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Epithelial Repair and Regeneration

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Introduction

Contact with the environment positions the respiratory epithelium at risk for acute and chronic injury from infectious pathogens, noxious agents, and inflammatory processes. Thus, to protect gas transfer within the lung the epithelium is programmed for routine maintenance and repair. Programs for repair are directed by epithelial, mesenchymal, and inflammatory signals that collectively constitute highly regulated networks. Principal components of the repair network are developmental morphogens, integrin and growth factor signaling molecules, and transcription factors. The epithelium responds to these signals with a remarkable plasticity and is bulwarked by a population of lung progenitor cells to ensure maintenance and repair for fluid balance and host defense functions.

Insight into mechanisms of injury response and epithelial cell repair comes from observations of human disease that have been tested using in vivo and in vitro models. Epithelial cell responses have been studied by numerous methods, initially based on morphologic image analysis, cell radiolabeling, and more recently by immunolabeling of molecular markers, receptors, ligands, and genetic tags for lineage analysis. Together, these data have been integrated with a classic injury-wound healing paradigm to create a multistage model (Figure 45.1). Although specific injury/repair responses initiate programs, the resulting steps of epithelial differentiation for repair recapitulate embryonic patterns. In the pathologic state, repair programs are perturbed, leading to states characterized by fibrosis, metaplasia, or carcinogenesis.

Repair mechanisms discussed in this chapter encompass mainly the airway and alveolar epithelium of the lung. The proximal airway epithelium lines the trachea and bronchi and forms the attendant bronchial glands. The distal airway includes bronchiolar epithelium that extends to the junction of the alveolar ducts. Major epithelial types of the airway include ciliated, secretory (Clara), mucous (goblet), and basal cells. Neuroendocrine cells are also found in the epithelial layer. The alveolar epithelium covers the surfaces of the alveolar ducts and alveoli and is composed of primarily the type I and type II pneumocytes. Differences in cell populations and gene expression dictate some differences in repair mechanisms between these regions.

Steady-State Kinetics of Lung Epithelial Cells

Studies of pulmonary cell kinetics in rodents have demonstrated that the epithelial compartment in the adult lung is mitotically inactive, based on ^{3}H-thymidine labeling indices.[1] This overall low proliferation rate and the diverse number of cell types in the lung have made determination of steady-state kinetics difficult. Following postnatal growth, the mitotic index in the rodent lung was calculated to be less than 0.4% per day.[2] In tracheal epithelial cells, 4-week-old rats had a turnover of about 1 month, whereas very low mitotic activity has been identified in the epithelial cells of the postgrowth trachea and terminal bronchiolar epithelium.[3] Prolonged studies achieved by the addition of radiolabeled thymidine to drinking water have demonstrated that alveolar epithelial cells can survive for 125 days in young animals.[4] These observations were obscured by the prolonged period of postnatal alveolar growth that occurs in animals and humans. When labeling of alveolar wall cells was considered relative to age, cell turnover in the alveolar epithelium was noted to decrease even further.[5] Thus, in the absence of injury, the epithelial cells of all lung compartments are mitotically quiescent.

Steps in Injury and Repair

Classic wound healing has been investigated in great detail in the skin where a paradigm for repair that is similar to the lung has been established.[6] This regenera-

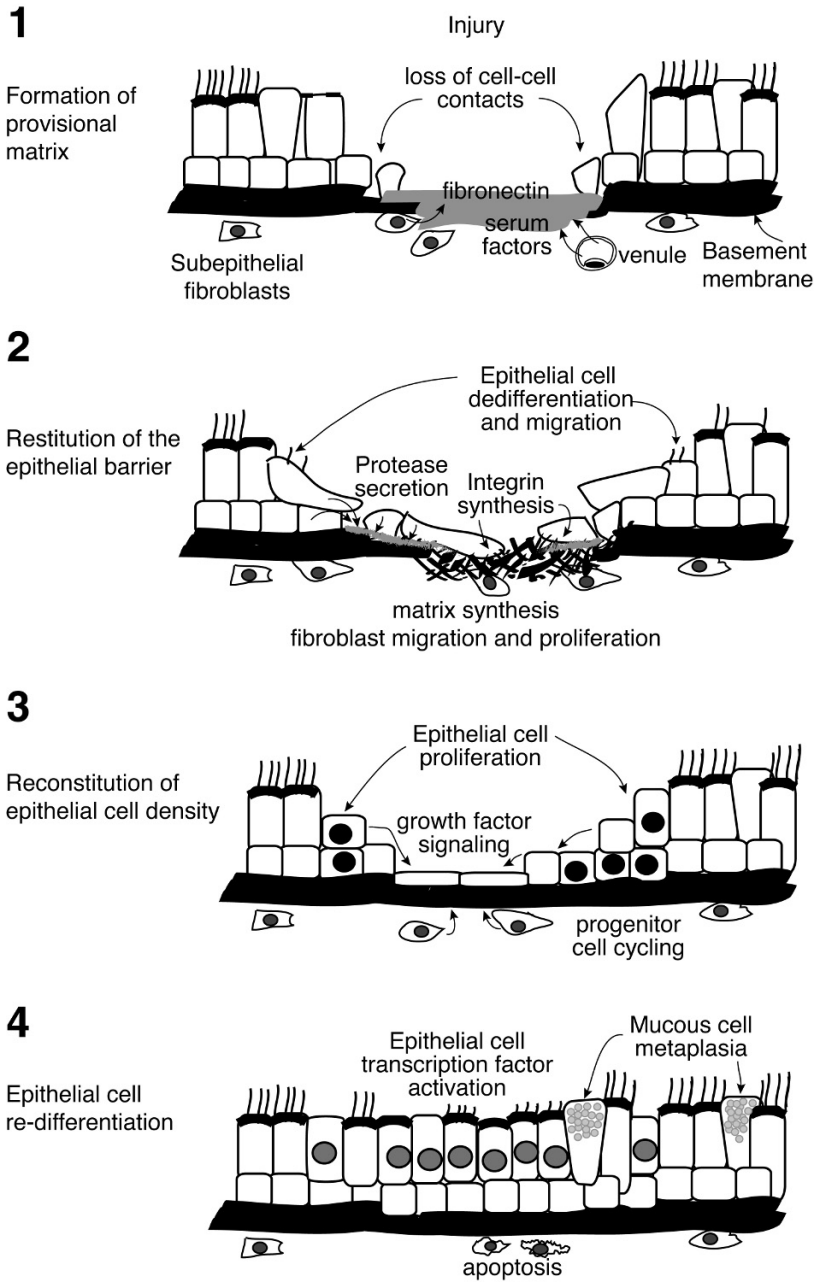

FIGURE 45.1. Steps in repair of airway epithelial cell injury. Conceptual stages of repair of the airway epithelium. See text and Table 45.1 for details of each step. Mitotic or label-retaining cells are indicated by dark nuclei.

tive response was observed over 50 years ago following direct wound injury of the tracheas of rats[7] and subsequently in large numbers of in vivo and in vitro models utilizing mechanical injury in airway and alveolar cell models.[8–15] A similar pattern of behavior is often observed in respiratory epithelial cells injured by chemical or infectious agents, suggesting a stereotypical repair program.[16–18] Although not a simple linear process, the programmed response to injury and repair can be condensed into a model, shown in Figure 45.1 and Table 45.1.

Although shown for an airway epithelial cell model, concepts are similar during alveolar epithelial cell repair.[15] Each of these stages involves participation of multiple factors that are directed by the mesenchymal and epithelial cells. For the purpose of this section, we discuss repair after classic wounding that includes loss of the epithelial cell barrier and, critically, disruption of the basement membrane and underlying interstitial matrix. However, injured epithelial cells may also be selectively removed without disruption of the basement membrane.

Table 45.1. Stages and components of repair after epithelial cell injury.

1. Formation of provisional matrix
Loss of epithelial cells during injury
Loss of normal basement membrane matrix
Vascular-derived factors for fibrin clot production
2. Restitution of the epithelial barrier
Epithelial cell dedifferentiation, spreading, and migration
Integrin-dependent epithelial cell signaling
Proteolytic degradation of intracellular adhesions
Subepithelial fibroblast migration and proliferation
3. Reconstitution of epithelial cell density
Growth factor–mediated epithelial cell proliferation
Progenitor and stem cell cycling
4. Epithelial cell redifferentiation
Growth/transcription factor–mediated epithelial cell differentiation
Metaplasia and hyperplasia of epithelial cell populations
Compensatory apoptosis of cell populations

Formation of A Provisional Matrix

Loss of Normal Basement Membrane

In the steady state, the basement membrane is composed of two parallel sheets of laminin and collagen. The uppermost layer (adjacent to the epithelial cells) is composed primarily of laminin-5 and laminin-10, while the lower layer is collagen IV.[19] The layers are connected by entactin/nidogen and multiple, highly charged, heparan sulfate proteoglycans (HSPGs) that are distributed throughout the basement membrane.[19,20] The underlying interstitial matrix contains fibroblasts in a fibrillar collagen (types I and III) matrix with additional HSPGs. Following disruption of the epithelial cell layer and basement membrane, a provisional matrix is formed predominantly by passive leakage of serum factors from local vasculature into the disrupted epithelial basement membrane and into the exposed airspace.[6,21] Cellular components including red blood cells (passively) and neutrophils (actively) move into the wound. The serum components form a fibrin clot that is present for up to 4 days.[7] In acute alveolar injury, the "hyaline membranes" seen in acute respiratory distress syndrome (ARDS) are an example of exuberant provisional matrix after breach of epithelial and endothelial barriers.[22] Inhibitors of fibrinolysis, such as serine protease inhibitors, are present at this point to stabilize the provisional matrix.[23] The passive provisional matrix formation is accompanied by active production of fibronectin by fibroblasts and epithelial cells.[24] Although many molecules compose the provisional matrix, the major factors are fibrin (most prominently), fibronectin, and vitronectin.

Functions of the Provisional Matrix

The provisional matrix provides a temporary barrier and contains multiple molecules to signal subsequent steps in repair. At the defect, a fibrin plug acts as a scaffold for fibronectin and vitronectin molecules but must be removed during reepithelialization.[25] The fibronectin and vitronectin contain multiple argininеglycine-aspartic acid (RGD) epitopes that are not present within the steady-state matrix. This peptide sequence provides sites for interaction of epithelial and fibroblast cell surface receptors (predominantly integrins) during cell migration and barrier restitution.[26–28] In addition to RGD epitopes, proinflammatory neoepitopes and exposed cryptic sites are present that function in growth factor signaling and immune cell recruitment.[29] Furthermore, alterations in matrix-associated HSPGs result in liberation of sequestered growth factors and cytokines.[30] Also, inflammatory and epithelial cells secrete platelet-derived growth factor that acts as both a mitogen and motogen (stimulating cell motility) for fibroblasts[31] that subsequently restore normal matrix components. Once established, the provisional matrix provides a rich source of matrix epitopes and sequestered growth factors that function as a scaffold and signaling unit for future reepithelialization.

Restitution of the Epithelial Barrier

Processes to return basement membrane to a fully established barrier covered with a layer of epithelial cells are initiated almost immediately following injury by several simultaneous processes. First, as discussed earlier, a change in basement membrane composition as mediated by epithelial and mesenchymal cell secretion occurs rapidly to provide barrier protection and matrix-dependent signals for epithelial cells and fibroblasts.[32] Second, epithelial cells, in response to matrix and growth factors, dedifferentiate, stretch, and migrate to cover the basement membrane for reestablishment of functional epithelial cells for barrier, host defense, and fluid equilibrium. Third, proteolytic cleavage of the provisional matrix returns the steady-state composition.[33] Using morphologic methods, these changes are observed to occur within hours following mechanical injury.[8,11]

Epithelial Cell Dedifferentiation

Soon after injury (1–2 hr), the epithelial cells at the edge of the wound undergo dynamic cytoskeletal rearrangement resulting in changes in morphology, featuring formation of lamellipodia.[14] This is the result of the loss of components of cell–matrix and cell–cell junctions leading to dissolution of focal adhesions, adherens junctions, and hemidesmosomes, untethering of intermediate filaments, and thus reorganization of the actin cytoskeleton. The change in morphology is accompanied by loss of features of differentiation (e.g. microvilli) as cells spread over the newly formed surface of provisional matrix. Following mechanical or naphthalene (selectively toxic for Clara

cells) injury, the ciliated cells assume a squamous cell-like form and lose cilia.[7,8,16] Molecular marker studies indicate that following naphthalene injury virtually all of the cells participating in the squamous cell-like barrier are from endodermally derived epithelial cells that were present at the time of injury.[34,35]

Signals for Dedifferentiation

Regulation of cell dedifferentiation is thought to result from alterations in matrix–integrin binding, growth factor secretion, and loss of cell–cell junctions. The Wingless (Wnt)/β-catenin pathway is intimately related to cell–cell junction complexes and epithelial cell differentiation.[36] Following injury, the disassembly of E-cadherin at cell junctions releases β-catenin to the cytoplasm where it can be translocated to the nucleus in a Wnt-dependent process.[37] Nuclear β-catenin activates signaling via the transcription factor lymphoid enhancer factor-1/T-cell factor. Consistent with this, after naphthalene-induced injury, β-catenin is diffusely upregulated in the cytoplasm of epithelial cells that are dedifferentiating to cover the provisional matix.[38]

Epithelial Cell Migration

In large wounds, both cell extension and migration occur. The rate of cell migration based on a moving front of cells has been determined to be 1–3 μm/min over the first 8 hr of mechanical injury.[8] Migration is dependent on growth factor activation, matrix–epithelial integrin binding, and matrix metalloprotease expression. Migration can be activated by growth factors such as epidermal growth factor (EGF) and trefoil factor in airway cell models; however, it is likely that other growth factors in serum also potentiate cell movement.[39,40] The predominant cell that migrates varies with the injury model, the cell type targeted, and the animal species studied.[17,35,41] In all cases, the matrix integrins play a critical role in directing epithelial adhesion and fibroblast movement into the wound. Simultaneously, proteases from epithelial and mesenchymal sources release cell–cell and cell–matrix contacts for epithelial cell cytoskeletal reorganization and migration.

Matrix–Epithelial Cell Interactions During Migration

Migrating cells must alter surface receptors for adhesion and traction across the provisional matrix. Integrins are heterodimeric proteins that adhere to many matrix substrates[27] but also transmit signals after ligation of substrate.[42] Alveolar and bronchiolar epithelial cells demonstrate directional migration (both chemotactic and haptotactic) toward many matrix proteins. Fibronectin is the most potent promigratory substance for these cells.[12,28] There is a switch in the expression of integrin receptors on epithelial cells during injury that facilitates migration over the provisional matrix. During steady state, the matrix-associated receptors in lung epithelial cells are predominantly collagen and laminin binding integrins (α2β1, α3β1, and α6β4) but α5 and αv are not present.[43] With injury, integrins α5 and αv are expressed on epithelial cells at the wound edge.[43] These integrins are specific receptors for RGD epitopes in fibronectin and vitronectin and are required for migration.[12,28] Fibroblasts also express α5 to facilitate migration on fibronectin. In addition to integrins, cell surface proteoglycan adhesion molecules such as syndecan and CD44 are also increased on migrating fibroblasts to bind components of provision matrix.[44,45]

Protease Functions in Epithelial Cell Migration

Two major categories of proteases are involved in cellular spreading and migration, matrix metalloproteases (MMPs) and serine. These proteases function by releasing epithelial cell–cell junctions and primordial contacts with the matrix. Of the MMPs, both MMP-7 and MMP-9 are required for normal airway epithelial cell migration.[46,47] Migrating epithelial cells secrete MMP-7 (matrilysin) in a basolateral direction to degrade the extracellular domain of E-cadherin,[46] contributing to cell detachment and spreading at the wound edge. Gelatinase B (MMP-9) releases lamellipodia of migrating cells from the matrix surface to allow extension.[47,48] Tissue inhibitor of matrix metalloproteinase (TIMP)-1 present in early wounding inhibits all secreted MMPs (including MMP-7 and MMP-9). A deficiency of this antiprotease accelerates wound closure.[49] Serum-derived serine protease plasminogen within the wound is activated to plasmin by uroplasminogen activator secreted by bronchial epithelial cells.[50] The primary role of plasmin is fibrinolysis of the provisional fibrin plug. Plasmin also promotes MMP synthesis and activation.[48] Plasmin activator inhibitor-1 is also present and blocks migration by binding to and obscuring vitronectin epitopes.[51] Fibroblasts also require proteases for migration, and membrane type 1 MMP (MT1-MMP, MMP-14) may be crucial for migration through fibrillar collagen and fibrin.[52] Additionally, inflammatory cells present in the wound can alter the activation of these proteases and alter the epitopes present in the provisional matrix.[53]

Reestablishment of the Normal Basement Membrane

Epithelial cells synthesize and remodel basement membrane components during migration. Migrating

epithelial cells grown on glass slides secrete a trail of synthesized basement membrane composed of fibronectin, laminin, collagen IV, and factors that newly arriving cells can use as a path for repairing a wound.[54] Other matrix components such as HSPGs, entactin/nidogen, and fibrillar collagens are supplied by the underlying fibroblasts. Restitution of basement membrane also requires removal of the provisional matrix. Failure to do so may result in prolonged signaling, recruitment of fibroblasts, and scar formation.[55] Furthermore, reestablishment of stable cell–matrix adhesions is not passive, and some proteases must continue to be expressed for proteolytic modification of the basement membrane.[54,56] For instance, laminin-5, the major matrix component of hemidesmosomes, requires proteolytic cleavage by serine proteases for enucleation of the hemidesmosomes.[56] Once the basement membrane and extracellular matrix are in the mature, stable confirmation, promigratory signals are quenched.

Reconstitution of Epithelial Cell Density

Epithelial Cell Proliferation

Following epithelial cell stretch and migration to cover a reestablished basement membrane, the epithelium must replace the cells lost during injury. A major mediator of proliferation during this stage is EGF and related family members; however, other growth factors, including fibroblast growth factor (FGF)-7 and hepatocyte growth factor (HGF), contribute to this function (Table 45.2) and are discussed later.[57–59] Epithelial cell proliferation has been noted to occur within 24–48 hr of mechanical and inhalation injury (e.g., ozone, nitrogen dioxide).[7,8,13,18] After injury, 20%–30% of undifferentiated epithelial cells covering the wound contain proliferation markers at 24–48 hr, followed by a marked decrease by 72 hr.[8,13,41,60] After nitrogen dioxide injury, an increase from 1% to 24% labeling index (proportion of labeled cells) of Clara cells and type II cells is observed at 24 hr.[41] The appearance of differentiated epithelial cells follows the peak of mitotic activity (the final stage in the repair model).[10]

Lung Progenitor and Lung Epithelial Stem Cells Roles

Observations many decades ago, based on thymidine labeling during development and after injury, suggested that the basal cell was the progenitor cell of the tracheal epithelium, the Clara cell provided this function in the distal airway,[61,62] and type II cells proliferated and gave rise to type I pneumocytes in the alveoli.[18,63] Recent injury–repair models and genetic approaches have confirmed many of these observations. Lineage tagging by conditional recombination in mice engineered with airway and alveolar epithelial cell specific promoters that drive cell markers has identified a single pool of progenitor epithelial cells present during early lung development.[64] Within fully formed adult lung, injury models reveal regional niches (proximal airway, distal airway, alveolar) for progenitor cells.[34,64–66] These lung cells are considered to be slow cycling but can give rise to progenitor cells that are capable of further cell division (transit amplifying cells). In the proximal airway, a niche of label-retaining cells has been localized within the submucosal glands and basal cells of the mouse trachea.[65,67] The expression of cell cycle protein Ki-67 in basal cells of cystic fibrosis bronchi identifies a similar proliferating population in human disease.[68]

In the distal airways, naphthalene injury models suggest that pulmonary neuroendocrine cells provide a protective niche for label-retaining Clara-like cells that are capable of epithelial reconstitution.[35] Similar studies identified injury-resistant cells expressing Clara cell secretory protein (CCSP) at the bronchoalveolar duct junction that are reparative.[34] Consistent with this, in the terminal airways of humans, 20%–40% of the proliferating cells express CCSP.[69] Cells expressing ciliated cell markers (e.g., transcription factor Foxj1) were not observed to proliferate in naphthalene or virus injury models.[17,38] Progenitor cells that repair the alveolar epithelium are less well defined. During alveolar injury, labeling studies show that type II but not type I cells proliferate and that type II can generate type I cells after lung injury.[70] Subpopulations of type II alveolar epithelial

Table 45.2. Growth factors in epithelial cell repair.

Ligand	Major sources	Key targets	Proliferation	Migration	Differentiation	Reference
EGF family	Epi	Epi	+	+	+	57
TGF-β	Epi, Fib, Mϕ	Epi, Fib	–	–	+	58
FGF-7 (KGF)	Fib, SM	Epi	+	+	+	59
HGF	Fib, En, Epi, Mϕ	Epi	+	+	–	59
PDGF	Mϕ, Epi	Fib	+	+	–	31

Note: EGF, epidermal growth factor; En, endothelial; Epi, epithelial; FGF, fibroblast growth factor; Fib, fibroblast; HGF, hepatocyte growth factor; KGF, keratinocyte growth factor; Mϕ, macrophage; PDGF, platelet-derived growth factor; SM, smooth muscle; TGF, transforming growth factor.

cells resistant to injury and capable of proliferation have been identified in vitro,[71] but compelling data suggests that a bronchoalveolar duct cell may function as a progenitor for both alveolar type II pneumocytes and Clara cells.[72]

Bone Marrow Stem Cells in Repair

New information is evolving concerning the reparative role of bone marrow–derived cells in lung injury repair (see Chapter 47). Some evidence suggests that adult stem cells can function to reconstitute at least a small percentage of the airway and alveolar epithelium of an injured lung.[66]

Growth Factor Functions in Epithelial Cell Reconstitution

Signaling between cells of endodermal and mesenchymal origin is a fundamental process in development that is shared with repair and is predominantly mediated by growth factors (see Table 45.2 and later discussion).[73,74] Growth factors that play key roles in epithelial cell repair include EGF, transforming growth factor (TGF)-β, FGF-7, platelet-derived growth factor, and HGF family members. Other growth factors are known to be critical in lung development, but specific roles in repair are not described.[73,74] Growth factors function during epithelial cell repair and reconstitution by motogenic, mitogenic, and prodifferentiation effects.

Epithelial Cell Redifferentiation

Differentiating epithelial cells within a repairing wound arise primarily from three different populations: dedifferentiated cells, proliferating cells, and multipotent stem or progenitor cells. In turn, differentiation is regulated by three major groups of molecules: secreted growth factors and other developmental signaling molecules, transcription factors, and extracellular matrix proteins (discussed earlier). In addition, multiple cofactors play important roles in differentiation, including retinoic acid[75] and those known to be critical for in vitro differentiation of primary epithelial cells such as insulin, epinephrine, thyroid hormone, and corticosteroids.[70,76] Epithelial cell differentiation during repair relies on programs similar to those used during lung development.[17,38,73]

Epithelial Cell Redifferentiation and Transdifferentiation

Morphologic evidence of redifferentiation commences at 3–10 days in vivo depending on wound model.[7,8,11] Multiple epithelial cell populations contribute to the redifferentiating airway epithelia within the repairing wound.[34,38,62,67,77] First, a large number of the redifferentiated cells arise from previously differentiated cells that have dedifferentiated to cover the provisional matrix. It has been observed that, following naphthalene injury, former ciliated cells that squamate to cover a wound continue to express the Foxj1 transcription factor for subsequent ciliated cell redifferentiation.[38] Second, proliferating cells also become differentiated cells. As noted earlier, these are either of Clara cell or basal cell origin in the airway or of type II cell origin in the alveolar compartment.[78,79] Third, it is likely that there is a multipotent or progenitor cell that can contribute to lung-specific lineages that is capable of resupplying all epithelial cell types. Here, genetic tagging and clonogenic studies have indicated that niches of specialized cells can generate multiple differentiated cell types.[65,67,72]

Developmental Signaling Pathways Mediating Differentiation

Developmental signaling proteins that are members of the Wnt, Sonic hedgehog (Shh), and bone morphogenic protein (BMP) pathways play essential roles in differentiation.[73,74] Each pathway contains multiple members and receptors that are functionally interdependent. Although these families have been studied in detail during lung development (specifically in branching morphogenesis), their roles in repair are not as well defined.

Wnt signaling regulates cell proliferation, migration, and differentiation.[36,80] The canonical Wnt/β-catenin pathway involves the binding of Wnt ligands to the receptor complex and regulation of β-catenin. Noncanonical Wnt signaling, such as utilized by Wnt5, is required for normal alveolar differentiation and alters expression of Shh and BMP4.[80] Sonic hedgehog is expressed in developing lung epithelium where it signals through its receptor Patch to activate the transcription factor Gli. Gli1 and Gli2 are expressed in developing lung mesenchymal cells budding from the foregut and instruct the tracheal epithelial cells during branching morphogenesis.[81] However, Shh and Gli are also highly expressed in the epithelium following naphthalene injury and are upregulated in small cell lung cancer.[82] Overactivation of the Shh pathway results in proliferation and perturbation of differentiation, notably in epithelial neuroendocrine cells of the lung, but the role in normal lung epithelial cells is unclear.[82] BMP4 is expressed in the lung epithelium, regulates proximal–distal patterning, and is required for normal alveolar epithelial cell differentiation.[83] This pathway may be interrupted in inflammatory states where activation of transcription factor NF-κB has been shown to alter epithelial–mesenchymal signaling through interruption of BMP4 in a chick lung model.[84]

Transcription Factors Mediating Differentiation Following Injury

Transcription factor expression in epithelial cells during injury and repair also recapitulates developmental sequences.[17,38] Transcription factors with central roles in lung epithelial cell differentiation are homeodomain thyroid transcription factor 1 (TITFI or TTF-1; Nkx2.1), forkhead family members (Foxa1, Foxa2, Foxj1), and GATA-6.[85] Lung morphogenesis requires both Gli and TITF-1.[86,87] Molecular analysis of mice deficient in TITF-1 showed a requirement for this factor in differentiation of epithelial cells of the distal airways and alveoli.[86] Thyroid transcription factor 1 and Foxa2 are expressed in epithelium in early development, persist in the adult, and activate epithelial cell-specific genes such as surfactant proteins and CCSP, suggesting major roles in epithelial differentiation.[85] Persistent Foxa2 and TITF-1 expression during injury in airway and alveolar (type II) epithelial cells may be due to a relative resistance of some cell populations and indicates that early programs of epithelial cell differentiation remain intact.[17,38,78] Selective cytotoxic injury of alveolar epithelial cells markedly increased transient expression of Foxn1 in airway and alveolar proliferating cells; however, a specific role for this factor in differentiation has not been described. Foxj1 is expressed later in repair when it is required for ciliogenesis.[17,38,88] Similarly, GATA-6 is required for differentiation of alveolar epithelial cells and the transition of type II to type I cells.[89]

Metaplasia and Compensatory Apoptosis of Epithelial Cell Populations

Mucous cell metaplasia commonly occurs during abnormal airway repair. Injury-associated inflammatory cells secrete proteases (neutrophil elastase) and cytokines (e.g., IL-13 and IL-9) to induce mucous cell metaplasia in animal models and human disease.[90–93] Metaplasia may be persistent or be remedied through apoptosis.[94] In normal repair of alveolar epithelium, type II cell hyperplasia after lung injury is followed by compensatory apoptosis.[95,96]

Roles for Growth Factors in Epithelial Cell Repair

Growth factor–mediated signaling between cells is a constant feature of repair and does not exist as a discrete stage of repair; however, specific growth factors may dominate at one point. Several growth factors with roles in injury an repair are noted in Table 45.2 and discussed in this section.

Epidermal Growth Factor Family

Epidermal growth factor is the prototypic member of the EGF family of ligands.[57] Epidermal growth ligands are key factors in airway and alveolar epithelial repair through their activation of epithelial cell migration, proliferation, and differentiation.[15,39,40,92] The EGF receptor Her1 (EGFR, ErbB1) and other family members (Her2–4) are receptor tyrosine kinases with affinity for multiple EGF ligands, including TGF-α, heparin binding EGF, amphiregulin, epiregulin, and neuregulin. Signaling occurs after homo- or heterodimerization of receptor family members. At steady state, EGF receptors are localized predominantly on the basolateral surface of epithelial cells, providing ligand–receptor exclusion. Following injury, there is redistribution of receptors to apical cell surfaces, enhancing ligand-receptor interaction and subsequent repair.[79,92,97] Epidermal growth factor, TGF-α, and other ligands are elevated in human disease (e.g., cystic fibrosis, asthma) and cell and animal models of airway and alveolar epithelial cell injury.[68,97–99]

Transforming Growth Factor-β Family

Transforming growth factor-β functions in both epithelial maintenance and injury–repair.[21,58] The three TGF-β forms all signal through the same receptor but have unique functions.[100] Abundant latent TGF-β is stored within the extracellular matrix at steady state and is activated after injury through proteolytic and nonproteolytic mechanisms.[58] Transforming growth factor-β activation results in increased synthesis of matrix factors by epithelial cells and fibroblasts, differentiation of epithelial cells, enhanced survival and proliferation of fibroblasts, inhibition and apoptosis of inflammatory cells, and maturation of certain inflammatory cell subsets. Failure to activate TGF-β in the lung matrix results in excessive inflammation and lung destruction.[101] Excessive or prolonged TGF-β activation leads to fibrosis (see Chapter 46). It also prevents normal epithelial cell differentiation due to TGF-β signaling via Smad that inhibits TITF-1 to subsequently reduce surfactant protein gene expression.[102] Additionally, TGF-β promotes epithelial-to-mesenchymal transdifferentiation.[103]

Fibroblast Growth Factor

Fibroblast growth factor-7 (keratinocyte growth factor [KGF]) functions as a potent mitogen for both Clara and type II cells.[104,105] In lung injury, FGF-7 is produced by fibroblasts and vascular smooth muscle cells and has high affinity for only the FGFR2-IIIb splice variant that is

expressed on airway and alveolar epithelia.[59] Proinflammatory cytokines stimulate fibroblast production of FGF-7 in vitro.[106] Fibroblast growth factor-7 enhances epithelial cell spreading and motility[107] by increasing MMP-9 and uroplasminogen activator secretion from wounded epithelial cells.[108] It also enhances differentiation and surfactant synthesis by alveolar type II cells.[109] Fibroblast growth factor-7 is elevated in human ARDS bronchoalveolar lavage fluid and in models of acute lung injury.[59] Exogenous intratracheal or systemic delivery prior to lung injury ameliorates damage in animal models.[59]

Hepatocyte Growth Factor

Hepatocyte growth factor is a mitogen and motogen for alveolar type II cells.[59] Hepatocyte growth factor is synthesized by fibroblasts, bronchial epithelial cells, endothelial cells, and alveolar macrophages as a precursor that is proteolytically processed.[110] The HGF receptor c-Met is a receptor tyrosine kinase that is present on epithelial cells, fibroblasts, endothelial cells, and hematopoietic cells. Hepatocyte growth factor is increased in human and animal models of lung injury, in part through proinflammatory cytokines (e.g., IL-1 and IL-6) that stimulate HGF secretion from fibroblasts.[59] Following alveolar epithelial cell injury, HGF secretion precedes FGF-7[111] and is temporally related to type II cell DNA synthesis during the cell proliferation phase, suggesting that HGF is a prominent mitogen in ARDS.[59]

Epithelial Cell Repair Following Prototypic Injuries

Human diseases characterized by respiratory epithelial cell injury provide insight into normal and abnormal repair mechanisms. Epithelial injury and repair occur commonly after respiratory viral and bacterial infections but may also occur with acid aspiration or toxic gas or steam inhalation. In contrast, chronic injury associated with cigarette smoking, asthma, and chronic bronchitis results in an interruption of normal repair. Normal and pathogenic mechanisms that follow acute respiratory epithelial injury in human disease demonstrate the importance of each stage of the injury–repair model described earlier (see Figure 45.1). Below, events occurring during repair of the airway after respiratory virus infection (Figure 45.2) and of the alveolar epithelium in ARDS demonstrate the relevance of the repair model.

Airway Epithelial Cell Repair Following Respiratory Virus Infection

Reestablishment of epithelium following viral injury mirrors the stages of the classic wound injury–repair model (compare Figures 45.1 and 45.2). Information regarding airway epithelial injury induced by respiratory viruses has been derived from natural human infections, but especially from studies of experimental infections in animals with paramyxovirus (e.g., parainfluenza and respiratory syncytial virus) and influenza virus. These infections result in loss of cilia and ciliated cells (based on virus-specific receptor targeting) and metaplasia of epithelial cells during repair.[17,112–115]

Morphologic Events Following Respiratory Virus Infection

Morphologic events of injury and repair following respiratory virus infection, as observed by electron and light microscopy studies, are similar across several animal infection models (see Figure 45.2A). A common initial event is sloughing of epithelial cells, resulting in denuded basement membrane in airways and alveoli.[17,112] This is followed by cell dedifferentiation, marked particularly by cell elongation covering the provisional basement membrane and by ciliary shorting and loss.[17,112,113] Concomitant are subepithelial cell inflammatory infiltrates and/or alveolar pneumonitis.[113] Virus clearance is coupled with regeneration of the epithelium, typically at days 5–8 postinoculation. In contrast to mechanical injury, experimental virus infection in animals showed that airway and alveolar epithelial cell proliferation occurred at 5 days after inoculation.[17,113] In the Sendai virus–infected mouse, bromodeoxyuridine labeling peaked at day 12 and was absent by day 21.[17] Airway and alveolar epithelial cell differentiation normalizes within 3–4 weeks postinoculation.[17,112,113] Functionally, injury is marked by decreased bacterial clearance and secondary bacterial infections, and repair tracks with measured mucociliary clearance.[17,113]

Molecular Correlates of Morphologic Events

Molecular markers of epithelial cell differentiation characterized in the developing lung have been assessed in postinfection repair (see Figure 45.2B).[17,85] As in naphthalene injury, throughout virus injury and repair Foxa2 and TITF-1 remain expressed.[38] Depressed expression of ciliated cell marker Foxj1 and Clara cell marker CCSP during injury and early repair reflects a relatively undifferentiated state of the epithelium as has been observed following human respiratory syncytial virus infection.[17] A relative absence of CCSP in epithelial cells postinjury suggests it is unlikely that mature Clara cells are functioning as a reservoir for new differentiated cells. Foxj1 expression was not detected in proliferating cells identified by bromodeoxyuridine labeling,[17] suggesting mature ciliated cells are nonmitotic. Instead, it is more probable that Foxj1 is important for late-stage ciliogenesis.[88] The

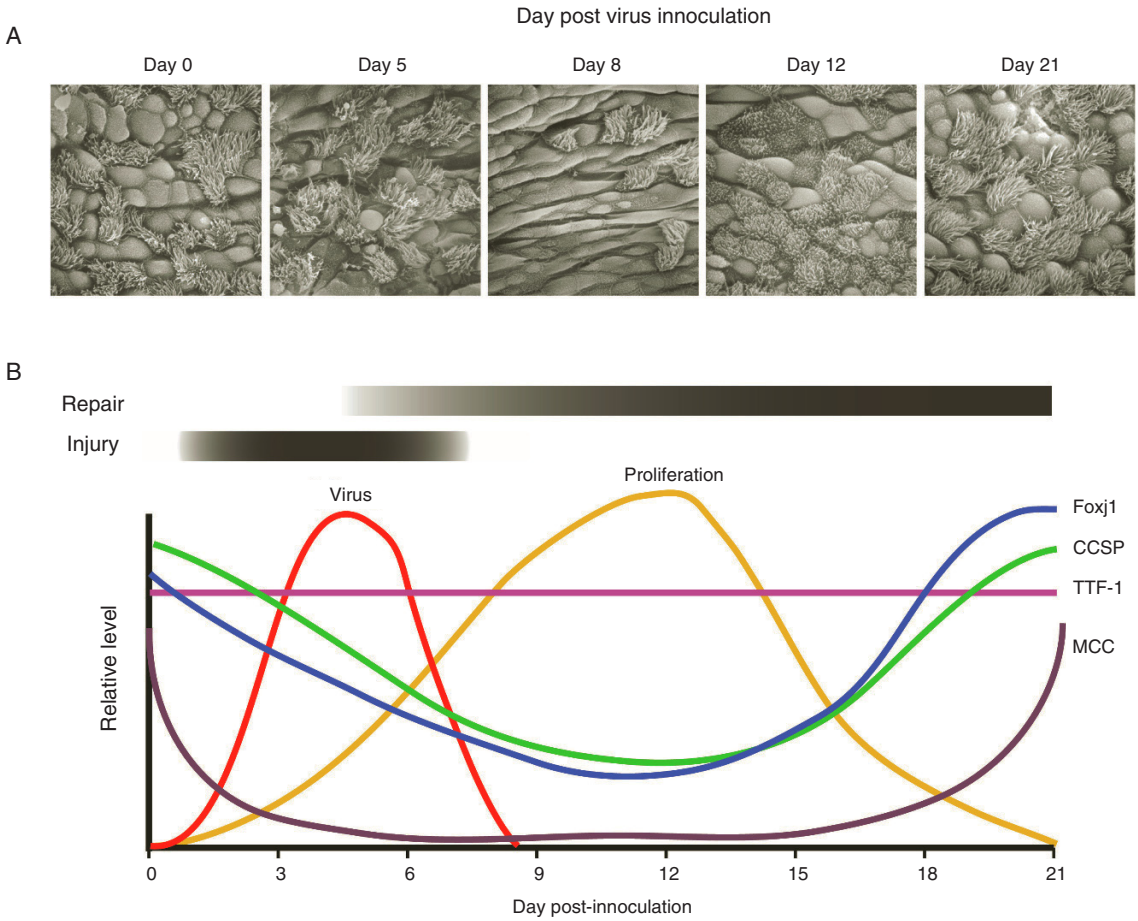

FIGURE 45.2. Airway epithelial cell repair following respiratory viral injury. **(A)** Scanning electron micrographs of mouse trachea following in vivo infection with Sendai paramyxovirus. (Reprinted from Am J Pathol 2001;159:2055–2069, with permission of the American Society for Investigative Pathology.) **(B)** Molecular and functional events following the respiratory virus infection shown in A. Relative changes in expression of airway epithelial cell markers and transcription factors. CCSP, Clara cell secretory protein; Foxj1, transcription factor; MCC, mucociliary clearance; TITF-1 (also known as TITF1, Nkx2.1), thyroid transcription factor 1.

sequence of differentiation during the repair phase, including the lag of CCSP expression relative to the appearance of cilia markers, is similar to that seen in the developing lung, supporting the idea that repair recapitulates development.[85]

Mucous Cell Metaplasia Following Respiratory Virus Infection

Mucous cell (goblet cell) metaplasia is a feature of chronic airway diseases including asthma and COPD. These diseases have been linked to preceding respiratory virus infections and have implicated several factors including aberrant EGF receptor and IL-13 signaling.[92] Mouse models of respiratory virus infection show mucous cell metaplasia in the late repair phase that persists despite virus clearance, suggesting that the virus reprograms epithelial cell responses through persistent immune responses.[92,93,116,117]

Alveolar Epithelial Cell Repair Following Acute Respiratory Distress Syndrome

Acute lung injury and the clinical manifestation ARDS are a classic example of alveolar injury and repair. Diffuse alveolar damage, the histologic correlate of acute lung injury/ARDS has been subdivided into three phases that parallel the wound–repair model.[22] The early exudative phase (days 1–7) is characterized by necrosis of pneumocytes and endothelial cells and by interstitial and alveolar edema with hemorrhage and hyaline membranes. The ensuing proliferative phase (days 7–21) is marked by type II cell hyperplasia, fibroblast migration into organizing areas of luminal fibrosis, and inflammation. The fibrotic

phase entails fibrosis with variable degrees of architectural remodeling.

Establishment of a Provisional Matrix in Acute Respiratory Distress Syndrome

The hallmark of the early exudative phase is the leakage of serum proteins, including serum albumin, β_2-microglobulin, ceruloplasmin and fibrinogen, into the alveolar space for provisional matrix formation.[22,118] An influx of platelets and inflammatory cells also occurs by passive and active mechanisms. Antiprotease production in this phase favors stabilization of the provisional matrix by inhibition of fibrinolytic activity.[23] Gene profiling of animals in this phase of acute lung injury suggests that three major cellular processes occur: (1) loss of the functional activity of differentiated type II cells, (2) production of inhibitors of the serine and matrix metalloproteinase family, and (3) production of cytokines and growth factors.[23] The loss of differentiated alveolar epithelial cells (type II cells) is likely due to injury and dedifferentiation to replace necrotic type I cells and is marked by decreased ion channel and surfactant-associated gene production.[23] Transforming growth factor-β_1-induced genes are rapidly upregulated within 72 hr after alveolar epithelial damage.[23,119] Blockade of TGF-β signaling in the exudative phase prevents alveolar edema, suggesting that TGF-β activation inhibits alveolar epithelial repair during this stage.[120]

Restitution and Reconstitution of the Epithelial Cell Barrier

The proliferative phase is characterized by migration of alveolar epithelial cells over the provisional matrix and type II cell proliferation. These processes are responsive to growth factors EGF and KGF as well as to matrix RGD epitopes.[28] Also during this phase, fibroblasts and myofibroblasts migrate toward the fibronectin cross-linked within the fibrin of the provisional matrix.[25] Failure of repair, associated with inability to reconstitute the epithelial barrier, results in fibroblast/myofibroblast production of collagen and fibronectin, contraction, and fibrosis.

Summary: Epithelial Cell Repair Following Injury

Repair of the epithelium can be conceptualized as a stage-dependent process, but lines of division of biologic activities are not discrete. Instead, an orchestration of mesenchymal and epithelial interactions directed by matrix and epithelial signaling in response to fundamental development, growth, and matrix factors results in the repair process. Models of repair after respiratory viral infection and acute lung injury provide useful information for understanding the molecular basis of repair in human lung diseases.

References

1. Kauffman SL. Cell proliferation in the mammalian lung. Int Rev Exp Pathol 1980;22:131–191.
2. Enesco M, Leblond CP. Increase in cell number as a factor in the growth of the young male rat. J Embryol Exp Morphol 1962;10:530–562.
3. Kauffman SL. Alteration in cell proliferation in mouse lung following urethane exposure. II. Effects of chronic exposure on terminal bronchiolar epithelium. Am J Pathol 1971;64(3):531–538.
4. Cameron IL. Cell renewal in the organs and tissues of the nongrowing adult mouse. Tex Rep Biol Med 1970; 28(3):203–248.
5. Simnett JD, Heppleston AG. Cell renewal in the mouse lung. The influence of sex, strain, and age. Lab Invest 1966; 15(11):1793–1801.
6. Clark RAF. The Molecular and Cellular Biology of Wound Repair, 2nd ed. New York: Plenum Press; 1996.
7. Wilhelm DL. Regeneration of tracheal epithelium. J Pathol Bacteriol 1953;65(2):543–550.
8. Erjefalt JS, Erjefalt I, Sundler F, Persson CG. In vivo restitution of airway epithelium. Cell Tissue Res 1995;281 (2):305–316.
9. Keenan KP, Combs JW, McDowell EM. Regeneration of hamster tracheal epithelium after mechanical injury. I. Focal lesions: quantitative morphologic study of cell proliferation. Virchows Arch B Cell Pathol Incl Mol Pathol 1982;41(3):193–214.
10. Lane BP, Gordon R. Regeneration of rat tracheal epithelium after mechanical injury. I. The relationship between mitotic activity and cellular differentiation. Proc Soc Exp Biol Med 1974;145(4):1139–1144.
11. Gordon RE, Lane BP. Regeneration of rat tracheal epithelium after mechanical injury. II. Restoration of surface integrity during the early hours after injury. Am Rev Respir Dis 1976;113(6):799–807.
12. Rickard KA, Taylor J, Rennard SI, Spurzem JR. Migration of bovine bronchial epithelial cells to extracellular matrix components. Am J Respir Cell Mol Biol 1993;8(1): 63–68.
13. Shimizu T, Nishihara M, Kawaguchi S, Sakakura Y. Expression of phenotypic markers during regeneration of rat tracheal epithelium following mechanical injury. Am J Respir Cell Mol Biol 1994;11(1):85–94.
14. Zahm JM, Chevillard M, Puchelle E. Wound repair of human surface respiratory epithelium. Am J Respir Cell Mol Biol 1991;5(3):242–248.
15. Kheradmand F, Folkesson HG, Shum L, et al. Transforming growth factor-alpha enhances alveolar epithelial cell repair in a new in vitro model. Am J Physiol 1994;267(6 Pt 1):L728–L738.
16. Lawson GW, Van Winkle LS, Toskala E, et al. Mouse strain modulates the role of the ciliated cell in acute tra-

cheobronchial airway injury—distal airways. Am J Pathol 2002;160(1):315–327.
17. Look DC, Walter MJ, Williamson MR, et al. Effects of paramyxoviral infection on airway epithelial cell Foxj1 expression, ciliogenesis, and mucociliary function. Am J Pathol 2001;159(6):2055–2069.
18. Adamson IY, Bowden DH. The type 2 cell as progenitor of alveolar epithelial regeneration. A cytodynamic study in mice after exposure to oxygen. Lab Invest 1974; 30(1):35–42.
19. Sannes PL, Burch KK, Khosla J, et al. Immunohistochemical localization of chondroitin sulfate, chondroitin sulfate proteoglycan, heparan sulfate proteoglycan, entactin, and laminin in basement membranes of postnatal developing and adult rat lungs. Am J Respir Cell Mol Biol 1993; 8(3):245–251.
20. Yurchenco PD, Amenta PS, Patton BL. Basement membrane assembly, stability and activities observed through a developmental lens. Matrix Biol 2004;22(7):521–538.
21. McGowan SE. Extracellular matrix and the regulation of lung development and repair. FASEB J 1992;6(11):2895–2904.
22. Tomashefski JF Jr. Pulmonary pathology of acute respiratory distress syndrome. Clin Chest Med 2000;21(3):435–466.
23. Wesselkamper SC, Case LM, Henning LN, et al. Gene expression changes during the development of acute lung injury: role of transforming growth factor beta. Am J Respir Crit Care Med 2005;172(11):1399–1411.
24. Rosi E, Beckmann JD, Pladsen P, et al. Modulation of human bronchial epithelial cell IIICS fibronectin mRNA in vitro. Eur Respir J 1996;9(3):549–555.
25. Greiling D, Clark RA. Fibronectin provides a conduit for fibroblast transmigration from collagenous stroma into fibrin clot provisional matrix. J Cell Sci 1997;110(Pt 7):861–870.
26. Hocking DC, Chang CH. Fibronectin matrix polymerization regulates small airway epithelial cell migration. Am J Physiol Lung Cell Mol Physiol 2003;285(1):L169–L179.
27. Sheppard D. Functions of pulmonary epithelial integrins: from development to disease. Physiol Rev 2003;83(3):673–686.
28. Kim HJ, Henke CA, Savik SK, Ingbar DH. Integrin mediation of alveolar epithelial cell migration on fibronectin and type I collagen. Am J Physiol 1997;273(1 Pt 1):L134–L41.
29. Laskin DL, Kimura T, Sakakibara S, et al. Chemotactic activity of collagen-like polypeptides for human peripheral blood neutrophils. J Leuk Biol 1986;39(3):255–266.
30. Li Q, Park PW, Wilson CL, Parks WC. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. Cell 2002;111(5):635–646.
31. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. Cytokine Growth Factor Rev 2004;15(4): 255–273.
32. Clark RA. Fibrin is a many splendored thing. J Invest Dermatol 2003;121(5):XXI–XXII.
33. Van Leer C, Stutz M, Haeberli A, Geiser T. Urokinase plasminogen activator released by alveolar epithelial cells modulates alveolar epithelial repair in vitro. Thromb Haemost 2005;94(6):1257–1264.
34. Giangreco A, Reynolds SD, Stripp BR. Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. Am J Pathol 2002;161(1):173–182.
35. Hong Ku, Reynolds SD, Giangreco A, et al. Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are critical for epithelial renewal after progenitor cell depletion. Am J Respir Cell Mol Biol 2001;24(6): 671–681.
36. Seto ES, Bellen HJ. The ins and outs of Wingless signaling. Trends Cell Biol 2004;14(1):45–53.
37. Orsulic S, Huber O, Aberle H, et al. E-cadherin binding prevents beta-catenin nuclear localization and beta-catenin/LEF-1–mediated transactivation. J Cell Sci 1999; 112(Pt 8):1237–1245.
38. Park KS, Wells JM, Zorn AM, et al. Transdifferentiation of ciliated cells during repair of the respiratory epithelium. Am J Respir Cell Mol Biol 2006;34(2):151–157.
39. Oertel M, Graness A, Thim L, et al. Trefoil factor family-peptides promote migration of human bronchial epithelial cells: synergistic effect with epidermal growth factor. Am J Respir Cell Mol Biol 2001;25(4):418–424.
40. Kim JS, McKinnis VS, Nawrocki A, White SR. Stimulation of migration and wound repair of guinea-pig airway epithelial cells in response to epidermal growth factor. Am J Respir Cell Mol Biol 1998;18(1):66–74.
41. Evans MJ, Stephens RJ, Freeman G. Effects of nitrogen dioxide on cell renewal in the rat lung. Arch Intern Med 1971;128(1):57–60.
42. Akiyama SK. Integrins in cell adhesion and signaling. Hum Cell 1996;9(3):181–186.
43. Pilewski JM, Latoche JD, Arcasoy SM, Albelda SM. Expression of integrin cell adhesion receptors during human airway epithelial repair in vivo. Am J Physiol 1997;273(1 Pt 1):L256–L263.
44. Clark RA, Lin F, Greiling D, et al. Fibroblast invasive migration into fibronectin/fibrin gels requires a previously uncharacterized dermatan sulfate-CD44 proteoglycan. J Invest Dermatol 2004;122(2):266–277.
45. Lin F, Ren XD, Doris G, Clark RA. Three-dimensional migration of human adult dermal fibroblasts from collagen lattices into fibrin/fibronectin gels requires syndecan-4 proteoglycan. J Invest Dermatol 2005;124(5):906–913.
46. McGuire JK, Li Q, Parks WC. Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium. Am J Pathol 2003;162(6): 1831–1843.
47. Legrand C, Gilles C, Zahm JM, et al. Airway epithelial cell migration dynamics. MMP-9 role in cell–extracellular matrix remodeling. J Cell Biol 1999;146(2):517–529.
48. Legrand C, Polette M, Tournier JM, et al. UPA/plasmin system–mediated MMP-9 activation is implicated in bronchial epithelial cell migration. Exp Cell Res 2001;264(2): 326–36.
49. Chen P, Farivar AS, Mulligan MS, Madtes DK. tissue inhibitor of metalloproteinase-1 deficiency abrogates obliterative airway disease after heterotopic tracheal

transplantation. Am J Respir Cell Mol Biol 2006;34(4): 464–472.
50. Chu EK, Cheng J, Foley JS, et al. Induction of the plasminogen activator system by mechanical stimulation of human bronchial epithelial cells. Am J Respir Cell Mol Biol 2006;35(6):628–638.
51. Lazar MH, Christensen PJ, Du M, et al. Plasminogen activator inhibitor-1 impairs alveolar epithelial repair by binding to vitronectin. Am J Respir Cell Mol Biol 2004; 31(6):672–678.
52. Hotary KB, Yana I, Sabeh F, et al. Matrix metalloproteinases (MMPs) regulate fibrin-invasive activity via MT1-MMP-dependent and -independent processes. J Exp Med 2002;195(3):295–308.
53. Parks WC, Shapiro SD. Matrix metalloproteinases in lung biology. Respir Res 2001;2(1):10–19.
54. Hintermann E, Quaranta V. Epithelial cell motility on laminin-5: regulation by matrix assembly, proteolysis, integrins and ErbB receptors. Matrix Biol 2004;23(2):75–85.
55. De Giorgio-Miller A, Bottoms S, Laurent G, et al. Fibrin-induced skin fibrosis in mice deficient in tissue plasminogen activator. Am J Pathol 2005;167(3):721–732.
56. Baker SE, Hopkinson SB, Fitchmun M, et al. Laminin-5 and hemidesmosomes: role of the alpha 3 chain subunit in hemidesmosome stability and assembly. J Cell Sci 1996;109 (Pt 10):2509–2520.
57. Citri A, Yarden Y. EGF-ErbB Signalling: towards the systems level. Nat Rev Mol Cell Biol 2006;7(7):505–516.
58. Sheppard D. Transforming growth factor-beta: a central modulator of pulmonary and airway inflammation and fibrosis. Proc Am Thorac Soc 2006;3(5):413–417.
59. Ware LB, Matthay MA. Keratinocyte and hepatocyte growth factors in the lung: roles in lung development, inflammation, and repair. Am J Physiol Lung Cell Mol Physiol 2002;282(5):L924–L40.
60. Evans MJ, Johnson LV, Stephens RJ, Freeman G. Cell renewal in the lungs of rats exposed to low levels of ozone. Exp Mol Pathol 1976;24(1):70–83.
61. Bindreiter M, Schuppler J, Stockinger L. Cell proliferation and differentiation in the tracheal epithelium of the rat. Exp Cell Res 1968;50(2):377–382.
62. Evans MJ, Cabral-Anderson LJ, Freeman G. Role of the Clara cell in renewal of the bronchiolar epithelium. Lab Invest 1978;38(6):648–653.
63. Evans MJ, Cabral LJ, Stephens RJ, Freeman G. Transformation of alveolar type 2 cells to type 1 cells following exposure to NO_2. Exp Mol Pathol 1975;22(1):142–150.
64. Perl AK, Wert SE, Loudy DE, et al. Conditional recombination reveals distinct subsets of epithelial cells in trachea, bronchi, and alveoli. Am J Respir Cell Mol Biol 2005;33(5):455–462.
65. Hong Ku, Reynolds SD, Watkins S, et al. Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. Am J Pathol 2004;164(2):577–588.
66. Neuringer IP, Randell SH. Stem cells and repair of lung injuries. Respir Res 2004;5(1):6.
67. Borthwick DW, Shahbazian M, Krantz QT, et al. Evidence for stem-cell niches in the tracheal epithelium. Am J Respir Cell Mol Biol 2001;24(6):662–670.
68. Voynow JA, Fischer BM, Roberts BC, Proia AD. Basal-like cells constitute the proliferating cell population in cystic fibrosis airways. Am J Respir Crit Care Med 2005;172(8):1013–1018.
69. Boers JE, Ambergen AW, Thunnissen FB. Number and proliferation of Clara cells in normal human airway epithelium. Am J Respir Crit Care Med 1999;159(5 Pt 1): 1585–1591.
70. Danto SI, Shannon JM, Borok Z, et al. Reversible transdifferentiation of alveolar epithelial cells. Am J Respir Cell Mol Biol 1995;12(5):497–502.
71. Reddy R, Buckley S, Doerken M, et al. Isolation of a putative progenitor subpopulation of alveolar epithelial type 2 cells. Am J Physiol Lung Cell Mol Physiol 2004;286(4): L658–L667.
72. Kim CF, Jackson EL, Woolfenden AE, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 2005;121(6):823–835.
73. Demayo F, Minoo P, Plopper CG, et al. Mesenchymal–epithelial interactions in lung development and repair: are modeling and remodeling the same process? Am J Physiol Lung Cell Mol Physiol 2002;283(3):L510–L517.
74. Shannon JM, Hyatt BA. Epithelial–mesenchymal interactions in the developing lung. Annu Rev Physiol 2004;66: 625–645.
75. Cardoso WV, Williams MC, Mitsialis SA, et al. Retinoic acid induces changes in the pattern of airway branching and alters epithelial cell differentiation in the developing lung in vitro. Am J Respir Cell Mol Biol 1995;12(5):464–476.
76. Lechner JF, Haugen A, Autrup H, et al. Clonal growth of epithelial cells from normal adult human bronchus. Cancer Res 1981;41(6):2294–2304.
77. Reynolds SD, Hong KU, Giangreco A, et al. Conditional Clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells. Am J Physiol Lung Cell Mol Physiol 2000;278(6):L1256–L1263.
78. Kalinichenko VV, Lim L, Shin B, Costa RH. Differential expression of forkhead box transcription factors following butylated hydroxytoluene lung injury. Am J Physiol Lung Cell Mol Physiol 2001;280(4):L695–L704.
79. Casalino-Matsuda SM, Monzon ME, Forteza RM. Epidermal growth factor receptor activation by epidermal growth factor mediates oxidant-induced goblet cell metaplasia in human airway epithelium. Am J Respir Cell Mol Biol 2006;34(5):581–591.
80. Li C, Xiao J, Hormi K, et al. Wnt5a participates in distal lung morphogenesis. Dev Biol 2002;248(1):68–81.
81. Motoyama J, Liu J, Mo R, et al. Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. Nat Genet 1998;20(1):54–57.
82. Watkins DN, Berman DM, Burkholder SG, et al. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 2003;422(6929):313–337.
83. Weaver M, Yingling JM, Dunn NR, et al. BMP signaling regulates proximal–distal differentiation of endoderm in mouse lung development. Development 1999;126(18): 4005–4015.
84. Muraoka RS, Bushdid PB, Brantley DM, et al. Mesenchymal expression of nuclear factor-kappaB inhibits epithelial

growth and branching in the embryonic chick lung. Dev Biol 2000;225(2):322–338.
85. Costa RH, Kalinichenko VV, Lim L. Transcription factors in mouse lung development and function. Am J Physiol Lung Cell Mol Physiol 2001;280(5):L823–L838.
86. Minoo P, Su G, Drum H, et al. Defects in tracheoesophageal and lung morphogenesis in Nkx2.1(–/–) mouse embryos. Dev Biol 1999;209(1):60–71.
87. Pepicelli CV, Lewis PM, McMahon AP. Sonic hedgehog regulates branching morphogenesis in the mammalian lung. Curr Biol 1998;8(19):1083–1086.
88. Brody SL, Yan XH, Wuerffel MK, et al. Ciliogenesis and left-right axis defects in forkhead factor Hfh-4-null mice. Am J Respir Cell Mol Biol 2000;23(1):45–51.
89. Yang H, Lu MM, Zhang L, et al. GATA6 regulates differentiation of distal lung epithelium. Development 2002; 129(9):2233–2246.
90. Fischer BM, Voynow JA. Neutrophil elastase induces MUC5AC gene expression in airway epithelium via a pathway involving reactive oxygen species. Am J Respir Cell Mol Biol 2002;26(4):447–452.
91. Longphre M, Li D, Gallup M, et al. Allergen-induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells. J Clin Invest 1999;104(10):1375–1382.
92. Tyner JW, Kim EY, Ide K, et al. Blocking airway mucous cell metaplasia by inhibiting EGFR antiapoptosis and IL-13 transdifferentiation signals. J Clin Invest 2006;116(2): 309–321.
93. Walter MJ, Morton JD, Kajiwara N, et al. viral induction of a chronic asthma phenotype and genetic segregation from the acute response. J Clin Invest 2002;110(2):165–175.
94. Tesfaigzi Y. Roles of apoptosis in airway epithelia. Am J Respir Cell Mol Biol 2006;34(5):537–547.
95. Bardales RH, Xie SS, Schaefer RF, Hsu SM. Apoptosis is a major pathway responsible for the resolution of type II pneumocytes in acute lung injury. Am J Pathol 1996; 149(3):845–852.
96. Fehrenbach H, Kasper M, Koslowski R, et al. Alveolar epithelial type II cell apoptosis in vivo during resolution of keratinocyte growth factor–induced hyperplasia in the rat. Histochem Cell Biol 2000;114(1):49–61.
97. Vermeer PD, Einwalter LA, Moninger TO, et al. Segregation of receptor and ligand regulates activation of epithelial growth factor receptor. Nature 2003;422(6929): 322–326.
98. Madtes DK, Busby HK, Strandjord TP, Clark JG. Expression of transforming growth factor-alpha and epidermal growth factor receptor is increased following bleomycin-induced lung injury in rats. Am J Respir Cell Mol Biol 1994;11(5):540–551.
99. Amishima M, Munakata M, Nasuhara Y, et al. Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. Am J Respir Crit Care Med 1998;157(6 Pt 1):1907–1912.
100. Bandyopadhyay B, Fan J, Guan S, et al. A "traffic control" role for TGFbeta3: orchestrating dermal and epidermal cell motility during wound healing. J Cell Biol 2006; 172(7):1093–1105.
101. Morris DG, Huang X, Kaminski N, et al. Loss of integrin alpha(V)beta6-mediated TGF-beta activation causes MMP12-dependent emphysema. Nature 2003;422(6928): 169–173.
102. Kumar AS, Gonzales LW, Ballard PL. Transforming growth factor-beta(1) regulation of surfactant protein B gene expression is mediated by protein kinase-dependent intracellular translocation of thyroid transcription factor-1 and hepatocyte nuclear factor 3. Biochim Biophys Acta 2000;1492(1):45–55.
103. Willis BC, Liebler JM, Luby-Phelps K, et al. Induction of epithelial–mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. Am J Pathol 2005;166(5): 1321–1332.
104. Fehrenbach H, Fehrenbach A, Pan T, et al. Keratinocyte growth factor–induced proliferation of rat airway epithelium is restricted to Clara cells in vivo. Eur Respir J 2002;20(5):1185–1197.
105. Yano T, Mason RJ, Pan T, et al. KGF regulates pulmonary epithelial proliferation and surfactant protein gene expression in adult rat lung. Am J Physiol Lung Cell Mol Physiol 2000;279(6):L1146–L1158.
106. Chedid M, Rubin JS, Csaky KG, Aaronson SA. Regulation of keratinocyte growth factor gene expression by interleukin 1. J Biol Chem 1994;269(14):10753–10757.
107. Waters CM, Savla U. Keratinocyte growth factor accelerates wound closure in airway epithelium during cyclic mechanical strain. J Cell Physiol 1999;181(3): 424–432.
108. Putnins EE, Firth JD, Uitto VJ. Keratinocyte growth factor stimulation of gelatinase (matrix metalloproteinase-9) and plasminogen activator in histiotypic epithelial cell culture. J Invest Dermatol 1995;104(6):989–994.
109. Chelly N, Henrion A, Pinteur C, et al. Role of keratinocyte growth factor in the control of surfactant synthesis by fetal lung mesenchyme. Endocrinology 2001;142(5):1814–1819.
110. Miyazawa K, Shimomura T, Naka D, Kitamura N. Proteolytic activation of hepatocyte growth factor in response to tissue injury. J Biol Chem 1994;269(12):8966–8970.
111. Sakai T, Satoh K, Matsushima K, et al. Hepatocyte growth factor in bronchoalveolar lavage fluids and cells in patients with inflammatory chest diseases of the lower respiratory tract: detection by RIA and in situ hybridization. Am J Respir Cell Mol Biol 1997;16(4):388–397.
112. Bryson Dg, McNulty MS, McCracken RM, Cush PF. Ultrastructural features of experimental parainfluenza type 3 virus pneumonia in calves. J Comp Pathol 1983; 93(3):397–414.
113. Castleman WL, Chandler SK, Slauson DO. Experimental bovine respiratory syncytial virus infection in conventional calves: ultrastructural respiratory lesions. Am J Vet Res 1985;46(3):554–560.
114. Ibricevic A, Pekosz A, Walter MJ, et al. Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. J Virol 2006;80(15):7469–7480.
115. Aherne W, Bird T, Court SD, et al. Pathological changes in virus infections of the lower respiratory tract in children. J Clin Pathol 1970;23(1):7–18.

116. Park JW, Taube C, Yang ES, et al. Respiratory syncytial virus–induced airway hyperresponsiveness is independent of IL-13 compared with that induced by allergen. J Allergy Clin Immunol 2003;112(6):1078–1087.
117. Hashimoto K, Graham BS, Ho SB, et al. Respiratory syncytial virus in allergic lung inflammation increases MUC5AC and GOB-5. Am J Respir Crit Care Med 2004; 170(3):306–312.
118. Schnapp LM, Donohoe S, Chen J, et al. Mining the acute respiratory distress syndrome proteome: identification of the insulin-like growth factor (IGF)/IGF-binding protein-3 pathway in acute lung injury. Am J Pathol 2006;169(1): 86–95.
119. Kaminski N, Allard JD, Pittet JF, et al. Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. Proc Natl Acad Sci USA 2000;97(4):1778–1783.
120. Pittet JF, Griffiths MJ, Geiser T, et al. TGF-beta is a critical mediator of acute lung injury. J Clin Invest 2001;107(12): 1537–1544.