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Human lung stem cells: Oh, the places you'll go!

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Chronic lung diseases (CLD), including chronic obstructive pulmonary disease (COPD), asthma, lung cancer, interstitial lung disease or pulmonary hypertension, are the second leading cause of death worldwide and a significant global health problem. Only limited effective causal therapies have been developed thus far for CLD, as such, lung transplantation remains the only available therapeutic option with the potential of long-term survival for most patients with end-stage CLD. CLD develop in the interface between genetic susceptibilities and environmental aspects, initiated and perpetuated by ill-defined lung injury and failed repair mechanisms, and ultimately lead to perturbed lung architecture incompatible with normal respiratory function.

In this setting, the controllable permanent renewal of resident cells, which are lost in CLD, to maintain the delicate lung architecture would be a highly desirable option. Consequently, the option for possible stem cell-based therapies that result in lung repair and regeneration has led to a unique search for resident and non-resident lung stem cells originating from various sources, including embryo, foetus and bone marrow, but also from the adult lung itself (Weiss et al, 2011). Up to now, resident lung progenitor cells have only been unequivocally identified in the mouse lung and were mainly restricted to the epithelial cell lineage: Epithelial progenitor cells within the distal buds have been identified during branching morphogenesis. Clara cells, bronchial basal cells and alveolar epithelial type II cells have been described to serve as progenitor cells postnatally. Lately, a cell population at the bronchoalveolar duct junction coexpressing Clara cell and alveolar epithelial cell marker proteins and named 'bronchoalveolar stem cells' (BASCs) has been reported to exhibit oligopotency. Overall, however, the lineage specificity of these putative cell populations and regulation thereof remains elusive (Rawlins, 2008).

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In the May 12, 2011 issue of the New England Journal of Medicine, Piero Anversa and his colleagues now presented the first evidence for the existence of multipotent resident lung stem cells in the adult human lung, which were able to induce lung repair after injury (Kajstura et al, 2011). The authors have identified a distinct cell population in the adult human lung that exhibits several important characteristics of stem cells, such as the ability for self-renewal, clonogenicity and multipotency. The investigators isolated a putative stem cell population from normal adult human lung expressing the cell-surface marker c-kit. Extensive flow cytometric analysis demonstrated that these cells lacked the expression of a variety of haematopoietic, epithelial, endothelial or mesenchymal lineage markers, corroborating their 'stem-ness'. Importantly, these characteristics remained stable upon clonal expansion of the isolated cell fraction.

To further prove their stem cell behaviour *in vivo*, human lung stem cells (clonal or non-clonal) were locally injected into damaged mouse lungs. The authors were able to demonstrate that some of the cells remained within the lung and maintained their stem cell phenotype. These cells have then been harvested and re-implanted into a second damaged lung, corroborating their self-renewal capacity.

Oh, the Places you'll go. The most intriguing finding, which the authors reported in this *in vivo* setup, is that adult human lung stem cells gave rise to newly formed airway structures, as well as vasculature, thereby, demonstrating the multiple lineage potential that these stem cells harbour. Further, human lung stem cells were capable of acquiring multiple lineages and differentiating into alveoli, bronchioles as well as vessels, after application to a severely cryo-damaged lung, thereby, re-building damaged lung tissue *in vivo*. It has to be pointed out that the human cell

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population underwent *ex vivo* expansion before implantation of c-kit-positive clones into an injured mouse lung; however, similar results have been described with non-clonal cells.

Next to the places these lung stem cells go, the authors investigated the niches in which lung stem cells resided. Interestingly, these c-kit-positive lung stem cells have been detected in a variety of compartments: they are detected in close proximity to known progenitor cells, that is near bronchial basal cells or alveolar epithelial cells, as well as differentiated cells, such as smooth muscle cells or fibroblasts. Future studies will now need to address the specificity of these niches, the characteristics of their microenvironment and whether these cells also may circulate (Montani et al, 2011).

While Kajstura et al have provided compelling evidence for the origin of resident stem cells in human lungs, the definitive conclusions of this study are exciting yet limiting and replication of the most significant observations in a second, independent group of subjects is now required. Kajstura et al demonstrated that human lung stem cells gave rise to newly formed airway structures in a humanized xenograft transplantation model, but precise lineage tracing experiments in a mouse model system will now prove useful to gain a more in-depth understanding of these resident lung stem cells in the future. Moreover, the findings thus far will clearly benefit from the therapeutic application of human lung stem cells into animal models more closely mimicking human CLD, such as lung fibrosis or COPD. Finally, it remains unclear whether the obtained results of lung stem cell multipotency can be translated to human CLD. If so, the underlying molecular mechanisms promoting cellular replacement and regeneration by lung stem cells is still undefined and will give a multitude of further questions for future studies.

This study contains a number of additional interesting aspects: First, the findings that neither cardiac stem cells nor haematopoietic stem cells, which exhibit similar stem cell characteristics, failed to repair lung injury demands attention. While we are left with the knowledge that c-kit-positivity defines the stem-ness of all of the above preparations, and we have a clear pictures of the markers that are used for negative selection (i.e. markers that these cells do *not* express), we have very limited knowledge about why these cells are committed to repair a specific organ only. What makes the cells different from each other and selective for repairing only a specific organ? Here, further in-depth analysis of the cellular genotype/phenotype and their interaction with the cellular and non-cellular microenvironment in the respective organ is of utmost interest to further understand this dependency.

Second, Kajstura et al identified resident lung stem cells, which exhibit several advantages over non-resident stem cells. In particular, the use of cells derived from human embryos or cells reprogrammed from adult somatic cells will raise ethical concerns and may exhibit a higher tumourigenic potential. Further, cells derived from bone marrow or peripheral blood, such as haematopoietic stem cells, mesenchymal stem cells or endothelial progenitor cells harbour the benefit of autologous transplantation and lack of immunogenicity, but mostly require *ex vivo* expansion or are limited in the supply of the adequate cell population. Therefore, resident adult lung stem cells, in comparison to non-resident stem cells, may have the potential to provide lung-specific matched stem cells with promising capacity of exogenous, as well as endogenous, *in vivo* differentiation for cell replacement therapies.

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In conclusion, Kajstura et al convincingly reported the existence of resident human adult lung stem cells and that these are a potential source for regenerative medicine in the lung (Fig 1). These findings manifest an exciting field for further research. Clearly, the question whether these cell populations or their functions are altered in CLD and how these cells change in different diseases in terms of numbers, markers or location now define exciting areas of research. If these cells are capable of repairing or regenerating injured lung tissue, why does this not at all happen endogenously in CLD or only to a very limited extent? Which stimuli are needed to activate possible niches to induce endogenous repair and regenerative processes of the adult diseased lung? At this point, we also do not know whether these cells may turn to places where they should not be found. To elucidate if the stem cell population of Kajstura et al is involved in initiation and progression of CLD, further studies are clearly required. In addition, inappropriate restore mechanisms, improper terminal differentiation or apoptosis of lung stem cells may be a reason for pathology of CLD.

Lung progenitor cells have been suggested to be involved in cancer development, as such it is reasonable to argue that cancer stem cells may originate from resident lung stem cells, thereby, driving lung cancer formation (Giangreco et al, 2007). Clearly, we need to understand the complexity of these cells much more in detail. Along this line, accumulative evidence on the cellular plasticity of previously thought fully differentiated resident lung cells (epithelial, endothelial as well as mesenchymal cell populations) highlight the dynamic cell–cell and cell–matrix interactions in CLD (Königshoff et al, 2009). Dramatic changes in the cellular phenotype and function, such as epithelial-to-mesenchymal transition has been demonstrated in CLD and certainly influences the homeostasis and activity of possible resident lung stem cells.

Given the availability and potential isolation of these cells, which can be harvested from human lung biopsies, adult human lung stem cells could be engineered *ex vivo* and re-implanted into a patient's lung, giving hope to truly personalized medicine. Furthermore, lung-specific resident stem cells offer the possibility to stimulate their *in vivo* differentiation and proliferation to improve endogenous regeneration of the injured lung tissue. These stem cells could serve as a delivery system for expressing therapeutic molecules in distinct damaged areas of the lung. Cell-specific targeting of injured, de-differentiated or malignant cells, that provide

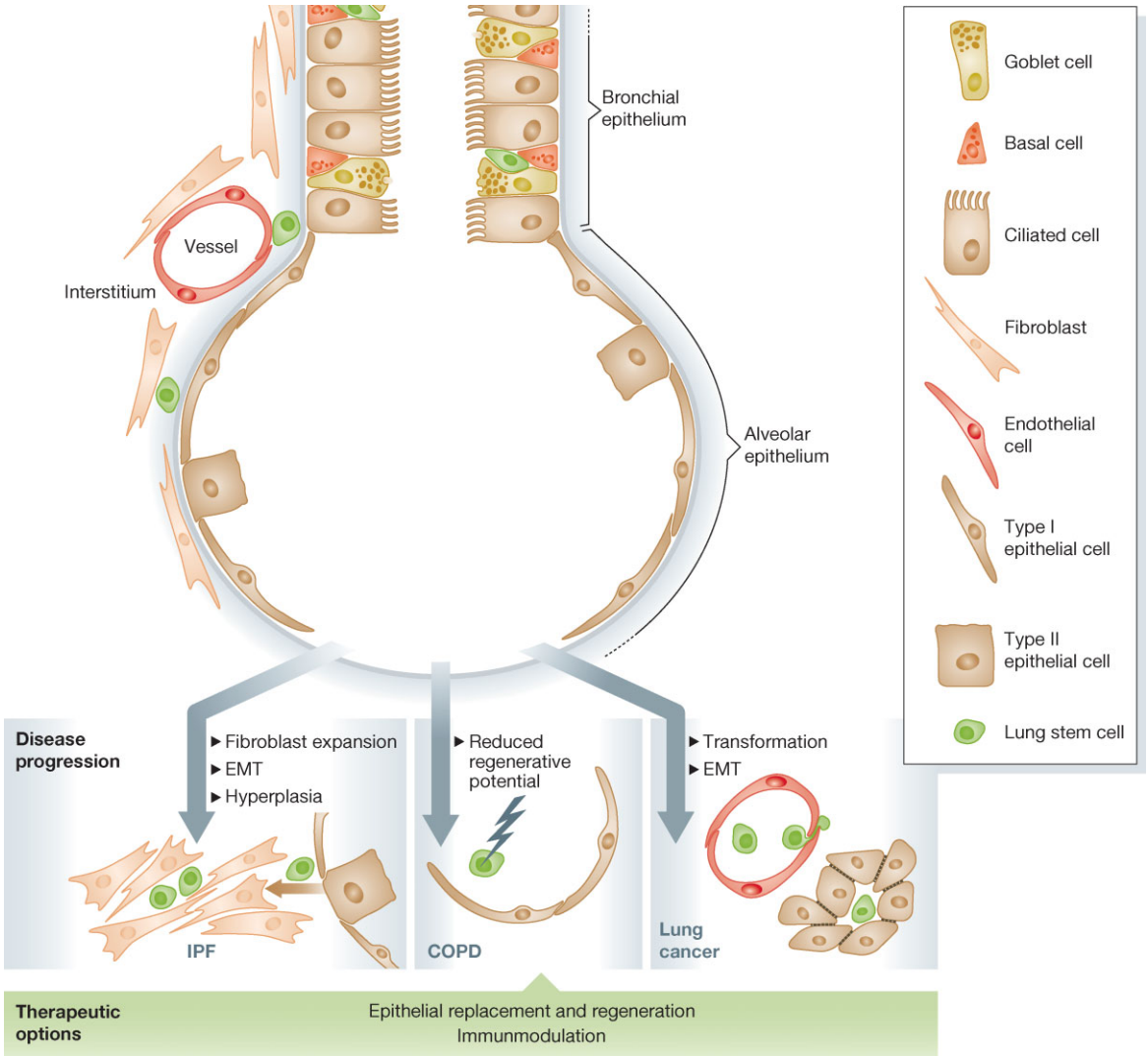

Figure 1. Human lung stem cells in CLD progression and therapy. Multipotent resident lung stem cells (green) originate from a variety of compartments and may contribute to the progression of several CLD. On the other hand, adult human lung stem cells could be used to exploit different therapeutic options.

critical functions in disease initiation and progression, would provide further therapeutic options.

In light of the devastating course of CLD, the goal to (do nothing less but) rebuild the diseased human lung is ambitious, but also imperative. The existence of resident human lung stem cells now opens up new possibilities and questions, the answers of which will bring us closer to deliver much needed new therapeutic options for patients suffering from CLD.

The authors declare that they have no conflict of interest.

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