliferation, angiogenesis, oncogenesis, and other cancerous symptoms. EGFR mutation is mostly happened when there's a deletion in exon 19 or a missense in exon 21 (Fig 2). In the missense mutation circumstance, one of the thymine molecules is substituted by a guanine molecule (Koo et al. 2017).

Figure 2. Mutation of EGFR gene due to the missense of exon 21. The thymine molecule is replaced by a guanine molecule which turn the code for amino acid leucine into arginine.

2.1.2 EML4-ALK Fusion Gene

Anaplastic lymphoma kinase (ALK) is a type of enzyme discovered from chromosomal translocation that will induce the formation of fusion protein. In this fusion, COOH-terminal donated by ALK is combined with a NH2 terminal from other genes. Recently, it is discovered that the fusion of ALK with echinoderm microtubule- associated protein-like 4 (EML4) might lead to the onset of lung cancer. Similarly, both ALK and EML4 are located at chromosome 2 and are separated by 12Mb (millions of base pairs). There're a variety of types of variants of EML4-ALK (Fig 3). For instance, when exon 20-29 of ALK is fused with either exon 1-13 or exon 1-20 of EMLK. The previous one is referred to variant 1 while the latter one is referred to variant 2. Both of the variants are responsible for initiation and maintenance of lung cancer. Expression of EML4-ALK gene was inhibited by injection TAE684 which is an ALK kinase inhibitor in mice sample. This causes a cease in the size of lung tumor which further suggests that lung cancer is related with the mutation of EML4-ALK gene.

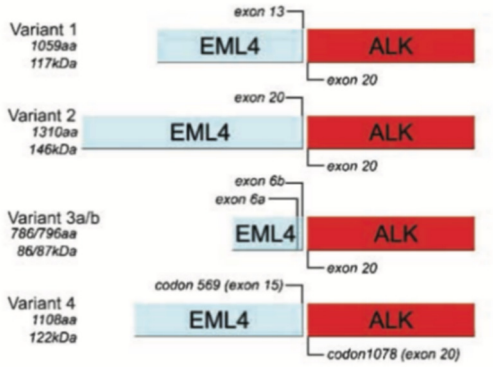

Figure 3: Example of 4 different types of EML4-ALK fusion gene variants with their mutated site and specific locations.

2.2 Tumor Suppressor Gene

Tumor suppressor gene is a type of gene that can prevent the growth of tumor. Inactivation of tumor suppressor gene will lead to a rapid growth of cancer cells.

2.2.1 TSLC1

A majority of tumor suppressor activities are located at a 100-kb segment 11q23.2. Kuramochi et al. conducted a genetic research by adopting yeast artificial chromosome (YACs) at chromosome 11 (Kuramochi et al. 2001). The study aimed to localize the existence of tumor suppressor genes in a small section at 11q23.2. By transferring the overlapped YACs region from human and nude mice, researchers have successfully localized a tumor suppressor gene at the central 700-kb segment of a 1.6-Mb YAC which is known as TSLC1. Scientists have found that the expression of TSLC1 is either reduced or absent in NSCLC as well as several other types of lung cancer cell lines. Loss of 11q23.2 which cause the deletion of one allele of TSLC1 was found in 40% cases of SCLC. The expression of TSLC1 is even more inhibited with the promoter methylation in those cell lines. As expected, by reactivating TSLC1, there's a significant suppression of malignant phenotype in lung cancer cells.

2.2.2 p107 and p130

Ng, S. R. et al. (2020) aimed to use CRISPR system to model lung cancers that are caused by the mutation of tumor suppressor gene by targeting and reactivating tumor suppressor gene that are once dormant. To do this, they target 2 members of retinoblastoma protein, p107 and p130. The mutation of retinoblastoma protein is found to happen in 6% of SCLC patients. Researcher made sgRNA to target p107 and p130 genes. The validity of those sgRNAs was tested in vitro by Western blot to ensure if there's a decrease in retinoblastoma protein level.

To test whether the system is worked in mice or not, researcher infect mouses with Ad5-USEC vectors which will express the sgRNA that target p107 or p130 gene. Then, they conducted a in vivo bioluminescence imaging to monitor the circumstance of lung tumor in animal's body. After a period of time, researchers detected an acute level of luciferase activity in both p107 infected and p130 infected animals which is accordant with the accelerated tumor progression. The average survival rate within those mouses have decreases significantly, too. This verifies the tumor suppressing function of p107 and p130 gene.

2.3 Insensitivity toward Chemotherapeutic Drugs

In order to treat cancer, chemotherapy is an effective and frequently used therapeutic method. Unfortunately, the resistance of patient's body toward chemotherapeutic drugs has increased a lot from the past few years. In such condition, some of the previously prevailed market drugs are not as effective as they were used to be For instance, cisplatin-containing regimens is a commonly used chemotherapeutic method to treat lung cancer, while the sensitivity of this cytotoxic drug to NSCLC patients still remain indistinct. Morodomi et al. conducted a research to investigate the sensitivity of chemotherapeutic drugs toward lung cancer patient with different types of gene mutation (Morodomi et al. 2014). The study found that patients with EML4-ALK fusion gene is less sensitive to cytotoxic chemotherapy compared with patient with EFGR gene mutation. By injecting chemotherapeutic drugs to patients with different gene mutations, it is found that the response rate is highest in patients with EGFR and lowest in patients with EML4-ALK fusion gene.

2.4 Single Agent Chemotherapy and Combination Chemotherapy

Maio et al. conducted a meta-analysis aimed to compare the effectiveness of single agent chemotherapy and combination chemotherapy as a second-line treatment (Di Maio et al. 2009). The result shows that as a second-line treatment, the response rate of patients toward combination chemotherapy is significantly higher than who received single agent chemotherapy. However, the overall surviving rate was not improved. In addition, most of the patients will end up by developing resistance to those drugs. Therefore, an innovative and effective way of treating lung cancer is in urgent needs.

3 TRADITIONAL TREATMENTS

Due to lung cancer's high prevalence rate among the world, a variety of innovative and effective therapies are in urgent need.

3.1 Chemotherapy

Chemotherapy is the initial treatment that SCLC patient usually received. It is effective in relieving various lung cancer complications like bronchial obstruction, pleural effusion, tumor metastasis and so on. It is a preferable palliative treatment. Platinum drugs are the most commonly used chemotherapeutic drugs on treating lung cancer. It works by bind with the N7 atom on adenine and guanine to stop DNA replication and in this way, induce the process of apoptosis (Rossi & Di 2016). There're several types of chemotherapy.

4 ADOPT CRISPR SYSTEM IN THE TREATMENT OF LUNG CANCER

CRISPR Cas9 technology is a revolution technique that allows scientists to target and edit a specific sequence of genes precisely (Jiang & Doudna 2017). Theoretically, it's possible to delete any cancerous genes by adopting CRISPR technology. This fresh idea of treating lung cancer by using "genetic scissors" has become to a hot topic to conduct research about recently. In the following section, different mechanisms of using CRISPR technology to edit cancer genes will be discussed.

4.1 Delete Cancer Gene

Crispr system takes advantages from those characteristics of cancer to fight against lung cancer. By deleting oncogene, activating tumor suppresser gene, or enhancing the sensitivity of chemotherapeutic drugs, the syndrome of lung cancer can be greatly relieved.

In order to use CRISPR Cas9 system to target this mutated gene, it's necessary to make an assumption that there's a protospacer- adjacent motif (PAM) sequence which is a nucleotide sequence targeted by Cas9 nuclease beside the missense gene. By

transferring an oncogenic mutant specific Cas9 using adenovirus as a media, the mutated gene can be targeted and deleted with high accuracy, which leads to a significant reduction of tumor production.

4.1.1 Repairing EGFR Gene

In order to treat patients by repairing EGFR gene, the biopsy sample of patient's tumor will be first obtained. The mutated EGFR gene will be identified from the biopsy and the correspondent single guide RNA (sgRNA) which is the RNA that will guide the endonuclease will be designed. The designed sgRNA will target a specific region on the mutated exon, e.g., L858R in exon 21, E19del in exon 19 and so on. Then, the sgRNA will guide Cas9 nickase to the target region which will make a single strand breaks in on each opposite side of the mutated exon (Fig 4). Next, the donated healthy DNA sequence will be substituted to the removed sequence by homology-directed repair (HDR) where its left and right arms are connected with the cut, mutated DNA sequence. The deletion of mutated exon and replacement with healthy DNA sequence will deracinate the mutated sequence and thus, stop the progression of lung cancer. While it is also possible to conduct a non-homologous end-joining (NHEJ) in which the mutated sequence is deleted, and rest of the DNA sequences are rejoined. By those two means, a stop codon introduced by HDR or indel made by NHEJ will destroy the translation of EGFR protein. As the result, the translated protein will lose its normal function, and therefore will not cause any oncogenic symptoms (Tang & Shrager 2016).

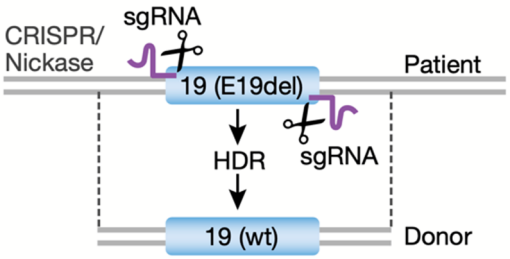

Figure 4: CRISPR Cas9 nickase cutting the opposite side of the mutated sequence.

4.1.2 Rearranging EML4-ALK Fusion Gene

Maddalo et al. (2014) conducted an experiment on adult mice aimed to investigate the efficiency of CRISPR Cas9 system on rearranging EML4–ALK oncogene. The study is unusual since it did the verification in a regressive way rather than a progression way. This means that instead of removing the EML4–ALK oncogene in mouses with lung cancer, they chose to introduce lung tumor into mouses body. If researcher can indeed add EML4-ALK fusion gene into mouses genome, it implies that it's also possible to remove the oncogene away from their genome.

They've genetically engineered mouses genome to simulate the most common type of EML4–ALK variant in NSCLC cases. They did this by introducing a double stranded DNA that breaks at specific regions in this case, intron 14 of EML4 gene and intron 19 of ALK oncogene. Next, in order to express CAS9 and sgRNA, researcher genetically engineered the plasmid started from tandem U6 promoters. Then, they made a recombinant adenovirus (Ad-EA) by introducing the CAS9 nuclease and sgRNA into an adenoviral shuttle vector to target the EML4 and ALK loci. Numerous mouses were infected by Ad-EA which leads to a speedy production of EML4-ALK inversion. This is exactly the pathological causes of NSCLC. After a month of infection, mouses lungs started to appear several small lesions. By the time of 6-8 weeks infection, the lungs tumor was large enough to be easily seen by necropsy and micro-computed tomography (Fig 5). The result shows that there's an obvious appearance and enlargement of lungs tumor in mouses lungs. This indicated the succession of rearranging EML4-ALK fusion gene. It also implies the feasibility of treating lung cancer by rearranging EML4-ALK oncogene in a positive way.

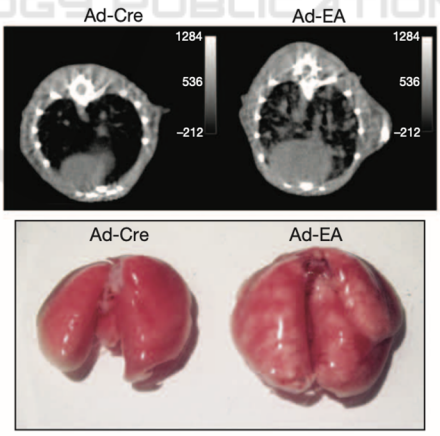

Figure 5: The above shows the micro-computed tomography of mouses lung tumor after 6-8 weeks of Ad-EA infection, while the below image shows the necropsy of mouses lung tumor. Ad-Cre is another type of infection that has the similar mechanism with Ad-EA infection.

5 CONCLUSIONS

Overall, CRISPR technology is a booming and innovative gene editing technology. It has an excellent applicational potential on treating and investigating lung cancer. CRISPR system can help scientists to construct tumor model, to study the pathology of lung cancer, to discover certain drug resistance and so on. It also enables researcher to target specific oncogene or tumor suppressor gene. By deleting or reactivating those genes, cancer symptoms can be greatly relieved. Recently, there're a huge amount of research of adopting CRISPR system on treating mouses with lung cancer. In order to apply this system on curing genuine human cases, more studies are in urgent needs. CRISPR system must pass several strict assessments before they're applied into clinical trials. In addition, there're a lot of ethical issues raised in testing the efficiency of CRISPR system on human samples. More animal studies are required before it is used in clinical circumstances.

However, it is necessary to aware the potential defects of CRISPR technology. Since human genome is an extremely large system, it is possible for CRISPR system to cut other genes that are not targeted by the nucleases which might cause an unpredictable effect.

This off-targeted effect can be reduced by improving guide RNA. Studies show that the length of guide RNA may induce certain type of mutations. Appropriate length of guide RNA is necessary for an optimum genome-editing efficiency. Research also reveals that specific chemical modification of guide RNA, like introducing 2′-O-methyl-3′-phosphonoacetate in the sugar backbone of guide RNA, can significantly reduce the rate of off target cleavage.

It is also possible to reduce off-target effect by improving the delivery system of CRISPR technology. For instance, adeno viruses have the potential of integrating into target cell genome in a more meager way, which will restrict off-target influence. In addition, deliver CAS9 protein and guide RNA together as a ribonucleoprotein complex will reduce the probability of missing the target gene, too. Yet, those methods are only be proven by a few studies. In order to further decrease the rate of off-target effect, more empirical evidence is needed.

Until now, substantial amount of research has done on animal sample. Once the technique has proven to be safe enough, clinical research should be conducted on human sample in order to adopt CRISPR technology on treating genuine human lung cancer. However, researchers should always be aware of the ethics involved in clinical research and should strictly follow the ethical guidelines.

REFERENCES

Di Maio, M., Chiodini, P., Georgoulias, V., Hatzidaki, D., Takeda, K., Wachters, F. M., ... & Gridelli, C. (2009). Meta-analysis of single-agent chemotherapy compared with combination chemotherapy as second-line treatment of advanced non-small-cell lung cancer. J Clin Oncol, 27(11), 1836-1843.

Jiang, C., Lin, X., & Zhao, Z. (2019). Applications of CRISPR/Cas9 technology in the treatment of lung cancer. Trends in molecular medicine, 25(11), 1039-1049.

Jiang, F., & Doudna, J. A. (2017). CRISPR–Cas9 structures and mechanisms. Annual review of biophysics, 46, 505-529.

Koivunen, J. P., Mermel, C., Zejnullahu, K., Murphy, C., Lifshits, E., Holmes, A. J., ... & Jänne, P. A. (2008). EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clinical cancer research, 14(13), 4275-4283.

Koo, T., Yoon, A. R., Cho, H. Y., Bae, S., Yun, C. O., & Kim, J. S. (2017). Selective disruption of an oncogenic mutant allele by CRISPR/Cas9 induces efficient tumor regression. Nucleic acids research, 45(13), 7897-7908.

Kuramochi, M., Fukuhara, H., Nobukuni, T., Kanbe, T., Maruyama, T., Ghosh, H. P., ... & Murakami, Y. (2001). TSLC1 is a tumor-suppressor gene in human non-small-cell lung cancer. Nature genetics, 27(4), 427-430.

Maddalo, D., Manchado, E., Concepcion, C. P., Bonetti, C., Vidigal, J. A., Han, Y. C., ... & Ventura, A. (2014). In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature, 516(7531), 423-427.

Morodomi, Y., Takenoyama, M., Inamasu, E., Toyozawa, R., Kojo, M., Toyokawa, G., ... & Ichinose, Y. (2014). Non-small cell lung cancer patients with EML4-ALK fusion gene are insensitive to cytotoxic chemotherapy. Anticancer research, 34(7), 3825-3830.

Ng, S. R., Rideout, W. M., Akama-Garren, E. H., Bhutkar, A., Mercer, K. L., Schenkel, J. M., ... & Jacks, T. (2020). CRISPR-mediated modeling and functional validation of candidate tumor suppressor genes in small cell lung cancer. Proceedings of the National Academy of Sciences, 117(1), 513-521.

Niederhuber JE, et al., eds. Cancer of the lung: Non-small cell lung cancer and small cell lung cancer. In: Abeloff's Clinical Oncology. 6th ed. Elsevier; 2020. https://www.clinicalkey.com. Accessed Jan. 13, 2020.

Non-small cell lung cancer. National Comprehensive Cancer Network. https://www.nccn.org/professionals/physician_gls/default.aspx. Accessed Jan. 13, 2020.

Rossi, A., & Di Maio, M. (2016). Platinum-based chemotherapy in advanced non-small-cell lung cancer: optimal number of treatment cycles. Expert review of anticancer therapy, 16(6), 653-660.

Soda, M., Choi, Y. L., Enomoto, M., Takada, S., Yamashita, Y., Ishikawa, S., ... & Mano, H. (2007). Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer. Nature, 448(7153), 561-566.

Tang, H., & Shrager, J. B. (2016). CRISPR/Cas‐mediated genome editing to treat EGFR‐mutant lung cancer: a personalized molecular surgical therapy. EMBO molecular medicine, 8(2), 83-85.