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Embryonic stem cells for lung fibrosis Is it the Prometheus myth or the Pandora's Box?

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Three thousand years ago the Greek epic narrative poet Hesiod introduced to humanity his earliest work called *"Theogony"* where he narrates the myth of "Prometheus" who provoked Zeus' wrath by stealing fire from the Gods and delivering it to humans. Zeus punished Prometheus by chaining him to mountain Caucasus and sent an eagle to eat his immortal liver, which constantly replenished itself, indicating human's immortal hidden nature and ultimate mission. In an alternative version of the myth, included in the poet *"Works and Days"*, Hesiod relates Zeus vengeance to Prometheus and humanity with the creation of the woman Pandora who was sent down to Epimetheus who, though warned by Prometheus, married her. Pandora took the great lid off the jar she carried, and evils, hard work, and disease flew out to plague humanity. Hope alone remained within.

In a relative manner with the liver, human lung is characterized by a tremendous repairing capacity allowing a complete renewal of the entire pulmonary parenchyma almost 300 times till the age of 75 years. The latter process constitutes a miraculous defensive mechanism against constant exogenous and endogenous injuries and is mainly based on niches of multipotent resident stem cells (1) as well as bone marrow derived progenitors (2). Unfortunately, genetic predisposition and age related epigenetic modifications and risk factors including smoking, infections and environmental or other toxic pollutants significantly affect in a cumulative way lung's regenerative properties. As a major consequence a significant proportion of patients develop an aberrant wound healing response leading to abnormal reepithelization, fibroblast proliferation, extracellular matrix deposition and progressive lung scarring, pathogenetic events characteristic of idiopathic pulmonary fibrosis (IPF) (3). The latter represents a worryingly increasing cause of morbidity and mortality worldwide with a considerable human, societal and financial burden (4). Despite extensive research efforts, IPF still symbolizes a disease paradigm with pathogenesis elusive (5) and treatment ineffective (6). Therefore, there is an urgent need for alternative more beneficial therapeutic applications with regenerative medicine and stem cell-based therapies being such promising options.

The past 10 years we witnessed an explosion of experimental data relating to safety and efficacy of stem cell application in different models of lung inflammation and fibrosis (2,7,8). While safety evidence seems reassuring, at present there is no available pre-clinical data reporting complete recovery of established fibrotic lung disease. So far published data suggests that early administration of stem cells in patients with IPF may exert optimal therapeutic effects (9,10). A phase I clinical trial estimating safety profile of endobronchially infused autologous adipose derived stem cells in patients with IPF has been recently published and preliminary safety results indicate reassurance (11,12). Unfortunately, substan-

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tial safety concerns and dilemmas whether stem cell intervention could attenuate or accelerate fibrotic cascade still remain unanswered.

In this issue of *"Sarcoidosis Vasculitis and Diffuse Lung Diseases"* journal, Spitalieri and colleagues, following already established protocols by their research group (13), demonstrate through a series of elegant experiments the beneficial effects of the intratracheal administration of human embryonic stem cells pre-differentiated into alveolar type II epithelial cells (HUES-ATII) in the bleomycin-model of lung fibrosis. Bearing in mind that pre-clinical studies of lung fibrosis are problematic given limitations of currently available models authors applied a more representative to human IPF experimental model consisting of daily (repetitive) administered doses of bleomycin over a period of 14 days. At the end of bleomycin administration period 2.5 x10^6 (6) HUES were firstly cultured into small airway growth medium to direct their in-vitro differentiation towards alveolar epithelial cell lineage and then intratracheally instilled. Intriguingly, a pure population of ATII epithelial progenitors was derived resulting into a significant reduction of collagen deposition and inflammatory cytokine milieu within cell-treated animal lungs indicating potential anti-fibrotic and anti-inflammatory properties. Further extending the above series of experiments a thorough histological and molecular analysis was followed and revealed indices of human cells' presence, ie. coexpression of surfactant protein C and human nuclear antigen, within murine lung tissue for up to 10 weeks after stem cell treatment. The latter observation is of major significance since it provides us with the first hints of long-term stem cell engraftment within the injured airway epithelium suggesting the scenario that therapeutic outcome may have arised not only via paracrine immunomodulatory activity but may also reflect increased differentiative capacity and structural replenishment. At the same time, gradual consumption of bleomycin-induced increased CXCL12 concentrations within murine lung after HUES-ATII delivery strengthens the notion of stem cell homing in response to repeated injurious stimuli. Finally, cell-treated mice exhibited a significant improvement of oxygen saturation percentage accompanied by survival benefit, evidence that further extends efficacious outcomes of stem cell therapy from histological and molecular to functional and clinical phenotype. The above data seems quite promising especially considering the fact that it was combined with encouraging safety results revealing the absence of teratogenicity on a longitudinal basis (one year follow-up).

This is the first preclinical study in the therapeutic field of lung fibrosis emphasizing not only on stem cell paracrine activity but also on their reparative properties through differentiation into alveolar progenitor cells. The selected time point of stem cell administration at the interim between inflammatory and fibrotic phase of the modeled disease was also crucial since it was neither too early to study only anti-inflammatory effects of cellular treatment nor too late to minimize therapeutic potential during irreversible fibrotic phases, as it happens in clinical practice. Furthermore, several previous investigations starkly reported that mesenchymal stem cells (MSCs) of different cellular origin (umbilical cord or bone marrow) may attenuate bleomycin-induced lung injury mainly through their pleiotropic anti-inflammatory, anti-apoptotic and anti-fibrotic properties (9,10,14). Nevertheless, none of them reported truly phenotypic differentiation into precursors of alveolar epithelial cells.

Moodley et al. (9) identified migration of intravenously delivered umbilical cord MSCs into sites of fibroblast proliferation 14 days after bleomycin administration but cells disappeared two weeks later. Additionally, no evidence of differentiation towards alveolar epithelial cell lineage was documented. So far, the only study demonstrating derivation of lung epithelium from ex vivo differentiated MSCs was published by Sueblinvong et al. (15). Nevertheless, only a minority of these cells engrafted to intact murine airway epithelium indicating that most of the cells only temporarily lodged in the capillary beds of pulmonary vasculature and then either cleared or migrated to other sites. However, authors have utilized, so far, adult stem cells that are inferior in terms of potency compared to HUES. In the light of these elegant studies it seems provocative, but also rationale, to speculate that ex vivo manipulation of HUES within a specific culture microenvironment that will safely direct their selective differentiation towards either epithelial or endothelial cell lineage may facilitate their structural engraftment thus improving their regenerative capacity. The latter premise presents with crucial translational implica-

tions since optimized cell bioprocessing protocols may enhance the efficacy of stem cell therapeutics overcoming (16), so far, disappointing results arising from large multicentre clinical trials including patients with chronic lung diseases, such as chronic obstructive lung disease (17).

Nonetheless, despite relative enthusiasm arising from these important features there is a number of safety concerns that should be treated cautiously before extrapolating the above observations into everyday clinical practice. Authors administered an excessive amount of HUES ($2.5x10^6$) relative to the animal body weight (20gr approximately) i.e., $125x10^6$ per kgr of body weight. Such stem cell numbers may harbor potential risks, including faulty or aberrant engraftment and lethal pulmonary emboli that should not be underestimated. So far, pilot phase I safety studies overcome this barrier by applying a more conservative therapeutic regimen of one till up to four infusions of 1.5 $x10^6$ per kg of body weight. On the other hand, there is significant lack of knowledge regarding the exact mechanisms of actions of these cells and their fate within chronically injured lung. The possibility that stem cells could be differentiated into fibroblasts given their common mesodermal origin or even promote tumorigenesis within a pro-fibrotic and potential dysplastic microenvironment of a patient with IPF represent two of the greatest concerns of the respiratory scientific community. Moreover, although embryonic or adult stem cells themselves appear to escape immune recognition, those cultured in medium containing excipients, such as bovine serum, can produce immune reactions in patients receiving repeated administrations of these cells. Finally, Spitalieri et al. corroborated earlier findings (9,10) by reporting attenuation and not complete recovery of established fibrotic changes.

In conclusion, Promethean effect of reinforcing tissue replenishment may alternatively lead to the opening of the "Pandora's box", leading to detrimental side effects of carcinogenesis and immunogenicity. It is in the hands of clinicians to carefully apply and not overestimate cell based therapies in order for hope only to be unleashed.

References

1. Kajstura, J., M. Rota, S. R. Hall, T. Hosoda, D. D'Amario, F. Sanada, H. Zheng, B. Ogorek, C. Rondon-Clavo, J. Ferreira-Martins, et al. 2011. Evidence for human lung stem cells. N.Engl.J Med 364:1795-1806.
2. Weiss, D. J., I. Bertoncello, Z. Borok, C. Kim, A. Panoskaltsis-Mortari, S. Reynolds, M. Rojas, B. Stripp, D. Warburton, and D. J. Prockop. 2011. Stem cells and cell therapies in lung biology and lung diseases. Proc.Am.Thorac.Soc. 8:223-272.
3. Selman, M., A. Pardo, and N. Kaminski. 2008. Idiopathic pulmonary fibrosis: aberrant recapitulation of developmental programs? PLoS.Med. 5:e62.
4. Raghu, G., H. R. Collard, J. J. Egan, F. J. Martinez, J. Behr, K. K. Brown, T. V. Colby, J. F. Cordier, K. R. Flaherty, J. A. Lasky, et al. 2011. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 183:788-824.
5. Wuyts, W. A., C. Agostini, K. Antoniou, D. Bouros, R. Chambers, V. Cottin, J. Egan, B. Lambrecht, R. Lories, H. Parfrey, et al. 2013. The pathogenesis of pulmonary fibrosis: a moving target. Eur.Respir.J. 41:1207-18
6. Bouros, D. 2011. Pirfenidone for idiopathic pulmonary fibrosis. Lancet 377:1727-1729.
7. Tzouvelekis, A., A. Antoniadis, and D. Bouros. 2011. Stem cell therapy in pulmonary fibrosis. Curr.Opin.Pulm.Med 17:368-373.
8. Tzouvelekis, A., P. Ntolios, and D. Bouros. 2013. Stem Cell Treatment for Chronic Lung Diseases. Respiration 85:179-92.
9. Moodley, Y., D. Atienza, U. Manuelpillai, C. S. Samuel, J. Tchongue, S. Ilancheran, R. Boyd, and A. Trounson. 2009. Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. Am J Pathol. 175:303-313.
10. Ortiz, L. A., F. Gambelli, C. McBride, D. Gaupp, M. Baddoo, N. Kaminski, and D. G. Phinney. 2003. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc.Natl.Acad.Sci.U.S.A 100:8407-8411.
11. Tzouvelekis A, Paspaliaris V, Koliakos G, Ntolios P, et al. A prospective, non randomized, no placebo-controlled, phase Ib clinical trial to study the safety of the adipose-derived stromal cells-stromal vascular fraction in idiopathic pulmonary fibrosis. J Transl Med 2013; 11: 171 doi: 10.1186/1479-5876-11-171.
12. Tzouvelekis, A., G. Koliakos, P. Ntolios, I. Baira, E. Bouros, A. Oikonomou, A. Zissimopoulos, G. Kolios, D. Kakagia, V. Paspaliaris, et al. 2011. Stem cell therapy for idiopathic pulmonary fibrosis: a protocol proposal. J.Transl.Med. 9:182.
13. Spitalieri, P., M. C. Quitadamo, A. Orlandi, L. Guerra, E. Giardina, V. Casavola, G. Novelli, C. Saltini, and F. Sangiuolo. 2012. Rescue of murine silica-induced lung injury and fibrosis by human embryonic stem cells. Eur.Respir.J. 39:446-457.
14. Kumamoto, M., T. Nishiwaki, N. Matsuo, H. Kimura, and K. Matsushima. 2009. Minimally cultured bone marrow mesenchymal stem cells ameliorate fibrotic lung injury. Eur.Respir.J. 34:740-748.
15. Sueblinvong, V., R. Loi, P. L. Eisenhauer, I. M. Bernstein, B. T. Suratt, J. L. Spees, and D. J. Weiss. 2008. Derivation of lung epithelium from human cord blood-derived mesenchymal stem cells. Am J Respir Crit Care Med 177:701-711.
16. Huang SXL, Islam MN, O'Neill J, Hu Z, Yang Y-G, et al. Efficient generation of lung and airway epithelial cells from human pluripotent stem cells. Nature Biotechnology 2013; doi: 10.1038/nbt.2754.
17. Weiss, D. J., R. Casaburi, R. Flannery, M. Leroux-Williams, and D. P. Tashkin. 2012. A Placebo-Controlled Randomized Trial of Mesenchymal Stem Cells in Chronic Obstructive Pulmonary Disease. Chest (in press). doi: 10.1378/chest.12-2094.