Human Epidermal Growth Factor Receptor Signaling in Acute Lung Injury

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Acute lung injury (ALI) is a syndrome marked by increased permeability across the pulmonary epithelium resulting in pulmonary edema. Recent evidence suggests that members of the human epidermal growth factor receptor (HER) family are activated in alveolar epithelial cells during ALI and regulate alveolar epithelial barrier function. These tyrosine kinase receptors, which also participate in the pathophysiology of pulmonary epithelial malignancies, regulate cell growth, differentiation, and migration as well as cell-cell adhesion, all processes that influence epithelial injury and repair. In this review we outline mechanisms of epithelial injury and repair in ALI, activation patterns of this receptor family in pulmonary epithelial cells as a consequence injury, how receptor activation alters alveolar permeability, and the possible intracellular signaling pathways involved. Finally, we propose a theoretical model for how HER-mediated modulation of alveolar permeability might affect lung injury and repair. Understanding how these receptors signal has direct therapeutic implications in lung injury and other diseases characterized by altered epithelial barrier function.

Keywords: acute lung injury; human epidermal growth factor receptor; inflammation; alveolar epithelial cell

Acute lung injury (ALI) is a devastating illness with an annual incidence of approximately 200,000 in the United States and a mortality rate between 25 and 40% (1). A complex inflammatory syndrome, ALI is marked by leukocyte recruitment and activation; release of cytotoxic reactive oxygen species, proteolytic enzymes, and cytokines; and activation of the coagulation cascade (2, 3). The net result is damage to the alveolar capillary barrier leading to air-space flooding with protein-rich fluid (4–6). Radiographically, this is manifested as pulmonary edema in the absence of left ventricular dysfunction. Clinically, ALI presents as life-threatening hypoxemia (7). Despite recent advances in understanding the pathogenic mechanisms of ALI, no specific pharmacologic therapy exists, and treatment remains supportive. Pulmonary epithelial cell damage resulting in loss of alveolar epithelial barrier function with increased permeability across the alveolo-capillary membrane is an initial step in ALI development. Rapid and efficient restoration of the epithelial barrier is essential to repair of the injured lung (4). These facts highlight the importance of understanding the processes that

CLINICAL RELEVANCE

This review highlights and synthesizes recent findings regarding the role of human epidermal growth factor receptor (HER) family of epithelial receptors in acute lung injury. Readers of this article will gain a better understanding of epithelial injury and repair processes and how the HER family influences epithelial biology in acute lung injury.

regulate epithelial cell injury and repair in ALI. Increasing evidence suggests that members of the Human Epidermal Growth Factor Receptor (HER) family, which are expressed by alveolar and airway epithelial cells, are activated in the setting of ALI and regulate epithelial barrier function during injury and repair. This review outlines the current understanding of the HER family's role in epithelial biology during ALI.

ALVEOLAR EPITHELIAL INJURY AND REPAIR

The alveolar epithelial surface is comprised of alveolar type I (ATI) and type II (ATII) cells, and injury to these cells is a pathological hallmark of ALI (8, 9). A functioning, intact alveolar epithelium is involved in ion transport and production of surfactant and serves as a physical barrier, all of which are essential to maintaining normal pulmonary homeostasis and fluid balance. Epithelial cell injury resulting in loss of cell-cell adhesion and increased paracellular permeability is central to the pathogenesis of ALI. The lung's epithelial barrier provides a greater resistance to protein and fluid flux than the capillary endothelium, and the alveolar epithelium is responsible for the active removal of edema fluid from the distal air spaces of the lung (10–12). Indeed, damage to the endothelium alone is insufficient to cause pulmonary edema, whereas concomitant injury to the alveolar epithelium results in severe lung injury (13–15). The epithelial cell barrier consists of airway epithelial cells joined by a belt-like apical junctional complex (AJC). The AJC is responsible for lateral adhesion between cells and acts as a barrier to diffusion between the interstitium and alveolar space through its major constituents, tight junctions and adherens junctions (16, 17). The tight junction acts as a gate, regulating solute flux, and as a fence preventing diffusion of proteins and lipids between the outer leaflet of the apical and basolateral plasma membrane domains. The adherens junction is essential for cell-cell adhesion. Alveolar epithelial injury in the setting of ALI results in disruption of the AJC, with loss of cell-cell adhesion leading to edema, alveolar epithelial cell apoptosis, and/or necrosis, and sloughing followed by regeneration of the epithelium or replacement with fibrous hyaline membranes.

Mechanisms to repair the alveolar epithelial barrier and restore a competent monolayer are initiated immediately in

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response to tissue damage. Initial epithelial repair events in ALI include proliferation, spreading, and migration of ATII cells to cover the denuded alveolar basement membrane (18, 19). ATII cells (20), and possibly distal airway cells (21), serve as progenitor cells to repopulate the exposed alveolar surface. Recovery is marked by early ATII proliferation and migration followed by differentiation into ATI cells (22, 23). Simultaneously, subepithelial fibroblasts proliferate to form a layer of granulation tissue. In contrast, recurrent injury results in a disorganized repair process marked by ATII cell proliferation as well as recruitment and expansion of fibroblasts and myofibroblasts (20, 24). The result is destruction of the original tissue architecture and deposition of excessive and disorganized extracellular matrix (ECM), including collagen and fibronectin. This dysregulated fibroblast proliferation and ECM production can lead to the development of more diffuse pulmonary fibrosis with compromised lung function. It is apparent that deficient or delayed reepithelialization of the basement membrane promotes fibrosis (25, 26), whereas rapid and complete reepithelialization inhibits fibrosis (27). Fibrosis is marked by a failure of normal ATII regeneration and differentiation into ATI cells, resulting in an abnormal epithelial cell phenotype, a lack of surfactant production, and alveolar collapse (28). In experimental models, targeted injury to ATII cells results in accumulation of myofibroblasts and pulmonary fibrosis (29, 30). Our understanding of the factors that drive the repair process from an orderly reconstitution of the alveolar epithelium and restoration of normal lung function to a pathological process characterized by excessive deposition of ECM with pulmonary fibrosis remains incomplete. Because efficient repair of the alveolar epithelium is critical to the restoration of lung function and prevention of fibrosis in the setting of ALI, understanding the signaling that regulates restoration of epithelial barrier function is pivotal.

Growth factors and their receptors, such as members of the receptor tyrosine kinase (RTK) HER family, have important functional effects on pulmonary epithelial cells. The effects of HER receptors on cell growth and transformation in epithelial malignancies, in particular of the breast and lung, are well known (31, 32) and have led to the development and widespread use of tyrosine kinase inhibitors (TKIs) or antibodies targeting HER receptors as focused chemotherapy for breast and lung cancer (33, 34). However, increasing evidence suggests that this receptor family is critical in the regulation of processes central to lung injury and repair, including alveolar epithelial cell–cell adhesion as well as epithelial migration, growth, and differentiation (35).

THE HER RECEPTOR FAMILY

The HER system has evolved into a multifaceted network of ligands, receptors, and internal signaling networks, which affords significant complexity. In *Caenorhabditis elegans*, the HER family includes a single ligand–receptor pair. In humans, the HER family consists of four type I, membrane bound, RTKs: HER1 or epidermal growth factor receptor (EGFR), HER2, HER3, and HER4. The HER family has also been referred to as the ErbB family since a series of discoveries in the 1980s demonstrated that the avian erythroblastosis virus gene encoded a mutant form of EGFR/HER1 (36–38). These receptors share a similar structure, including an extracellular ligand binding domain, a single transmembrane domain, an intracellular tyrosine kinase domain, and a cytoplasmic tail (39). Receptor function is characterized by homo/heterodimerization in response to ligand binding, kinase domain activation, autophosphorylation of C-terminal tyrosine residues, and tyrosine phosphorylation of docking proteins to initiate downstream signaling (39). Control of HER family signaling exists at multiple levels, including

specific cellular patterns of ligand and receptor expression, sub-cellular segregation of ligands and receptors, and the diversity of intracellular receptor binding proteins that can amplify or attenuate receptor signaling.

HER family ligands are synthesized as transmembrane peptide precursors that contain an EGF-like domain comprised of six cysteines folding into a complex tertiary structure (40). This domain contains all of the known biologic activity of the ligand. Surface ligands are processed through proteolytic shedding of the extracellular domain to produce a free active ligand. These soluble growth factor ligands then bind their cognate receptor in an autocrine or paracrine fashion. There are at least 10 known EGFR/HER family ligands that are commonly divided into three groups (Table 1). Group 1 includes epidermal growth factor (EGF), transforming growth factor (TGF)- α , and amphiregulin, which bind EGFR/HER1. The second group contains betacellulin, heparin-binding EGF (HB-EGF), and epiregulin, all of which can bind EGFR/HER1 or HER4. The third group includes the neuregulins (NRGs). There are four known NRG proteins (NRG-1, -2, -3, -4); NRG-1 and NRG-2 primarily bind HER3, whereas NRG-1, -2, -3, and -4 can bind HER4. HER2 has no known ligand and requires dimerization with another HER receptor for activation (41). In contrast, HER3 lacks a functional tyrosine kinase domain and also only signals as part of a heterodimer with another HER member (Figure 1) (42). Members of the HER ligand–receptor family are expressed by a variety of cells, including pulmonary alveolar epithelial cells (43).

In the quiescent state, HER family ligands are expressed at the apical surface of polarized epithelia, whereas expression of their cognate receptors is restricted to the basolateral aspect of cells, creating a natural segregation of ligands and receptors that favors receptor inactivation (43–45). Given that receptor activation influences cell proliferation, this spatial separation maintains cells in a dormant, differentiated state. However, injury allows for receptor activation through simultaneous ligand shedding and disruption of the epithelial barrier and cell polarity, making receptors available to bind free ligand, resulting in cell proliferation, migration, and differentiation. This creates an internal repair mechanism for epithelia in which receptor activation in wounded cells initiates necessary repair mechanisms. In addition, basolateral expression of receptors on epithelia allows for binding of ligands that originate from the mesenchyme, allowing for paracrine transactivation, possibly accounting for receptor activation in the absence of epithelial injury. Finally, ligand-independent HER activation, via intracellular kinases such as Src, provides an additional mechanism of driving HER signaling (46).

Internal adapter proteins and signaling molecules translate receptor activation into diverse intracellular responses, including gene transcription, cell migration, and cell–cell adhesion.

TABLE 1. HER FAMILY RECEPTORS AND LIGANDS

Receptor	Ligand
HER1/EGFR	EGF
	TGF- α
	Amphiregulin
	Betacellulin
	Heparin-binding EGF
HER2	Epiregulin
	No ligand
	Neuregulin 1, 2
	Betacellulin
HER3	Heparin-binding EGF
	Epiregulin
	Neuregulin 1–4
HER4

Figure 1. Human epidermal growth factor receptor (HER) Signaling. HER ligands exist as surface-bound proteins, which are proteolytically shed to produce EGF-like domain containing free ligand (EGF has an EGF domain). The free growth factors are then able to bind individual receptors that homo- (HER1/HER1) or hetero- (HER2/3) dimerize, autophosphorylate, and initiate downstream intracellular signaling. Particularities of the HER family include HER2, which has no known ligand (orphan receptor), and HER3, which has a catalytically inactive tyrosine kinase domain.

Differential interaction between receptors and possible cytoplasmic binding partners influences the cellular response and the intensity of the signal. For example, EGF binding to HER1 and NRG-1-bound HER4 partnering with HER1 induce HER1 binding to the adaptor protein Shc; however, HER1 additionally binds to Grb only after activation by EGF (47). This response is likely the result of differences in the site of HER1 phosphorylation that is determined by ligand and dimerization partner. In addition, tissue-specific expression of different ligand and receptor isoforms adds to the complexity of HER family signaling. For example, HER4 has at least four known isoforms as a result of alternative RNA splicing, resulting in variation in the extracellular and cytoplasmic domains that influence signaling. The presence of a 16-amino-acid region within the cytoplasmic tail of HER4 determines the ability to bind and activate PI (3) kinase (48, 49). This receptor isoform has high expression in the heart, and an isoform that lacks this region, and therefore does not activate PI (3) kinase, is expressed in the brain. Differential processing of transcripts for HER ligands also results in isoform variation. For example, the NRG-1 gene can generate at least 15 different protein isoforms through alternative splicing. The resulting isoforms can vary in the protein's EGF-like domain or N-terminal sequence. Isoform variation can also affect protein localization, as the protein can be synthesized as a transmembrane or nontransmembrane form (50, 51).

HER SIGNALING AND LUNG DEVELOPMENT

Organized epithelialization of premature bronchial tubes and alveoli is required for effective lung development (52, 53). There is overlap between the signaling events governing epithelial proliferation, migration, and differentiation during lung development and those that are activated during lung injury and

repair. Consequently, lung development represents a paradigm for understanding the cellular mechanisms of epithelial injury and repair in the mature lung. In this regard, HER receptors have been identified as central regulators of alveolar epithelial cell growth and differentiation during lung development. EGF-EGFR signaling enhances ATII differentiation and maturation (54–56), and, conversely, lungs of EGFR-deficient mice (EGFR^{-/-}) are marked by defects in epithelial growth. EGFR^{-/-} mice display deficient pulmonary alveolarization, loss of surfactant expression, and alveolar collapse, similar to the pathology of neonatal respiratory distress syndrome (57). Conversely, administration of EGF stimulates proliferation of fetal lung cultures (58) and lung maturation (59) and rescues premature fetal lambs from respiratory distress syndrome (60). In addition, NRG-1, NRG-2, HER2, and HER3 are expressed in the human fetal lung (61–63). NRG-1 administration leads to HER2/3 activation, enhancing epithelial proliferation and differentiation (64) and stimulating fetal lung growth (63) via the MAPK and Akt pathways (65). In the fetal lung, HER4 activation promotes ATII proliferation (66) and fetal lung growth, and genetic deletion of HER4 results in hypoplastic lungs similar to those observed in bronchopulmonary dysplasia (67). Surfactant production, a key physiological function of ATII cells, is regulated by HER4 in the developing lung (66, 68). Given these critical roles for HER receptors in pulmonary organogenesis, these pathways are likely to play important roles in the setting of ALI and repair.

HER ACTIVATION AS A REGULATOR OF EPITHELIAL BARRIER FUNCTION

EGFR signaling has been implicated in the restoration of epithelial cell barrier function after injury in numerous tissue beds including skin (69), intestine (70), cornea (71–73), and the lung

(74). The EGFR ligand, TGF- α , is present in edema fluid from patients with ALI (75), and exogenous TGF- α enhances wound closure in cultured ATII cells. Wound closure involves loss of cell-cell adhesion followed by cell proliferation and migration, and HER receptors participate in all of these processes. In cultured ATII cells, TGF- α , EGF, and HB-EGF induce cell proliferation (76, 77), spreading, and motility (78). However, in an *in vitro* model of ATII wound healing, TGF- α and EGF enhanced wound healing primarily through increased cell migration as opposed to proliferation (78). Keratinocyte growth factor also enhanced wound healing in ATII cells in an EGFR-dependent manner primarily through cell migration without a significant effect on proliferation (79, 80). This effect is likely the result of keratinocyte growth factor-mediated increases in TGF- α shedding, leading to EGFR activation (79, 81).

The effect of EGFR activation in bronchial epithelial cells mimics that observed in the alveolar epithelium, suggesting conserved signaling processes throughout the respiratory tree. Exogenous EGF enhances repair of wounded bronchial epithelial monolayers in an EGFR-dependent fashion (82). In addition, injury induces shedding of EGFR ligands, resulting in EGFR activation. In an *in vitro* scratch model, injured bronchial epithelial cells exhibited EGFR activation, and wound repair was delayed in the presence of a specific EGFR inhibitor. Mechanical stress, which has been shown to alter pulmonary epithelial repair after wounding (83), also induces shedding of HB-EGF and subsequent EGFR activation (84).

Similar to EGFR, HER2 signaling, in partnership with HER3, can influence pulmonary epithelial barrier function. Exogenous NRG-1 increases proliferation in pulmonary epithelial cells that is dependent on HER2-mediated activation of the JAK-STAT pathway (64, 85). Using a scratch model, Vermeer and colleagues demonstrated that NRG-1 was shed from pulmonary epithelial cells, resulting in HER3 binding, HER2/3 complex formation, and HER2 activation at the wound edge (44). When HER2 activation was blocked using antibodies to NRG-1 or HER2, wound closure was significantly delayed, suggesting that HER2 activation is a compensatory response to injury to help reconstitute an intact epithelium.

HER RECEPTORS AS INTEGRATORS OF THE INFLAMMATORY RESPONSE

ALI is marked by an acute inflammatory response, and increases in the lung of cytokines such as IL-1 β and IL-6 play a key role in the pathogenesis of epithelial injury in this setting. Members of the HER family can serve as signaling intermediaries for extracellular stimuli such as hormones, neurotransmitters, and cytokines, translating them into an intracellular response. For example, IL-1 β , a central regulator of epithelial barrier function in ALI (43, 86–93), enhances repair of wounded epithelium in an EGFR-dependent fashion. In rat ATII monolayers wounded in a scratch model, exogenous IL-1 β hastens wound closure through IL-1 β -mediated TGF- α and EGF release, with subsequent EGFR activation leading to cell spreading (18). Similarly, pulmonary edema fluid from patients with ALI has high IL-1 β levels and improves pulmonary epithelial wound closure in an IL-1 β -dependent manner (94).

HER2 also participates in models of pulmonary epithelial barrier disruption by inflammatory cytokines. IL-1 β activates HER2 in airway and alveolar epithelial cells (43). This occurs through IL-1 β -mediated shedding of the HER3 ligand, NRG-1, from pulmonary epithelial cells, resulting in HER2/3 activation and HER2-dependent loss of epithelial barrier function. Down-regulation of this pathway targeting NRG-1 or HER2 significantly attenuates IL-1 β -induced pulmonary epithelial barrier dysfunction *in vitro*,

whereas exogenous NRG-1 activates HER2 and increases pulmonary epithelial permeability (43). This is in contrast to the barrier protective effect of NRG-1 and HER2 in epithelial monolayers mechanically wounded in a scratch model (44). These apparently discrepant effects of HER activation on barrier function (disruption versus barrier enhancement) *in vitro* likely reflect differences in the injury models. Experiments in which HER activation is barrier protective use a scratch model, which results in an immediate wound defined by a leading edge of epithelial cells where compartmentalized HER receptor activation takes place. In contrast, in the context of a diffuse injury to a monolayer using IL-1 β , HER family activation is generalized, leading to disruption of the epithelial barrier and increased paracellular permeability. Given these contrasting responses, HER activation likely participates in epithelial injury as well as recovery, depending on the nature of injury and state of the epithelial barrier. HER2 activation in an injured epithelium leads to barrier restoration, whereas receptor activation in an intact monolayer results in barrier disruption.

In vivo studies provide additional evidence for HER family activation in lung injury. In a murine model of ALI induced by high tidal volume mechanical ventilation (95) and in *in vitro* cyclic stretch injury of alveolar epithelial cells (96), EGFR is activated. Pharmacological inhibition of EGFR activation attenuates lung injury, including alveolar leak in ventilator-induced lung injury. However, activation of EGFR with exogenous EGF fails to recapitulate injury in mice (95), suggesting that EGFR activation is necessary but not sufficient to induce ALI (or that spatially restricted release of EGF might be important). In contrast, TGF- α overexpression in ATII cells induces EGFR-dependent pulmonary fibrosis in mice (97), and EGFR blockade decreases pulmonary fibrosis in a bleomycin model (98), further suggesting a role for this receptor in alveolar injury. There are conflicting reports regarding the effect of the EGFR inhibitor gefitinib on bleomycin-induced fibrosis in mice. Suzuki and colleagues reported increased fibrosis after gefitinib treatment (99, 100), whereas Ishii and colleagues observed decreased fibrosis (98). The causes of this discrepancy are unclear, but variations in mouse strain and duration of gefitinib treatment might account for the differences.

Animal studies reveal that the NRG-1–HER2 pathway is activated in murine models of ALI, with increased bronchoalveolar lavage NRG1 and pulmonary HER2 activation after bleomycin or lipopolysaccharide (43, 101, 102). Using a bleomycin lung injury model, transgenic mice unable to activate HER2 in pulmonary epithelial cells have decreased alveolar leak, attenuated fibrosis, and improved survival compared with wild-type mice (101). Pharmacological blockade of HER2 *in vivo* confirmed a protective effect in murine ALI models (102). In humans with ALI, NRG-1 is elevated in pulmonary edema fluid (43) and bronchoalveolar lavage fluid (103) compared with controls and correlates with the severity of lung injury, suggesting that this pathway is activated in patients.

THE ROLE OF PROTEASES IN HER FAMILY SIGNALING AND ALI

Proteases, through shedding of surface-bound ligands, serve a central role in RTK activation by extracellular stimuli and therefore would be expected to participate in regulating epithelial HER receptor activation. Indeed, ADAM17, a member of the ADAM (A Disintegrin And Metalloprotease) protease family, a group of multidomain, type I transmembrane proteins, is involved in shedding of various surface molecules (104), including HER ligands. Also known as TNF- α converting enzyme (TACE), ADAM17 was first described as the protease responsible for shedding the membrane-bound form of TNF- α (105). Insights into ADAM17 function have been obtained through

the analysis of mice lacking the TACE zinc-binding domain, a mutation that inactivates the protease (TACE^{ΔZn/ΔZn}) (106). These mice have a targeted deletion in exon 11 that encodes the catalytic active site of the TACE metalloprotease domain, resulting in a lack of enzymatic activity (106). TACE^{ΔZn/ΔZn} animals have substantial perinatal lethality with several phenotypic defects characteristic of loss of signaling through the HER family, specifically loss of EGFR signaling (57, 107, 108), including the open-eye phenotype, altered eyelid, hair and whisker development (109, 110), aberrant heart valve development (111, 112), and defects in mammary morphogenesis (113). Subsequently, numerous HER family ligands have been identified as ADAM17 targets, including NRG-1, TGF-α, heparin-binding EGF, and amphiregulin (43, 114–116).

ADAM17 has been linked to diverse inflammatory diseases (117, 118) and to diseases characterized by altered epithelial permeability (73, 119, 120), including ALI. Blocking ADAM17 attenuates IL-1β-mediated increases in epithelial permeability through decreasing NRG-1 shedding and subsequent HER2 activation (43). Targeting NRG-1 shedding using a pharmacologic ADAM17 blocker decreases NRG-1 shedding, HER2 activation, and lung injury in mice (43). In contrast, mice lacking tissue inhibitor of metalloprotease 3, the endogenous inhibitor of ADAM17, exhibit delayed resolution of lung injury (121), supporting the concept that enhanced ADAM17 function can be pathogenic in ALI.

HER SIGNALING AND MECHANISMS REGULATING EPITHELIAL PERMEABILITY

The mechanisms through which HER family proteins influence epithelial repair are unclear; however, knowledge of HER-mediated signaling in other systems suggests several potentially relevant pathways. HER family receptors participate in the pathophysiology of many epithelial malignancies. Given that epithelial cancers and inflammatory injury to the epithelium are marked by loss of epithelial cell polarity, alterations in cell–cell adhesion, increased cell migration, enhanced cell proliferation, and epithelial–fibroblast cross talk (119, 122), signaling identified in the process of HER-mediated oncogenesis might be instructive in identifying key signaling mechanisms through which these receptors participate in epithelial injury and repair in ALI. Two proteins that modulate epithelial cell–cell adhesion and permeability, the Rho GTPase Rac and transforming growth factor (TGF)-β, have been implicated in HER signaling in malignancies, suggesting that these proteins might participate in HER signaling in nontransformed cells.

The Rho family of GTPases has been linked to processes central to epithelial barrier function and wound healing, including cell–cell adhesion, cell proliferation, and cell migration (123). This group of GTPases is a subfamily of the Ras family of GTPases (124). Although there are numerous members of the Rho family, the most widely studied include RhoA, Rac, and cdc42. In breast cancer cells, HER2 activation leads to Rac activation, which drives cell proliferation (125). Exogenous NRG-1 activates Rac in an HER2-dependent fashion, and Rac inhibition blocks NRG-1-mediated cell migration (126). Montero and colleagues demonstrated that NRG-1-mediated Rac activation is via the upstream activating protein P-Rex1, and down-regulating P-Rex1 blocked NRG-1-mediated Rac activation as well as cell migration and proliferation (127). Similarly, EGFR activation leads to Rac activation and Rac-dependent cell migration (128). Additionally, Rho activation has been observed after HER family activation, particularly during wound closure. In a corneal epithelial wound model, scratch injury triggers Rho activation through EGFR signaling. Rho inhibition decreases cell migration and proliferation, resulting in delayed wound closure (129).

The cytokine TGF-β is another potential signaling intermediary for HERs, specifically HER2, and epithelial injury. TGF-β is a growth factor that, like HER2, regulates numerous processes relevant to epithelial cell permeability, including proliferation, apoptosis, and migration (74). Human and murine studies have demonstrated a connection between TGF-β and HER2 signaling to ALI and fibrotic lung disease pathophysiology (130, 131). Like HER2, TGF-β increases pulmonary epithelial permeability and is required for the increased epithelial permeability after IL-1β (43, 86, 132). Mechanistic studies demonstrate that HER2 and TGF-β synergize to induce an augmented, promigratory phenotype in epithelial cells (133). Exogenous TGF-β or expression of a constitutively active TGF-β receptor increases HER2 activation (134), and TGF-β-induced cell motility and wound closure in epithelial cells is HER2 dependent (135). This effect requires the HER2 adapter protein ShcA (136) as well as enhanced TGF-β–HER2-mediated PI3K activation and involves colocalization of HER2 with Rac1, Pak1, cell surface integrins, and the actin cytoskeleton at the leading edge of cell membrane protrusions in migrating cells (137, 138). This effect is, at least partly, the result of TGF-β-mediated ADAM17 activation resulting in the release of HER family ligands with subsequent HER2 activation (134). The signaling appears bidirectional because epithelial cells expressing a constitutively active HER2 mutant have up-regulated TGF-β expression and signaling, an effect that is Rac dependent (139). These cells also have altered morphology consistent with epithelial to mesenchymal transition, a cellular phenotype that is associated with a fibroproliferative response. Further studies are required to determine whether HER2 and TGF-β cooperate in the setting of lung injury and repair.

At the cell junction, evidence suggests that HER receptors might influence cell–cell adhesion through interacting with AJC proteins. Activation of EGFR/HER1 leads to changes in claudin expression and to rearrangement of AJC proteins, including claudins and ZO-1 (140, 141). NRG-1–HER2-mediated changes in epithelial permeability are also associated with alterations in cellular localization of the tight junction proteins ZO-1 and occludin (142, 143). Integrin β4, an adhesion molecule that regulates cell–cell junctions (144) and epithelial proliferation (145), amplifies HER2 activation in epithelial cells to activate STAT and disrupt cell–cell adhesion (146).

Conclusions

In ALI, current evidence demonstrates that HER activation in epithelial cells regulates processes central to barrier function, including cell–cell adhesion, proliferation, migration, and differentiation. This evidence allows us to propose a model whereby inflammatory cytokines such as IL-1β induce protease-mediated ligand shedding, which is followed by autocrine or paracrine HER receptor activation in the epithelium. This activates signaling pathways, possibly involving Rac and TGF-β, with subsequent modulation of AJC proteins and epithelial cell–cell adhesion (Figure 2).

Several questions regarding the role of HER pathways in ALI remain unanswered. Perhaps most pressing is whether alveolar epithelial HER activation in ALI exacerbates injury resulting in increased permeability and fibrosis or is a compensatory response that promotes wound repair. One possible answer is that HER activation can lead to a restorative or an injurious phenotype, depending on the degree of activation (controlled versus unregulated) and the timing of activation in the injury process. Alternatively, it is possible that HER activation increases epithelial permeability as an initial, required step in the process of epithelial wound repair. Wound healing in ALI requires coordinated cellular responses, including proliferation, migration, and differentiation, to repopulate the alveolar epithelium.

Figure 2. Proposed model of HER2/3 signaling in epithelial cells. Injurious stimuli, including IL-1 β , induce protease-mediated ligand shedding, which is followed by autocrine or paracrine epithelial HER receptor activation. This induces activation of intracellular signaling pathways, including TGF- β and Rac, with subsequent modulation of tight junction and adherens junction proteins and regulation of epithelial cell–cell adhesion. IL-1 β activation of HER2/3 through shedding ADAM17-induced shedding of neuregulin (NRG)-1 serves as a prototypical pathway.

HER activation leads to cell migration and proliferation, both of which require an initial loss of cell–cell adhesion. Although alterations in cell–cell adhesion might exacerbate edema in an injured lung, these events may be a required first step in wound repair to restore barrier function. Loss of epithelial barrier function and edema might further promote wound repair by allowing passage of other cell types and proteins into the wounded area. Increased epithelial permeability permits migration of neutrophils, which stimulates repair of the pulmonary epithelium (19). NRG-1–HER2 signaling also increases expression of the water channel aquaporin 5 in pulmonary epithelial cells (147), suggesting a possible role in fluid reabsorption in the injured lung. Although initial increases in permeability might be tolerable in certain tissues (e.g., skin), the lung is more sensitive to the effects of edema with resultant hypoxemia and organ dysfunction. Further studies are required to delineate the timing of alveolar epithelia HER activation in ALI and how HER signaling at different time points influences repair and restoration lung function.

Additional important areas of uncertainty include whether different cell types, such as neutrophils, macrophages, fibroblasts, and endothelial cells, can serve as sources of HER ligands and affect receptor activation. How proteases such as ADAM17, which cleaves and sheds multiple HER ligands, are activated and what determines protease–ligand specificity in the instances where multiple ligands are expressed by a given cell type is largely unknown. Finally, whether therapies targeting the protease, the ligand, or the receptor would be beneficial in patients

with ALI remains unstudied. Recent discoveries suggest that targeting RTKs can be beneficial in previously untreatable lung diseases, including pulmonary fibrosis (147). Drug treatments of ALI are extremely limited. Confirmation of HER pathway activation in ALI would immediately open the door to a host of existing pharmacological agents targeting the different HER receptors and their pathways.

Author disclosures are available with the text of this article at www.atsjournals.org.

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