Potential therapeutic application of adult stem cells in acute respiratory distress syndrome

JIANG Jian-xin 蒋建新 and LI Li 李力

Acute respiratory distress syndrome (ARDS) remains a poor prognosis in spite of the recent development of new therapeutic strategies. Cell-based therapy with stem cells has been considered as a promising way for the treatment of vital organ damage. Putative endogenous stem cells have been shown to be located within the adult lung in the basal layer of the upper airways, within or near pulmonary neuroendocrine cell rests, at the bronchoalveolar junction, as well as within the alveolar epithelium. These stem cells are hypothesized to be the source of lung regeneration and repair.

But this mechanism seems to be insufficient after lung injury. There is increasing excitement over the last few years with the suggestion that exogenous stem cells may offer new treatment options for ARDS. Exogenous stem cells have the ability to differentiate and function as both airway and lung parenchymal epithelial cells in both in vitro and increasingly in vivo experiments. However, there is great controversy concerning the repair effect of adult stem cells in lung injury. This review evaluates the advances in endogenous respiratory stem cells, and assesses the evidence for the use of stem cells in the repair of lung injury.

Key words: Respiratory distress syndrome, adult; Stem cells; Lung injury

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State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Surgery Research, Third Military Medical University, Chongqing 400042, China (Jiang JX and Li L)
*Corresponding author: Tel: 86-23-68757460, E-mail: jiangjx@cta.cq.cn

Acute lung injury and acute respiratory distress syndrome (ARDS) occur most commonly in patients with major trauma and all other critical illness. The estimated annual frequency of ARDS is reported as 75 cases per 10,000 population. Mortality rates remain in the range of 30%-40%, which increase with advanced age. It still has a poor prognosis in spite of the recent development of new therapeutic strategies. The major reason is that the treatments currently widely used in clinic could mainly act as functional support to the injured lungs. The pathological basis for ARDS is characterized with severe and diffuse injury to the alveolar-capillary membrane (the air sacs and small blood vessels) of the lung. Therefore, it would be an ideal therapeutic target to decrease the high mortality of ARDS by structural repair and functional recovery. Stem cells are undifferentiated cells capable of renewing itself throughout its life and of generating one or more types of differentiated cells. It is believed that cell-based therapy with stem cells can be perspective for the treatment of vital organ damage. This review will describe the current advances in endogenous respiratory stem cells and application of stem cell therapy in lung repair, and evaluate the therapeutic potential of adult stem cells in organ repair.

Endogenous respiratory stem/progenitor cells

Stem cells are typically categorized into embryonic stem cells and adult stem cells, with varying differentiation abilities or potencies. Embryonic stem cells are derived from blastocysts in a developing embryo and have the ability to differentiate into all cells of the three germ layers (endoderm, mesoderm and ectoderm). This ability is defined as totipotency or pluripotency. However, researches on embryonic stem cells have been greatly hampered due to its ethical and moral problems. Adult stem cells are found in adult tissues, commonly in discrete niches, and are thought to help repairing damaged tissues by replenishing specialized cells. The differentiated progeny of these adult stem cells are classically thought to be restricted to a specific cell lineage.
appropriate to its location. Adult stem cells are either multipotent, with an ability to differentiate into a limited range of cells (e.g. adult haematopoietic stem cells), or unipotent, with an ability to generate only one type of cells (e.g. type II alveolar cells). Unlike tumor cells and embryonic stem cells, adult stem cells are not immortal and show decreasing telomere length with increased age. Adult stem cells are vital for continuously renewing tissues such as the bone marrow and intestine, and play an important role in recovery from injury in tissues.4

While bone marrow, intestine mucosa and skin have well-known stem cell populations, other organs, such as lungs, previously thought to be "post-mitotic" and unable to regenerate, also have the defined stem cell populations.4 The endogenous stem cells in the respiratory system are complex. It has been considered that different regions of the respiratory tract rely on different populations of cells for repair.5, 6 The endogenous stem cells are more easily understood by dividing the respiratory system into the large airways, small airways and lung parenchyma. The trachea and primary bronchi contain ciliated cells, Clara-like cells, and basal, neuroendocrine and submucosal cells. Both the basal and Clara-like cells in the proximal airway might be able to function as local endogenous stem cells both in the stable state and in response to injury.7 In the small airways, there are no basal cells and the epithelium is made up of Clara, ciliated and neuroendocrine cells. In this region, subpopulations of Clara cells and bronchoalveolar stem cells (BASCs) at the bronchoalveolar junction have been considered as the cells involved in proximal small airway repair and distal epithelial reconstitution after injury, respectively.8 BASCs have been demonstrated to express both alveolar (surfactant protein C, SP-C) and bronchiolar (Clara cell-specific protein, CCSP) epithelial markers. Isolation of these cells has demonstrated their stem cell properties and their ability to differentiate into Clara cells and type II pneumocytes in vitro. In vivo, they appear to play a role both in bronchiolar and alveolar repair and homoeostasis.9 The alveolar epithelium consists of thin-walled type I pneumocytes involved predominantly in gas exchange, and cuboidal type II pneumocytes concerned with many metabolic functions in the airspace. Rapid repair of the denuded alveolar surface after injury is crucial for survival. The alveolar surface is very large (i.e. 70 m²) in human adults. The size and spatial restrictions of the alveolar surface suggest that at least one progenitor cell per alveolus be required to achieve rapid coverage and repair of alveolar epithelial leak. At least two kinds of putative endogenous respiratory stem/progenitor cells might exist in lung tissues. One of them might be located within the alveolar epithelium.

Driscoll et al9 indicated that the expression of telomerase, a stem/progenitor cell marker, is widely up-regulated in alveolar epithelial cells (AEC) during the recovery phase after acute oxygen injury. The putative AEC progenitor cells can be sorted out by fluorescence-activated cell sorter (FACS) from primary AEC populations isolated from rat lungs during the recovery phase after sublethal hyperoxia. These AECs obtained from FACS are shown to be relatively more proliferative and relatively resistant to injury-induced apoptosis. It suggests that this subpopulation may be responsible for the proliferative phase of repopulation of the injured alveolar epithelium.10 However, it does not exclude the possibility that the majority of alveolar epithelial cells might undergo reactivation or reprogramming into a progenitor-like state in response to injury. The type II pneumocytes might also be responsible for the putative AEC progenitors due to their ability to produce type I pneumocytes.10

Another population of stem cells responsible for alveolar repair might be BASCs. The existence of these two putative endogenous alveolar stem cell populations may provide a target for directed regenerative therapies in the lung. The relative contributions and relationship between type II pneumocytes and BASCs in alveolar homoeostasis and repair have not been fully elucidated. In addition, resident lung side population cells have recently been reported.11, 12 They appear to have both mesenchymal and epithelial potential and are characterized by their ability to efflux Hoescht dye. Their roles in endogenous repair is not clear.

**Therapeutic application of adult stem cells in ARDS**

The endogenous repair and continual tissue replacement by local stem cells are effective in some organs, such as the skin, gut mucosa and bone marrow. But this mechanism is not effective in the respiratory tract and is insufficient to prevent many progressive respiratory diseases even several kinds of endogenous stem/progenitor cells might exist in lung tissues. There is no
significant restoration of the gas exchange surface if it is disrupted by a disease. In view of this, there are increasing efforts to investigate the possibility of enhancing stem cell repair using exogenous stem cells.

The idea of enhancing repair using exogenous stem cells has grown over the last decade. Adult stem cells are attractive for several reasons. They avoid many ethical and moral problems pertaining to the use of embryonic stem cells. Furthermore, they allow for the possibility of expansion of a patient’s own cells before transplantation. This delivery of autologous adult stem cells would help prevent immunological rejection by the host. More evidences have indicated that the lineage specificity of adult stem cells is not as rigid as we thought before. The adult stem cells are able to cross lineage barriers and adopt the functional phenotypes of other tissues, which coincides with the concept of adult stem cell plasticity and provides an important theoretical basis for stem cell therapy.

Bone marrow stem cells are the best defined adult stem cell population. They are easily accessible and well understood. The bone marrow contains haematopoietic stem cells (HSCs), which can differentiate into all the mature blood cells and endothelial cell progenitors and mesenchymal stem cells (MSCs), which differentiate into the stromal supporting tissue, including fat, bone and cartilage. There are increasing evidences to suggest that transplanted cells after lung injury have some effects on the disease process. Systemic MSC administration following bleomycin-induced lung fibrosis results in a reduction in fibrosis and collagen deposition in murine models. A report from Mei et al. indicated that the treatment with MSCs could significantly reduce LPS-induced acute pulmonary inflammation in mice. An animal model of emphysema, with intranasal elastase, was used to demonstrate an improvement of outcomes when endogenous bone marrow stem cells were augmented by all-trans retinoic acid or granulocyte colony-stimulating factor. Suppressing a normal bone marrow response with busulphan or radiation led to an increased mortality in mice subjected to lung insults, which could be reversed with an infusion of bone marrow stem cells.

Bone marrow stem cells have been demonstrated to contribute to different lung cell types, including type II and type I pneumocytes, tracheal epithelial cells, fibroblasts and endothelial cell progenitors. Some in vivo experiments also indicated that exogenous stem cells homed in lung tissues could express markers of lung epithelial and endothelial cells. This effect of adult bone marrow stem cells has been considered to be engraftment. Similar results were obtained in human subjects that had undergone sex-mismatched transplants. Male patients who had received female lung transplants were shown to have a proportion of male lung epithelial cells on lung biopsy. Conversely, female patients with male bone marrow allograft transplants were similarly shown to have a degree of pulmonary chimerism, with Y-chromosome containing epithelial and endothelial pulmonary cells. It suggests that the adult stem cells in bone marrow were actively recruited by, and homed to injured tissues. Soluble factors or chemokines are implicated in the homing of bone marrow stem cells to the damaged lung tissue. Damaged lung produces chemokines such as CXCL12 (stromal-derived factor 1) and secondary lymphoid chemokine, which are ligands for CXCR4 and CCR7 receptors found on certain bone marrow-derived stem cells.

In addition to engraftment, fusion of exogenous stem cells and host epithelial cells has been also considered as the mechanism for colocalization of histochemical markers. Embryonic cells and adult somatic cells fuse in vitro, causing a change in the phenotypic properties of cells. In vivo, fusion has been shown to be important in engraftment of some organs (e.g. the liver). However, an elegant set of experiments using a Cre/lox system with β-galactosidase and GFP transgenic mice illustrated that this was not the mechanism in lung engraftment. However, there are also increasing evidences indicating that bone marrow stem cells might ameliorate pulmonary injury via a pseudo-pharmaceutical effect, with the release of cytokines and growth factors and possible stimulation of endogenous repair, rather than by engraftment and tissue regeneration per se. However, there is a great controversy concerning repair effects of bone marrow stem cells in lung diseases, with some groups showing no contribution of adult bone marrow cells to lung epithelial cells. This might be due to great variability in the type of bone marrow stem cells used, such as single bone marrow cells, whole bone marrow cells, HSCs, MSCs, side population cells and MAPCs (multipotent adult progenitor cells). In addition, different conditions might also be responsible for this variation of results, including the type of
lung injury (e.g. radiation, bleomycin, lipopolysaccharide, elastase and paracetamol) and the amount of irradiation pre-infusion of the transplanted cells.

Exogenous stem/progenitor cells may be delivered into the lung intravenously, or intratracheally, or by direct injection. Since the alveolar capillary bed is perfused by the entire right cardiac output and is very narrow (5-7 µm), it serves as a sieve to trap larger, more rigid cells than the standard red or white blood cells. Thus, after intravenous administration, the immediate efficiency of exogenous stem/progenitor cell arrival and trapping in the lung is very high. However, diapedesis of these cells into the lung parenchyma and eventual integration into lung cell lineages are extremely inefficient in the absence of lung injury. Even in the presence of injury, the efficiency of integration relative to the entire lung cell population is quite low (<2%). In this case, intratracheal administration of stem cells might be a better way for lung repair.

Possible negative effects for stem cell therapy

In contrast with their apparent positive effect in lung repair after injury, adult stem cells have also been shown to be implicated in worsening disease. Compared with epithelial engraftment, there have been more robust evidences for the contribution of bone marrow-derived cells such as fibroblasts or myofibroblasts in disease. There is an increasing appreciation in fibrosis that the effector cells, the fibroblasts and/or myofibroblasts may not just be of resident tissue origin. These cells, which are thought to have an essential role in fibrotic lesions, have been shown to have a bone marrow derivation and typically express cell-surface markers typical of HSCs (e.g. CD34 and CD45). In an asthma animal model, labeled bone marrow stem cells were shown to participate, as myofibroblasts, in bronchial subepithelial fibrosis, which is thought to be one of the mechanisms contributing to airway remodeling in asthma. Similar evidence indicates that bone marrow stem cells play a role in the development of pulmonary vascular remodeling in chronic pulmonary hypertension. In this condition, the cells are thought to contribute to the vascular fibrosis directly by the production of collagens, and to have a paracrine effect of releasing cytokines that cause the activation of resident fibroblasts. Research using a bleomycin model of lung fibrosis has shown that up to 80% of the collagen-producing fibroblasts in the lung have a bone marrow origin. A separate study showed that the addition of intravenous MSCs could also contribute to fibroblasts and myofibroblasts in lung fibrosis models. The negative effect of the bone marrow-derived cells was also demonstrated in vivo, whereby the removal of the chemotactic gradient, with antibodies to CXCL12, reduced the bone marrow-derived component to lung fibroblasts and the overall fibrotic response after injury.

Another negative effect of the stem cell therapy is shown to be tumor genesis. Evidence is available for the development of karyotype abnormalities of bone marrow stem cells during in vitro passage. Furthermore, MSC delivery in a mouse model has been associated with sarcoma development in vivo. Bone marrow stem cells have also been implicated in the development of a gastric carcinoma in mice chronically infected with Helicobacter felis.

Conclusions

The endogenous stem cells in the respiratory system are complex. The basal cells in the proximal airway, Clara cells in the small airways, BASCs at the bronchioalveolar junction and the type II pneumocytes in the alveolar epithelium have been considered to be the endogenous respiratory stem cells, being responsible for repair of different regions of the respiratory system after injury. But this endogenous repair mechanism is not sufficient. How to find a way to enhance the endogenous stem cell repair after lung injury is important in this field. Bone marrow stem cells are characterized with a strong plasticity. They are easily accessible and have a very low antigenicity, providing ideal cell source for stem cell therapy. Bone marrow stem cells are able to home to injured lung tissues and play a positive role in tissue repair through direct engraftment, cell fusion or paracrine effects. However, there is a great inconsistency in literature with use of exogenous stem cells. This might be due to significant numbers of uncontrolled variables, including the type of stem cells used, the timing and the injury model.

In addition to positive effects, administration of bone marrow stem cells might have some negative effects, for example, it could lead to lung fibrosis and tumor. In order to fully assess the positive and negative implications of bone marrow stem cells, it is important to characterize the individual effects of fractionated, universally defined subgroups of bone marrow cells in well-designed studies.
**REFERENCES**


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