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EVIDENCE FOR THE PRESENCE OF LIPOFIBROBLASTS IN HUMAN LUNG

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□ *The lipid-containing alveolar interstitial fibroblast (lipofibroblast) is known to be critically involved in rodent lung development, homeostasis, and injury/repair. However, there is lack of information on their presence and function in the human lung. Based on a number of morphological (lipid staining), molecular (presence of characteristic lipogenic and absence of myogenic markers), and functional (triglyceride uptake) characteristics that are the hallmarks of the rodent lung lipofibroblast, using human lung fibroblasts of embryonic (WI-38) and adult origin and lung tissue from human autopsy specimens, the authors for the first time clearly demonstrate the presence of lipofibroblasts in the human lung.*

Keywords lipofibroblast, lung fibroblast, myofibroblast, parathyroid hormone–related protein, peroxisome proliferator–activated receptor γ

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There is a growing body of evidence suggesting that mesenchymal interstitial fibroblasts play a critical role in normal development and injury/repair mechanisms in many organ systems, including the lung [1–6]. During the process of lung development, there is coordinate signaling between the epithelial cells and fibroblasts that determines alveolar development, maintains normal alveolar homeostasis, and mediates injury/repair mechanisms [1, 2, 7–12]. However, it is now amply clear that based on their structural morphology, lipid content, location, and molecular characteristics, the interstitial fibroblast population is heterogenous [2, 6, 13–17]. Based on their lipid content, pulmonary interstitial fibroblasts have been classified into 2 populations: the lipid droplet-containing lipofibroblast (also known as lipid interstitial cell), and the nonlipid interstitial cell, which lacks the characteristic lipid droplets and is located more peripherally in the alveolar septum. The lipid-containing interstitial cells account for about 50% of resident alveolar wall cells in the immature rodent lung [13]. The lipid-containing and non–lipid-containing intersitial cells seem to have quite distinct and unique functions during lung development, homeostasis, and injury/repair [2]. Because of its essential role during normal rodent lung development, the lipofibroblast has been extensively characterized in the rat, and has also been observed in mice and hamsters [2, 13–18]. But data supporting the presence of lipofibroblasts in the human lung and their role in human lung development and injury/repair are scant. Therefore, the purpose of this report is to document the presence and functional characteristics of fibroblasts of human origin indicative of their lipofibroblastic nature. We predict that, similar to their role in the rodent lung, lipofibroblasts play a critical role during normal human lung development, homeostasis, and injury/repair.

MATERIALS AND METHODS

Cell Culture

Human embryonic lung fibroblasts (WI-38), obtained from The American Tissue Culture Collection (Rockville, MD), and adult human lung fibroblasts (HLFs), obtained from adults undergoing lung biopsies for suspected chronic lung diseases who did not have idiopathic pulmonary fibrosis, were cultured in minimum essential medium (MEM) (GIBCO) with 10% fetal bovine serum (Gemini) and antibiotics (10,000 U/mL penicillin G sodium, 10,000 μg/mL streptomycin sulfate, and 25 μg/mL amphotericin B as Fungizone in 0.85% saline) (GIBCO). Adult human lung fibroblasts were isolated from open lung biopsy specimens as previously described [19] and with appropriate institutional review board (IRB) approval. These were grown to 80% confluence and passaged. At the time

of the 4th passage, pulmonary fibroblast purity was >99% as determined by the absence of nonspecific esterase, factor VIII–related antigen, or cytokeratin immunostaining. The cells were >90% positive for vimentin, laminin, and fibronectin, and were >90% negative for α-smooth muscle actin and desmin.

When ready, WI-38 cells and HLFs were grown in 6-well plates, 2-well slides, 60-mm, and 100-mm culture dishes as needed and were incubated at 37°C in 5% CO_2/air in a standard incubator until approximately 80% confluent and then were either treated with parathyroid hormone–related protein (PTHrP) (1–34) (Bachem Biosciences) at graded concentrations (1×10^{-9}, 1×10^{-7}, and 5×10^{-7} M), or maintained without PTHrP (controls) for 24 hours.

To unequivocally demonstrate the presence of lipofibroblasts in the human lung, lung tissue sections (autopsy) from infants and adults dying from nonrespiratory causes were stained for the presence of lipid droplets and adipocyte differentiation-related protein (ADRP), the signature lipofibroblast markers. The lung specimens were collected and kindly provided by Dr. Kay Washington, Department of Pathology, Vanderbilt University, Nashville, Tennessee.

Oil Red O Staining

The slides mounted with 5-μm human lung tissue sections were fixed in 10% formalin, and then rapidly rinsed in distilled water. After draining water off, the slides were immersed in 100% propylene glycol for 5 minutes ×2. Oil Red O was prepared by slowly dissolving in propylene glycol (0.7 g/100 mL), while heating to 100°C, but not over 110°C, for a few minutes. At the same time, it was stirred constantly. This was then filtered (Whatman no. 2 filter paper) and cooled, and then filtered again. The slides were then dipped in Oil Red O for 7 minutes, with occasional agitation, following which these were dipped in 85% propylene glycol for 3 minutes. Subsequent to Oil Red O staining, slides were rinsed in distilled water and then allowed to react with hematoxylin for 1 minute, washed in water, and then mounted with glycerol.

Triglyceride Uptake Assay

The method used to quantitate triglyceride uptake by lung fibroblasts has previously been described [20]. Briefly, culture medium was supplemented with [^{3}H]triolein (5 μCi/mL) for the last 4 hours of the experiment and cells continued to be incubated at 37°C in 5% CO_2–balance air. At the termination of the incubation, the medium was decanted; the cells were rinsed twice with 1 mL of ice-cold MEM, and were removed from the

culture plate after a 5- to 10-minute incubation with 2 mL of a 0.05% trypsin solution. An aliquot of the cell suspension was taken for protein assay (BioRad), and the remaining cell suspension was extracted for neutral lipid content. Thin layer chromatography, as previously described [20], was performed on the extracted neutral lipids to separate and quantitate the specific lipid.

Isolation of Total Cellular RNA and Semiquantitative Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from WI-38s and HLFs with a single step extraction method (UltraSpec, Biotecx). Integrity of RNA was assessed from the visual appearance of the ethidium bromide–stained ribosomal RNA bands following fractionation on a 1.2% (*w*/*v*) agarose-formaldehyde gel and quantitated by absorbance at 260 nm. Equal amount of RNA from each sample was reverse transcribed and then amplified by PCR, with primers specific for human PTHrP receptor: 5′ ATGTGGATGTAGTTGCGCGTG CAGT3′ and 3′GGAAGCCCAGGAAAGATAAGGCAT5′ (445 bp); peroxisome proliferator–activated receptor (PPAR)γ: 5′CCCTCATGGCAATTGA ATGTCGTG3′ and 3′TCGCAGGCTCTTTAGAAACTCCCT5′ (757 bp); ADRP: 5′GTTGCAGTTGATCCACAACCG3′ and 3′TGGTAGACAGGGATCC CAGTC5′ (666 bp); α-smooth muscle actin (αSMA): 5′CGCAAATATTCTGT CTGGATCG3′ and 3′TCACAGTTGTGTGCTAGAGACA5′ (167 bp); and 18s: 5′TTAAGCCATGCATGTCTAAGTAC3′ and 3′TGTTATTTTTCGT CACTACCTCC5′ (489 bp). Complementary DNA (cDNA) was synthesized from 1 μg total RNA by RT using 100 U Superscript reverse transcriptase II (Invitrogen, Carlsbad, CA) and random primers (Invitrogen) in a 20 μL reaction containing 1× Superscript buffer (Invitrogen), 1 mM deoxy-NTP mix, 10 mM dithiothreitol, and 40 U ribonuclease inhibitor. Total RNA and random primers were incubated at 65°C for 5 minutes followed by 42°C for 50 minutes. Incubation with a denaturing enzyme at 70°C for 15 minutes terminated the reaction. For PCR amplification, 1 μL cDNA was added to 25 μL of a reaction mix containing 0.2 μM of each primer, 0.2 mM deoxy-NTP mix, 0.5 U AccuPrime Taq DNA Polymerase (Invitrogen), and 1× reaction buffer. PCR was performed in a RoboCycler (Stratagene, La Jolla, CA). The PCR products were visualized on 2% agarose gels by ethidium bromide staining, and gels were photographed under ultraviolet (UV) lights. Band densities were quantified using the Eagle Eye II System (Stratagene). The expression of different mRNAs was normalized to 18s mRNA levels. The amplification conditions were 32 cycles for PTHrP receptor, 35 cycles for PPARγ, 28 cycles for ADRP, 27 cycles for αSMA, 25 cycles for 18s of denaturation for 30 seconds at 94°C, annealing for 45 seconds at 55°C, and extension for 1 minute at 72°C. These

conditions were shown in preliminary studies to result in amplification within a linear range.

Protein Determination and Western Blot Analysis

Protein determination was made using the Bradford dye-binding-method [21]. Western blotting was performed according to the following protocol. Briefly, cells were lysed using an extraction buffer (10 mM tris (hydroxymethyl)aminomethane [Tris; pH 7.5], 0.25 M sucrose, 1 mM ethylenediaminetetraacetic acid, 5 mM benzamidine, 2 mM phenylmethylsulfonyl fluoride, and 10 μg/mL each of pepstatin A, aprotinin, and leupeptin), and centrifuged at 140 × *g* for 10 minutes (4°C). Equal amounts of the protein (25 μg) from the supernatants were added in sodium dodecyl sulfate (SDS) sample buffer and were subjected to SDS-polyacrylamide (4% to 12% gradient) gel electrophoresis followed by electrophoretic transfer to a nitrocellulose membrane. Nonspecific binding of antibody was blocked by 1× Tris-buffered saline (TBS) containing 0.1% Tween 20 with (TBST) 5% nonfat milk for 1 hour. The blot was then incubated in TBST with specific primary antibodies (PPARγ 1:2000, Alexis Biochemicals, San Diego, CA; αSMA 1:100,000, Sigma, St. Louis, MO: ADRP 1:3000, a kind gift from Dr. Constantine Londos, NIDDK; and Thy-1 1:500, BD Biosciences Pharmingen, San Diego, CA) overnight at 4°C. Blots were then washed in TBST, and incubated for 1 hour in secondary antibody, washed, and developed with a chemiluminescent substrate (enhanced chemiluminescence; Amersham, Arlington Heights, IL) following the manufacturer's protocol. The densities of the specific protein bands were quantified using a scanning densitometer (Eagle Eye II still video system; Stratagene, La Jolla, CA). The blots were subsequently stripped and reprobed with anti-GAPDH (1:5000; Chemicon, Temecula, CA) antibody to confirm equal gel loading of samples.

Adipocyte Differentiation-Related Protein Immunostaining

Paraffin-fixed 5-μm human lung tissue sections were deparaffinized in xylene for 10 minutes ×3 followed by hydration through a series of decreasing ethanol concentrations and then placed in TBS buffer. Endogenous peroxide was quenched and nonspecific staining blocked according to instructions of the Dalco LSAB-2 detection kit using diaminobenzidine as the chromogen (catalog number K073; Dalco Cytomation, Carpinteria, CA). Slides were then exposed to primary ADRP antibody (1:750) at room temperature for 1 hour followed by the secondary antibody (1:1000) for 2 hours. Slides were then counterstained with hematoxylin,

dehydrated through increasing ethanol concentrations in xylene, and coverslipped using Permount before visualization.

Immunofluorescence Double Staining

Lipogenic and myogenic status of cultured WI-38 cells was assessed by simultaneous staining for lipid droplets and αSMA. Lipids were stained using Oil Red O staining and αSMA expression was assessed by using anti-αSMA (1:1000, mouse monoclonal IgG_{2a}; Sigma, catalog number A2547) primary antibody. In brief, cells were cultured on Lab-Tek 2-chamber slides and when ready were fixed in freshly prepared 4% paraformaldehyde. Fixed slides were washed in phosphate-buffered saline (PBS), blocked with 3% normal goat serum (Jackson Immunoresearch Lab, West Grove, PA,) in PBS for 30 minutes at room temperature to block nonspecific binding, and then incubated in primary antibody overnight at 4°C. Secondary goat anti-mouse IgG_{2a}–conjugated fluorescein isothiocyanate (FITC) was used at 1:200 dilution for 30 minutes. The slides were then washed 3 times with PBS, with double distilled water 2 times, and were then incubated with Oil Red O (Sigma, St. Louis, MO) for 15 to 30 minutes. Slides were rinsed 3 times for 5 minutes and then mounted and coverslipped with Vestashield mounting medium with DAPI (Vector Laboratories, CA) for visualization under fluorescence microscope.

Statistical Analysis

Analysis of variance with Newman-Keuls post hoc analysis was used to analyze the experimental data. $P < .05$ was considered to indicate significant differences in the expression of the various molecules examined among the treatment groups.

RESULTS

Documentation of Lipid Droplets and Absence of α-SMA Staining in Human Lung Fibroblasts

Initially, we examined the presence of lipid droplets in WI-38 cells and HLFs as indicated by staining with Oil Red O. Both WI-38 cells and HLFs showed abundant staining for lipid droplets (Figure 1). As a further proof that these cell types exhibited lipogenic characteristics, both WI-38 cells and HLFs stained only minimally for α-SMA (Figure 1), which is a signature marker of myofibroblasts, and is characteristically absent in lipofibroblasts [1]. Figure 2 clearly shows abundant cytoplasmic lipid droplet staining in

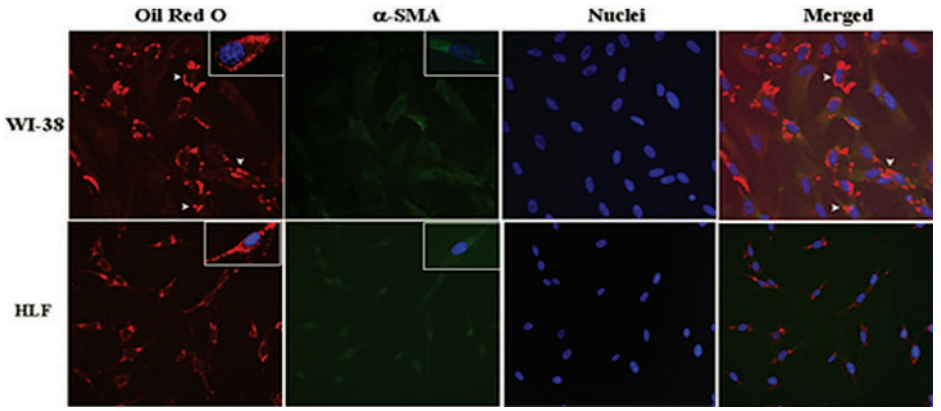

FIGURE 1 Representative immunofluorescence staining for lipid droplets (*red*), αSMA (*green*), and nucleus (*blue*) in early passage (3 to 4) human embryonic lung WI-38 fibroblasts and adult human lung fibroblasts, cultured under basal conditions (MEM + 10% FBS) for 24 hours. Both WI-38 and adult human lung fibroblasts clearly stained positive for lipid droplets using Oil Red O and only minimally for αSMA using a specific-monoclonal antibody. Insets show a magnified WI38 fibroblast and an adult human lung lipofibroblast stained intensely for lipid droplets and minimally for αSMA.

interstitial cells in a lung section from an autopsy specimen from a human adult.

Documentation of Characteristic Functional Lipogenic Molecular Markers for Lipofibroblasts in Human Lung Fibroblasts

As our previous studies have demonstrated the presence of characteristic functional lipogenic molecular markers such as PTHrP receptor,

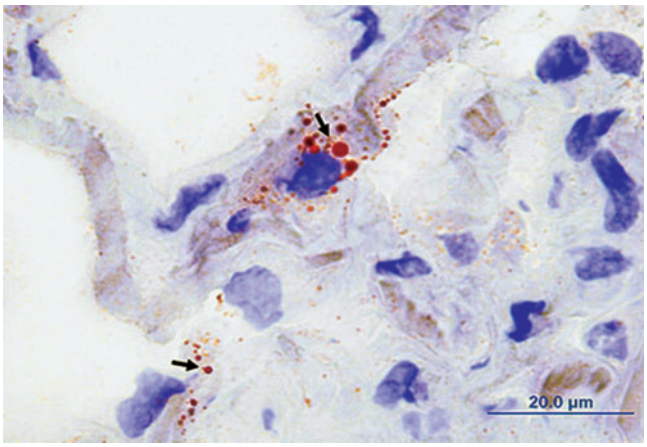

FIGURE 2 Representative lung tissue section from a human adult (autopsy) staining positive for cytoplasmic lipid droplets (*black arrows*) in alveolar interstitial cells using Oil Red O stain.

PPARγ, and ADRP in rat lung fibroblasts, we examined WI-38 cells and HLFs for the presence of these functional markers using both RT-PCR and Western hybridization. Furthermore, as these markers are the functional intermediates that determine the PTHrP-mediated lipogenic response in the rat lung fibroblast, we determined their response to PTHrP stimulation based on the expression of PTHrP receptor, PPARγ, and ADRP mRNA and protein. PTHrP receptor, PPARγ, and ADRP mRNA were all expressed in both WI-38 cells and HLFs. In general, both cell-types showed dose-dependent increases in PTHrP receptor, PPARγ, and ADRP mRNA expression upon treatment with increasing doses of PTHrP (1×10^{-9}, 1×10^{-7}, 5×10^{-7} M) (Figure 3). Similar data for baseline and PTHrP-stimulated protein content were observed for PTHrP receptor, PPARγ, and ADRP protein (Figure 4). The presence of lipid-containing interstitial fibroblasts in the human lung was further confirmed by the presence of lipid (data not shown) and ADRP (Figure 5) positive stained alveolar interstitial cells in lung tissue sections from both neonatal (Figure 5*A*) and adult (Figure 5*B*) autopsy specimens.

Documentation of the Functional Features of Human Lung Fibroblasts Consistent with Lipofibroblasts

As one of the hallmarks of pulmonary lipofibroblasts is their capacity to take up lipid, triolein uptake upon PTHrP stimulation was examined. Both WI-38 cells and HLFs showed dose-dependent increases in triglyceride uptake upon PTHrP stimulation (Figure 6).

Finally, because the expression of the glycophosphatidylinositol-linked protein Thy-1 has been described to discriminate between a lipogenic and a myogenic fibroblast, we next examined Thy-1 expression by WI-38 cells and HLFs and how it changes in response to PTHrP stimulation. Both WI-38 cells and HLFs expressed Thy-1, which was unaffected by PTHrP stimulation for 24 hours (Figure 7).

DISCUSSION

There is extensive experimental evidence to indicate that lipid-containing alveolar interstitial fibroblasts play a critical role in normal lung development, homeostasis, and injury/repair [1, 2, 6–12, 22]. However, most of this evidence is based upon data from rodents [2, 13–18, 23, 24], and in fact there is a lack of clear evidence to support the existence of such lipid-containing alveolar interstitial fibroblasts in the human lung demonstrating the morphological, molecular, and functional characteristics described for the rodent lipofibroblast. Using cultured lung fibroblasts of human origin and human lung tissue from autopsy specimens, our data,

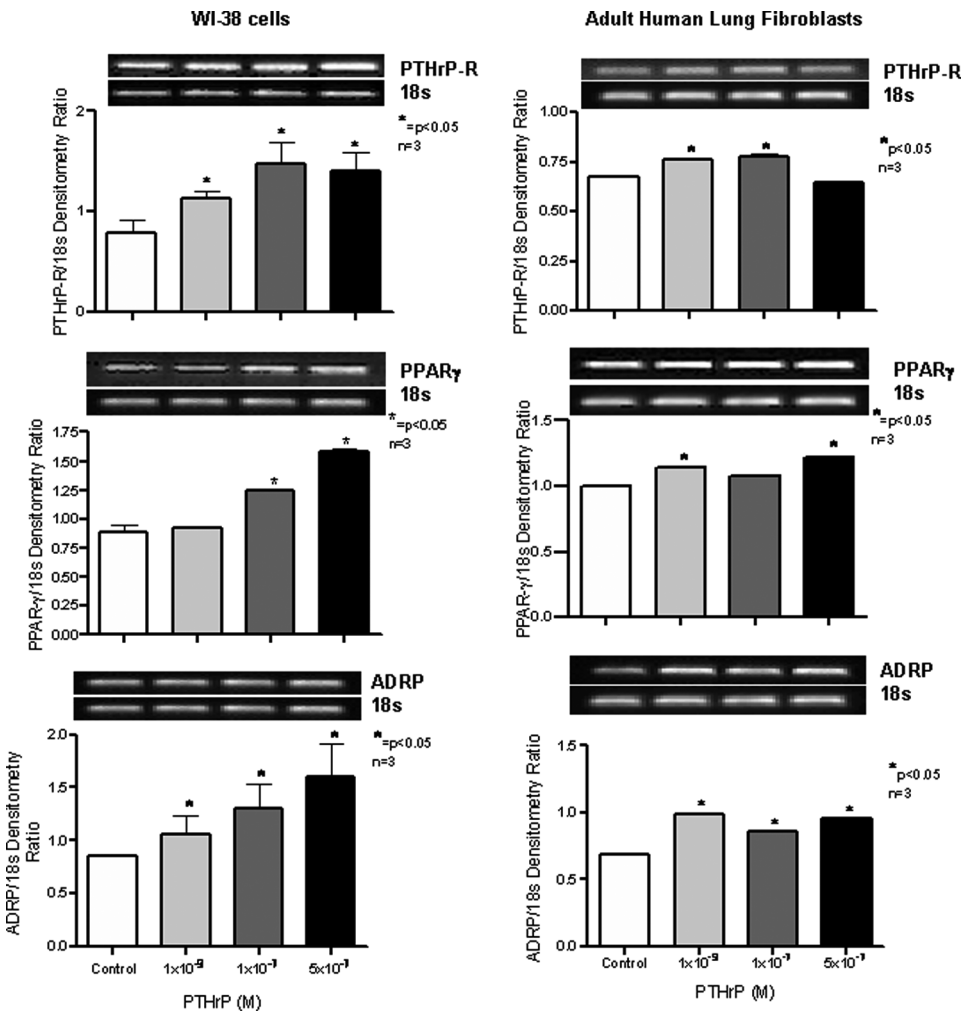

FIGURE 3 Effect of PTHrP (1–34) agonist stimulation at graded concentrations (1×10^{-9}, 1×10^{-7}, and 5×10^{-7} M) for 24 hours on mRNA expression of the fibroblast lipogenic markers PTHrP-R, PPAR-γ, and ADRP in human embryonic lung WI-38 fibroblasts and adult human lung fibroblasts. Representative RT-PCR gels and density histograms of specific mRNA expression normalized to 18s expression from 3 independent experiments are shown. In both WI-38 and adult human lung fibroblasts, there were significant increases in the mRNA expression of all 3 lipogenic markers examined (values are mean ± SEM; $^*P < .05$).

for the first time, clearly documents the presence of lipofibroblasts in the human lung. Cultured lung fibroblasts of human origin, i.e., WI-38 cells and adult HLFs, demonstrated the morphological, molecular, and functional characteristics that are the hallmarks of the rat lung lipofibroblast. These fibroblasts stain positively for neutral lipids with Oil Red O, and demonstrate the expression of ADRP, which mediates the uptake of

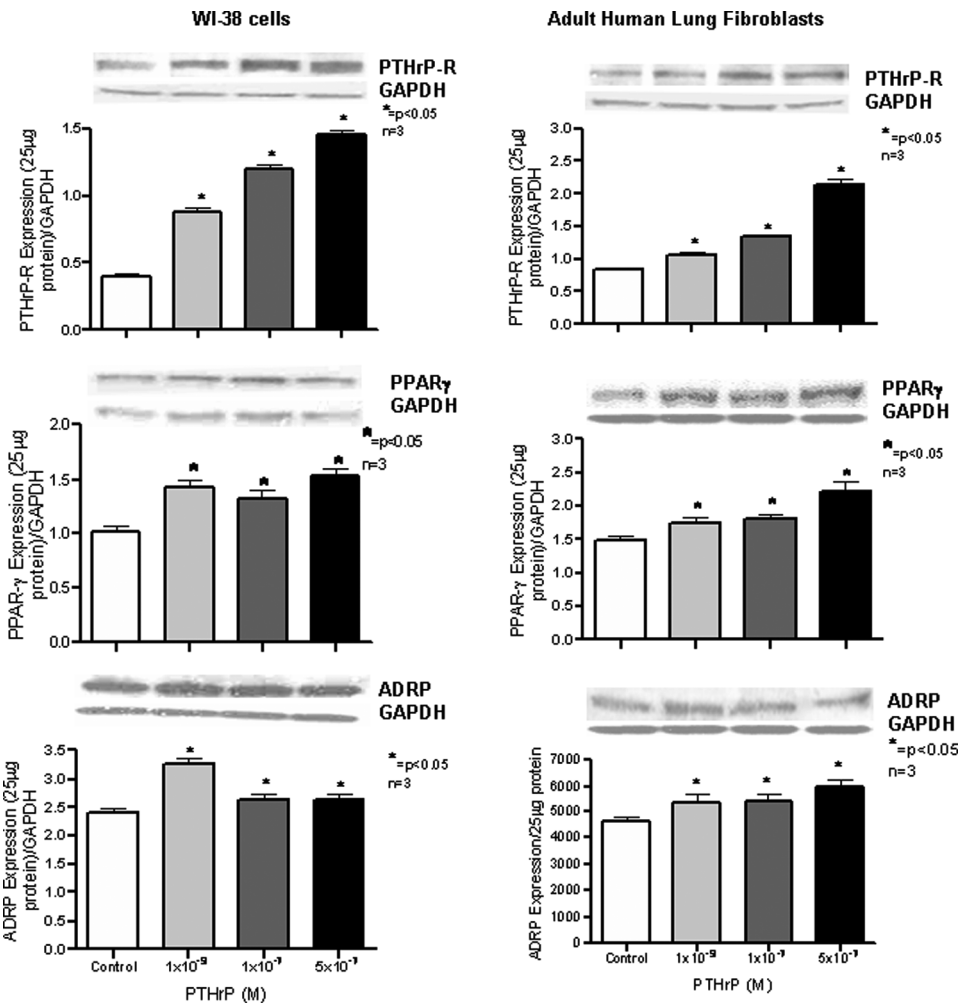

FIGURE 4 Effect of PTHrP (1–34) agonist stimulation at graded concentrations (0, 1×10^{-9}, 1×10^{-7}, and 5×10^{-7} M) for 24 hours on the protein expression of the fibroblast lipogenic markers PTHrP-R, PPAR-γ, and ADRP in human embryonic lung WI-38 fibroblasts and adult human lung fibroblasts. Representative Western blots and density histograms of specific protein expression normalized to GAPDH expression from 3 independent experiments are shown. Similar to the mRNA data, in both human embryonic lung WI-38 fibroblasts and adult human lung fibroblasts, there were significant increases in the protein expression of all 3 lipogenic markers examined (values are mean $\pm$ SEM; $^{*}P < .05$).

neutral lipids by the alveolar interstitial cell, and its trafficking to the alveolar type II cells [25]. These fibroblasts not only express lipogenic genes such as PTHrP receptor, PPARγ, and ADRP, but also respond to PTHrP stimulation by increasing their lipogenic characteristics, a feature highly characteristic of the rat lung lipofibroblast [1, 2, 13–15, 26]. Furthermore, these lipofibroblasts show an absence of staining for αSMA,

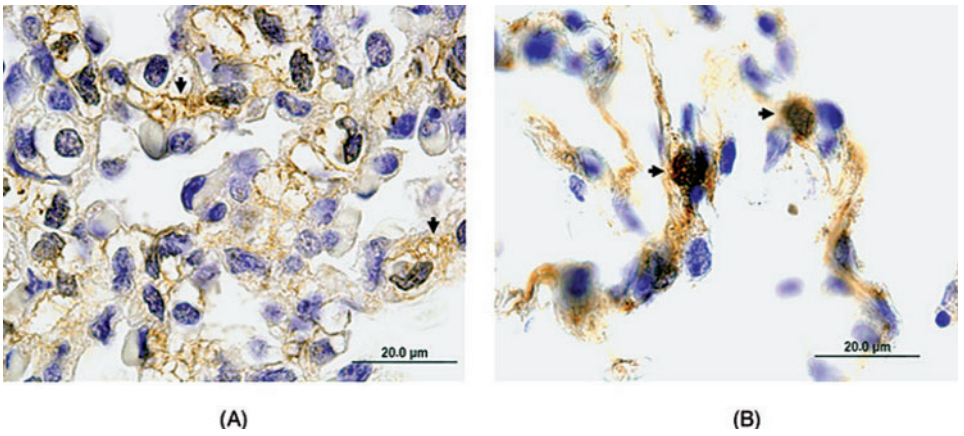

FIGURE 5 Lung tissue sections (autopsy) from a human infant (36 weeks' gestation age) (*A*) and a human adult (*B*), who died from nonrespiratory causes, clearly show alveolar interstitial cells staining positively for adipocyte differentiation-related protein (*black arrow heads*).

a myogenic marker, validating that these cells are of a lipogenic and not a myogenic phenotype. Positive staining for neutral lipids and ADRP by the alveolar interstitial fibroblasts from the lung tissue sections from both neonatal and adult lungs further confirms the lipogenic nature of these fibroblasts.

When lipid-containing pulmonary intersitial cells were first observed in the rodent lung, Vaccaro and Brody termed them lipid interstitial cells in 1978 [18]. Lipofibroblasts are evident in rat lungs at gestational day 16, and the triglyceride content of the whole lung increases 3-fold between

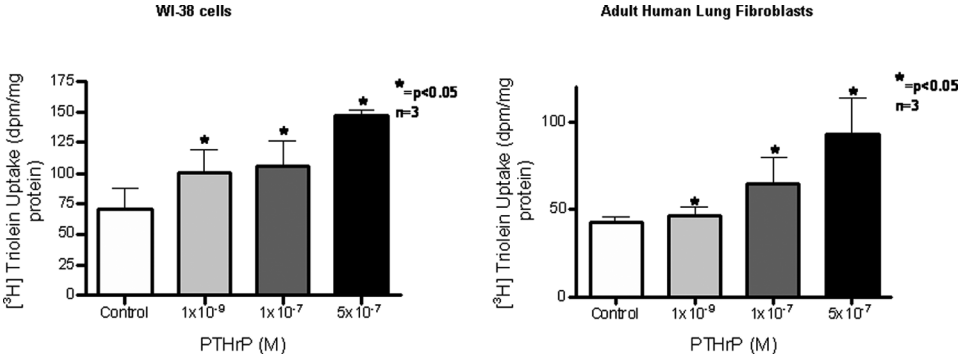

FIGURE 6 As a functional assessment, the effect of PTHrP (1–34) agonist stimulation at graded concentrations (1×10^{-9}, 1×10^{-7}, and 5×10^{-7} M) for 24 hours on [^{3}H]triolein uptake by human embryonic lung WI-38 fibroblasts and adult human lung fibroblasts is shown. In both WI-38 and adult human lung fibroblasts, PTHrP stimulation resulted in a dose-dependent increase in [^{3}H]triolein uptake (values are mean ± SEM, $^*P < .05$).

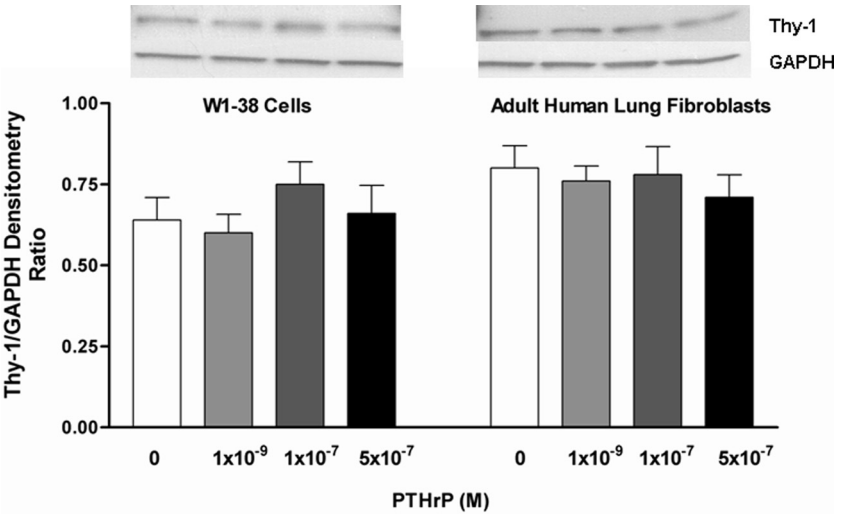

FIGURE 7 Thy-1 expression in the basal state by human embryonic lung WI-38 cells and adult human lung fibroblasts, and upon stimulation with graded concentrations (1×10^{-9}, 1×10^{-7}, and 5×10^{-7} M) of PTHrP (1–34) for 24 hours. Representative Western blots and density histograms normalized to GAPDH expression from 3 independent experiments are shown. In both WI-38 human embryonic lung fibroblasts and adult human lung fibroblasts, Thy-1 is well expressed in the basal state, but on PTHrP stimulation, this expression did not change significantly (values are mean ± SEM, $^{*}P < .05$).

gestational days 17 and 19, and another 2.5-fold between gestational day 21 and postnatal day 1, peaking during the 2nd postnatal week [27]. The abundance of lipofibroblasts follows the same time course. The lipid droplets of lipofibroblasts primarily contain neutral lipids (65% triglycerides, 14% cholesterol esters, 7% free fatty acids and cholesterol) [14]. Recently, we have described the molecular markers characterizing these lipofibroblasts [1]. These molecular markers include the expression of PTHrP receptor, PPARγ, and ADRP. Through these molecular intermediates, lipofibroblasts interact with adjacent alveolar type II cells to stimulate surfactant synthesis by these cells [1, 28]. Under the influence of cyclic stretch, e.g., during normal breathing, PTHrP is secreted by the alveolar type II cell, which binds to its cognate receptor on the lipofibroblast, activating the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA)-mediated lipogenic pathway [28], up-regulating PPARγ and its downstream target, ADRP, which facilitates triglyceride uptake by the lipofibroblast [25]. The triglycerides taken up by the lipofibroblast are then trafficked to the alveolar type II cell as substrate for surfactant phospholipid synthesis [20]. In fact, for physiologic surfactant phospholipid synthesis, alveolar type II cells are essentially dependent upon lipofibroblasts to recruit neutral lipids from the circulation to be trafficked to the alveolar type II cells and incorporated into surfactant phospholipids.

In addition to its role in surfactant phospholipid synthesis, we have recently also demonstrated that the triglyceride content of the rat lung lipofibroblast plays a critical role in providing cytoprotection against oxidant injury [29]. Furthermore, rat lung lipofibroblasts have been shown to store retinoids during perinatal lung development, which are known to regulate many retinoid-responsive genes crucial for alveolar type II cell differentiation and proliferation, and hence alveolarization [30]. Given these critical functions of the lipofibroblast in normal lung biology, it is not surprising that we have observed that disturbance of normal lipofibroblast functioning as a consequence of disruption in normal epithelial-mesenchymal signaling, e.g., due to prematurity, volutrauma, hyperoxia, and/or inflammatory cytokines, leads to significant alveolar molecular and histopathological changes that are characteristic of bronchopulmonary dysplasia in the newborn [1, 31–33]. In fact, we have proposed that transdifferentiation of lipofibroblasts-to-myofibroblasts is a central event in the pathogenesis of bronchopulmonary dysplasia in the newborn and chronic lung disease in the adult [1, 33]. Transdifferentiated alveolar interstitial fibroblasts (i.e., myofibroblasts) are unable to maintain pulmonary alveolar homeostasis [1, 31–33], which results in pulmonary fibrosis. More importantly, we have observed that the alveolar fibroblast transdifferentiation process is not a terminal event, and under appropriate conditions is reversible [1]. Expression of the nuclear transcription factor PPARγ seems to be the key to whether the fibroblast will be of lipogenic or myogenic phenotype, and augmentation of PPARγ not only prevents alveolar interstitial fibroblast to myofibroblast transdifferentiation, but may also reverse it, providing a novel approach to treat and reverse chronic lung disease [1, 34].

Despite the extensive literature on the presence and functional significance of lipofibroblasts in the rodent lung, as alluded to above, there is rather scant information on their significance and even their existence in the human lung. In fact, to our knowledge, this report provides the first documentation of their existence and molecular characterization in the human lung, although their exact functional significance in the human lung remains to be determined. The expression of Thy-1 protein by both WI-38 and adult human lung fibroblasts is interesting and consistent with the data from Hagood and colleagues, who have shown that normal lung fibroblasts, in both mice and humans, are Thy-1 positive. Thy-1 expression is a critical biologic response modifier that renders Thy-1 positive fibroblasts resistant to fibrogenic stimuli [17]. However, in contrast, Koumas and colleagues have shown that only Thy-1–positive human myometrial and orbital fibroblasts are capable of myofibroblast differentiation following treatment with fibrogenic stimuli such as transforming growth factor beta (TGFβ). Further, on stimulation with a PPARγ agonist, such as prostaglandin J_2, only Thy-1–negative, but not Thy-1–positive, fibroblasts

differentiated to lipofibroblasts [16]. Clearly, the role of Thy-1 expression in the human lung, particularly during lung development and injury, remains to be further elucidated.

In conclusion, based on immunohistochemistry, RT-PCR, Western hybridization, and triglyceride uptake, we provide unequivocal evidence for the existence of lipofibroblasts in both the embryonic and the adult human lung, under both in vitro and in situ conditions. We predict that similar to its role in the rodent lung, lipofibroblasts in the human lung play a critical role during normal lung development, homeostasis, and injury/repair.

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