Transdifferentiation of Ciliated Cells during Repair of the Respiratory Epithelium

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Since the lung is repeatedly subjected to injury by pathogens and toxicants, maintenance of pulmonary homeostasis requires rapid repair of its epithelial surfaces. Ciliated bronchiolar epithelial cells, previously considered as terminally differentiated, underwent squamous cell metaplasia within hours after bronchiolar injury with naphthalene. Expression of transcription factors active in morphogenesis and differentiation of the embryonic lung, including β -catenin, Foxa2, Foxj1, and Sox family members (Sox17 and Sox2), was dynamically regulated during repair and redifferentiation of the bronchiolar epithelium after naphthalene injury. Squamous cells derived from ciliated cells spread beneath injured Clara cells within 6–12 h after injury, maintaining the integrity of the epithelium. Dynamic changes in cell shape and gene expression, indicating cell plasticity, accompanied the transition from squamous to cuboidal to columnar cell types as differentiation-specific cell markers typical of the mature airway were restored. Similar dynamic changes in the expression of these transcription factors occurred in ciliated and Clara cells during regeneration of the lung after unilateral pneumonectomy. Taken together, these findings demonstrate that ciliated epithelial cells spread and transdifferentiate into distinct epithelial cell types to repair the airway epithelium.

Keywords: naphthalene; lung injury; transcription; pneumonectomy; bronchiole

The respiratory tract has an extensive cell surface that is directly exposed to inhaled gases, particles, and pathogens. A complex epithelium lines the airways, mediating gas exchange, mucociliary clearance, host defense, and surfactant homeostasis to maintain lung sterility and stability. While the adult lung is not mitotically active, respiratory epithelial cells can proliferate rapidly after injury to maintain lung structure and function.

Models in which relatively rare subsets of nonciliated respiratory epithelial cells located in unique environments play critical roles in lung repair have been proposed (1–5). Krause and co-workers have provided evidence that extrapulmonary, bone marrow–derived cells migrate to the lung, contributing to the repair of the respiratory epithelium after injury (6). From a stochastic view, however, models in which rare progenitor cells account for the rapid and extensive repair of the lung are not compatible with the observed short period of proliferation and rapid restoration of epithelial surfaces that is observed after catastrophic injury caused by infection or toxicants. Rather, the remarkable repair capacity of the lung is more consistent with a model in

which relatively abundant or multiple cells participate in repair of the respiratory epithelium. *In vitro* studies support the concept that both basal and nonciliated (Clara) respiratory epithelial cells in the conducting airways, and type II cells in the alveoli, maintain proliferative capacity (7–9). Indeed, widespread proliferation of type II epithelial cells accompanies growth of the remaining lung after unilateral pneumonectomy (10). Injury induced by hyperoxia or SO₂ causes proliferation of type II and nonciliated airway epithelial cells (11, 12).

In general, type I and ciliated cells have been considered terminally differentiated cells that do not contribute substantially to proliferation in the normal lung (11–14). However, dynamic changes in the morphology and proliferation of ciliated cells were demonstrated in naphthalene injury (15, 16), supporting their potential for repair of the bronchiolar epithelium. The role played by various cell types in repair of the lung, as well as the nature of the genetic programs regulating epithelial cell differentiation during the repair process, remain poorly defined.

To repair the respiratory epithelium while maintaining lung function requires a rapid cellular response to maintain or restore permeability barriers and to initiate proliferative responses, and redifferentiation of the diverse epithelial cell types characteristic of the normal lung. Many of the concepts regarding lung cell differentiation and proliferation are derived from developmental studies. Signaling via various growth factors and cytokines have been implicated in both lung morphogenesis and repair (see Refs. 17 and 18 for review). Transcription factors, such as TTF-1, Fox family members (including Foxa1, Foxa2, and Foxj1), GATA-6, and β -catenin/TCF (or LEF), influence genetic programs critical for lung morphogenesis, differentiation, and pulmonary homeostasis (see Refs. 18 and 19 for review). These transcription factors control the expression of genes that are critical for differentiation of the distinct subsets of cells characteristic of the mature lung, and regulate surfactant homeostasis, fluid transport, mucociliary clearance, and host defense, processes critical for pulmonary homeostasis. It is possible that the molecular mechanisms regulating proliferation and differentiation during development also function during regeneration of the lung after injury or resection.

The present study was undertaken to identify the cellular and transcriptional programs mediating repair or regeneration of the lung. Expression of a number of transcription factors, critical for formation and differentiation of the fetal lung, was dynamically regulated in ciliated cells as they spread and redifferentiated into both Clara cells and ciliated cells characteristic of the adult airway.

MATERIALS AND METHODS

Transgenic Mice and Naphthalene Treatment

Female FVB/N mice (12 wk old) were obtained from Charles River (Wilmington, MA) and housed under pathogen-free conditions. Naphthalene (Sigma Chemical Co., St Louis, MO) was dissolved in corn oil at a concentration of 30 mg/ml and administered to mice (275 mg/kg) via intraperitoneal injection (2). Control mice received corn oil. The

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triple transgenic mice *hSP-C-rtTA/(tetO)₂Cre/ZEG(lacZ/EGFP)* were generated by crossing three different transgenic lines of 3.7 *hSP-C-rtTA*, *(tetO)₂Cre*, and *ZEG* as previously described (20). When these mice are treated with doxycycline throughout gestation, *rtTA* is expressed under control of 3.7 kb *human SP-C* promoter activating Cre recombinase expression. Subsequently, Cre-mediated recombination of the floxed enhanced green fluorescent protein (EGFP) gene induces expression of EGFP in lung epithelial progenitor cells whose descendants, including ciliated and nonciliated cells lining intrapulmonary bronchioles and type II and type I cells lining the alveoli, are permanently labeled. Dams bearing triple transgenic pups were treated with doxycycline from Embryonic Day 0.5 to birth. The triple transgenic mice were maintained on doxycycline until administration of naphthalene and killing for analysis. Mice used in this study were housed and maintained in pathogen-free conditions according to protocols approved by the Institutional Animal Care and Use Committee at Cincinnati Children's Hospital Research Foundation. Mice were anesthetized with a mixture of ketamine, acepromazine, and xylazine, and exsanguinated by severing the inferior vena cava and descending aorta. Pneumectomy was performed in adult mice essentially as previously described (10). Sham-operated controls and pneumectomy mice were killed ($n = 4$ per group) for analysis.

Immunohistochemistry

Lungs of embryonic and adult mice were fixed in 4% paraformaldehyde/phosphate-buffered saline for 15–24 h at 4°C and processed according to standard methods for paraffin-embedded blocks. Immunohistochemistry was performed on 5- μ m-thick sections using antibodies against FoxJ1, TTF-1, CCSP, β -tubulin IV, and β -catenin as previously described (21–23). Guinea pig anti-Sox17 antibody was raised against a synthetic peptide composed of a.a. 249–400 of mouse Sox17. Rabbit polyclonal antibodies against Sox2 and phosphohistone-3 (pH-3) were used at dilution of 1:200 and 1:500, respectively (Santa Cruz Biotech, Santa Cruz, CA, and US Biological, Swampscott, MA). For dual immunolabeling, antibodies from two different species were used as follows: anti-Sox17, 1:100; anti-Sox2, 1:20; anti-CCSP, 1:500; anti- β -tubulin IV, 1:50; anti-Foxa2, 1:100; and anti-pH-3, 1:50. BrdU labeling was performed using the labeling kit according to the manufacturer's protocol (Zymed Laboratories Inc., South San Francisco, CA). Goat or donkey secondary antibodies were conjugated with Alexa Fluor 568 (red) or Alexa Fluor 488 (green) fluorochrome (Molecular Probes, Eugene, OR). Samples were mounted with anti-fade reagent containing DAPI (Vecta Shields, Burlingame, CA).

Transmission Electron Microscopy

Adult mouse lungs were inflation-fixed via a tracheal cannula at 25 cm of water pressure with modified Karnovsky's fixative (2% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer [SCB] containing 0.1% calcium chloride [pH 7.3]). Tissue was post-fixed with 1% osmium tetroxide (reduced with 1.5% potassium ferrocyanide), stained *en bloc* with aqueous 4% uranyl acetate, and processed for electron microscopy.

RESULTS

Ciliated Cells Undergo Squamous Metaplasia and Redifferentiate during Repair of the Bronchiolar Epithelium

To identify cells participating in repair of the bronchiolar epithelium, adult mice were treated with naphthalene by intraperitoneal injection. Naphthalene is concentrated in nonciliated bronchiolar epithelial cells (Clara cells) that are enriched in P450 enzymes (CYP 2F2). Metabolism of naphthalene generates toxic metabolites resulting in selective injury of nonciliated cells (24). Twenty-four hours after injection of naphthalene, the bronchiolar surface appeared to be denuded at the light microscopic level (Figure 1A). However, the conducting airways were actually lined by a homogenous population of remarkably thin squamous cells. Because of their attenuation, the squamous cells were not readily identified at the light microscopic level. The presence and endodermal origin of these cells was clarified using *hSP-C-*

Figure 1. Lung epithelial origin and ultrastructure of squamous progenitor cell. (A) Hematoxylin-eosin staining of mouse bronchioles 24 h after administration of naphthalene demonstrates exfoliation of the epithelium. (B) GFP was observed in the squamous progenitor cells lining the conducting airway in *hSP-C-rtTA/(tetO)₂CMVCre/ZEG* mouse 24 h after naphthalene treatment. The GFP-positive cells in peripheral lung parenchyma (white arrow) are alveolar type II cells. (C) Electron microscopy on mouse bronchioles 24 h after naphthalene treatment demonstrated squamous cells with reduced numbers of short cilia (black arrowhead). A necrotic, nonciliated cell (asterisk) is observed in airway lumen. Arrow indicates tight junctions. (D) Basal bodies (white arrowhead) and internalized cilia are observed within squamous cells lining the bronchioles. AL: airway lumen. Scale bars: A and B, 50 μ m; C and D, 2 μ m.

rtTA/(tetO)₂Cre/ZEG mice. When these mice are treated with doxycycline throughout gestation, descendants of lung epithelial progenitors, including ciliated and nonciliated cells in intrapulmonary bronchioles and type II and type I cells in alveolar region, are permanently labeled by GFP (20). After naphthalene injury to the adult lung, virtually all squamous cells lining the “denuded” bronchioles were fluorescent, indicating their origin from prelabeled endodermally derived bronchiolar epithelial cells (Figure 1B). Electron microscopy confirmed the presence of the squamous cells lining the airways. These cells maintained some characteristics of ciliated cells, including disorganized cilia, basal bodies, and intracellular ciliary structures (Figures 1C and 1D). These findings demonstrate that ciliated cell progenitors undergo squamous shape changes and that the airway surface is not denuded. Squamous metaplasia of the ciliated cells occurred within 6–12 h and preceded sloughing of the nonciliated cells. The basal regions of ciliated cells extended beneath the injured Clara cells, the latter identified by CCSP staining (Figure 2), indicating that the bronchiolar surface is covered by a thin squamous epithelium as the Clara cells are being sloughed. Clara cells, identified by immunostaining with anti-CCSP antibody, were selectively shed into the airway lumen and the remaining squamous cells expressed both β -tubulin IV and Foxj1, indicating their origin from ciliated cells (Figures 2 and 3). These squamous cells exhibited reduced numbers of organized cilia on the cell surface as well as cytoplasmic fragments of internalized cilia; these squamous cells did not stain for CCSP 6–24 h after injury (Figures 2 and 3B). Phospho-histone-3-stained epithelial cells were not observed at 24 h, but were detected 2–4 d after injury, indicating that epithelial integrity was initially maintained by extension and migration of the squamous cells in a process that preceded proliferation (Figure 4). BrdU labeling confirmed the

Figure 2. Spreading of ciliated cells during squamous metaplasia following naphthalene injury. Double immunolabeling for CCSP (red) and β -tubulin IV (green) was performed on lung sections of uninjured control (0 h) and naphthalene-treated mice 6, 12, and 24 h after injury. Sections were counterstained with DAPI (blue). Columnar Clara cells and ciliated cells lined normal bronchiolar airway before injury (0 h). The basal region of neighboring ciliated cells (arrow) extended beneath the injured Clara cells (asterisk) that were now cuboidal or round (6 h). Ciliated cells became squamous and spread to cover the regions where Clara cells had detached (12 h). Twenty-four hours later, only β -tubulin-positive squamous cells (green) lines the injured airway, while residual Clara cells (red) were sloughed into the airway lumen. The dashed white line marks the basal lamina. Arrowheads indicate nuclei of serosal cells underneath basal lamina.

lack of proliferation of the squamous cells and enhancement of proliferation 2–4 d after injury (data not shown). Thus, the bronchioles were not denuded by naphthalene, but covered by extension of existing ciliated cells. Within 48 h after injury, the squamous cells lining the injured bronchioles had transformed to a relatively homogenous population of cuboidal cells. β -Tubulin IV and Foxj1 (ciliated cell markers), but not CCSP, were detected in these transitional cuboidal cells (Figures 3B and 3G). Again, in the squamous cells, β -tubulin IV staining was localized in the intracellular compartments and organized cilia were not seen in the squamous and transitional cuboidal cells (Figures 1C and 1D). Clara cells were not observed until 4–7 d after injury, at which time CCSP was detected, albeit at low levels, in subsets of airway cells (Figure 3D). Fourteen days after injury, morphology of the bronchiolar epithelium and the distinct pattern of staining of CCSP (in Clara cells), as well as Foxj1 and β -tubulin (in ciliated cells) was substantially restored (Figures 3E and 3J). These findings demonstrate that after naphthalene injury, ciliated cells undergo squamous metaplasia, extend to cover the epithelial surface, and redifferentiate into both ciliated and nonciliated cell types.

Transcriptional Reprogramming after Epithelial Cell Injury

Since molecular mechanisms regulating fetal lung morphogenesis and differentiation might be involved in repair of adult lung, expression of transcription factors known to be critical for fetal lung morphogenesis was assessed during recovery from naphthalene injury. In the normal lung, Foxa1 and Foxa2 expression was enhanced selectively in ciliated epithelial cells lining the bronchioles (Figure 5), while TTF-1 staining was more widespread (data not shown). After injury, squamous and transitional cuboidal cells all stained intensely for Foxa1 and Foxa2 (24–48 h after the injury), and their expression became increasingly restricted to subsets of ciliated cells during redifferentiation as seen on Day 4 (Figure 5) and Day 14 (data not shown). This expression pattern of Foxa1 and Foxa2 coincided with that of Foxj1, a ciliated cell-specific transcription factor (Figure 3).

Figure 3. Ciliated cells undergo squamous metaplasia and redifferentiate during repair of airway epithelium. Double immunolabeling for CCSP (red) and β -tubulin (green) (A–E), and Foxj1 staining (F–J) was performed on lung sections of uninjured control (A, F) and naphthalene-treated mice 1–14 d after injection. Sections were counterstained with DAPI (blue, A–E). One day after injury, squamous cells lining injured airways stained for β -tubulin IV and Foxj1, but not CCSP (B), while exfoliated cells in the lumen stained for CCSP. Two days after injury, Foxj1 was detected in nuclei of cuboidal cells that now lined the bronchioles wherein β -tubulin staining was primarily intracellular and disorganized. CCSP staining was not detected (C, H). Four days after injury, a subset of epithelial cells was weakly stained for CCSP (D). Fourteen days after injury, the normal staining pattern of CCSP and β -tubulin was restored, although the intensity of CCSP staining remained less than controls (E). Foxj1 was restricted to a subset of cells 4 and 14 d after injury (I, J). Figures are representative of $n \geq 5$ individual animals. Scale bars: 20 μ m.

Because β -catenin is required for lung branching morphogenesis and differentiation of respiratory epithelium, we examined the expression pattern of β -catenin during repair of the bronchiolar epithelium. In the normal adult lung, β -catenin staining is normally membrane-associated and rarely observed in nuclei of airway epithelial cells (Figure 5). Twenty-four to 48 h after injury, nuclear and cytoplasmic staining for β -catenin was markedly increased in the squamous and cuboidal cells lining the bronchioles (Figure 5). Four days after injury and afterward, β -catenin staining decreased and was restored to the pattern seen in the normal adult lung (Figure 5). These findings suggest that a dynamic transcriptional program, similar to that observed during normal lung morphogenesis, accompanies squamous metaplasia and redifferentiation of the ciliated cells after naphthalene injury.

Expression of Sox17 and Sox2 in the Squamous Cells Lining the Airway Epithelium after Injury

The observation that ciliated cells underwent squamous metaplasia and redifferentiated after injury led us to hypothesize that

Figure 4. pH-3 staining after naphthalene injury. To determine cell proliferation, pH-3 staining was performed on lung sections of uninjured control and naphthalene-treated mice 1–4 d after injection. Before injury, pH-3 staining was not detected (*upper left panel*). One day after naphthalene injection, squamous cells lining the injured bronchiolar airways did not stain for pH-3, while exfoliated cells in airway lumen stained nonspecifically (*upper right panel and insets*). Two days after injection, a number of cuboidal cells in small and large bronchioles stained for pH-3 (*lower left panel and insets*). Four days after injury, the number of pH-3–positive cells was decreased (*lower right panel*). Figures are representative of $n \geq 5$ individual animals at each time point. Scale bar: 50 μm .

transcription factors specific to ciliated bronchiolar cells may play an important role in the repair process. Since Foxa1 and Foxa2 are regulated by interaction of Sox17 and β -catenin in early *Xenopus* endoderm, and Sox proteins are involved in regulating homeostasis of progenitor cells in a number of tissues, the cellular localization of Sox17 and Sox2 was determined in the adult mouse lung. Nuclear staining of Sox2 and Sox17 was selec-

tively, but not exclusively, observed in ciliated respiratory epithelial cells, the most intense staining being colocalized with β -tubulin IV (Figure 6, *insets*). Intense Sox17 and Sox2 staining was observed in all of the squamous and cuboidal cells lining the bronchioles 24–48 h after injury (Figure 6). Four days after injury and thereafter, Sox17 and Sox2 staining was again increasingly restricted to ciliated cells, a pattern similar to that of β -tubulin IV, Foxa1, Foxa2, and Foxj1 (Figure 6). These findings suggest that Sox proteins influence expression of genes in ciliated cells, or the progenitor cells derived from them, during repair of the bronchiolar epithelium.

Induction of β -catenin, Foxa2, Sox2, Sox17, and Foxj1 during Lung Regeneration after Unilateral Pneumonectomy

Compensatory lung growth occurs in conducting airways as well as lung parenchyma following unilateral pneumonectomy (25, 26). We determined whether the regrowth of bronchiolar epithelium was associated with similar changes in transcription proteins that were observed in ciliated cells during repair after naphthalene injury. Marked hyperplasia of both peripheral (alveolar) and bronchiolar epithelium was observed after pneumonectomy, was most evident 7 d after surgery, and was decreased by 14 d, consistent with previous observations in this model (27, 28) (Figure 7 and data not shown). The extent and intensity of Sox17, Sox2, and Foxj1 staining were increased after pneumonectomy (Figures 7B, 7D, and 7I). Likewise, β -catenin and Foxa2 staining was enhanced (Figure 7). In this model, bronchiolar hyperplasia was associated with increased numbers of both ciliated and Clara cells (Figure 7J). pH-3 staining was readily detected on Day 7, but not on Day 3 after surgery, and was observed in multiple cell types, including ciliated and Clara cells in the bronchioles (Figure 7I, *inset*, and data not shown) and type II cells in the alveoli (data not shown). Thus, a transcriptional program similar to that observed during repair of the bronchiolar epithelium was induced during regeneration after unilateral pneumonectomy.

DISCUSSION

The respiratory epithelium is lined by diverse cell types that vary along the cephalo-caudal axis during development and after

Figure 5. Dynamic changes in expression of Foxa1, Foxa2, and β -catenin during repair. Before injury, Foxa2 and Foxa1 were detected primarily in a subset of bronchiolar epithelial cells (*black arrow*), while β -catenin was widely expressed and membrane-associated (d0). Dual labeling for Foxa2 (*red*) and β -tubulin (*green*) demonstrated that Foxa2 was most intense in β -tubulin–positive ciliated cells (d0, *inset*, *white arrow*). Twenty four to 48 h after injury, all squamous and cuboidal cells were positive for Foxa2 and Foxa1 (d1, d2) while β -catenin staining was markedly increased. Four days after injury, Foxa2 and Foxa1 staining was most intense in ciliated cells and β -catenin staining was restored to normal pattern. Clara cells are indicated by *white arrow-head* in the *inset* (d0). Scale bars: 20 μm .

Figure 6. Sox2 and Sox17 staining during repair of the airway epithelium. Before injury (d0), Sox17 (red) and Sox2 (red) were detected in β -tubulin IV (green)-positive ciliated cells (insets). Sox17 and Sox2 staining was observed in squamous cells 1 d after injury (B, F) and in cuboidal cells 2 d after injury (C, G). On Day 4, Sox17 and Sox2 staining became increasingly restricted to subsets of cells (D, H). The normal pattern of staining was substantially restored from Day 4–14 (data not shown). Figures are representative of $n \geq 5$ individual animals at each time point. Scale bars: 20 μ m.

acute and chronic injuries. The mechanisms controlling formation and repair of this cellular and functional diversity are relatively unknown at present. This study demonstrates that ciliated epithelial cells are capable of remarkable phenotypic plasticity, rapidly undergoing squamous metaplasia and redifferentiating into cuboidal and then columnar cell types to contribute to the restoration of the complex airway epithelium after an acute Clara cell injury to the bronchioles. These findings demonstrate that the early repair process is independent of cell proliferation. The findings are not consistent with a significant role for extrapulmonary cells (e.g., bone marrow-derived cells or other mesenchymal derived cells) in repair of the respiratory epithelium after injury. These findings also challenge the view that ciliated cells are “terminally” differentiated cell type. The potential role of ciliated cells in repair of bronchiolar epithelium was further supported by the finding that, in the pneumonectomy model, ciliated cells were among epithelial cells participating in compensatory growth of the respiratory epithelium. The concept that this relatively abundant subset of cells (ciliated cells) can rapidly spread and redifferentiate to participate in repair of the complex airway epithelium provides a basis for the rapid repair of the lung after infection, exposure to toxicants, or lung resection.

After naphthalene injury, ciliated cells sequentially underwent squamous to cuboidal to columnar morphologic transition as the complex bronchiolar epithelium was restored. Rapid spreading of ciliated cells that occurred after injury likely plays a critical role in maintaining an intact epithelial barrier. Subsequently, the squamous cells differentiated into cuboidal and columnar cells (both ciliated and nonciliated cells), demonstrating

Figure 7. Expression of Sox2, Sox17, β -catenin, Foxa2, Foxj1, and CCSP/ β -tubulin during compensatory growth after unilateral pneumonectomy. Immunohistochemistry was performed on the right lung 3 d (A, C, E, G) and 7 d (B, D, F, H, I, J) after left pneumonectomy. Numbers of cells that stained for Sox17, Sox2, β -catenin, Foxa2, and Foxj1, were increased in the bronchiolar epithelium 7 d after pneumonectomy (B, D, F, H, I). pH-3 immunostaining (red, inset in I) was detected in β -tubulin (green)-positive ciliated cells (arrow, inset in I). Adjacent nonciliated cells (arrowhead) showed no staining or were less intensely labeled with pH-3 antibody (inset in I). The numbers of β -tubulin-positive ciliated cells and CCSP-positive Clara cells were increased (J). Scale bars: 20 μ m.

remarkable plasticity during the process of redifferentiation. Thus ciliated cells underwent transdifferentiation during repair of the bronchiolar epithelium. Squamous metaplasia and spreading of once ciliated cells occurred before proliferation, which was maximal 2–4 d after injury. Regeneration of the bronchiolar epithelium after naphthalene injury is thought to be completed after 14–20 d (1, 15), and is also associated with proliferation and migration of naphthalene-resistant Clara cells from protected niches near airway branch points (1–3, 5). From a stochastic view, however, it is not likely that the rapid restoration of the bronchiolar surface results from proliferation of rare naphthalene-resistant Clara cells, but primarily from spreading of squamous progenitor cells that were derived from ciliated cells. Formal proof for this concept will require cell-specific lineage tracing that is not feasible at present. The present study demonstrates that the early rapid restoration of the bronchiolar epithelium after Clara cell injury is mediated by spreading and squamous metaplasia of ciliated cells, which maintain the epithelial barrier during repair. Thus, repair of naphthalene injured bronchiolar epithelium consists of two major phases: (1) an early phase during which ciliated cells transdifferentiate to maintain and

restore the bronchiolar epithelium, and (2) a proliferative phase during which cell number and differentiated phenotypes are restored.

The regeneration of bronchiolar epithelium in both naphthalene injury and pneumonectomy models was accompanied by dynamic changes in Sox family proteins, Sox17 and Sox2, and transcription factors known to play important roles in lung morphogenesis and cell differentiation, including Foxa1, Foxa2, Foxj1, TTF-1, and β -catenin (22, 23, 29–33). In naphthalene-induced injury, selective loss of nonciliated bronchiolar cells occurs without apparent injury to or proliferation of alveolar epithelial cells, whereas in the pneumonectomy model marked hyperplasia and proliferation occurs in both airways and alveoli, involving multiple epithelial and nonepithelial cell types (25, 26). Nevertheless, dynamic changes in the same transcription factors were associated with regeneration of the bronchiolar epithelium after both naphthalene injury and pneumonectomy models. These observations support the concept that the initial repair of the bronchiolar epithelium, at least in part, recapitulates transcriptional programs that coordinate respiratory epithelial cell differentiation during normal lung normal development. Since multiple cell types proliferate and differentiate after injury or during compensatory growth, it is anticipated that distinct transcriptional programs will influence these processes in diverse cell types.

Immunostaining showed that expression of Sox17 and Sox2 was selectively enhanced in ciliated cells before injury and that the Sox proteins and β -catenin were coexpressed in the squamous and cuboidal cells during repair of epithelium. Multiple Sox proteins, including Sox2 and Sox17, interact with Wnt/ β -catenin signaling to regulate diverse developmental processes, including cell type specification and stem/progenitor cell maintenance. Sox2 was shown to interact with β -catenin to regulate Wnt/ β -catenin signaling in the differentiation of osteoblast (34). Sox17 and β -catenin are known to interact to regulate a subset of genes, including Foxa1 and Foxa2, in the early endoderm (35). Foxa1, Foxa2, and Foxj1 were dynamically regulated after injury and restoration of the bronchiolar epithelium, the highest levels of expression being observed in ciliated cells and their derivatives early in the repair process. These Fox transcription factors are known to influence gene expression and epithelial cell differentiation in the lung (23, 30–33).

The present study provides cellular evidence that ciliated cells actively participate in repair of the bronchiolar epithelium after acute injury through rapid squamous metaplasia and redifferentiation into mature, columnar cell types. Thus, ciliated cells are capable of remarkable plasticity, undergoing dynamic changes in cell shape and gene expression during repair. These findings support previous electronmicroscopic studies demonstrating rapid spreading of ciliated cells after naphthalene exposure (15, 16). Taken together, repair of the bronchiolar epithelium after naphthalene share biological processes with repair of other tissue. Cell spreading/migration, redifferentiation, and proliferation play a critical role in wound healing of the skin, wherein keratinocytes migrate to denuded areas, and undergo cell shape changes that precede proliferation (36, 37).

Conflict of Interest Statement: K.-S.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.M.W. was a consultant for CyThera 2004–2005 and has received a total of \$3,000 of consulting fees during this time. A.M.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.E.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. V.E.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. L.G.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.A.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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