Pulmonary interstitial glycogenosis cells express mesenchymal stem cell markers

To the Editor:

Pulmonary interstitial glycogenosis (PIG) was first defined as a distinct neonatal interstitial lung disease of unknown aetiology that presents in neonates and young infants with mild to severe hypoxic lung disease [1]. Characterised clinically by unexplained respiratory distress and cyanosis with an onset during early infancy, PIG was primarily defined by the presence of distinct and unusual-appearing cells contained within the interstitium that were characterised by a widened interstitium containing variable numbers of immature-appearing, polygonal-to-spindle shaped cells, which may contribute to impaired gas exchange. The most unique feature of PIG cells is the widespread presence of non-membrane bound, periodic acid-Schiff stain-positive, mono-particulate glycogen in the cytoplasm, for which the disease was named ("glycogenosis") [1]. By ultrastructure, PIG cells are considered primitive due to the presence of only sparse organelles and a lack of specific features that indicate differentiation towards any well-characterised pulmonary cell line, including lymphocytes or macrophages [1].

Observations from this landmark paper led to subsequent studies that further characterised PIG, using the presence of these novel and atypical appearing cells as the pathognomonic diagnostic hallmark [2–5]. Although initially considered a distinct disease, recent studies have identified histologic evidence of PIG in association with diverse cardiopulmonary disorders, including childhood interstitial lung disorders, congenital heart disease, bronchopulmonary dysplasia, congenital airway malformations, pulmonary hypertension, neuroendocrine cell hyperplasia of infancy and congenital lobar emphysema [2–5]. Long-term outcomes for infants with histologic findings of PIG are highly variable and may be linked with the severity of the underlying disease process associated with PIG histology; however, fatal cases have been reported even in the absence of other known primary diagnoses [2–5]. Clinical, imaging, bronchoscopic and genetic findings are not specific for PIG and diagnosis still requires lung biopsy.

The ultrastructural appearance of PIG cells and the association of PIG with early infancy strongly suggest that these cells reflect early disruption of lung development or a poorly understood response to lung injury. The nature and origin of this unique cell population and their link with diverse diseases, however, remain speculative at this time. PIG cells stain strongly for vimentin, supporting a likely mesenchymal origin, perhaps as the result of abnormal differentiation of interstitial fibroblasts or lipofibroblasts [4, 6–9]. In fact, an early paper originally suggested that PIG cells may represent persistence of normal fetal lung mesenchyme [5].

Given their primitive mesenchymal appearance and strong link with several developmental lung diseases, we hypothesised that PIG cells are lung-resident mesenchymal stem cells (LR-MSC). LR-MSC have been previously identified in adult and fetal lungs and have a primitive ultrastructural appearance with glycogen as observed with PIG cells [9–14]. LR-MSC are capable of self-renewal, multi-lineage differentiation and are thought to play a crucial role in lung homeostasis and repair [8, 9]. Defining features of isolated MSC from diverse sources have been established and include intense adherence to the culture dish wall, expression of CD44, CD73, CD90 and CD105, the lack of expression of haematopoietic markers and the ability to differentiate into multiple cell types, such as adipocytes, osteocytes and chondrocytes [6–9]. Whether PIG cells represent LR-MSC and their role during lung development remains speculative.

To begin to address the possible MSC nature of PIG cells, we studied lung tissue from five cases of PIG (ages between 1 and 7 months) that covered a wide spectrum of associated diseases, including prematurity,

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Pulmonary interstitial glycogenesis (PIG) is characterised by a unique but poorly characterised cell population in developmental lung disease. This study reports that PIG cells express cellular markers, suggesting a mesenchymal stem cell lineage. https://bit.ly/2YNdDIY

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congenital heart disease (hypoplastic left heart), congenital pulmonary airway malformation type 3, bronchial atresia and a genetic mitochondrial syndrome with severe pulmonary hypertension. We performed histologic, ultrastructural and immunohistochemical studies to determine the presence of defining markers of MSC, including CD44, CD90 and CD105. CD10 expression was examined due to its common expression in mesenchymal cells during lung development and past use as a cell surface marker of LR-MSC [8]. CD34, a traditional marker of bone marrow-derived haematopoietic MSC, was used as a negative LR-MSC marker. Histologic and ultrastructural studies confirmed PIG cells in all cases as based on the classic and typical primitive appearance with sparse organelles and mono-particulate glycogen [1]. PIG cells from all cases were consistently and strongly marked by CD44, CD105 and CD10 immuno-positive staining but negative for CD34. The EM studies of PIG cells showed rare fat droplets. PIG cells were strikingly positive for CD90 in one subject (figure 1).

Overall, these findings provide strong evidence that PIG cells express MSC biomarkers. Their capacity for multi-lineage differentiation and adherence in cell culture remains to be tested. However, studying PIG cells *in vitro* is a considerable challenge, as there is currently no animal model for PIG and the only potential source of PIG cells is from the diagnostic lung biopsy. However, infant lung biopsy is extremely limited in size and provides numerous technical barriers for harvesting adequate numbers of PIG cells for sufficient *in vitro* studies, further limiting studies to archived samples.

Based on these findings, we speculate that PIG cells represent endogenous MSC, or lung-resident MSC (LR-MSC) within the lung interstitium, which may with further differentiation, potentially contribute to diverse cell types that may contribute lung development or pathobiology [7, 9, 11–13]. A critical question remains as to whether PIG cells reflect impaired MSC development or are actively recruited to the sites of disrupted lung development for repair. It is also possible that PIG cells are dysfunctional LR-MSC that are unable to further differentiate into a pre-programmed lung cell type, but may differentiate into diverse cell types as determined perhaps by environmental cues, such as lipofibroblasts, that may enhance surfactant production by alveolar type II cells [6]. Older studies have not found fat droplets in most PIG cells [1–5], however, a recent study reports positive fat staining in PIG cells, suggesting their ability to form lipofibroblasts [6]. Other possible roles include differentiation into interstitial fibroblasts that are involved in distal lung growth, pro-fibrotic diseases or aberrant vascular remodelling with increased smooth muscle cell growth in various types of neonatal pulmonary hypertension.

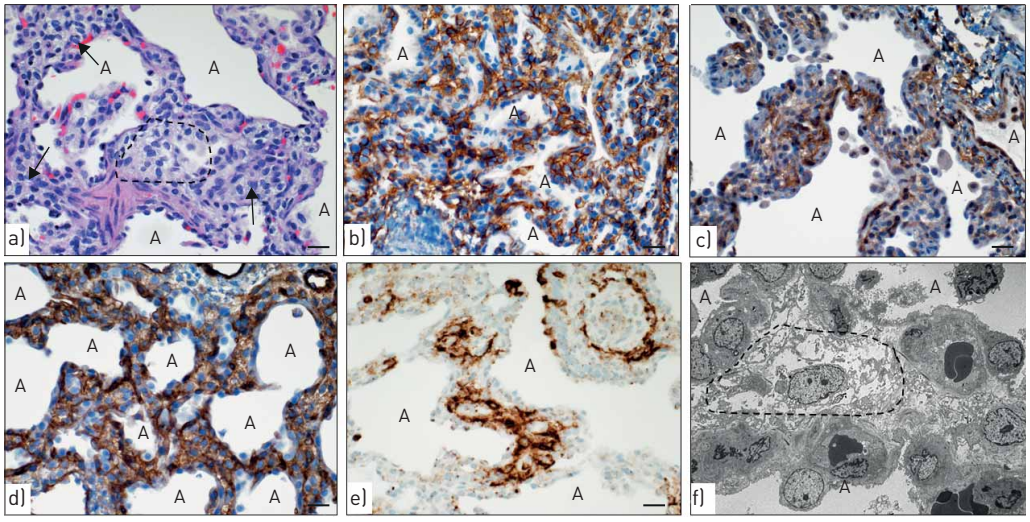

FIGURE 1 a) Haematoxylin and eosin staining (40×) highlights the classic polygonal, spindle shaped pulmonary interstitial glycogenosis (PIG) cells with pale cytoplasm diffusely expanding the interstitium (dashed circle and arrows). Mesenchymal stem cell markers, including b) CD44 (40×), d) CD105 (40×) and e) CD10 (40×) immunostains strongly and diffusely label the interstitial PIG cells with a combination of membranous and cytoplasmic fashion in all cases. c) CD90 immunostaining intensely decorates the PIG cells, as illustrated (40×). CD34, a haematopoietic cell marker, is negative in PIG cells from all cases (not shown). f) Ultrastructural examination diagnostically confirms the presence of interstitial PIG cells in each case (highlighted with a dashed outline), which show the classic primitive cells with sparse organelles and non-membrane bound cytoplasmic mono-particulate glycogen in all cases. Three of the five cases contained lipid droplets as well. "A" denotes alveolar spaces. Scale bars represent 20 μm.

Although an important case study reported that PIG cells "disappeared" upon repeat lung biopsy with concurrent improvement in lung function after high-dose steroid therapy [15], it remains unclear whether PIG cells influenced or simply reflected clinical improvement. Indeed, the steroid response is highly variable in infants with diverse diseases associated with PIG [2–4] and further studies are needed to better understand the relationship of PIG cells and mechanisms underlying their impact on normal and abnormal lung structure and function.

Over the past decade, MSC from multiple sources have been considered to have a potential role in cell-based therapy of lung disease, yet the potential role of endogenous MSC during normal development is unknown. Our findings suggest that the presence of endogenous MSCs are associated with diverse lung diseases and we speculate that these cells may contribute to aberrant structure and the response to injury in the developing lung. As recently reviewed, lung MSCs have the potential for diverse functions, including modulation of lung cell growth and function, and modulation of immunologic, inflammatory and fibrotic pathways [16].

We propose that insights into the PIG cell, its potential characterisation as a type of LR-MSC and mechanisms underlying LR-MSC function and regulation will enhance our understanding of normal lung growth and development, regenerative medicine and perhaps provide insights into potential beneficial or adverse effects of MSC therapies. We further speculate that more precise characterisation of LR-MSC during lung development in animal models, enhanced marker identification of LR-MSC differentiation and function, and improved techniques for MSC lineage tracing may ultimately lead to a better understanding of normal and aberrant lung development and reveal novel therapeutic interventions for diverse developmental lung diseases.

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