Review Article

Repair of the airway epithelium: Potential opportunities for therapeutic intervention in airway disease

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ABSTRACT

The epithelium of the airway has considerable capacity to undergo repair. These repair processes are responsible for maintaining and restoring function after injury of the airways, which likely occurs often as a result of infectious, toxic or other exposures. However, repair processes need not be fully effective. Inadequate repair can take a number of forms and may result in metaplasia, hyperplasia or fibrotic scarring. It is likely that repair processes that lead to tissue disruption play an important role in the chronic airways diseases, such as asthma and bronchitis. Through delineation of these processes, it may become feasible to target therapeutic interventions in order to achieve more effective maintenance of tissue structure.

Key words: fibrosis, hyperplasia, metaplasia, transforming growth factor-β.

INTRODUCTION

The airway epithelium possesses considerable capacity to repair following injury. This repair involves an orderly sequence of events. As demonstrated by Keenan et al. and by Lane, within 12 h of mechanical abrasion of the trachea the denuded airway epithelial surface is recovered by epithelial cells. These cells are markedly different from normal epithelial cells; rather than being columnar or cuboidal, they are exceedingly flattened. Following this initial re-epithelialization, cellular proliferation begins. In contrast with the relatively low rate of cellular proliferation in the normal epithelium, where 5% or less of cells may be dividing, nearly every cell in an epithelial wound may be dividing at 24 h. As a result, cells accumulate and differentiation begins. The cells acquire a columnar phenotype over 3 days and eventually acquire features of the differentiated pseudostratified columnar epithelium, including secretory granules and cilia.

The initial cells present in the wound possess basal cell markers. These markers are expressed by cells that proliferate and by columnar cells present at early stages in the differentiation process. However, over 2 weeks cytokeratin 14, which is a marker of basal cells, becomes restricted to cells that line the basement membrane. In contrast, the columnar cells that are present by day 3 gradually assume positive staining for cytokeratin 18, a marker of mature columnar cells, while losing positivity for cytokeratin 14. This progression suggests that basal cells are recruited into a wound where they de-differentiate to cover the wound defect, proliferate and then subsequently differentiate into both basal cells and normal columnar epithelial cells. However, the alternative possibility that columnar cells on the edge of the wound de-differentiate, acquire basal cell markers and migrate to participate in wound repair is also consistent with available data.

That airway epithelial cells have the capacity to migrate into a wound is consistent with in vitro observations on the chemotactic migration of these cells. Airway epithelial cells, for example, are capable of migration towards a number of stimuli, including insulin, fibronectin and fragments of extracellular matrix (ECM). Fibronectin is a product of airway epithelial cells and this creates the possibility that airway epithelial cells can produce a chemotactic factor for neighboring epithelial cells. The ability of airway epithelial cells to produce fibronectin can be modulated by transforming growth factor (TGF)-β,
which can increase fibronectin production by epithelial cells by five- to 10-fold.\textsuperscript{8,9} Because airway epithelial cells can both produce TGF-\(\beta\)\textsuperscript{10-12} and, at least in vitro, release it in its active form,\textsuperscript{10} this creates the possibility that airway epithelial cells can release TGF-\(\beta\) that can function in an autocrine or paracrine manner to stimulate epithelial cell fibronectin production which, in turn, can lead to the chemotactic recruitment of neighboring epithelial cells into a wound (Fig. 1).

The migration of airway epithelial cells is a complex process that takes place on an ECM surface. That is, airway epithelial cells must be attached to a surface over which they must crawl.\textsuperscript{7} Therefore, migration of the cells involves the ability of the leading edge of the cells to advance, spread and attach to ECM components while the trailing edge must de-attach. The nature of the surface can affect cell migration. Specifically, epithelial cells migrate more rapidly over interstitial ECM components than they do over basement membrane components.\textsuperscript{7} While the processes by which epithelial cells attach to ECM are both complex and incompletely understood, it is likely that cell surface integrins play a prominent role.\textsuperscript{13-15} Consistent with this, migrating epithelial cells differentially express integrins during and following wound repair.\textsuperscript{16} It is also of interest that TGF-\(\beta\) can dramatically affect cell surface integrin expression, increasing the expression of these adhesive receptors.\textsuperscript{17} Moreover, TGF-\(\beta\)-exposed airway epithelial cells are markedly more adhesive than are control cells.\textsuperscript{18} Interestingly, TGF-\(\beta\)-exposed cells are also significantly less responsive to chemotactic stimuli.\textsuperscript{19} This suggests the very interesting possibility that TGF-\(\beta\) released centrally in a wound could function as an autocrine or paracrine factor to cause the adhesive spreading of cells within the wound, preventing them from exposing more epithelium while at the same time driving these cells to produce fibronectin, thus mediating the recruitment of additional cells to help repair a defect (Fig. 1).

The ability of epithelial cells to respond in the repair process can be modulated by other cytokines present in the inflammatory milieu. In this respect, interleukin (IL)-4, a characteristic Th2-derived cytokine, can inhibit TGF-\(\beta\)-induced production of fibronectin.\textsuperscript{19} In contrast, cytokines derived from cultured mononuclear phagocytes render airway epithelial cells more responsive to chemotactic stimuli.\textsuperscript{20} Thus, the nature of the repair response in the airway epithelium likely will depend not only on the airway epithelial cells, but also on the presence and state of activation of other inflammatory cells.

Once recruited to sites of injury, airway epithelial cells must proliferate. In this regard, epithelial cells will proliferate in response to a number of growth factors\textsuperscript{21,22} and

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Fig. 1 Model of epithelial repair following injury. Following loss of epithelial cells, basal cells remaining within the wound produce active transforming growth factor (TGF)-\(\beta\) (a). This TGF-\(\beta\) functions to increase the adhesivity of nearby cells within the wound, inducing them to spread and cover the wounded area. Transforming growth factor-\(\beta\) also induces the production of increased amounts of fibronectin (b). This fibronectin, in turn, can recruit cells from the edge of the wound that can also assist in repair of the defect (c). Following this recruitment process, cells can proliferate and differentiate (see text for details).
\end{center}
their proliferation can be driven by supernatant medium from cultured mononuclear phagocytes or from fibroblasts. This suggests that, as with chemotaxis, the proliferative response of airway epithelial cells is likely to be regulated by a number of components within an inflammatory/repairative milieu. Proliferation of airway epithelial cells is also regulated by the ECM. In this regard, the composition of the ECM may be an important determinant of both the rate of epithelial cell proliferation and the responsiveness of epithelial cells to growth factors. Following accumulation, airway epithelial cells must differentiate. This process likely involves the selective loss of cells through apoptosis, a process that can be modulated by epithelial cell–matrix interactions. The precise mechanisms that govern airway epithelial cell differentiation remain to be determined, but it is likely that interactions with ECM, the presence of cytokines and, possibly, the presence of the air interface on the airway surface all contribute. If differentiation proceeds satisfactorily, airway epithelial integrity can be restored. In contrast, if re-epithelialization is incompletely effective, hyperplastic and metaplastic lesions can develop.

In addition to airway epithelial cells, the mesenchymal cells within the airway wall can participate in the repair response. Overexuberant recruitment, proliferation and activation of these cells can result in the accumulation of peribronchiolar connective tissue. Like scar tissue anywhere, these tissues can contract and can result in airway narrowing. This peribronchiolar fibrosis is thought to be a major anatomic correlate of airflow limitation in individuals with moderately severe chronic obstructive airway disease (COPD). Similar lesions may account for fixed airflow limitation in patients with chronic asthma. Taken together, these processes suggest a number of potential opportunities to intervene in order to achieve a more favorable outcome in airway disease. Specifically, it may be possible to augment the normal reparative process leading to restoration of epithelial integrity. Alternatively, it may be possible to inhibit the development of peribronchiolar fibrosis. With respect to this latter possibility, peribronchiolar fibrosis could be blocked either by inhibiting the release of mediators that drive the fibrotic response or by directly inhibiting fibroblast responses. In vitro evidence suggests that several agents commonly used to treat airway disease may be active in such pathways.

Many cells can release mediators that can drive fibrotic responses, including lymphocytes, mononuclear phagocytes, mast cells, fibroblasts and epithelial cells. In addition, profibrotic mediators can be generated by activation of mediators present in the extracellular milieu. Among the cells that have the capability of participating in driving a fibrotic response are the epithelial cells, which appear to play an important role in normal repair as well. Airway epithelial cells can release mediators that can drive many aspects of the fibrotic response, including fibroblast recruitment, proliferation, matrix production and matrix remodeling (Fig. 2).

Fibronectin released by airway epithelial cells can recruit fibroblasts as well as other epithelial cells. It is possible that this pathway could be susceptible to therapeutic manipulation. Specifically, agents that increase fibroblast cAMP, including prostaglandin E (PGE) and β-adrenergic agonists, appear to block the chemotactic response of fibroblasts towards fibronectin. Inhibiting fibroblast recruitment may have a number of advantages as this recruitment process may select for fibroblasts with specific synthetic capabilities. In vitro systems, fibroblasts that migrate towards fibronectin are enriched for α-smooth muscle actin and, thus, have properties of myofibroblasts.

Epithelial cells also have the capability of releasing activities that can drive fibroblast proliferation. These activities appear to be heterogeneous and several growth factors are likely to be involved. The ability of fibroblasts to respond to these growth factors can be blocked directly by PGE. The release of these factors can be attenuated by glucocorticoids.

Airway epithelial cells can also drive fibroblast matrix production, at least in part, by releasing TGF-β. The production of matrix components can also be inhibited. Again, agents that increase fibroblast cAMP levels have an anti-fibrotic effect, inhibiting the production of type I collagen. Glucocorticoids also have a modest effect in inhibiting type I collagen produced by fibroblasts under basal conditions, although they appear to have little effect on fibronectin production (SI Rennard, unpubl. obs., 1994). In contrast to their modest effect on basal rates of fibroblast type I collagen production, glucocorticoids can completely inhibit the TGF-β-driven augmentation of fibroblast type I collagen production (SI Rennard, unpubl. obs., 1994). Interestingly, glucocorticoids appear to have no effect on epithelial cell-driven fibroblast fibronectin production (DJ Romberger, unpubl. obs., 1994). This raises the possibility that glucocorticoids may change the relative composition of ECM by changing the ratio of matrix components.
Fig. 2  Epithelial cell-derived factors and peribronchiolar fibrosis. Mediators released by epithelial cells are capable of driving all aspects of the fibrotic process, including fibroblast recruitment, proliferation and matrix production and remodeling. Interestingly, some of the same mediators involved in epithelial repair, such as fibronectin and transforming growth factor (TGF)-β can also lead to mesenchymal cell recruitment and activation. The effects of potential therapeutic agents can be variable. Glucocorticoids, for example, have no effect on fibroblast recruitment, can inhibit the release of growth factors from epithelial cells, can inhibit the TGF-β-induced stimulation of collagen production by fibroblasts and can augment fibroblast induced matrix contraction (see text for details).

Once ECM components are produced, they can undergo extensive remodeling. This remodeling process, which is believed to be mediated at least in part by the ability of mesenchymal cells to contract the ECM, is a process that can also be regulated by exogenous factors. Transforming growth factor-β derived from airway epithelial cells, for example, can result in augmented contraction. β-Adrenergic agonists can inhibit this contraction. Interestingly, glucocorticoids augment fibroblast mediated contraction of ECM. Thus, β-adrenergic agonists and other agents that increase fibroblast cAMP appear to inhibit all aspects of fibroblast responses that may be considered pro-fibrotic. Glucocorticoids, in contrast, do not appear to affect chemotactic recruitment, while they can inhibit proliferation. Glucocorticoids can inhibit the production of type I collagen, but not of other matrix components, such as fibronectin. Finally, glucocorticoids can exert a pro-fibrotic response augmenting fibroblast-mediated collagen gel contraction. This suggests that the effects of some agents, such as glucocorticoids, on the fibrotic response may differ for various parts of the fibrotic response (Fig. 2). Perhaps it is such mixed effects that account for the variable therapeutic utility of glucocorticoids in fibrotic conditions.

The assessment of any therapeutic intervention in modulating peribronchiolar fibrosis in chronic obstructive pulmonary diseases will be exceedingly difficult to demonstrate clinically. This is due to the relatively slowly progressive nature of these disorders and the relatively imprecise measurements that can be made in order to follow progression; that is, forced expiratory volume in one second (FEV₁). It has been estimated that, in order to demonstrate a therapeutic benefit of an intervention to modify the natural history of COPD, over 1000 patients would have to be followed for at least 3 years at a cost of greater than Canadian $20 million. For this reason, the ability to assess intermediate end-points will have great utility in designing clinical strategies. With regard to the
fibrotic response, this raises a number of potential points of interest. Specifically, the production of mediators relevant to the fibrotic response may be addressed in tissue samples by either immunohistochemical or in situ hybridization methods. Similarly, the responsiveness of cells to pro-fibrotic mediators could be assessed using either markers of cellular proliferation or markers of matrix production, such as immunohistochemistry for procollagen peptides. Assessment of soluble markers of matrix biosynthesis would also be a reasonable possibility.

The ability to manipulate the cellular and biochemical responses that underlie both normal and abnormal repair in the airway are beyond current clinical capabilities. Nevertheless, these represent important avenues for future investigations. Current understanding of these processes can permit the in vitro assessment of potential therapeutic interventions. Recent advances along these lines raise the possibility that such potential interventions could be tested, at least with respect to proof concept, in human disease in vivo. Ultimately, however, it will be necessary for such interventions to be tested in rigorous clinical trials. Considering that no effective therapies are currently available that can alter the relentlessly progressive natural history of COPD, the development of such therapeutic interventions should remain a high priority.

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