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Review Article: Lung Cancer Pathophysiology using CRISPR Technology

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ABSTRACT

According to the World Health Organization, respiratory disorders, such as "influenza infection, acute tracheal bronchitis, TB, chronic obstructive pulmonary disease, lung cancer, and nasopharyngeal carcinoma", have a major influence on human health. While environmental and socioeconomic variables might impact the pathogenesis of lung and respiratory tract diseases, it is nevertheless important to further investigate genetic and epigenetic reasons since a great many respiratory illnesses have a genetic or epigenetic basis. CRISPR is made up of interspaced, regularly-spaced palindromic repeated sequences and related proteins that carry out the CRISPR system's duties, which are found in prokaryotes' immune systems. This technology may be used to target, alter, and control genes, making it essential in respiratory research. Cas9 systems enable preclinical modelling of causative variables implicated in respiratory disorders, to generate fresh insights into its operations. CRISPR is also used to hunt for respiratory functions and pathology-associated genes. Which may lead to the discovery of new disease causes or therapeutic targets. The genetic and epigenetic mutations and the disease-associated mutations could be edited using CRISPR/Cas9. This kind of personalised medicine, which might be combined with stem cell reprogramming and transplantation are additional methods, that support embryonic stem cell expansion, might lead to the creation of novel respiratory illness treatment options. The new and developing area of investigation of CRISPR gene editing is one that requires further study the challenges of its specialty and the need for effective and safe delivery strategies. In respiratory health research and treatment, CRISPR systems represent an important step forward, and the discoveries made possible by this technology are likely to continue.

Keywords- CRISPR, Cas9, pathology associated genes, lung cancer.

I. INTRODUCTION

Many individuals have issues with their quality of life due to upper respiratory tract issues, including nose, throat, bronchi, lungs, and the pleural cavity. Common respiratory diseases fall into three broad categories, Examples of relatively mild disorders include "Chronic obstructive lung disease (COPD), acute bacterial and viral infections, asthma and lung and nasopharyngeal malignancies". Asthma, lung and nasopharyngeal malignancies are instances of considerably harsher situations, such as acute bacterium and viral infection (COPD), chronic respiratory illness[1-3]. Surfactant protein deficiency, CF, AAT and a number of other unique monogene illnesses have little or no influence on health and life of the patient. Environmental variables such as cigarettes tobacco, air pollution, infections, somatic or inherited genetic abnormalities and the effect of ageing are all issues to consider because of the vast variety of diseases that may emerge. As genetic risk factors contributing to the respiratory disease increase the knowledge[4-6], new technologies are needed to alter the genetic material and modulate endogenous gene expression. The availability of gene editing techniques enables preclinical models to examine the role of certain genes in respiratory physiology and pathology, which may lead to future therapies for respiratory illnesses.

However, newly emerging technologies, including homologous recombination and site-directed nucleases, have been based on small nucleic acid molecules that are able to bind specific DNA sequences, such as ZFNs and TALEN.

One newer system, the CRISPR-Cassystem, has added a significant dimension to the genetic-modification technology. Prokaryotic adaptive immune systems CRISPR functions as an immune system that detects and eliminates viruses and plasmids[7-9], using an RNA-guided method of target recognition. Cas9 system found in Streptococcus pyogenesand described by study authors as a "programmable, efficient, and specific DNA editing"

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might be reused for a number of uses. For some time, this has been implemented for the purpose of editing the endogenous genes of different cell types and species. To take use of the wide variety of CRISPR systems found in species other than S. pyogenes[10], many of which provide different advantages in terms of gene editing specificity, targeting flexibility, and delivery simplicity, researchers are only starting to do so. Other than the already mentioned use of CRISPR systems[11-13], the methods included using the Cas9 system for gene editing. CRISPR systems were employed for the transformation and confirmation of the relevance of gene variations associated with respiratory illnesses, as well as for the modulation of gene sequences and the rapeuticalexpression[14-15]. CRISPR also has the potential to be widely used in respiratory research in the future, particularly for disease-associated genes, since it is a quick and easy technique to screen for them. The following portion will focus on how CRISPR systems in prokaryotes are detected and functioned and how they have been transformed into a gene editing tool[16-18]. In the publication, we analyse uses of CRISPR editing in breathing research and potential hindrances to their wider use, especially the CRISPR methodology.

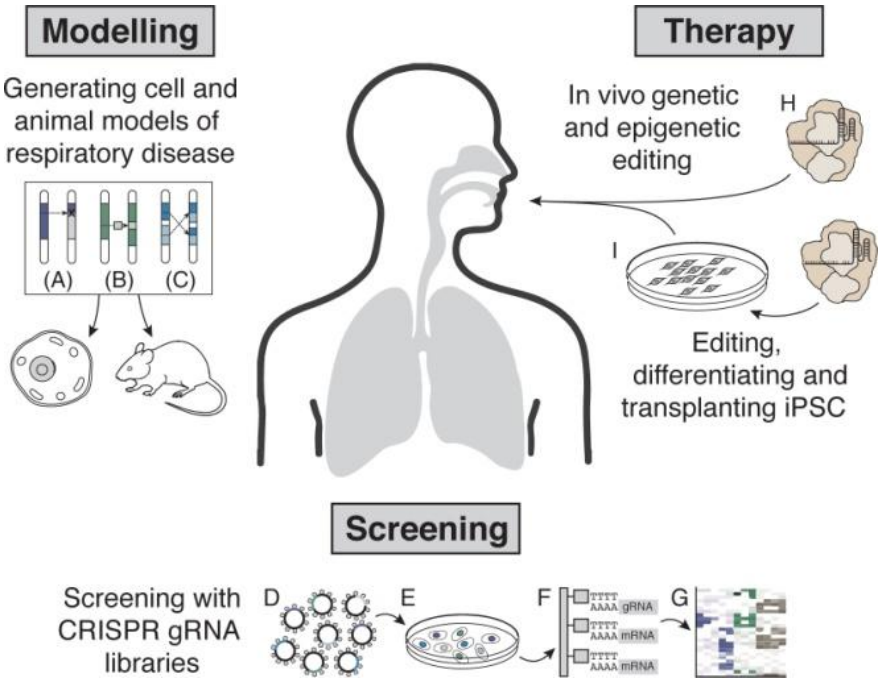

Figure 1: CRISPR systems with recurs frequently, although the repeats are separated by gaps that are palindromic have been used to treat respiratory illness.

II. METHODOLOGY OF CRISPRSYSTEMS

When a new pathogen is detected, proto-spacers made up of the pathogen's nucleic acid fragments, also referred to as protospacers, are incorporated into CRISPR adaptation proteins edit the bacterial host genome, resulting in a CRISPR array. As the virus enters the second stage of infection, to make CRISPR RNA, the whole array is transcribed and processed (crRNA)[19-21]. Each array incorporates a unique complementary RNA (cRNA) that makes it possible for the host to identify pathogen nucleic acids that have a similar sequence to the RNA first introduced into the array. Target sequence binding (through Watson–Crick base pairing) initiates Cas protein nuclease cleavage of foreign DNA or RNA elements, as is shown in the pathogen Cas end nuclease binding to crRNA and crRNA binding corresponding target sequences.

Although CRISPR systems of different kinds exist, the endonuclease Cas9 from the bacteria Staphylococcus pyogenes is the most widely used for gene editing applications thus far[22]. The Cas9 complex has the crRNA ligated to a transactivatingcrRNA, both of which are delivered to the target DNA regions by the short mRNA molecule referred to as a "transactivatingcrRNA" (tracrRNA)[23-25]. This Cas9 protein only detects and is programmed to cleave DNA targets that complement the crRNA and are located just upstream of the PAM consists of NGG. Cas9 cuts the DNA when it detects the proper DNA target, causing a DNA DSB three base pairs upstream of the PAM. To make matters

simpler, the non-coding RNA strand, known as tracrRNA, was replaced with a chimeric single-guide RNA molecule[26-28], which is more easily designed and synthesised than the complicated non-coding RNA strand, known as crRNA-tracrRNA. The DNA cutting enzyme Cas9 protein and a targeting oligonucleotide produced a nuclease that is designed to induce a DSB throughout the genome, with an accuracy comparable to the NGG.

Involving the elucidation of the mechanism of the S. pyogenes Cas9, which allows it to target bacterial cells, later it was discovered that the S. pyogenes Cas9 system may also be used for gene editing in human cells[29-31]. Cas9-triggered DNA double-strand breaks (DSBs) may be used for gene editing via two different routes. Non-homologous end joining (NHEJ) is the most often utilised DNA repair mechanism. The faulty ones are naturally error-prone and are very likely to produce random insertions and deletions. We may infer that CRISPR-mediated gene re-parations often produce nonsense, since these examples have resulted in functional gene deletion.HDR is a method of DNA repair which utilises the cell's existing templates as donors, and the pathways of which it is a part when a cell has an accessible DNA donor[32-36]. This method includes progression modifications that are taken from applied to the host DNA using the donor template. Cas9, artificial DNA donor molecules, and sgRNA are all delivered together with the possibility of specific sequence target identification and potential for more gene editing using Cas9[37]. In addition, the newly reported Cas13 proteins may be utilised to specifically target RNA for RNA interference modifications and the number of unintended changes is theoretically smaller than for traditional RNA interference[38]. Despite the many CRISPR systems discovered, the biological variety that they represent has yet to be completely investigated and will no doubt provide many new innovations in the near future.

Crispr systems have played a critical role in molecular biology, contributing much to the advancements in this area. Each new DNS goal sequence has to be designed, manufactured, selected and validated for new DNA-binding and time demanding protein domains[39-41], for ZFN and for TALEN to find targets via protein-DNA interactions. A simple, additional base pair between the crRNA and a CRISPR system DNA strand is used for the identification of the target, while the non-complementary basis pairing between crRNA and DNA strand is needed to regulate a crRNA-reliant[42-43] constraint in Cas systems. Different Cas proteins may be employed with different DNA target sequences using the same Cas protein with each new target simply needing the production of a fresh crRNA or sgRNA. The introduction of this capability has dramatically decreased the time, cost, and expertise necessary to carry out gene editing workflows[44]. This section summarises how CRISPR systems have been used in respiratory research and medicine and screening and therapeutic applications.

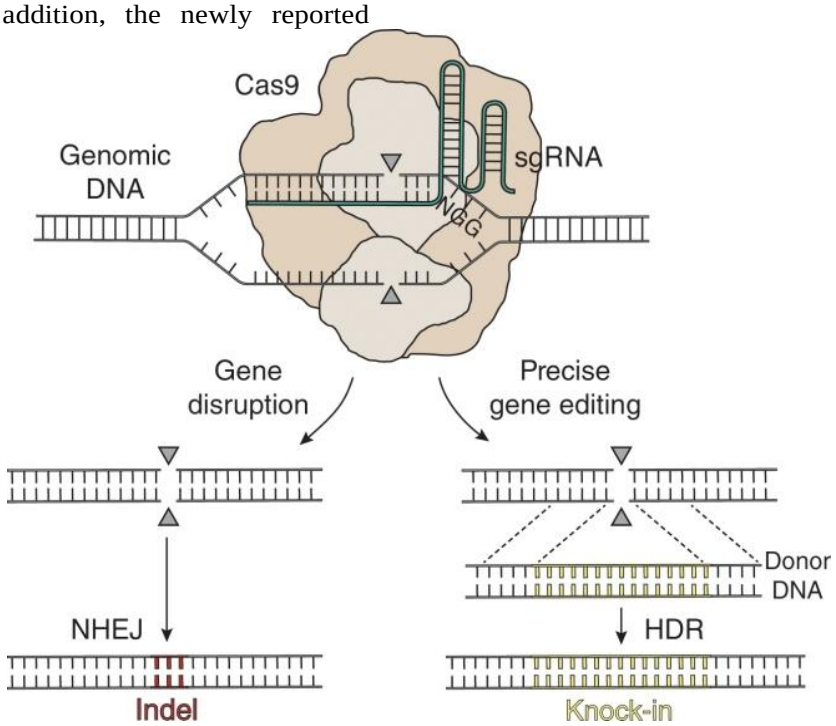

Figure 2: The CRISPR Next-generation gene editing technologies uses arranged at regular intervals and punctuated by palindromic repeating groups repeats.

III. LUNG CANCER MODELLING

When it comes to using computer modelling to represent and study the characteristics of cancerous lungs, the complexity has always been a substantial obstacle. That said, new methods that use CRISPR have helped researchers produce more customizable and models that may be trusted for research use[45-47]. Tumor suppression may be knocked down by an anticancer drug that depletes tumour suppressor proteins led to the development of molecularly modelled carcinomas, which are physically and functionally analogous to genuine tumours. The use of Cas9 for gene deletion has proven particularly successful utilising NHEJ. AsgRNA library to carry the afore described sgRNAs was successfully removed from all tumour-suppressor genes to inactivate the genes. In addition to being deployed on its own[48-49], CRISPR was utilised alongside other gene editing methods, such as Crerecombinase. In these experiments, researchers activated the KrasG12D gene with Cre expression, which provided KrasG12D-expressing cells to the mice[50]. Next, the researchers edited major tumour suppressor genes using CRISPR-based gene editing, and this activation system successfully created functional adenocarcinomas in the mice's lungs.

Table 1: Various methods have been used to simulate lung cancer in vitro and in vivo

Target cell	Approach	Disease	Gene	Alteration	Delivery
h HEK-293	In-vitro	Lung Adenocarcinoma	CD74-ROS1, EML4- ALK, KIF5B-RET	Chromosomal rearrangement	Plasmid transfection
Mouse lung cells	In-vivo	Lung Adenocarcinoma	Nkx2.1, Pten, Apc	Loss of function	Lentiviral
Mouse lung cells	In-vivo	NSCLC	GeCKO library	Loss of function	Lentiviral
Endothelial cells and neurons	In-vivo	Lung Adenocarcinoma	Kras, p53, Lkb1	Loss of function and site- directed mutagenesis	Lentiviral, AAV
Mouse lung cells	In-vivo	NSCLC	Eml-Alk	Chromosomal rearrangement	Adenoviral
Mouse lung cells	In-vivo	NSCLC	Eml-Alk	Chromosomal rearrangement	Lentiviral

Clinical studies of Lung cancer Crispr-based gene therapy

CRISPR is a tremendously strong and potentially groundbreaking gene therapy technology. Although CRISPR genome editing is very effective, few current human clinical studies are employing it, mainly because few current projects have been given the go-ahead to perform clinical trials. Until far, CRISPR has mostly been employed in the laboratory to change the genomes of cells in a test tube[51-53]. Despite the 13 published clinical studies throughout the globe using CRISPR for the treatment of cancer, there are still a number of these studies going on that have not yet been reported. Only one research on cancer cell gene editing has been conducted[54]. That was done by giving CRISPR Systems to the body, and a systematic review of all studies confirms that in vivo treatment did successfully alter all mentioned cancer cell genes. In addition, the other twelve Cas9-mediated Cancers clinical trials were tested as a cancer treatment. Two techniques have been frequently used in ex vivo gene editing investigations. It aims to improve T-cell efficacy by removing genes from T-lymphocytes[55] that impede their targeting efficiency. The second strategy involves attaching chimeric antigen receptors to the surfaces of T-lymphocytes, which are specific to antigens found on malignant cells, in order to enhance targeting specificity and efficiency.

The PD-1 knockout engineered T cells study, which Sichuan University is doing, is presently ongoing and is evaluating the safety of the PD-1 knockout T cells in treating metastatic non-small cell lung cancer[56-58]. This research may be placed into one of the previously indicated categories. At the core of this work is the PD-1, programmed gene that only expresses cell death as an immunological checkpoint and has only been proven to activated T-cells. When PD-1 interacts with PD-1 ligands, the process of T-cell death is triggered[59-60]. Antigen presenting cells are usually discovered to have PD-1 ligands on them. The PD-1 pathway interferes with T cell receptor signalling to prevent an overreaction of the immune system. Since knocking down the PD-1 gene will expand the T-cells' longevity and avoid their demise when activated, by removing the T-cell cycle checkpoint inhibitor[61-63], it can be concluded that doing so would help prolong the life of the T-cells. In addition, it would thus have the effect of boosting the number of T-cells that are active in the blood, so boosting the tumor's susceptibility.

The researchers would take autologous T-cells from the peripheral blood and use the CRISPR9 technology to selectively knock off the PD-1 gene in vitro for this study[64]. When they've done that, the researchers will increase the quantity of autologous PD-1 knockout T-lymphocytes and choose a group of people from the initial participants who aren't responding to the treatments. The initial stage in the treatment would be to provide Cyclophosphamide three days before T-cell infusion. Three test groups (each with a different amount of PD-1 knockout T cells) would be used to divide the patients into three groups, each receiving 1×10^7 per kg PD-1 knockout T cells, 2×10^7 per kg PD-1 knockout T cells, and 4×10^7 per kg PD-1 knockout T cells. In order to conclude therapy[65], there would be two further cycles in total. The research would go on to examine the responses of the patients to the therapy and any side effects for an additional period of time after the conclusion of therapy.

Etiology of Lung Cancer

For more than 50 years, researchers have observed a link between tobacco use and lung cancer, and this link continues to dominate the etiologic milieu of this malignant illness. Lung cancer has also been shown to be caused by a variety of other chemicals, many of which were identified in the work environment. It has long been assumed that there is an inherited propensity to the illness, and current research has identified numerous plausible causes as well as a probable method of inheritance for the condition. There has been significant progress in understanding the molecular abnormalities that are present in lung cancer cells in recent years. Both of these well-known models of lung carcinogenesis have been modified to include the results of contemporary research[66]. As the specifics of the carcinogenic process are uncovered, one objective is to find intermediate (preneoplastic) signs of exposure and inherent propensity that will aid in the assessment of the risk of lung cancer in both individuals and populations.

Pathogenesis of lung cancer

The development of lung cancer is similar to that of other cancers, in which start events triggered by carcinogens result in the first stages of promotion and advancement of the disease, with each step building on the previous. Cigarette smoking both begins and accelerates the development of cancer. The initiating event occurs early in the smoking process, as indicated by the presence of comparable genetic alterations in the present and past smokers. As a consequence, smoking produces a "field impact" on the lung epithelium, resulting in a large number of initiated cells and a higher chance of transformation. As a consequence of the stimulation given by chronic irritation and promoters inherent in cigarette smoke, continued cigarette smoke exposure allows for the accumulation of new mutations[67]. Cancer normally develops after a 20-25-year period of time has passed after the beginning of smoking. After quitting smoking, the chance of developing cancer diminishes, but cancer cells that have already been begun may advance if another carcinogen continues the process.

It is necessary to differentiate between SCLC and NSCLC due to the fact thosethem: i) start from separate cells, (ii) go through distinct pathogenesis processes, and (iii) acquire various genetic alterations. “MYC, BCL2, c-KIT, p53, and RB are often mutated in SCLC, while EGFR, KRAS, CD44, and p16 are often mutated in NSCLC”. Depending on their function, all of these genes are tumor suppressors or ontogenesis.

Classification of invasive lung cancer

This categorization is based on the appearance of the tumor cells under a microscopical microscope. It is critical to distinguish between these two forms of cancer because they develop, spread, and respond to treatment in very different ways.

SCLC accounts for around 10 percent to 15 percent of all lung malignancies. This is the most aggressive and rapidly growing kind of lung cancer among all sorts. Cigarette smoking is highly associated with the development of SCLC. SCLCs proliferate swiftly to several areas throughout the body, and they are most typically detected after they have disseminated far.

The most prevalent kind of lung cancer, NSCLC, accounts for around 85 percent of all occurrences[68]. It is possible to distinguish three forms of NSCLC based on the kind of cells that are detected in the tumor. They are as follows:

• ***Adenocarcinomas*** are the most frequent kind of non-small cell lung cancer in the United States, accounting for up to 40% of all lung cancer occurrences. However, unlike other types of lung cancers, adenocarcinomas may occur in people who do not smoke, particularly in women. Lung adenocarcinomas are more often seen in the outside or peripheral regions of the lungs. In addition, they have a predilection for lymphatic node expansion and elsewhere. Adenocarcinoma in situ is a kind of adenocarcinoma that occurs in various locations in the lungs and spreads across the previously developed alveolar walls. A chest X-ray may also reveal what seems to be pneumonia. It is becoming increasingly frequent, and it affects more women than it does males. People diagnosed with such lung cancer are more likely than those diagnosed with other kinds.

- Formerly, squamous cell carcinomas were predominant over adenocarcinomas, but now account for around 25%-30% of all cases of lung cancer. Squamous cell malignancies are more common in the middle chest region, namely in the bronchial passages[69]. The majority of the time, this kind of lung cancer remains inside the lung, spreads to lymph nodes, and develops rather big, producing a hollow.

• ***Large cell carcinomas*****,** also known as undifferentiated carcinomas, are the least prevalent kind of non-small cell

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lung cancer (NSCLC), accounting for 10 percent to 15 percent of all lung malignancies. This kind of cancer is quite likely to spread to, among others, the lymph nodes and other distant regions.

Treating and interrogating lung tumori genes is with crispr

CRISPR can do numerous sophisticated chromosomal recombinations in addition to knockin and knockouts. This model was used to study the aspects of the rearrangement of Eml4-Alk genes reported for non-small cell lung carcinomas (NSCLC). The ablation of PTPN2 boosted the effectiveness of immunotherapy by boosting the interferon-mediated effects on growth suppression and antigen presentation. Upregulation of a deubiquitination enzyme, which has been linked to early phases of lung epithelial cell transition and cancer[70], is also a result of the deletion. The regulation of tumor suppressor miR584-3p by FECR in SCLC and NSCLC tissues is a significant factor in lung cancer spreading. The suppression of the miR585-ROCK1 pathway by CRISpr Cas9-based FLI1 gene knock-out resulted in a stop in tumour development.

Another effort led in clinical research at the University of Pennsylvania presently involves the use of autologous NYCE T cells. The NYCE T-cell operation is being carried out. In preclinical in vitro research for this clinical investigation, the human lung cancer cell line A549-ESO-CBG was employed[71]. The efficacy of p53 reactivating chemicals is heavily reliant on the drug's resistance in the patient, which may be addressed by confirming the target using CRISPR-Cas9. Drug resistance is a major challenge for doctors when treating patients with lung cancer. The uPAR (Urokinase plasminogen activator receptor) receptor was knocked out, and lung cancer cells were less resistant to treatments, including 5-FU, Doxorubicin, Cisplatin, and Docetaxel.

The use of Cas13 to pick exceedingly tiny amounts of DNA or RNA is another sector developing. Mutations were discovered using rapid paper-based testing in the liquid sample of non-small cell lung carcinoma patients.

Future Perspective

The CRISPR device has a bright future ahead of it in cancer biology because it is an adaptive, simple, convenient, and efficient technology. The technology presents a unique approach to cancer therapy that was previously impossible to implement by allowing for genomic alterations in target cells. The variety, efficacy, and adaptability of the technology will make it the ideal type of cancer treatment in the future[72]. In the future, researchers will have an influence on Cancer Biology as a whole by developing well structured strategies and instruments to provide the target cell or tissue with the technical technology and efficient ways and instructions to manage and eliminate off-target impacts of the technology.

IV. CONCLUSION

Conventional approaches based on genetic treatment can enable novel discoveries being a very accurate and effective technique in cancer research and CRISPR, can transform them into useful medicines. At the moment, it is thought that CRISPR has several benefits over the other major gene psychotherapy approaches, including “ZFN and TALENs”. Though currently existing treatments are only short-term for the body[73], and gradually wear off over time, gene therapy being genetically engineered allows for permanent treatment delivery and is comparable to hard-coding the treatment into the body. This has the potential to generate vaccinations and therapies that last for a lifetime[74]. As of now, CRISPR is only being used in the clinical setting for drug therapies, but in the future, CRISPR might be used to examine the way cells function. Among all the varieties of Cas9, such as dead Cas9 and dead nCas9, we believe that “dead Cas9 and dead nCas9” are especially useful for the testing of cancer cell growth and proliferation pathways and processes. Based on gene therapy techniques, lung cancer has received a significant amount of attention in the medical research community. However, due of the ethical issues that occur with these approaches, the number of therapy options for this condition is still quite limited[75]. The translatability of gene therapy research is anticipated to increase as governments adopt new laws and regulations, paving the way for the launch of a new generation of cancer-fighting medications.

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