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Pluripotent Stem Cells to Cure and Study Pulmonary Fibrosis

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Abstract: Pulmonary fibrosis, a devastating lung disease characterized by scarring and dysfunction of the lung tissue, lacks effective treatments and a comprehensive understanding of its pathogenesis. Pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), offer a promising approach for both therapeutic interventions and the use of pluripotent stem cells. Pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), offer a promising approach for both therapeutic interventions and disease modeling. These cells can be differentiated into lung-specific cell types, such as alveolar epithelial cells and airway epithelial cells, which can be used to study the mechanisms of pulmonary fibrosis and test potential anti-fibrotic drugs. In vitro models derived from pluripotent stem cells, such as lung organoids and three-dimensional cell cultures, recapitulate the complex cellular and extracellular structures. the complex cellular and extracellular matrix interactions of the lung, providing a more accurate representation of the human disease state than traditional animal models. Furthermore, recent advances in directed differentiation and bioengineering techniques have enabled the generation of lung tissue-like structures that could potentially reduce the risk of lung cancer. Furthermore, recent advances in directed differentiation and bioengineering techniques have enabled the generation of lung tissue-like structures that could potentially be used for regenerative medicine to repair or replace damaged lung tissue in pulmonary fibrosis patients. patients.

Keywords: Pulmonary Fibrosis; Pluripotent Stem Cells; Embryonic Stem Cells; Induced Pluripotent Stem Cells.

1. Introduction

Pulmonary fibrosis is a chronic and progressive respiratory disease characterized by scarring, thickening and loss of elasticity of lung tissue, which in turn affects gas exchange. As the disease worsens, overgrowth of connective tissue in the lungs may lead to life-threatening respiratory failure. Currently, there is no cure, and available medications only relieve symptoms and do not stop the progression of the disease. Although lung transplantation can improve the quality of life and life expectancy of some patients, it is not a universally feasible solution due to organ donor limitations [1,2] . Therefore, there is an urgent need to find alternative therapeutic strategies to deal with this complex disease3

In exploring new treatments, cell therapy and gene therapy show potential. In particular, stem cell therapy, such as mesenchymal stem cell transplantation, is able to differentiate into alveolar epithelial cells to promote lung repair. In addition, gene editing techniques, such as CRISPR-Cas9, promise to slow fibrosis by repairing abnormal genes. However, these approaches are still in the early stages of research.

Pluripotent stem cells, including embryonic stem cells and induced pluripotent stem cells, have become a focus of research for the treatment of pulmonary fibrosis because of their ability to self-renew and differentiate. These cells can not only be used directly for treatment, but can also differentiate into other cell types or be used as in vitro models to help understand disease mechanisms and screen drugs.

The aim of this review is to explore the promise of pluripotent stem cells in pulmonary fibrosis treatment and research. Stem cell technology may improve patient conditions by repairing damaged lung tissues, modulating immune responses, and slowing down the fibrotic process. The paper analyzes recent advances in the field of pluripotent stem cells in the treatment of pulmonary fibrosis, discusses the challenges of clinical applications and future research directions, and aims to provide a comprehensive perspective for researchers at to stimulate more innovations and to find new strategies for the treatment of this disease.

2. Progress of Pluripotent Stem Cells and Pluripotent Stem Cell-Derived Cells in PF Treatment and Research

Studies have shown that treatment with iPSCs can affect the IGF signaling pathway and inhibit BLM-induced overexpression of IGF1, IGF2, and Irs1 genes, which play a key role in pulmonary fibrosis [3] . iPSCs treatment also modulates the Wnt signaling pathway and reduces inflammation and fibrosis in the lung [4] . And by regulating the Wnt/β-catenin signaling pathway, it can promote cell differentiation towards type I alveolar epithelial cells, thus reducing collagen deposition and improving lung function in a mouse model of pulmonary fibrosis [5] . In addition, transplantation of iPSCs inhibited the TGF-β/Smad2/3 signaling pathway and reduced extracellular matrix deposition and EMT, whereas NO enhanced the proliferation and therapeutic effect of iPSCs [6] .

Some studies have shown that hESCs and iPSCs differentiated into LECs can increase survival and improve lung function when transplanted into damaged mice [7] . ATII cells are one of the main components of alveoli, and they are able to secrete surface-active substances to maintain alveolar stability and prevent alveolar atrophy. ATII cells are also able to proliferate and differentiate, replacing the damaged ATI

cells, and thus participate in lung tissue repair [8,9] . Therefore, ATII cells are considered to be the key cells for lung regeneration. A number of studies have now demonstrated the therapeutic potential of ATII cells derived from the differentiation of hESCs in animal models of pulmonary fibrosis [10] . Studies have utilized type II alveolar epithelial cells, HUES-3-ATII, differentiated from the human embryonic stem cell HUES-3 cell line, to treat nude mice with pulmonary fibrosis by tracheal administration, effectively repairing lung injury and fibrosis and exploring the possible mechanisms; the same method achieved similar therapeutic effects in mice with BLM-induced lung injury [11,12] . It has also been found that mouse-derived iPSCs can differentiate into alveolar structures in vitro and attenuate inflammation and fibrosis in a mouse model of lung injury [13] . hESCs can differentiate into ATII cells, which are useful for the repair of lung tissues in mice with pulmonary fibrosis after transplantation [14] . In addition, transplantation of hiPSCs-derived ATII cells restored lung function and prevented fibrosis without tumorigenic side effects [15] .

hESCs-derived IMRCs are cells with immunomodulatory and tissue repair functions similar to MSCs. IMRCs have a unique gene expression profile different from that of UCMSCs, exhibiting higher levels of expression of proliferative, immunomodulatory, and antifibrotic genes [16] . Treatment of bleomycin-induced pulmonary fibrosis mice by direct tail vein injection of hESC-IMRCs ameliorates BLM-induced pulmonary fibrosis and is superior to UCMSCs and PFD [16] . The mechanism of action may be its secretion of multiple growth factors, chemokines and cytokines, which affect the surrounding cells and tissues and exert remote therapeutic effects, thus improving lung function. The therapeutic efficacy and safety of hESC-IMRCs have now been explored for the treatment of pulmonary fibrosis in patients with neocoronary arthritis. It was found that hESC-IMRCs treatment resulted in significant improvement in dyspnea, pulmonary fibrosis lesion area, and inflammatory factor levels in patients, and there were no adverse or abnormal reactions [17] .

3. Advances in Secreted Substances from Pluripotent Stem Cells and Pluripotent Stem Cell-Derived Cells in PF Therapy and Research

Studies have shown that human foreskin fibroblast-transformed iPSC-cm reduced collagen content and expression of inflammatory and fibrotic factors in rats with bleomycin-induced pulmonary fibrosis, an effect that may be related to HGF in iPSC-cm [18] iPSC-cm also reduced TGF-β1 levels, inhibited the Smad signaling pathway, and deterred the transformation of the epithelial mesenchymal stroma, thereby attenuating pulmonary fibrosis [19] . iPSC-cm can inhibit TGF-β-induced EMT, also suggesting that iPSC may play a therapeutic role by secreting factors [20] .

Macrophages are thought to play an important role in the pathogenesis of IPF. Depending on the local micro environment, macrophages can be polarized into either a classically activated (M1) or alternatively activated (M2) phenotype. In general, M1 macrophages are responsible for wound healing after alveolar epithelial injury, whereas M2 macrophages are responsible for resolving the wound healing process or terminating the inflammatory response in the lung. Pulmonary fibrosis, on the other hand, is a pathological result of altered wound healing after sustained lung injury [21,22] . It has been found that iPSC-CM can regulate macrophage phenotype, thereby attenuating pulmonary fibrosis and promoting tissue repair and regeneration [23,24] . And it reveals the mechanism by which iPSC-exosomes inhibit M2-type macrophages through the miR-302a-3p/TET1 axis, thereby alleviating lung fibrosis [25] .

Experiments with the human type II alveolar epithelial cell line A549 showed that hESC-differentiated secretions contribute to wound repair, providing a new idea for the treatment of pulmonary fibrosis [26] . iPSCs and their culture medium have therapeutic effects on bleomycin-induced pulmonary fibrosis, and iPSC-CM avoids the complexity and uncertainty of cell transplantation [27] . Extracellular vesicles (EV), a bioactive substance secreted by stem cells, can promote regenerative repair of damaged tissues while avoiding the risks and limitations of cell transplantation therapy. Pulmonary fibrosis caused by radiation lung injury is a serious side effect when radiotherapy is used to treat lung cancer or other chest tumors, which can affect patients' quality of life and prognosis. Human embryonic stem cell (hESC)-derived EVs were found to treat radiological lung injury, ameliorate lung fibrosis, and improve survival in mice [28] . miR-17-5p in human embryonic stem cell exosomes (hESC-exo) is able to directly target platelet glycoprotein-2 (Thbs2), which regulates inflammation and fibrosis and achieves prevention of pulmonary fibrosis [29] .

Further studies found that the therapeutic effect of iPSC-AEC2 is dependent on its secretion rather than cell transplantation [30] . It has been found that the exosome miR-371b-5p secreted by the human ATIIC cell line A549 promotes the differentiation of pluripotent stem cells into ATIICs and increases the proliferative capacity of ATIICs by targeting PTEN to activate the PI3K/Akt signaling pathway [31] , which provides a new target for the currently incurable lung diseases. hESC-derived mesenchymal stem cell-like immune and stromal regulatory cells (IMRCs) and their cultures attenuate pulmonary fibrosis through antioxidant and anti-inflammatory effects [32] . IMRC-derived EVs are effective in the treatment of idiopathic pulmonary fibrosis (IPF), and different routes of administration also affect the therapeutic effect [33] .

4. Advances in Three-Dimensional Modeling of Pluripotent Stem Cell Sources for PF Treatment and Research

Pluripotent stem cell technology enables the creation of personalized in vitro models for organ transplantation, which is particularly important for lung disease research, as traditional models do not accurately reflect human disease characteristics [34,35] . And it is more convenient to study lung cell interactions [36] . This technique is useful for studying lung cell interactions, simulating tissue morphogenesis and function, and for modeling respiratory development, regeneration, and disease [37,38] . And human lung organs prepared using induced pluripotent stem cells or pluripotent stem cells derived from patient samples provide tools for understanding developmental processes and personalized medicine approaches [39] .

Early articles have reported efficient generation of lung and airway epithelial cells from human pluripotent stem cells (hPSCs). These cells have a wide range of applications in

regenerative medicine, lung disease modeling, drug screening, and human lung development studies. By manipulating developmental signaling pathways, successful differentiation of lung organ-like structures, called lung-like organs (HLOs), whose transcriptomes are similar to those of human fetal lungs, is an excellent model for studying lung development and disease [40,41] . Three-dimensional bioengineering technology can generate lung tissue models with different cell types to simulate the alveolar microenvironment and the pathological features of pulmonary fibrosis for the study of its mechanism and drug screening [42] . Using hESCs that can differentiate into lung-like organs expressing multiple cell types, the model that is closer to real organs helps to study respiratory diseases [43] . Classoid organs generated from pluripotent stem cells (hPSCs) and primary human fetal lung fibroblasts (HFLFs), called fibroblast-dependent alveolar classoid organs (FD-AOs), are able to recapitulate the multiple pathological changes of pulmonary fibrosis, and are an effective model for the study of pulmonary fibrosis [44-47] . TGF-β1-treated human pluripotent stem cells (hPSCs) generated alveolar organoids (AOs) capable of exhibiting distinct fibrotic features, including deposition of extracellular matrix (ECM), increase in fibroblasts, and onset of epithelial-mesenchymal transition (EMT), and thus can provide a reliable in vitro human organoid system for modeling PF and evaluating the antifibrotic mechanisms of potential drugs [48] . In addition, analysis of hiPSC-derived lung-like organs by microCT technology revealed that they express lung epithelial cell and alveolar macrophage-specific markers and display fibrosis-associated markers after treatment [49] . 3D minilungs models constructed using human embryonic stem cells (hESC) can mimic the structure and function of the original organ and are used to study lung fibrosis models and evaluate therapeutic agents [50] . There are also studies that generated spheroids expressing NKX2-1 by optimizing a directed differentiation paradigm, expanding into lung budgie cell-like organs for the study of lung development and cell therapy [51] .

In addition, in vivo transplantation of three-dimensional cells provides a new strategy for the treatment of pulmonary fibrosis. Human lung organoids can be transplanted into mammalian hosts. hPSC-derived epithelial bud-tip-like structures survive for a long period of time in vitro and were successfully transplanted into the airways of immunodeficient mice, demonstrating their ability to colonize and repair at the damaged site [52,53] . Bioengineered microporous poly(lactic-co-glycolic) scaffold ecological niche improves the survival of hPSCs-derived human lung organoids (HLOs) after in vivo transplantation and promotes the maturation of lung epithelium [54] . And lung organoids (HLOs) derived from human pluripotent stem cells (hPSCs) also mature at the molecular and structural levels after transplantation into immunodeficient mice [55] . This study also illustrates that in the in vivo environment HLOs have improved differentiation and adult-like structural features [54,55] .

5. Conclusion

In summary, pluripotent stem cells show remarkable potential in the treatment of pulmonary fibrosis. They not only provide direct treatment, but also differentiate into cell types such as AT II and IMRC and their secreted substances for repair. Alveolar epithelial cells, especially type II cells, play a key role in maintaining lung function. Studies have shown that type II cell injury is an early event in pulmonary fibrosis, and pluripotent stem cell differentiation of AT II cells is expected to be a cell replacement therapy.

In contrast to conventional therapies, pluripotent stem cells have the ability to self-renew and differentiate in multiple directions, providing a source of cells for tissue repair. Their secreted material can also serve as a drug delivery system. However, there are ethical, legal, and stability issues associated with the acquisition and culture of pluripotent stem cells, and in vivo implantation may also be challenging in terms of survival and tumorigenicity.

In addition, pluripotent stem cells can be differentiated into 3D cell models that more realistically simulate pulmonary fibrosis. Such models can help study complex disease mechanisms, improve the controllability and reproducibility of experiments, and accelerate drug development. Nevertheless, 3D models still have limitations in terms of technical complexity, cost, simulated human organ complexity and in vitro culture differences, which affect their large-scale application and prediction accuracy.

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