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Lung regeneration using amniotic fluid mesenchymal stem cells

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ABSTRACT

Respiratory diseases, such as chronic obstructive pulmonary disease (COPD), pulmonary hypertension and lung fibrosis, are yet a major challenge in the world and they result in irreversible structural lung damage. Lung transplantation as the only therapeutic option face some major challenges like graft rejection and cancer, arising as a result of immunosuppression. A low survival rate faced by lung transplantation patients is presently limited to approximately 5 years. Lungs shortage therefore calls for a mechanism that would increase the availability of suitable organs for transplantation. In this review, we give an update on the use of amniotic fluid mesenchymal stem cells (AFMSCs) as an optimal source for lungs scaffold re-cellularization, due to their limitless accessibility and possibility for proliferation and differentiation. Further studies will be required in tissue engineering (TE) and regenerative medicine (RM), especially shifting our focus towards AFMSCs as a cell source for this regeneration.

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Introduction

Chronic and acute lung diseases pose an increasing socio-economic problem [1]. The main cause of perinatal morbidity and mortality is preterm birth. It accounts for >85% of all death and complications of perinatal [2]. Perinatal care improvement has raised survival of extremely premature newborns [3]. These infants, nevertheless, are prone to long-term lung and brain injury [3]. Respiratory diseases cause differ, but the resulting damage of organ are comparable fibrosis and scaring, chronic inflammation results in loss of functional lungs tissue [4].

Recent TE and RM advancement has led to the synthesis of functional organs or tissues *in vitro* and their positive integration into human recipients without rejection [5,6]. TE aims at fabricating organs or tissues to replace injured or damage body parts. Major components of this strategy includes a biomaterials; as the scaffold upon which other components are built, tissue-specific cells and a suitable environment. An appropriate combination of these constituents can direct the TE of tissue construct *in vitro* and in addition promotes functional tissue substitutes development after implantation *in vivo* [7].

Studies have revealed some potential for the regeneration of adult and foetal lung after injury [8–11], failure of lung to regenerate frequently leads to chronic inflammation, disease progression and pathogenesis. Approaches to regenerate injured lungs, through resident lung stem cells activation [12–15], or through bone marrow-derived stem cells grafting [16–18], have shown some success but need to be further validated. A more direct strategy based on directing pluripotent stem cells differentiation towards different lung epithelial cell kinds is obviously promising. However, initial determinations to differentiate embryonic stem cells into those of the pulmonary cells showed inadequate achievement [19–22]. Recently, investigations have taken a stepwise strategies based on imitating the steps of lung development and have accomplished more favourable results.

The lung organogenesis

The lung is derived from the foregut endoderm. A quite number of patterning process cause the gut tube separation and other endodermal organs including pancreas, liver, eventually resulting in the generation of the lung buds and trachea [23,24]. Active reciprocal signalling between nearby mesenchyme and developing multipotent distal tip epithelium are necessary for lung buds stereotypical branching morphogenesis, likewise early differentiation events resulting in different cell lineages. The proximal epithelium was firstly formed from proximal progenitors with the emergence of neuroendocrine, basal, ciliated and secretory cells lining the maturing epithelium (Figure 1 and Table 1) [25]. As branching morphogenesis proceeds, bronchioles ultimately branch into alveoli or millions of terminal air sacs, where gaseous exchange took place after birth. The respiratory epithelium is mainly made of two cells before birth; for gaseous exchange-type-I alveolar epithelial cells, and for secreting surfactants-type-II cells needed to stimulate breathing at birth. Lung maturation accomplishment occurs post-natally. As it matures, the

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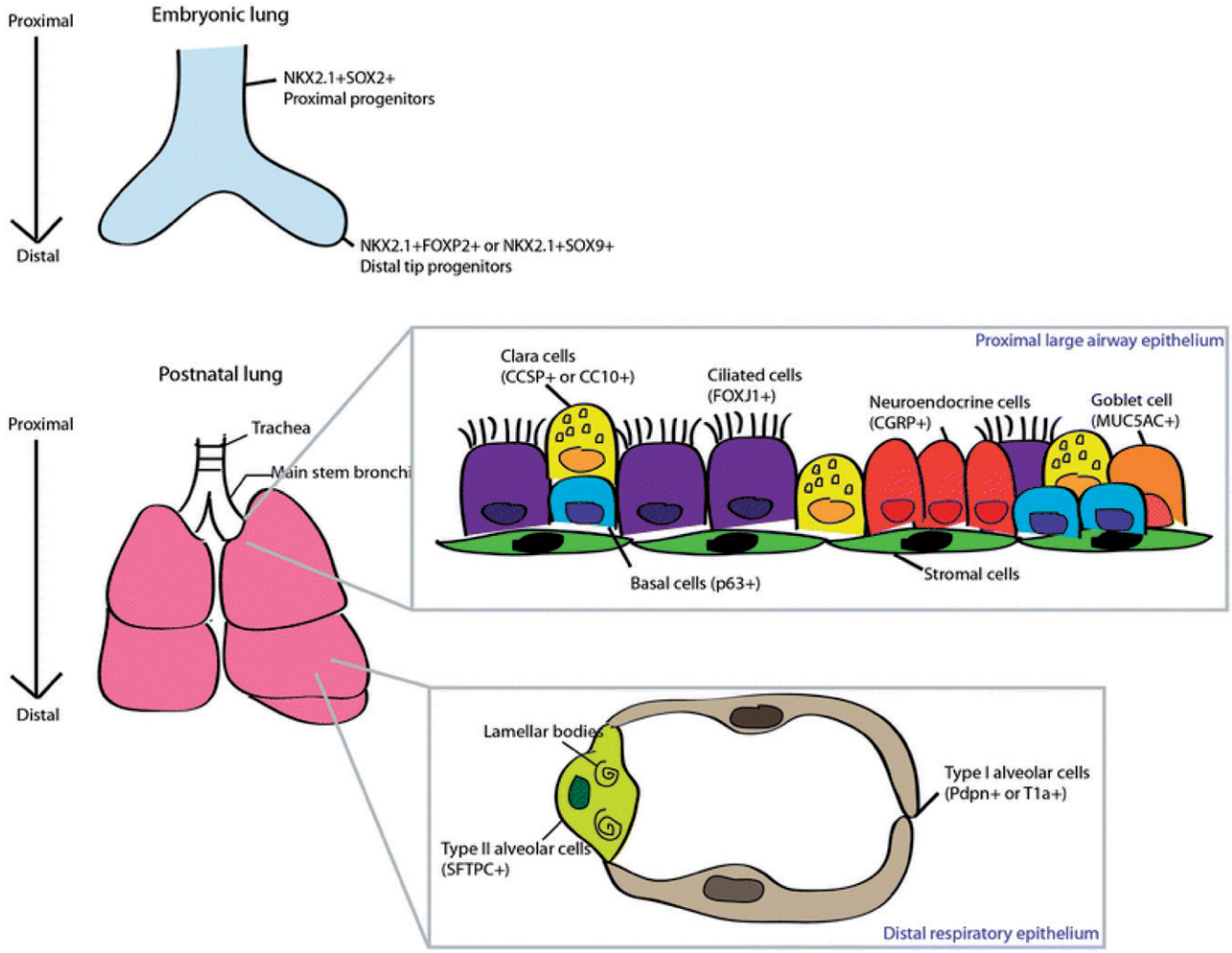

Figure 1. The diagram depict human lung at embryonic and adult stages. During lung development (upper panel), the proximal progenitors expressing NKX2.1 + SOX2 + develops to produce the proximal cells lineages seen in the adult epithelium (lower panel). The distal tip progenitors represented as NKX2.1 + SOX9 + or NKX2.1 + FOXP2 + give rise to the stereotypical branching morphogenesis, and thereafter the respiratory epithelial cells. This image was adapted from [25] with copyright permission.

Table 1. Formation of anterior foregot endoderm using several factors and cocktails.

References	Year	Aim	Inhibition	Induction factors	Remark
Green et al. [34]	2011	Differentiation of hESC into AFE	BMP and tgfβ using NOGGIN and SB431452	Wnt3a, FGF10, KGF, BMP4 and EGF	Sox2 was upregulated and CDX2 downregulated, generate up to 37% NKX2.1+
				+ RA	AFE enrichment Type-II ALVEOLAR CELL MARKER SP (SFTPC), and ciliated cell marker FOXJ1
Longmire et al. [35]	2012	Gene induction and modulation	NOGGIN and SB431452 alone		induce GFP expression in up to 21% of the cells
			NOGGIN and SB431452	Wnt3a, FGF10, KGF, BMP4, EGF and high conc. FGF2	upregulated both the thyroid and lung lineage genes expression
				Further differentiation of FGF10, FGF2, and mixture of dexamethasone, IBMX (DCI), FGF2, and cAMP.	downregulation of Nkx2.1 in about half of the cells
Longmire et al. [35]			SB431452 alone		10% of Nkx2.1
			BMP4, FGF2 and a GSK3 inhibitor		30% of Nkx2.1
			retinoic acid, BMP7, KGF, Wnt antagonism and MAPK/ERK inhibition		produced up to 18% Nkx2.1+ Sox2+ proximal progenitors

Table 2. Stepwise formation of lungs progenitor cells.

Procedure	Inferences	References
Ipsc or Esc + Activin A	Mesoendoderm	[34]
+ More Activin A	Definitive Endoderm	[36]
Inhibition Of Bmp and Tgf-B	Formation of anterior foregot endoderm (AFE) (Sox2 and FoxA2)	[34]
Wnt, FGF, EGF, KGF and BMP signalling	Formation of ventral AFE cells that express Nkx2.1	[37–41]
Treatment with FGF2 or FGF10 ligands	Promotes specification of lung progenitors, which express Nkx2.1, Sox2 and Sox9.	[42–46]

turnover of cell decreases and is very slow in the completely mature adult lung (Table 2).

Endodermal bronchial buds and subsequent airway branches grow into the mesenchyme surrounding the thoracic gut tube. Deficiencies or abnormalities in the branching of the respiratory tree are the basis of many forms of pulmonary hypoplasia. Studies over the past several decades have demonstrated that branching morphogenesis of the respiratory tree is regulated by reciprocal interaction between the endoderm and surrounding mesoderm. For example, when mesenchyme in the region of the bifurcating bronchial buds is replaced with mesenchyme from around the developing trachea, further branching is inhibited.

Conversely, replacement of tracheal mesenchyme with that from the region of the bifurcating bronchial buds stimulates ectopic tracheal budding and branching. Based on experiments such as these, components of the extracellular matrix and growth factors have both been implicated in the stimulation and inhibition of branching.

For example, *Collagens* types *IV* and *V, Laminin, Fibronectin,* and *Tenascin-all* components of the extracellular matrix-are thought to play either a permissive or a stimulatory role in branching of the bronchial buds. Likewise, the regulation of expression of receptors for these matrix components has also been implicated in control of branching morphogenesis.

Mesenchymal stem cells

There is limited knowledge on lung mesenchymal precursors. However, there is report that small populations of resident lung cells that expresses certain phenotypic characteristics of mesenchymal stem cells (MSCs) with progenitor capacity exist within the lung. For example, resident lung "side population" (SP) cells have been isolated, they possess both epithelial and mesenchymal potential [26–28]. These SP cells have been found to be resident at all levels of the airway tree, exhibiting a relatively constant phenotype, regardless of their source of isolation [28]. Even though, it has been confirmed that these SP cells are a source of adult lung MSCs [27], the role of SP cells in endogenous lung repair is yet to be fully understood. In addition, McQualter et al. described an endogenous population of fibroblastic progenitor cells with clonogenic ability in adult lung, which are mostly representative of MSC lineages [29].

Repopulating injured and defective lung

AF is regarded as a crucial tool for the diagnosis of foetus well-being in pregnancy because of the role they play in the development of foetus. However, AF can safely be isolated by amniocentesis before culturing. Several different types of cells arises from AF and recent studies are looking into their potential for the application of cell therapy. The potentials of multipotent and pluripotent cells obtained from AF has been investigated and *in vitro* differentiations into several types of cells have effectively been carried out. Human amnion epithelial cells (hAECs), AFSCs and placental mesenchymal stem cells (PMSCs), have demonstrated the ability to differentiate into various cell types osteogenic, adipogenic, endothelial, myogenic, neurogenic, pulmonary, hepatogenic, pancreatic and cardiac lineages. In addition, *in vivo* investigation is stressing the importance and mechanisms of AFMSCs for therapy, particularly for lung and kidney diseases. AF may represent a good precious source for safely and easily retrievable types of cell that may be employed for RM purposes [30].

With amniotic fluid mesenchymal stem cells

A novel stem cell source have been obtained from AF, AFMSCs is a promising source for regenerative medicine. These types of cells are of multipotent origin, having the potentials of differentiating into each of the embryonic germ layer cells. Carraro et al. [31] studied hAFSC potential to differentiate into pulmonary lineages and integrate into murine lung following injury. Results of their investigations showed that hAFSC plasticity to respond to various kinds of lung damage in different ways through alveolar versus bronchiolar epithelial cell lineage markers specific expression, depends on injury type to recipient lung.

With amnion epithelial cells

AEC is also a promising source of cell in disease treatment such as COPD, acute respiratory distress syndrome, pulmonary oedema, pulmonary fibrosis and pulmonary hypertension. The transplantation of AECs in animal model for lung disease decreased subsequent fibrosis and inflammation and eventually improves the function of the lung [4]. hAECs administration to bleomycin-induced lung diseased mice leads to a downregulation of interleukin-6, transforming growth factor-β, proinflammatory cytokines tumour necrosis factor-α, and interferon-γ, α-smooth muscle actin expression, reduced pulmonary collagen deposition and inflammatory cell infiltrate. Murphy et al. [32] lately showed that hAECs reacts with host macrophages to exercise their regenerative potentials, probably through macrophages induction into an alternatively activated phenotype. Recent investigations have induced hAECs for the expression of lung-specific proteins [33] like the ion channel cystic fibrosis transmembrane conductance regulator, signifying a crucial use of hAECs for cystic fibrosis

therapy. These significant investigations shows that hAECs is a potential tool for bioengineering and clinical applications of lung disease patients.

Conclusions

Stem cell research had a significant progress during the past years and had turn out to be one the most attractive aspect of modern biology. Thanks to several studies, stem cells hold a significant valuable possibility in supporting and protecting native tissues throughout inflammatory and ischaemic-induced damage. AFMSCs hold a great potential. This review proposes AFMSCs to be a better source of stem cell for lungs regeneration because it has the ability to differentiate to all forms of cells; however, this stem cell choice needs further laboratory investigations.

Disclosure statement

No potential conflict of interest was reported by the authors.

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