THE EFFECT OF PULMONARY FIBROSIS CAUSED BY NITROGEN DIOXIDE ON THE MORPHOLOGICAL PARAMETERS OF THE ESOPHAGEAL WALLS AND THEIR CORRECTION

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Annotation*. An understanding of the mechanisms underlying pulmonary fibrosis remains elusive. Once believed to result primarily from chronic inflammation, it is now clear that inflammation and chronic fibrosis, especially in diseases such as idiopathic pulmonary fibrosis/usual interstitial pneumonia, are often dissociated, and that inflammation is neither necessary nor sufficient to induce fibrosis. The origin of the primary effector cell of fibrosis in the lung, the myofibroblast, is not clearly established. Three potential sources have been hypothesized. Although conversion of resident fibroblasts and differentiation of circulating bone marrow–derived progenitors likely play a role, the possible contribution of alveolar epithelial cells (AECs), through a process termed "epithelial–mesenchymal transition" (EMT), has only recently received consideration. A process by which epithelial cells lose cell–cell attachment, polarity and epithelial-specific markers, undergo cytoskeletal remodeling, and gain a mesenchymal phenotype, EMT plays a prominent role in fibrogenesis in adult tissues such as the kidney. This review summarizes the evidence supporting a central role for EMT in the pathogenesis of lung fibrosis, the potential for EMT in AECs in vitro and in vivo and role of transforming growth factor-β1 in this process, and the implications of epithelium-driven fibrosis on future research and treatment. Potential pathways involved in EMT are also discussed. It is hoped that a major shift in current paradigms regarding the genesis of pulmonary fibrosis and dissection of the relevant pathways may allow development of targeted interventions that could potentially reverse the process and ameliorate the debilitating effects of abnormal repair and progressive fibrosis.*

Keywords: *alveolar epithelium; epithelial–mesenchymal transition; lung injury; transforming growth factor-β.*

АЗОТ ДИОКСИДИДАН КЕЛИБ ЧИҚҚАН ЎПКА ФИБРОЗИНИНГ ҚИЗИЛЎНГАЧ ДЕВОРЛАРИНИНГ МОРФОЛОГИК ПАРАМЕТРЛАРИГА ТАЪСИРИ ВА УЛАРНИ ТУЗАТИШ

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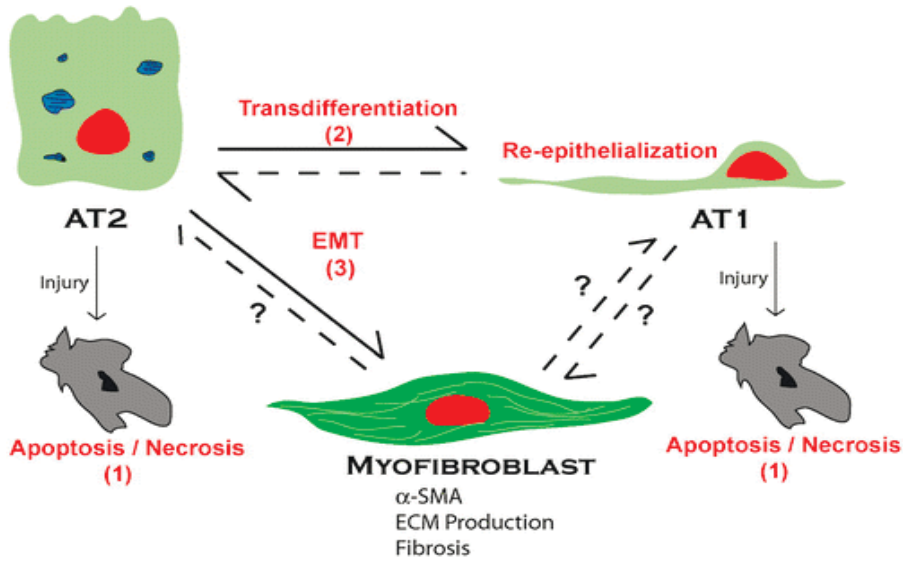
Аннотация. *Ўпка фибрози асосидаги механизмларни тушуниш қийин бўлиб қолмоқда. Бир пайтлар асосан сурункали яллиғланишдан келиб чиқади деб ишонилган бўлса, энди яллиғланиш ва сурункали фиброз, айниқса идиопатик ўпка фибрози одатдаги интерстициал пневмония каби касалликларда тез-тез ажралиб туриши ва яллиғланиш фиброзни қўзғатиш учун зарур эмас ва этарли эмаслиги аниқ. Ўпкада фибрознинг бирламчи эффектор ҳужайраси миофибробластнинг келиб чиқиши аниқ белгиланмаган. Учта потенциал манбалар фараз қилинган. Резидент фибробластларнинг конверсияси ва айланма суяк илигидан олинган прогениторларнинг дифференциацияси эҳтимол рол ўйнайди алвеоляр эпителия ҳужайралари (аесс), "эпителиал–мезенхимал ўтиш" (ЭМЎ) деб номланган жараён орқали яқинда кўриб чиқилди. Эпителиал ҳужайралар ҳужайра–ҳужайра бирикмасини, поларитесини ва эпителияга хос белгиларини йўқотадиган, ситоскелетал ремоделингдан ўтадиган ва мезенхимал фенотипга эга бўладиган жараён, ЕМТ буйрак каби катталар тўқималарида фиброгенезда муҳим рол ўйнайди. Ўпка фиброзининг генезиси ва тегишли йўлларни ажратиш билан боғлиқ ҳозирги парадигмаларнинг катта ўзгариши жараённи тескари йўналтириши ва ғайритабиий таъмирлаш ва прогрессив фибрознинг заифлаштирувчи таъсирини яхшилаши мумкин бўлган мақсадли аралашувларни ривожлантиришга имкон беради деб умид қиламиз.*

Калит сўзлар: *альвеоляр эпителий; эпителиал–мезенхимал ўтиш; ўпкалар шикастланиши; β-трансформацияловчи ўсиш омили.*

Changes in paradigms, although slow and usually difficult, are fundamental to most significant advances in science. Such a paradigm change may be underway in the current investigation into mechanisms of chronic injury and fibrosis in the lung. Historically, idiopathic pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP) has been viewed as the result of ongoing inflammation and cellular injury, with subsequent activation and proliferation of resident mesenchymal elements in the lung (1). Obser-

vations from both animal models of fibrosis and patients with IPF have led to recent reassessment of the concept that inflammation is the major pathogenic event in IPF. Inflammation is not a prominent feature in IPF biopsies and antiinflammatory therapies have shown little benefit in the treatment of IPF. αvβ6 integrin knockout mice that cannot activate transforming growth factor (TGF)-β1 develop an exaggerated inflammatory response to bleomycin, yet have near-complete attenuation of the fibrotic response, indicating that inflammation and fibrosis can be dissociated (2). These and other observations have contributed to the evolving view that IPF is a disorder that involves abnormal wound healing, and that ongoing epithelial injury and/or activation may lie at the heart of fibrogenesis and mesenchymal cell proliferation, independent of inflammation. This hypothesis has been advanced in a number of recent reviews (3, 4). This article outlines the evidence that supports a central role for the alveolar epithelium in the development of IPF and reviews recent data that suggest that, more than just serving as the pathogenic instigator, alveolar epithelial cells (AECs) themselves may acquire a mesenchymal phenotype and serve as an important source of fibroblasts and myofibroblasts through a process known as epithelial–mesenchymal transition (EMT). Under this new paradigm, the alveolar epithelium should be viewed as one of the key participants in fibrosis, serving as a "multipotent" progenitor with considerable plasticity and the capacity to participate in alternate pathways: re-epithelialization to restore normal alveolar architecture, apoptosis, or fibrogenesis through EMT. Understanding the factors that determine cell fate decisions of AECs along these pathways will be important in further elucidating the pathogenesis The myofibroblast is believed to play a central role in the pathogenesis of IPF. Increased numbers of fibroblastic foci are associated with disease progression and a worse prognosis in IPF/UIP (5), and the rapid development of fibrotic lesions composed of proliferating myofibroblasts and fibroblasts underlies the pathogenesis of irradiation-induced pulmonary fibrosis (6). These activated fibroblasts are characterized by a spindle or stellate morphology with intracytoplasmic stress fibers, a contractile phenotype, expression of various mesenchymal immunocytochemical markers (including, most reliably, α-smooth muscle actin [α-SMA]), and collagen production (7). They are the key mediators of extracellular matrix deposition, structural remodeling, and destruction of alveocapillary units during and after lung injury (8), and as such, knowledge of their cellular source is critical to the understanding of the pathogenesis of IPF in particular and fibrosis of the lung in general. Three hypotheses have been proposed with regard to the cellular origin of the myofibroblast. The first, and historically most prevalent, hypothesis postulates that resident intrapulmonary fibroblasts respond to a variety of stimuli during fibrogenic responses and differentiate into myofibroblasts (8). TGF-β1, a key regulator of fibrosis, induces transdifferentiation of fibroblasts in vitro through a Smad3-dependent mechanism (9). Although this hypothesis is tempting in its simplicity, an alternate hypothesis has recently been proposed that bone marrow–derived progenitors contribute to myofibroblast induction and proliferation during pulmonary fibrosis. Epperly and colleagues (6) demonstrated using transplantation of green fluorescent protein–positive bone marrow into wild-type mice that marrow-derived cells constitute 20 to 50% of cells in fibrotic areas during irradiation-induced fibrosis. Direkze and colleagues (10) demonstrated multiple organ engraftment by bone marrow–derived fibroblasts and myofibroblasts in mice after radiation injury. Consistent with these results, Hashimoto and colleagues (11) showed that collagen-producing lung fibroblasts in bleomycin-induced pulmonary fibrosis can be derived from bone marrow progenitor cells. However, these marrow-derived fibroblasts did not express α-SMA and were resistant to fibroblast–myofibroblast conversion by TGF-β1. A novel third possible source of fibroblasts and/or myofibroblasts in pulmonary fibrosis has recently been proposed: that AECs, through the process of EMT, also play a significant role. It is important to stress here that these potential sources of myofibroblasts are not mutually exclusive and the relative contribution of each source to the progression of fibrosis remains to be determined. EMT is a process by which fully differentiated epithelial cells undergo phenotypic transition to fully differentiated mesenchymal cells, often fibroblasts and myofibroblasts (12). This is a form of metaplasia, but does not always require cell division. For clarity, it is important, especially in the case of the alveolar epithelium, to distinguish this type of transition from epithelial–epithelial transdifferentiation processes, which classically refer to differentiated cells changing into other differentiated cells (13).



The process of EMT has long been known to play a pivotal role in cellular transdifferentiation during development and tumor progression. Epiblasts undergo EMT early in development to form primary mesenchyme. Secondary epithelia are created through mesenchymal–epithelial transitions. These secondary epithelia then differentiate to form fully differentiated adult epithelia, or can undergo a second round of EMT to form a variety of mesenchymal and connective tissue cells, such as adipocytes, chondrocytes, osteoblasts, myocytes, and fibroblasts (16). One of the critical aspects of EMT is the ability of epithelial cells to lose polarity, disassemble cell adhesion systems, produce cell-motility machinery, and move from one location to another (12).

Increasingly, it is being recognized that, in the adult, injury can induce epithelial cells to undergo transition to a mesenchymal phenotype, thereby contributing to fibrosis in a number of organs (17, 18). Fibroblasts and myofibroblasts that have differentiated from epithelium are commonly identified in these tissues through morphologic changes (e.g. a change from a cuboidal cell shape to an elongated or spindle-shaped form), the acquisition of fibroblast- or myofibroblast-specific markers (e.g., fibroblast-specific protein [FSP1] and α-SMA, respectively), and the loss of characteristic epithelial markers (e.g., E-cadherin and zonula occludens-1 [ZO-1]) (19). EMT has been most extensively investigated as a mechanism underlying fibrosis in renal and lens epithelium. Renal tubular epithelial cells express FSP1 (a member of the S100 family of calcium-binding proteins exclusively expressed in fibroblasts) early after injury during kidney fibrosis in transgenic mice, migrate through damaged basement membranes into the interstitium, and fully transdifferentiate into fibroblasts and myofibroblasts (16, 20). In this setting, 36% of new fibroblasts come from EMT of local epithelium (17). In vitro, rat kidney tubular epithelial cells treated with TGF-β1 lose expression of the epithelial marker E-cadherin, acquire an elongated shape, and increase expression of α-SMA (20). Lens epithelial cells have also been shown to undergo EMT in vivo and in response to TGF-β in vitro, likely via a Smad3-dependent pathway (18). Clearly, EMT may play a pivotal role in the normal differentiation processes of adult tissues and in response to injury.

In nearly every case of EMT in adult tissues, a crucial role for the stimulatory input of soluble growth factors and/or extracellular matrix components (usually collagen) has been demonstrated. Most commonly, epidermal growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factors (FGF), and especially the TGF-β family of factors are directly involved (16, 21). Under the influence of these factors, epithelial cells lose polarity, express basement membrane–degrading matrix metalloproteinases, undergo cytoskeletal rearrangements, and express machinery necessary for motility, which often leads to migration and complete transition to a mesenchymal phenotype (16, 19). Whether this transition is irreversible and represents true transdifferentiation has been debated, with some suggestion of an epithelial "phenocopy," termed "reversible scatter," being an alternative possibility (19). This reversible scatter is defined as epithelial cells undergoing partial dedifferentiation with loss of polarity and down-regulation of epithelial markers, but losing these characteristics once the inciting stimulus is removed. Importantly, cells undergoing "scatter" do not express appreciable amounts of mesenchymal markers. EGF, HGF, FGF, and TGF-β1 alone often induce a reversible scatter phenomenon. However, prolonged exposure to TGF-β1 (> 4–6 d) is a powerful inducer of complete EMT in many cases, often acting in conjunction with a variety of costimuli (including EGF and activation of the small GTPases Ras and RhoA) (22, 23). TGF-β has been implicated as a "master switch" in the induction of fibrosis in many organs, including the lung (24). Targeted expression of TGF-β1 alone in the lungs of newborn and adult rats induces a dramatic fibrotic response with minimal inflammation (25) and, as discussed above, inability to activate TGF-β1 affords significant protection

from bleomycin-induced fibrosis in transgenic mice (2). It makes intuitive sense that TGF-β1 would also play a pivotal role in the induction of EMT, given the progressively more apparent role of EMT in fibrotic processes, the key role of TGF-β1 in fibrosis, and the ability of TGF-β1 to promote loss of the epithelial phenotype. Most commonly, TGF-β1 stimulation of epithelial cells leads to the induction of Smad proteins, which serve both as transcription factors themselves and as inducers of other transcription factors, including Slug, Snail, Scatter, lymphoid enhancing factor-1, and β-catenin (16). These transcription factors lead to expression of the "EMT proteome," including the cellular machinery necessary for junctional disassembly, cytoskeletal rearrangement, and cellular motility (12). The majority of Smad-dependent target gene transcription is controlled by Smad3 (26), which partners with Smad4 on activation by TGF-β receptor serine/threonine kinases and translocates to the nucleus. EMT in many tissues, including retina, lens, and kidney, is dependent on Smad3 (18, 26).

TGF-β1 can also activate non–Smad-mediated cellular signaling pathways, most importantly involving Rho kinase, which directly activates the cellular machinery necessary for cytoskeletal rearrangement, basement membrane detachment, and E-cadherin down-regulation (27). In most cases, stimulation of these cooperative signaling pathways provides the important physiologic context that allows for induction and specification of EMT within particular tissues. Cross-talk between classical TGF-β pathways and Rho, as well as a host of other modulatory signaling molecules, including Ras, extracellular signal-related kinase (ERK), p38 mitogen-activated proteins kinase (MAPK), Notch, Wnt proteins, nuclear factor-κB, and PI3K, have all been demonstrated to affect the extent and reversibility of EMT (12).

Classically, the alveolar epithelium has been thought of as a passive bystander in the process of pulmonary fibrosis. Recently, there has been a return to the notion proposed by Adamson and colleagues (28) that ongoing AEC injury and retarded wound repair may be central to the pathogenesis of pulmonary fibrosis (3, 4). These authors demonstrated that epithelial injury and blunted epithelial repair is sufficient to promote pulmonary fibrotic processes (28). The extent of hyperoxia-induced fibrosis in cultured murine lung explants correlated directly with the degree of epithelial injury, and inflammatory mechanisms involving alveolar macrophages or polymorphonuclear cells were unnecessary. Consistent with this, AEC apoptosis is detected adjacent to myofibroblast-containing fibroblastic foci, the presumed primary sites of epithelial injury in IPF/UIP. Ongoing apoptosis is believed to be a key component in the progression of IPF/UIP (28, 29) and appears to be essential for the development of TGF-β1–induced lung fibrosis (30).

It has long been recognized that the epithelial cells overlying fibroblastic foci are hyper- and dysplastic, with abnormal morphology and gene expression patterns (1, 31). These cells secrete a variety of profibrotic cytokines, participating in a bidirectional communication network with neighboring fibroblasts whereby each cell type influences the proliferation/survival of the other. The alveolar epithelium serves as a major source of TGF-β1 and many other cytokines, including endothelin-1 and tumor necrosis factor-α, during lung injury and fibrosis (32–34), independent of proinflammatory mediators (35). Instead, changes in and activation of epithelial cells appear to be critical inciting factors in fibrosis initiation. The alveolar epithelium also regulates an intrinsic capacity to respond to TGF-β1 stimulation through differential expression of TGF-β1 receptor subtypes (36). Taken together, these data suggest that the alveolar epithelium plays a major role in the pathogenesis of lung fibrosis, with the capacity to both produce and respond to TGF-β1, regulate the function and differentiation of fibroblasts, and modify cell morphology and gene expression in response to injury, all independent of the degree of inflammation.

The exact nature of the epithelial injury in IPF/UIP is unknown, although it has been speculated that viral infection may play a role. As discussed below, for re-epithelialization to occur, AT2 cells must proliferate and differentiate into AT1 cells. In IPF, this process appears to be impaired, with detection of abnormal, hyperplastic AT2 cells with an intermediate phenotype overlying fibroblastic foci (31). Thus, depending perhaps on the degree and nature of the injury, extent of disruption of underlying basement membrane, and the exact cytokine milieu, injured AECs may face one of several choices: apoptosis; proliferation and differentiation into AT1 cells to effect re-epithelialization; or, as has been recently suggested, EMT, thereby contributing directly to fibrosis.

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ВЛИЯНИЕ ЛЕГОЧНОГО ФИБРОЗА, ВЫЗВАННОГО ДИОКСИДОМ АЗОТА, НА МОРФОЛОГИЧЕСКИЕ ПАРАМЕТРЫ СТЕНОК ПИЩЕВОДА И ИХ КОРРЕКЦИЮ

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Аннотация. *Понимание механизмов, лежащих в основе легочного фиброза, остается неясным. Когда-то считалось, что это в первую очередь результат хронического воспаления, теперь ясно, что воспаление и хронический фиброз, особенно при таких заболеваниях, как идиопатический легочный фиброз / обычная интерстициальная пневмония, часто диссоциированы, и что воспаление не является ни необходимым, ни достаточным для индуцирования фиброза. Происхождение первичной эффекторной клетки фиброза в легком, миофибробласта, четко не установлено. Были выдвинуты гипотезы о трех потенциальных источниках. Хотя конверсия резидентных фибробластов и дифференцировка циркулирующих предшественников, полученных из костного мозга, вероятно, играют определенную роль, возможный вклад альвеолярных эпителиальных клеток (AECs) посредством процесса, называемого "эпителиально–мезенхимальный переход" (ЭМП), был рассмотрен только недавно. Процесс, посредством которого эпителиальные клетки теряют межклеточную привязанность, полярность и эпителиоспецифические маркеры, подвергаются ремоделированию цитоскелета и приобретают мезенхимальный фенотип. ЭМП играет важную роль в фиброгенезе во взрослых тканях, таких как почки. В этом обзоре обобщены данные, подтверждающие центральную роль ЭМП в патогенезе фиброза легких, потенциал ЭМП при AECs in vitro и in vivo и роль трансформирующего фактора роста β1 в этом процессе, а также влияние эпителиального фиброза на будущие исследования и лечение. Также обсуждаются потенциальные пути, участвующие в ЭМП. Есть надежда, что серьезный сдвиг в существующих парадигмах относительно генеза легочного фиброза и рассечения соответствующих путей может позволить разработать целенаправленные вмешательства, которые потенциально могли бы обратить процесс вспять и ослабить изнурительные последствия аномальной репарации и прогрессирующего фиброза.*

Ключевые слова*: альвеолярный эпителий; эпителиально–мезенхимальный переход; повреждение легких; трансформирующий фактор роста-β*