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Review article

On the evolution of the pulmonary alveolar lipofibroblast

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ABSTRACT

The pulmonary alveolar lipofibroblast was first reported in 1970. Since then its development, structure, function and molecular characteristics have been determined. Its capacity to actively absorb, store and 'traffic' neutral lipid for protection of the alveolus against oxidant injury, and for the active supply of substrate for lung surfactant phospholipid production have offered the opportunity to identify a number of specialized functions of these strategically placed cells. Namely, Parathyroid Hormone-related Protein (PTHrP) signaling, expression of Adipocyte Differentiation Related Protein, leptin, peroxisome proliferator activator receptor gamma, and the prostaglandin E2 receptor EP2- which are all stretch-regulated, explaining how and why surfactant production is 'on-demand' in service to ventilation–perfusion matching. Because of the central role of the lipofibroblast in vertebrate lung physiologic evolution, it is a Rosetta Stone for understanding how and why the lung evolved in adaptation to terrestrial life, beginning with the duplication of the PTHrP Receptor some 300 mya. Moreover, such detailed knowledge of the workings of the lipofibroblast have provided insight to the etiology and effective treatment of Bronchopulmonary Dysplasia based on physiologic principles rather than on pharmacology.

Contents

1. Discovery of the pulmonary alveolar lipofibroblast

The presence of lipid droplets in pulmonary interstitial cells was first described by Hitchcock et al. in 1970 [1]. Vaccaro and Brody were the first to name the associated cell-type the lipid interstitial cell (LIC) [2]. They emphasized the abundance of lipid droplets, the high glycogen content, and localization of LICs to the central region of the alveolar septum [3]. LICs were later better characterized as differentiated mesenchymal cells, meriting their designation as Lipofibroblasts (LIFs) [4].

LIFs are evident in rat lung by gestational day 16, and the triglyceride content of whole lung tissue increases three-fold between gestational days 17 and 19 (=term) [5]. The lung triglyceride content then increases another 2.5-fold between gestational day 21 and postnatal day 1, peaking during the second postnatal week [5]. The abundance of LIFs in the lung follows the same time-course, as evidenced by the yield of LIFs isolated by centrifugal sedimentation at various postnatal ages [6]. The amount of lipid per cell appears to remain constant from postnatal day 4 through 8. The lipid droplets primarily contain neutral lipids —65% triglycerides, 14% cholesterol esters—and an additional 7% are free fatty acids and cholesterol [6]. Phospholipids comprise the

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remaining 14% of the cellular lipids. The number of LIFs decreases prior to weaning and appears result from both a decrease in cellular proliferation [7], and increased apoptosis [8].

LIFs are found in the lungs of mice and hamsters at postnatal day 8, although the volume density of lipid droplets is lower than in neonatal rats [9]. When identified by their signature location in the alveolar wall, LIFs are observed in adult rats, mice, and hamsters, although they contain far less lipid than in the neonate [9]. The volume density of the lipid droplets in lung fibroblasts is higher in the adult mouse than in the adult rat or hamster [9].

Surprisingly, the functional properties of the LIF did not advance for decades, until Torday et al. performed LIF co-culture experiments with alveolar type II (ATII) cells to determine the metabolic fate of the stored triglycerides [10]. The discovery of the robust transit of radiolabeled triglyceride from LIFs to ATIIs, termed Neutral Lipid Trafficking revealed the developmental, homeostatic, and ultimately the evolutionary significance of the LIF [10,11].

2. In the process of lung evolution, homologies run deep

The LIF is like an ontogenetic–phylogenetic 'Rosetta Stone' for the evolution of the lung [12], offering the opportunity to 'reverse-engineer' the processes by which the alveolus evolved to accommodate metabolic drive. The physiologic relevance of the LIF to alveolar growth, differentiation, homeostasis and repair has revealed such deep evolutionary homologs as [1] the peroxisome, which is thought to have evolved in response to the otherwise pathologic effects of Endoplasmic Reticulum stress in unicellular organisms; [2] Neutral Lipid Trafficking, encompassing lipid uptake and storage in defense against hyperoxia mediated by Adipocyte Differentiation Related Protein (ADRP), and release under the control of Prostaglandin E_2. This mechanism refers all the way back to the advent of cholesterol [13], and the evolution of the fat cell [14], producing the hormone leptin, ultimately coopted to regulate surfactant production by the Alveolar Type II Cell (ATII), coming full circle from the antioxidant property of the LIF. For orientation to these cellular–molecular evolutionary properties of the lung, the pathways for ontogeny, phylogeny and evolution of the LIF–ATII interactions are illustrated in Figs. 1 and 2 respectively.

LIFs in the alveolar wall of rat lung were first described by Hitchcock et al. [1], and extensively documented in rodent [2,6–9], and more recently in human [15] lung. However, their functional relevance to the alveolus was not determined for two more decades, though their cytoprotective nature was suggested by comparative studies of Frank et al. [16], who described the association between the LIFs and their putative role in overall antioxidant protection. These physiologic studies were paralleled by biochemical studies of triglyceride metabolism conducted by Mostello et al. [17]. The breakthrough in understanding the functional nature of these cells in lung alveolar physiology came with the co-culture of LIFs pre-labeled with radiolabeled triglyceride and naïve ATIIs, resulting in active uptake of the triglyceride by the ATIIs, and their subsequent robust incorporation into surfactant phospholipid by these cells [10], termed Neutral Lipid Trafficking. Experimentally, it was observed that LIFs could readily take up triglyceride and store it in a stable form; furthermore, this process could be stimulated by glucocorticoids, highlighting its regulated nature. Moreover, the presence of neutral lipid droplets in the LIFs protected them against oxidant injury [18], providing a physiologic function for these cells. This property of the LIF may have evolved from the myofibroblast in response to the rise in atmospheric oxygen during the Phanerozoic Era, as shown experimentally by Csete et al. [14], who found that if myocytes were cultured in 6%

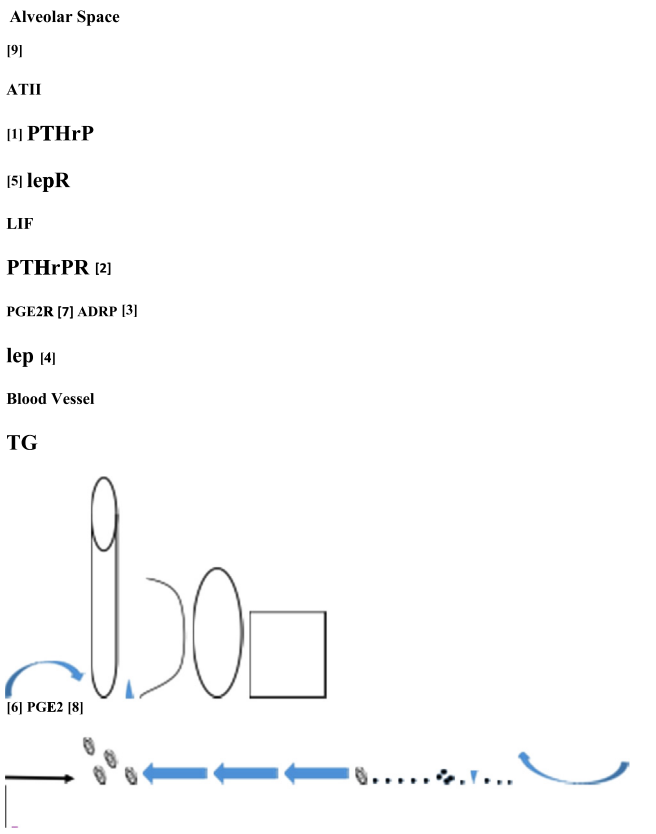

Fig. 1. Cell–cell interactions determine alveolar development. PTHrP secreted by the ATII [1] binds to its receptor on the LIF [2] initiating alternating signals between the ATII and LIF, beginning with the stimulation of ADRP [3], which is necessary for the 'trafficking' of triglyceride (TG) from the circulation to the LIF, and from the LIF to the ATII. PTHrP also stimulates leptin [4] expression by the LIF, which then binds to its receptor on the ATII [5], stimulating surfactant synthesis [8]. The ATIIs secrete surfactant into the alveolar space [9], reducing surface tension to prevent alveolar collapse.

oxygen they retained a myofibroblast phenotype, but in 21% oxygen they differentiated into adipocytes. It was subsequently determined that the uptake, storage and transfer of the neutral lipids was actively mediated by ADRP [19], a member of the PAT (Perilipin, Adipocyte Differentiation Related Protein, TIP47) family of proteins that mediate the trafficking and storage of neutral lipids throughout the body.

During the course of these studies, it was discovered empirically that isolated ATIIs could not absorb TGs, and isolated LIFs could not release them, implying the existence of active regulatory mechanisms for Neutral Lipid Trafficking. Such a mechanism had long been suspected since surfactant production is known to occur 'on demand' [20,21]. That led to the discovery that the prostaglandin PGE_2, secreted by ATIIs specifically causes PGE_2 -specific EP_2 receptor-mediated release of TGs by LIFs [22], and leptin produced by the LIFs facilitates the uptake of TGs by binding to its specific cell surface receptors on ATIIs [23].

*Please note that all but steps 1,3 and 9–11 are lipofibroblast specific genes.

3. Gene expression

The culmination of these cell–cell interactions mediating and facilitating the production of lung surfactant phospholipid production was the discovery that Neutral Lipid Trafficking is stretch-regulated, providing key insights to both the cellular–molecular basis for the mechanism of alveolar ventilation–perfusion