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Review

Regenerative activity of the lung after epithelial injury ☆

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ABSTRACT

Lung epithelial cells use remarkably adaptive sensing and signaling systems to maintain a physiological state supporting gas exchange and minimizing environmental insults. One facet of epithelial adaptability is the reversible acquisition of mesenchymal features, a process termed epithelial–mesenchymal transition (EMT). Although in the adult, permanent and complete EMT appears rare or non-existent, a growing body of evidence implicates a critical role for the activation of EMT signaling in tissue remodeling, including fibrotic lung disease. The specific phenotypes of cells undergoing EMT re-programming during epithelial responses to injury continue to be defined and are reviewed here. Several recent studies implicate epithelial expression of canonical EMT transcription factors, such as Snail and Twist1, with the acquisition of a less differentiated, more proliferative stem-like state, providing an additional link between activation of EMT signaling and tissue repair. In lung airways, proliferating variant clara cells rely upon Snail for effective epithelial repair, and in the breast, cells possessing the greatest regenerative capacity also express Snail2. The ongoing elucidation of signaling underlying epithelial stem/progenitor expansion coincides with recent discoveries implicating regenerative activity in the lung, possibly including de novo regeneration of airway and alveolar units. It remains largely unknown what signals drive organization of epithelial progenitor cells that expand after lung injury, to what degree such organization is ever functionally relevant, and whether the lung regenerative potential recently observed in mouse models extends to humans. Yet these unknowns with clinical potential bring future mechanistic studies of EMT and lung repair directly into the field of regenerative medicine. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

1. Introduction

In addition to its position in an architectural arrangement critical to gas exchange, the lung epithelium serves the major functions of maintenance of an effective barrier against untoward fluid accumulation and protection of the lung from environmental insult. This biology involves both local constitutive activities of the epithelium such as ciliated movement, secretion of surfactants, and anti-bacterial peptides as well as sensing functions that endow the lung with the capacity to respond to potentially injurious foreign agents. In the past decades, numerous pathways of epithelial signaling have been elucidated that help explain the capacity of the lung to mount host defense against such diverse insults as infectious agents, oxidative stress, particulate matter, and leakage of blood components into the airway and alveolar compartments [1–7]. Defects in any of the epithelial defense systems are a source of pathology. Indeed there is increasing attention on the epithelium as both a site of disease initiation and a driver of disease progression [8]. In no small part this attention derives from increased understanding of the molecular mechanisms by which the epithelium maintains homeostasis and how injury leads to dysfunction that promotes disease.

The multifaceted defense systems of the epithelium are fully integrated into the complicated architecture of the lung and the innate and adaptive immune systems that operate to both respond to the epithelium and to effect inflammatory responses designed to resist infection [5,9]. A number of recent reviews summarize current thinking about how the epithelium interfaces with the immune system in defense of the lung and its role in common disorders such as asthma [10–12]. There are also a number of comprehensive reviews of the diverse signaling pathways by which the lung epithelium responds to injurious signals, in part to promote repair [13–15]. An important and evolving area of epithelial biology in the adult is the degree to which epithelial plasticity contributes to tissue repair and remodeling. By epithelial plasticity is meant the capacity for epithelial cells to change from one state of differentiation to another, either transiently or more prolonged, in a regulated manner [16]. One such change is epithelial to mesenchymal transitions (EMT) in which epithelial cells lose strong mediators of cell:cell contacts such E-cadherin (adherens junctions) and ZO-1 (tight junctions) and instead begin to express mesenchymal

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proteins such as N-cadherin, fibronectin, and collagens [17,18]. In this review we will highlight recent studies that continue to define the biology of EMT as it relates to tissue repair, the relationship of EMT to epithelial stem/progenitor cell function, and consider recent evidence that lung injury resolution also includes regeneration of new airway and alveolar structures.

2. Evolution of the concept of epithelial–mesenchymal transition

2.1. Development

Though the phenomenon of epithelial cells acquiring mesenchymal features during embryogenesis has been noted for over a century, the term epithelial–mesenchymal transition (EMT) arose in the 1980s in reports primarily from the Elizabeth Hay lab [19]. A compelling example of EMT comes from studies of gastrulation, a stage in embryonic development transitioning from the single cell layer blastula to the gastrula, comprised of the 3 major cell layers for subsequent organogenesis: endoderm, ectoderm, and mesoderm [18]. A mesenchymal transdifferentiation of epiblasts (termed mesendodermal cells) of the early Drosophila blastula is needed to initiate the process of gastrulation. This is preceded by the appearance of the transcription factors Snail1 and then Twist on a restricted domain of the ventral blastula [20]. Snail1 was identified as a transcriptional repressor whose major function is to inhibit the expression of non-mesodermal elements such as E-cadherin, thereby defining the spatial territory of mesodermal initiation [21]. In the fly, expression of Twist is also required for gastrulation and the lineage switch of epithelial to mesenchymal cell fate. Twist is not only a repressor but also directly promotes activation of a number of mesenchymal genes [22]. Other transcriptional repressors such as goosecoid are expressed in this gastrulation organizer region and support this process [18]. The ingress of this collection of Snail-expressing cells into the primitive endodermal mass initiates the formation of the mesodermal layer and generation of almost the entirety of mesenchymal structures such as skeleton, cartilage, and bone in the mature organism. Snail and Twist empower the ingressing cells to dissociate and migrate to form mesodermal structures. Many of the cardinal events and signaling programs mediating gastrulation appear to be conserved from Drosophila to humans. But gastrulation is not the only developmental process requiring EMT. Formation of the cardiac valves has been well studied as a process involving an EMT of the epicardial cells (derived from the mesodermal layer). Mesenchymal elements such as the peripheral nervous system of the adult organism also derive from neural crest cells that are epithelial in nature and undergo EMT via signaling reminiscent of gastrulation [18]. Mice homozygous for deletion of Twist die at E11.5 with failure of neural tube closure and numerous mesenchymal defects [23]. A thorough discussion of the many facets of development involving EMT biology has been previously published [18,24]. The concept of EMT thus emerged as a developmental program for a complete transdifferentiation of cells from an epithelial to mesenchymal phenotype, sometimes temporary and sometimes permanent, but apparently crucial for organogenesis. Although signaling leading to induction and maintenance of EMT at the various stages of development is quite complicated, involving induction or repression of numerous transcription factors, a relatively small set of transcription factors can be viewed as principally driving EMT: Snail1, Snail2 (Slug), Twist1, Zeb1/2, E12/E47, and Goosecoid. It is worth noting that FGF receptor signaling is important for sustaining and organizing the mesoderm following mesodermal initiation and may have a similar function in adults [25,26]. There is no evidence of EMT during lung development and no known requirement for any of the principal EMT transcription factors for branching morphogenesis or maturation of airway epithelial lineages during development. Indeed sustained activation of TGFβ1, a major inducer of the EMT program in adults, markedly suppresses embryonic development of airways and alveoli [27].

2.2. Cancer and EMT

That EMT biology is relevant in the adult derives from a series of observations, beginning with a report in 2000, indicating that human carcinomas (epithelial tumors) frequently express one or more of the principal EMT transcription factors. Snail1 was initially identified in a number of epithelial tumor cell lines as a direct repressor of E-cadherin transcription [28]. Two groups then demonstrated that breast carcinomas frequently express Snail1 and moreover that the expression of Snail1 and downregulation of E-cadherin transcription developed in invasive regions of breast tumors and in metastatic nests within lymph nodes [29,30]. Snail1 directly suppresses E-cadherin transcription in carcinomas and in turn repression of E-cadherin correlates with and in part mediates the development of a mesenchymal phenotype. Expression of Snail1 and/or Snail2 (also termed Slug) is now linked to metastasis and outcome in numerous carcinomas including breast, ovary, colon, and lung [31–34]. Twist1, in addition to Snail, is also strongly linked to metastasis and outcome in numerous carcinomas [35]. Twist expression induces an EMT phenotype that includes not only loss of cell:cell contacts via disruption of adherens junctions but also the development of altered protease expression and cytoskeletal reorganization that collectively promote increased cell motility and tumor invasion across basement membranes [22]. There is accumulating evidence that the activation of an EMT program and acquisition of an EMT phenotype is important to tumor invasion and metastasis [17,36]. The functional effects of Snail1 and Twist on tumor cells is obviously reminiscent of their effects during mesodermal development, but permanent or even temporary complete transition of carcinomas to a mesenchymal cell appears to be unusual [37,38]. Much more common is the temporary acquisition of mesenchymal features, sometimes referred to as partial EMT, at sites of tumor invasion into surrounding normal tissue and the vasculature [39,40]. The loss of epithelial and acquisition of mesenchymal features in this context appears strongly dependent on the cardinal EMT transcription factors, justifying the continued use of the term EMT applied to tumor biology.

An important feature of carcinomas is the fibroblast-rich stromal milieu surrounding and inter-digitated with tumor cells. Consistent with enhanced plasticity rather than lineage switch as a primary consequence of activation of the EMT program in cancer, there is little evidence that cancer-associated fibroblasts derive from the epithelial tumors themselves. Rather, an extensive crosstalk between tumor cells and activated stromal fibroblasts contributes to the invasive phenotype of tumors [41]. On the one hand cytokines such as TGFβ1 and PDGF, derived either from tumor cells or macrophages recruited to the tumors, activate stromal fibroblasts to not only proliferate but also produce more TGFβ1, supporting further expansion of the stromal ECM. Cytokines derived from activated stromal fibroblasts such as hepatocyte growth factor and Wnts then directly promote growth and invasion of the tumors. The stiffness acquired by an expanding ECM appears to secondarily further promote tumor invasion as the extent of stromal collagen is a strong prognostic indicator for carcinomas [42]. The molecular details of tumor–stromal crosstalk may parallel the epithelial–stromal interactions found during tissue remodeling; certainly tumor–stromal interactions elucidated by studies of tumor progression are a roadmap for further inquiry into the molecular pathways by which epithelial cells regulate tissue remodeling.

2.2.1. EMT promotes cancer cell survival

One relevant pathway of activation of the tumor EMT program is signaling via PDGF receptors. PDGF binding to its receptor is reported to activate Src kinase within breast tumors that then induces expression of Twist [43]. Twist functions not only to induce loss of tumor epithelial markers and acquisition of a mesenchymal phenotype but also to promote tumor survival and senescence resistance. Twist at least in part is reported to block oncogene-induced premature senescence, a possible consequence of a burst of epithelial proliferation, by