Keratinocyte growth factor improves alveolar barrier function: keeping claudins in line

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Keratinocyte growth factor (KGF) has myriad effects on alveolar epithelial cells with the potential to prevent lung injury and improve repair including: mitogenic activity, stimulating cell migration, promoting surfactant production and improving lung fluid clearance (9, 24). In fact, KGF pretreatment has proven to have a protective effect in animal models of pulmonary fibrosis and acute lung injury. Thus, there has been considerable interest in pursuing KGF as a therapeutic approach to treating human lung disease.

In this issue, LaFemina et al. (17) demonstrated that KGF enhanced the barrier function of primary rat alveolar epithelial cells in culture. This effect was primarily due to cytoskeletal reorganization induced by KGF. Cells cultured in the absence of KGF had actin organized into stress fibers traversing through the cytoplasm, however, filamentous actin in cells treated with KGF was primarily peri-junctional (Figure 1). This has a two-pronged effect on alveolar epithelial barriers, since the disassembly of stress fibers helps stabilize intercellular cell contacts by reducing cell contractile forces and peri-junctional bundles of actin directly fortify intercellular junctions (15).

Several classes of intercellular junctions interact with actin, including tight junctions which directly regulate diffusion across paracellular barriers between cells (3). Peripheral scaffold proteins, primarily Zonula Occludens (ZO)-1 and ZO-2, act as intermediaries to crosslink actin with transmembrane tight junction proteins, including claudins and occludin (Figure 1). There are nearly two dozen mammalian claudins; the subset of claudins expressed by a given cell dictates the extent and charge selectivity of

tight junction permeability (4). However, claudins do not provide a functional paracellular barrier unless they are assembled into tight junctions tethered to the actin cytoskeleton (22, 27).

Alveolar epithelial cells have a particularly complex pattern of claudin expression (6, 16) which is regulated in response to sepsis, inflammation and acute lung injury (7, 11, 26). Given this, LaFemina et al. (17) performed a careful analysis of claudin expression using highly purified type I and type II alveolar epithelial cells isolated directly from rat lung. At the level of mRNA, the three major claudins expressed by both type I and type II cells were claudin-3, claudin-4 and claudin-18. However, at least seven different claudins were detectable by immunoblot. The pattern of claudin expression for alveolar epithelial cells derived from cultured type II cells was comparable to *bona fide* type I cells, which further confirms the utility of this approach to study most aspects of alveolar epithelial barrier function.

Importantly, the direct analysis of *bona fide* type I and type II cells enabled confirmation of previous reports that claudin-3 is highly enriched in type II cells as compared with type I cells (16, 23). In fact, by immunoblot, type II cells contained 17 times more claudin-3 protein than type I cells (17). The functional significance of enhanced claudin-3 expression by type II cells remains obscure at present. Considering that in the normal alveolus most tight junctions formed by type II cells will also involve type I cells, one intriguing possibility is that the presence of claudin-3 in heterotypic

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junctions might confer unique permeability characteristics for type II – type I cell junctions. Future work is needed to determine whether this is the case.

LaFemina et al (17) found that KGF significantly improved the barrier both to rapid ion diffusion (measured as increased transepithelial resistance (TER)) and to the paracellular diffusion pathway used by small molecules. Since TER is largely controlled by the claudin composition of tight junction strands, it was surprising that the increase in TER induced by KGF treated alveolar epithelial cells was not associated with any changes in claudin expression. The ability of KGF to enhance alveolar epithelial cell TER was also unexpected given previous studies demonstrating that KGF did not prevent a decrease in airway epithelial TER induced by hydrogen peroxide (5). However, KGF does enable airway epithelial cells treated with hydrogen peroxide to retain the barrier to paracellular albumin diffusion, by stabilizing the actin cytoskeleton (5, 25), comparable to the effect on alveolar epithelial cells (17).

One possible explanation for the effect of KGF on alveolar epithelial TER is that the increase in cortical actin induced by KGF may induce changes to the assembly of tight junctions with the potential to alter claudin function. However, these changes must be subtle, since they were undetectable by total cell immunoblot analysis of tight junction protein expression or at the level of immunofluorescence microscopy.

Moreover, actin remodeling and cell contractility through the Rho kinase and myosin light chain kinase (MLCK) pathways have an adverse effect on tight junctions

(15). In fact, KGF improves airway barrier function through inhibition of Rho kinase

activity (10). Whether KGF improves alveolar epithelial barrier function by inhibiting

Rho kinase remains to be determined. Recent studies where cultured human alveolar

epithelial cells were challenged with a mixture of pro-inflammatory hormones (cytomix)

to disrupt tight junctions suggest that MLCK may play a more prominent role than Rho

kinase in modulating alveolar barrier function (11). In that study, cytomix caused a

significant, specific increase in claudin-18 internalization by alveolar epithelial cells

which was antagonized by MLCK inhibitors, but not Rho kinase inhibitors.

KGF generally promotes maintenance of the type II cell phenotype (1, 14, 19, 20),

so it was unexpected that KGF did not induce cultured alveolar epithelial cells to have a

pattern of claudin expression more in line with type II cells; the comparable level of

claudin-3 expression in either the presence or absence of KGF was particularly striking

(17). However, since KGF preserved the expression of a type II cell marker (RT270),

and prevented expression of a type I cell marker (RT140), claudin expression is clearly

dissociable from other aspects of alveolar epithelial phenotype. This also underscores the

concept that type I and type II cells are likely to represent extreme endpoints of a

spectrum of alveolar epithelial phenotypes (12, 13) and that the effect of KGF on alveolar

epithelial cell phenotype will be influenced by cell microenvironment (e.g. extent and

type of injury) or as mimicked by cell culture conditions.

Studies using mesenchymal stem cells (MSCs) have provided another clue for the

potential of KGF to prevent acute lung injury. Using an isolated, perfused human lung

model challenged with endotoxin, MSCs administered intratracheally 1 hour after endotoxin prevented disruption of the pulmonary endothelial barrier and promoted ENaC mediated alveolar fluid clearance (18). Conditioned medium from MSCs had a similar effect. However, MSCs treated with siRNA to interfere with KGF production blunted this effect, consistent with a protective effect of KGF. The results of LaFemina et al. (17) suggest that KGF may also promote alveolar barrier function; whether this occurs in the intact, stressed lung remains to be determined. It will also be important to define the therapeutic window of opportunity for KGF to be effective, since clinical efficacy ideally requires patients to be treated hours to days after the initial onset of acute lung injury.

MSCs produce several other cytokines in addition to KGF (8, 21). In fact, a recent study identified a key role for angiopoietin-1 secreted by MSCs in protection of alveolar epithelial barrier function from the effects of pro-inflammatory hormones by stabilizing claudin-18 incorporation into tight junctions (11). This raises the possibility that KGF may require other co-factors for optimal activity. If this is the case, one can envision combination therapy using tailored hormone mixtures. However, the potential for MSCs to home to sites of active injury may provide a more effective approach to targeted, localized paracrine tissue repair (8, 21). Moreover, strategies based on engineering MSCs to have increased KGF secretion represent another alternative to systemic hormone treatment. Aguilar et al. (2) recently showed that MSCs transfected with a tetracycline inducible KGF construct partially protected mice from bleomycin induced pulmonary fibrosis; transfected hematopoetic stem cells had significantly higher protective potential. Given recent progress using natural MSCs, it is tempting to

speculate that MSCs engineered for inducible KGF expression might prove useful in
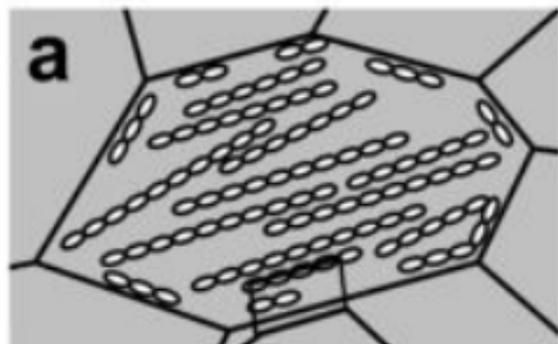
treatment of acute lung injury.

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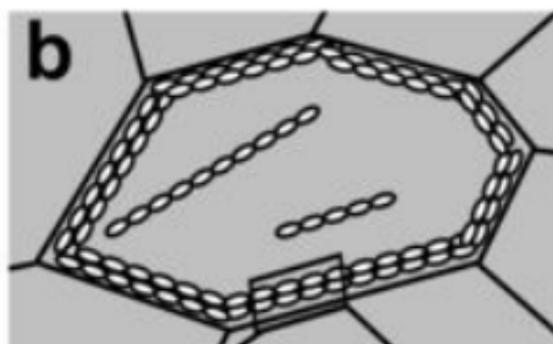
Figure Legend

Figure 1. Cytoskeletal rearrangement induced by KGF treatment of alveolar epithelial cells. a, b. Diagram indicating the orientation of actin filaments in cells cultured in control medium forming stress fibers (a) or in the presence of KGF which are predominantly peri-junctional, cortical actin (b). c,d. Attachment of transmembrane tight junction proteins (claudins, occludin) to the actin cytoskeleton via the scaffold protein ZO-1. Increased peri-junctional bundles of actin fibers provide more ZO-1 attachment sites which stabilize tight junction strands, thus promoting their barrier function. Not depicted in this diagram are other classes of scaffold and transmembrane proteins which also crosslink tight junctions to the cytoskeleton.

actin stress fibers

a

+ KGF
cortical actin

b

cell monolayer

c

cell 1

d

actin
ZO-1
claudins
occludin

cell 2

tight junction
complex