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Gene-Editing Technology in Lung Cancer: Models and Therapies

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Abstract. Cancer is a disease that develops when the body's cells expand unchecked. Lung cancer is a general term for cancer that first appears in the lungs. The most common cancer that is lethal is lung cancer. Lung cancer is the commonly diagnosed cancer diagnosed between both female and male in the US, only behind certain forms of skin cancer. Lung cancer is most often caused by smoking. Additionally, smoking other tobacco products (including pipes or cigars), breathing secondhand smoke, being linked to radon or radon at work or home, and lung cancer risk may be increased by having a history of the disease in your family. Furthermore, new developments in genome editing technology have dramatically increased the possibility of curing cancer at its origin. This article discusses potential gene editing methods for treating lung cancer and systematically identifies the locations of common gene changes in the condition.

Keywords: gene-editing technology, lung cancer, therapy

1. Introduction

Lung cancer, one of the most common types of cancer in the world today, is a malignant lung tumor brought on by the unregulated expansion of lung tissue cells. With five-year survival rates of 10–20%, it has the poorest prognosis of any tumor kind, ranging from 92% to 0% for the earliest and most advanced stages, respectively [1]. Technology for gene-editing that is based on synthetic or since the development of genome editing technologies, nearly all eukaryotic cells can now be directly targeted and modified. [2]. This method is based on manmade or bacterial nucleases. Finally, we describe clinical studies using genome editing platforms to treat lung cancer, as well as some uses, restrictions, and potential future uses for this technique.

1.1. Pathologies of lung cancer

It is widely recognized that the development of lung cancer is related to smoking, ionizing radiation, air pollution and nutritional status. Among them, smoking is the main cause of lung cancer. Nicotine or other harmful substances in tobacco can induce cellular carcinogenesis and aggravate the development of cancer. Ionizing radiation and the activation of some oncogenes in the patient can also induce cellular cancer or trigger the development of tumor.

Proto-oncogenes, tumor suppressor genes (TSGs), and DNA repair genes are three of the at least three classes of cellular genes that are implicated. TSGs are typically inactivated by loss of one parental allele coupled with a point or minor mutation, or by methylation inactivation of a target TSG in the remaining allele, whereas oncogenic activation frequently comes from point mutations, gene

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amplifications, or rearrangements. Dysregulated gene expression may result from as of yet unidentified processes. Simple repetitive sequences, however, show alterations. In lung cancer, aberrations of DNA repair genes, particularly DNA mismatch repair genes, have not yet been shown to have a significant effect. The replication error repair (RER+) phenotype frequently observed in tumors with mutations in DNA mismatch repair genes does not appear to be present in lung malignancies. In contrast to "RER+ laddering," the instability in lung cancer occurs in a single "shift" of individual allelic bands and impacts a comparably smaller number of markers. The lung cancer characteristic is hence known as "microsatellite alteration." There have been discoveries connecting different DNA repair processes to lung cancer. O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair gene, is sometimes made inactive by the epigenetic process of promoting hypermethylation, and a gene (OGG1) 8 implicated in the repair of oxidative DNA damage is infrequently altered [3].

1.2. Models of lung cancer

1.2.1. In vitro models. Smith et al. used the word "organoid," which means "resembling an organ," to describe an instance of cystic teratoma [4,5]. Currently, the word "organoids" is currently used to refer to three-dimensional (3D) structures made of several cell types from their in vivo counterparts that resemble the major tissue and are unique to the parental organ. Using an organoid model to analyze the mechanisms behind lung cancer is beneficial. Lung cancer cell primary cultures have received treatment using the cancer tissue-originating spheroid technique. Malignant pleural mesothelioma, small cell lung cancer (SCLC), and non-small cell lung cancer (NSCLC) make up 85% and 85% of cases, respectively, of the three most frequent subtypes of lung cancer that are generally detected. Large-cell carcinoma, lung adenocarcinoma, squamous cell carcinoma, and other less distinguishable kinds of the illness are further NSCLC subtypes. Spheroids of NSCLC tumors grown by patients have been created [4]. These patient-derived tumor spheroids had features with the matched patient tumor samples, such as tumor cells with high nuclear-cytoplasmic ratios, hyperchromatic nuclei, and irregular nuclear membranes. These tumor spheroids from patients have some tubular characteristics and branching morphogenesis when seen under an inverted microscope. The tumors are adenocarcinomas, as shown by Thyroid Transcription Factor 1 (TTF-1) labelling in patient-derived tumor spheroids. Additionally, it was shown via the evaluation of several patient-derived tumor spheroid passages that the clinical indications were consistently retained with repeated passaging and that the tumor cytological properties remained unchanged [6].

In addition to producing acinar or massive glandular patterns, they noticed that ADC organoids possessed the ADC markers TTF-1, napkin-A, and cytokeratin 7. LKB1 deletion has been associated with significant alterations in the tumor microenvironment, metabolism, cellular polarity, and differentiation. The inactivating hepatic kinase B1 is widely aberrant in ADC (LKB1). Lkb1 ablation in autochthonous and transplant models revealed lineage reversal from KRAS-positive ADC to SCC in KRAS-positive organoids [7]. Since pre-clinical research shows the effectiveness of treating the changed cellular pathways linked to LKB1 depletion, these adjustments together provide choices for therapy strategies. The histological characteristics of SCC organoids match those of SCC tissue, including clear cell boundaries, overexpression of cytokeratins 5/6 and cytoplasmic keratinization, and p63. BCL11A is an SCC oncogene, as shown by basal cell-derived organoids created from Bcl11a conditional-knockout rats. Organoids from SCLCs have characteristics of tiny cells. Alternately, organoids made from healthy tissue might be altered to resemble lung cancer. As an example, Park et al. produced human bronchus epithelial cell organoids and subsequently added genetic alterations, such as the upregulation of the B-cell leukemia/lymphoma 2 and myeloblastomatosis oncogenes on organ . The generated organoids gave rise to tumors that were very comparable to SCLC after being implanted into immunosuppressed animals [8].

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1.2.2. In vivo models. Many of the mutations observed in non-small cell lung cancer (NSCLC) in humans have been transferred to mice.

1.2.3. p53. It has been discovered that mice with p53 mutation had much higher lung tumor loads and multiplicity compared with wild type (WT) mice. p53 homozygous and heterozygous knockout mice had a faster rate of malignant development. The fact that p53val135/WT mice are extremely vulnerable to chemically inducing mouse lung cancers supports the critical function of p53 in the development of lung tumors. Clinically, lung cancer occurs very frequently in people with a p53 germline mutation, and it occurs much more frequently in smokers [9].

It is believed that the gene most frequently altered in human cancer is the tumor suppressor gene p53, which is frequently altered in a number of malignancies of the aerodigestive tract. The intimate function of the p53 in humans is confirmed by the elevated risk of lung cancer in people with Li-Fraumeni syndrome, who frequently have a germline mutation in the gene. On the other hand, the majority of spontaneous or chemically caused tumors in rodents lack p53 mutations. In order to investigate this, examined a transgenic mouse carrying the dominant-negative p53 gene mutation Arg135Val, which is then regulated by its own endogenous promoter [10]. The resulting animals had 2 healthy p53 alleles along with 3 copies of the mutant transgene.

1.2.4. KRAS. Ras gene alterations are seen in 30% or more of human cancers1,2. K-Ras is the gene in this family that is changed most often in human tumors, including carcinoma cells of the pancreas (incidence: 70–90%), and lung (incidence: 25–50%). This family also contains N- Ras and H- Ras. They developed mouse strains with K-ras oncogenic alleles that can only be triggered on a stochastic recombination event throughout the whole animal in order to develop mouse cancer models that include K- Ras. Here, we demonstrate how these alterations made mice very susceptible to many tumor forms, including lung cancer with an early onset.

The consequences of germline mutations in the oncogene p53, were examined to further support this notion. This method has an advantage over conventional transgenic methods since it is more similar to spontaneous cancer progression that occurs in clinical malignancies.

The K-rasLA1 and K-rasLA2 mutant cultures both exhibited similar tumour forms. All of the mice developed multifocal tumours in the lung, which is the most common location for tumour development. At one week of age, these tumours were first identified as tiny thoracic nodules. The observation that K-ras expression significantly rises in rat lungs shortly after birth10 is compatible with the absence of tumours or pre-neoplastic lesions in the lungs of one-day-old pups. Finally, the animal's respiratory issues and eventual death or sacrifice were caused by age-related increases in tumour size and proliferation. The lesions developed into tiny tumours known as pulmonary adenomas, which were mostly made of a single kind of airway epithelium and showed little histologic atypia. Areas of secretory differentiation and papillary architecture were seen in some alveolar adenomatous polyps. A well-differentiated human follicular adenocarcinoma, which includes nuclear expansion, evident nucleoli, and enhanced mitotic rate, was seen in around 5–10% of the lesions. On rare circumstances, lung tumours brought on by K-rasLA in older animals impacted the kidney, thoracic lymphatic vessels, and other internal tissues. Surfactant apoprotein-C and -A were found by immunohistochemical analysis in the lung tumours but not Clara cell antigen, indicating an alveolar type II cell lineage [11].

The stimulation of the PI3K pathway and further evidence of a synergistic interaction between mutant Kras and it imply that mutant Kras' ability to cause cancer rely on signalling via both channels [12]. Additionally, one of the most highly overexpressed microRNAs in human NSCLC, microRNA-21, inhibits the negative regulation of the Ras/MEK/Erk pathway and so encourages the development of KrasG12D initiated lung tumours [13]. Combinations of lesions may exhibit interdependence or synergistic effects that lead to different disease phenotypes or shorter or longer latency periods, making them highly instructive.

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2. Therapy

2.1. CRISPR-Cas9

The use of genetic modifications, notably the Cas9 system, for the study and therapy of cancer, particularly lung disease, has been the subject of recent research. CRISPR/Cas9 is a potent method for successfully altering a cell's genome. Following DNA repair, single-guide RNA (sgRNA)-directed Cas9 activity cleaves DNA at certain locations, allowing mammalian cells to alter the DNA at those locations. Any gene may be modified utilizing the straightforward, precise CRISPR/Cas9 genome - editing technique. There are also some advancements in the treatment of pulmonary cancer as a result of the use of gene editing technologies.

Theoretically, the use of CRISPR/Cas9 technologies to cure cancer relies on the silencing of a particular oncogene in tumor cells. Because of its fast recognition of DNA sequences, the gene-editing method CRISPR/Cas9 has been employed to selectively detect overexpressed or overactivated genes. This method offers innovative suggestions for the cancer treatment. In the course of cancer treatment, knocking out specific oncogenes that are mutated, overexpressed, or overactivated may be beneficial. fter the FAK gene has been knocked out using EGFR mutation-specific Cas9, which lowered tumor growth by 81.5% and 78.3% in contrast to alkaline buffered CRISPR/Cas9 technology, NSCLC cells with mutant KRAS may also be used to detect continuing DNA damage and irradiation sensitivity [14]. By preventing the epithelial-to-mesenchymal transition in A549 and H1299 cells, NESTIN deletion may increase apoptosis, reduce growth and engraftment, and reduce cell invasion [15].

Lung cancer's ability to spread aggressively is increased by the oncogene -catenin. Through the reduction of Wnt signalling 17, Lewis lung cells and a well-established xenograft lung cancer model in C57/B6 mice that had the CTNND2 gene were able to lose their ability to be tumorigenic and metastatic in vivo. The protein -catenin was reduced in cancer cells to achieve this. Erlotinib resistance was created after IGF1R knockdown by CRISPR/Cas9 in HCC827 NSCLC cells decreased mesenchymal cell marker levels and increased MET amplification 18. In terms of biological activity, erlotinib is a more potent EGFR-TKI than gefitinib. Overall, the data suggests that CRISPR/Cas9 gene editing technology and proto-oncogene editing may one day be used to treat lung cancer. Inactivation of tumor-suppressor genes also has a significant impact on carcinogenesis. Tumor-suppressor-expressing products may prevent the growth of tumors, promote cell differentiation, halt cell migration, and reduce cell proliferation. Tumor-suppressor gene deletion, mutation, or lack of function causes oncogenes to become active during the tumorigenic process. Several different cancer forms have been linked to mutated tumor-suppressor genes with low levels of expression. The CRISPR/Cas9 gene editing technology offers promising therapeutic options for these tumor suppressor genes. Tumor-suppressor genes may be repaired using CRISPR/Cas9 technology, regaining their activity and functionality to prevent the growth of malignancies. Lung cancer therapy may be significantly impacted by the targeted restoration of dormant tumor-suppressor genes using the CRISPR/Cas9 system.

CRISPR/Cas9 is now being used as a new therapeutic approach to study tumor suppressor genes. One such study used a KRAS-driven animal model of lung adenocarcinoma, and many studies have found that over-activation of Nrf2 causes and promotes tumor survival and growth in mice. Deletion of the tumor suppressor miR-1304 also promotes survival and growth of lung cancer cells as it may have the ability to enhance haem oxygenase-1 (HO-1) production. Tumor suppressor genes in lung cancer have not been fully investigated using CRISPR/Cas9 gene editing technology; nevertheless, it is definitely an important area for future research. This is because it may be able to restore and activate tumor suppressors that have been silenced using CRISPR/Cas9 gene editing techniques, offering the possibility and hope of a complete cure for cancer.

2.2. CAR-T

Adoptive cell therapy (CAR-T), one of the most advanced cancer treatments, uses T cells that have been genetically modified to express chimeric antigen receptors, has shown excellent results in the treatment of hematological malignancies. However, because to the potently immunosuppressive tumor milieu that

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blocks immune responses, CAR-T cell effectiveness in solid tumors is still fairly restricted. Clinically, the creation of next-generation, personalized CAR-T cells for the treatment of solid tumors is essential.

Mucin is a frequently used antigen in lung clinical studies and will play a main role in the study of the treatment of NSCLC (MUC1), which is usually expressed in large amounts in lung cancer. According to some theories, it can promote the development of precursor lung lesions to urothelial cancers. Mesothelin is the second most targeted molecule in CAR-T medical research in lung cancer. mesothelin is frequently expressed in advanced lung adenocarcinoma and this usually leads to a poorer prognosis [16]. In NSCLC, PD-L1 has recently been shown to be a promising research direction for CAR-T therapies. It is a common indicator of the efficacy of PD-L1/PD-1 immune checkpoint inhibitors. Some studies have shown that PD-1 axis blockers are proven effective therapies.

For now, some CAR-T cells that specifically target the PD-1/PD-L1 pathway have not been fully understood. These cells produce cytokines that have been shown to be specific and may activate specific immunity, providing ideas for limiting tumor progression. Although the expression of CTLA-4 in NSCLC is currently not considered to be of very clear predictive use, he provides ideas for future studies. Current phase I/II studies are dedicated to EGFR-CAR-Ts or meso CAR-Ts expressing anti-CTLA-4/PD-1.

Several HER2 mutations and aberrations have been found in NSCLC patients, adding to the body of evidence linking HER2 to the condition. As a result, mutant HER2 remains a potential therapeutic target for the treatment of NSCLC and a prognostic indicator, particularly for HER2 exon 20 mutations. Since EGFR is expressed by far more over 60% of NSCLC adenocarcinoma, it acts as the principal NSCLC tumor driver when activating alterations are prevalent. EGFR plays a significant role in the growth of NSCLC tumors and, hence, therapeutic resistance. Additionally, lung epithelial tissue carcinoma and lung malignancy have therapeutic value for the expression of the GPC3 protein [12]. In fact, individuals with lung cancer who had metastases and a pleomorphic disease showed a predictive significance for GPC-3 expression [17].

The current evidence provides strong support for the use of these tumor-specific peptides as tumor markers and development targets for CAR-T fibroblast treatment interventions for the preclinical creation of novel monoclonal antibodies for lung disease and other breast cancers. The discovery of innovative CAR molecular processing technologies that have the possibility to provide effective and lengthy tumor suppression is one of the most exciting advancements in the lung cancer treatment process. Novel preclinical and clinical approaches that enhance CAR-T cell penetration and longevity within the ecosystem of malignant tumors, particularly in lung cancer, are necessary for the successful treatment of solid tumors.

2.3. mRNA vaccine

Messenger RNA (mRNA) vaccines are currently a highly promising cancer treatment, involving delivering antigen-containing exogenous mRNA into the body, which causes cells to produce these antigens, which may ultimately cause an autoimmune reaction. Nanodrugs show better selectivity and availability than conventional drugs and have lower cytotoxicity to healthy tissue, increased drug load, longer half-life and specific drug release patterns making them a promising direction for clinical research. By far the most popular cancer therapeutic approach is the nanocarrier. Some studies have shown that mRNA vaccines created using nanocarriers have outstanding efficacy in treating cancer and are widely used. Nanofibres are lipid nanoparticles (LNP) are the most commonly used material [18].

However, preclinical investigation. This held true whether pemetrexed was administered in conjunction with local radiation treatment or not. 40% of participants had a functional CD4 and/or CD8 T cell count that was at least twice as high after vaccination as it was at baseline. The efficacy of BI1361849 (CV9202) and immunotherapy medications in combination for NSCLC patients was established by this therapy. Some of the same conclusions have now been reached in a number of relevant phase I/IIa studies, many of which have found that people tolerate allergen vaccines better and can improve immunotherapy in patients with stage IIIB/IV NSCLC. One study evaluated the effectiveness and potential harms of mRNA vaccines in combination with anti-CTLA-4 and anti-PD-

L1 checkpoint inhibitors in the treatment of NSCLC [19]. It concluded that the combination of immune checkpoint inhibitors and mRNA-based therapies would be a good approach for the treatment of NSCLC patients.

3. Conclusion

The corpus of scientific research has shown that the development of therapeutic strategies for a range of human illnesses has been considerably facilitated by genome editing techniques. Future research will be able to identify synthetic lethal interactions in the genome by combining pooled CRISPR screening with knowledge about the genetic and epigenetic properties of cancer cell lines and make it simpler to uncover novel treatment targets. The creation of medicinal drugs, epigenetic modification, gene expression regulation, cell imaging, and gene diagnostics has all benefited from the use of gene editing techniques. Clinic-ready genome editing technology is getting closer because to novel genome editing methods and more precisely targeted nanostructured delivery technologies that have boosted efficacy and lowered toxicity throughout the delivery process. However, there is still room for improvement in terms of how to use gene editing methods to lessen the off-target impact.

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