In search of the elusive lipofibroblast in human lungs

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Ahlbrecht K, McGowan SE. In search of the elusive lipofibroblast in human lungs. Am J Physiol Lung Cell Mol Physiol 307: L605–L608, 2014—Although the pulmonary interstitial lipofibroblast (LF) has been widely recognized in rat and mouse lungs, their presence in human lungs remains controversial. In a recent issue of the Journal, Tahedl and associates (Tahedl D, Wirkes A, Tschanz SA, Ochs M, Mühlfeld C. Am J Physiol Lung Cell Mol Physiol 307: L386–L394, 2014) address this controversy and provide the most detailed stereological analysis of LFs in mammals other than rodents. Strikingly, their observations demonstrate that LFs were only observed in rodents, which contrasts with earlier reports. This editorial reviews the anatomical, physiological, and biochemical characteristics of the LF to better understand the significance of LFs for lung development and disease. Although lipid droplets are a signature of the LF cell type, it remains unclear whether lipid storage is the defining characteristic of LFs, or whether other less overt properties determine the importance of LFs. Are lipid droplets an adaptation to the neonatal environment, or are LFs a surrogate for other properties that promote alveolar development, and do lipid droplets modify physiology or disease in adults?

Current Understanding of the Pulmonary Lipofibroblast in the Process of Alveolarization

Lipid droplets in pulmonary mesodermal cells were first described by O’Hare and Sheridan (42). This report was closely followed by a detailed analysis of the morphological and biochemical features of this mesenchymal cell type. Because of the prominent lipid vacuoles evident in lipofibroblast (LFs), the LFs were initially termed lipid interstitial cells (LICs, here referred to as LFs) (7, 8, 26, 33, 34, 42, 54, 55). During alveolarization [in the rat postnatally (P)5 until P15] interstitial mesenchymal cells with fibroblast characteristics were divided into two types. The LF cell type was located at the base of newly formed septa, in close proximity to the alveolar type II (ATII) cells and endothelial cells. LFs contained lipid vacuoles, few cytoplasmic organelles, and myofilaments, which were orientated perpendicular to the alveolar wall (7, 8, 55). The second cell type, termed a nonlipid interstitial cell (NLIC), was observed at the nascent septal termini, had features of active protein synthesis, and was located adjacent to collagen and elastic fibers (7, 34, 55). Myofilaments in the NLICs were positioned parallel to the alveolar wall and lipid droplets were absent (7, 34). From gestational day 17 until P10, the accumulation by LFs of di- and triacyl glycerides into vacuoles, which lacked a distinct membrane, closely paralleled the composition of these lipid moieties in the lung (34, 54, 55). Because of the proximity of LFs to ATII cells and capillaries, LFs were proposed to shuttle lipids from the capillaries to the ATII cells and regulate surfactant production (54). Testing this hypothesis, Torday and coworkers (52) demonstrated that cultured fetal rat lung fibroblasts absorbed [3H]triolein, a triglyceride (TG), and transferred [3H]triolein to neighboring ATII cells, which incorporated [3H]triolein into surfactant phospholipids. Because lipid vacuoles are a convenient marker, this feature was extensively used to characterize changes in the ontogeny and physiology of LFs during alveolarization (7, 27, 34). In the rat, the quantity of LFs increases by approximately twofold between P4 and P7, with an accompanying increase in the cellular lipid content (34). LFs decrease in abundance after P7 through diminished proliferation and after P14 owing to apoptosis; thus LFs are rarely observed in the adult rat (2, 27). The abundance and composition of the lipid droplets are strongly influenced by the cellular environment. For example, the lipid content of neonatal rat LFs mirrors the pattern of circulating TGs (34, 54). LFs lose lipid droplets in culture, but lipid droplets can be restored when LFs are exposed to neonatal rat serum that contains an abundance of chylomicrons (33). In obese adult rats, LFs accumulate lipids, which along with extracellular lipid deposits increase the septal thickness at the expense of adjacent capillaries (19). In adult mice, LF neutral lipid storage increases when there is a defect in sterol binding protein-mediated surfactant synthesis in ATII cells (4). These observations indicate that the ability to identify lipid droplets in LFs is strongly influenced by the availability of extracellular fatty acids and chylomicrons.

The relationship between the LF and NLIC has been examined to determine whether these two cell types represent different developmental stages [i.e., are LFs precursors to NLICs or myofibroblast (MF) cell types?]: do LFs and MFs share a common precursor cell, and how do LFs and MFs assume divergent phenotypes? Because culturing LFs on a rigid surface promotes a MF phenotype [loss of lipid droplets, increase in actin $\alpha_2$ (ACTA2), and sometimes elastic fibers], it cannot be concluded that most LFs transition to MFs in the lung. However, manipulating culture conditions can change the relative proportions of LFs and MFs (33, 36, 37, 53). TG uptake and storage increase after mechanical stretch or exposure to parathyroid hormone-related peptide (PTHrP), resulting in leptin production by LFs and increased surfactant phospholipid synthesis by ATII cells (53). Introducing the Thy-1 (CD90) antigen into cultured fibroblasts also promotes lipid accumulation (56). The MF phenotype can be reversed by exogenous peroxisome proliferator-activated receptor (PPAR)
Appearance and Detection of Lipofibroblasts in Human Lungs

The study by Tahedl and coworkers (51a) was undertaken to identify LFs, based on their characteristic lipid droplets, in a variety of mammalian species including humans. Lungs were analyzed by osmium tetroxide postfixation, transmission electron microscopy (TEM), and light microscopy (LM). Design-based stereology was applied with TEM (43) to obtain the volume fraction and total volume of lipid bodies, which was confirmed with Sudan Black staining and LM. Lipid bodies were confirmed in rodents but not in 14 mammalian species including humans. This contradicts former results demonstrating LFs in the human lung (47). In all species few lipid bodies were detected in ATII cells. Only the dog samples contained higher amount of lipid bodies in ATII cells.

Several considerations help rectify the opposing conclusions from the studies by Rehan et al. (47) and Tahedl and associates (51a). Although TEM enables precise identification and quantification of individual cells, including those with lipid droplets, it provides some challenges. Strengths of the study by Tahedl and associates are the design-based stereological approach, including multiple subjects from a variety of mammalian species, and imaging at both the electron and light microscopic levels. However, staining procedures need to be optimized to visualize hydrophobic lipids. Unlike the phospholipid surface, visualization of the dominant hydrophobic glyceride core requires that its unsaturated fatty acids react with osmium tetroxide to make these structures more electron dense and less soluble in organic solvents (1, 20, 21, 22a, 23, 51). Therefore, staining procedures need to be optimized to visualize hydrophobic lipids. Unlike the phospholipid surface, visualization of the dominant hydrophobic glyceride core requires that its unsaturated fatty acids react with osmium tetroxide to make these structures more electron dense and less soluble in organic solvents (1, 20, 21, 22a, 23, 51). Therefore, recognition of lipid droplets by TEM is dependent on the composition of unsaturated fatty acids, which may vary among species and differentially impact the detection of lipid bodies in rodents compared with higher mammals. The unsaturated fatty acids, palmitoleic, oleic, and linoleic acids comprise 62% of the triglyceride acids in rat LFs at P10, whereas palmitoleic, oleic, and linoleic acids comprise 43% of human infant subcutaneous fat (18, 34). Using subcutaneous fat as a surrogate, one might speculate that osmium tetroxide may less efficiently detect lipid droplets in humans, but this is unlikely to explain an absence of lipid droplets. Rather than solely visualizing lipid droplets, future studies could also identify LFs by enzymatic properties (such as hormone sensitive or lipoprotein lipase) (7, 26, 33, 34, 54, 55), other markers of an adipocyte-like phenotype (such as ADRP or PPARγ), characteristic cytokines (such as PTHrP or FGF-10), or cell surface antigens (such as Thy-1) (16, 24, 33, 34, 44, 56, 58). The ideal experimental design would include all of these identifying features, but it may be untenable when only archival human samples are available. When immunohistochemical or cytological approaches such as those of Rehan and associates are used at the LM level, negative selection markers should be
used to exclude lipids in macrophages or phospholipids in ATII cells. Alternatively, immunoelectron microscopy of lipid droplet-associated proteins such as ADRP might confirm that lipid droplets were in fibroblasts rather than macrophages or epithelial cells. Newer fluorescent light microscopic approaches should also be considered (14). Debarre and coworkers (14) used third harmonic generation (THG) microscopy to image lipid bodies in the lung based on their light backscattering in tissue that does not require fixation or exposure to organic solvents. By combining THG with second harmonic generation (SHG) and two-photon excited fluorescence (2PEF) microscopy, lipid droplets, collagen, and elastic fibers can all be visualized without encountering some of the limitations of TEM.

**Does Rodent Lung Development Model that in Humans, Where Lipofibroblasts May Be Absent?**

Rats, and more recently mice, have been extensively used to model alveolar septal development, and the process largely parallels that in humans (10). Apart from the differences in timing and scale, the processes of alveolarization in rats and humans are histologically similar. Although saccules—but not alveoli—are observed in rats at birth, the human gas-exchange surface is comprised of both saccules and alveoli, which contribute ~15% of the alveoli present in adults (15, 17, 29). In both neonatal rats and humans, thick septa contain a double capillary layer that merges into one layer as the interstitium thins and the epithelial surface increases (62, 63). The extensive increase in alveolar surface area results from outgrowth of new (secondary) alveolar septa occurs between postnatal days 4 and 13 in the rat but requires approximately 7 years in humans (9, 10, 31). As secondary septation decelerates, the septal microvascular surface increases through intussusceptive microvascular growth, which has been observed in both rats and humans (11, 41). Finally there is evidence for regrowth of the lung after pneumonectomy in both species (12, 28, 57, 61). Despite extensive similarities, the article by Tahedl and coworkers (51a) importantly illustrates that the composition of rodent and human lungs are not identical and that observations about alveolar development in mice or rats need to be verified in humans. Since ethical and practical constraints limit the availability of tissue from children, mice will remain valuable tools enabling genetic manipulations and identification of important developmental pathways. This enlightening study by Tahedl and associates also illustrates that a variety of technical and biological approaches are required to wed the findings in rodents with those made in humans.

**Possible Role of Lipofibroblasts in Lung Disease and Regeneration Future Perspectives**

Some studies have explored the potential contributions of LFs to pulmonary disease and regeneration. Bellusci and coworkers (49) demonstrated that the PPARγ agonist rosiglitazone completely blocked nicotine induced Wnt-activation in human embryonic fibroblasts. Using a murine model of pneumonectomy, Chen and coworkers (13) demonstrated that rosiglitazone inhibited α-smooth muscle actin expression in PDGF-Rα+ fibroblasts and the formation of new alveolar septa, suggesting that the activation of PPARγ may promote a LF phenotype rather than a MF phenotype. Taken together, there is a growing body of evidence supporting the importance of LFs in lung regeneration and disease, but further studies in human and rodent tissue are clearly needed to explore this idea. In vivo studies in transgenic mice using the Cre-recombinase loxP approach to target specific cell types might be beneficial to elucidate the origin and fate of the LF. Cell type-specific lineage tracing, cell depletion (6), and gene deletion in combination with suitable morphological assessment (43, 59) might represent useful tools to identify potential therapeutic targets, which will then need to be validated by histological and cellular studies in humans (46).

**REFERENCES**


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