Has HGF met other partners? Met-independent epithelial morphogenesis induced by HGF.

Focus on “Hepatocyte growth factor induces MDCK cell morphogenesis without causing loss of tight junction functional integrity”

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Polarized epithelial cells line the majority of vertebrate organs (including the urinary, digestive, and respiratory tracts), where they are responsible for life-sustaining vectorial transport and barrier functions. Morphogenesis of the epithelial cell phenotype is a complex phenomenon, and several decades of pioneering multidisciplinary studies have now begun to yield important clues toward our understanding of this process. Developmental and molecular biological investigations have identified critical growth and transcription factors that direct the biogenesis of epithelial organs. Concurrently, cell biological studies of epithelia cultured on two-dimensional filter supports or within three-dimensional extracellular matrix (ECM) systems have provided us with novel insights into the mechanisms by which these factors orchestrate epithelial morphogenesis. These approaches have recently led to the proposal of a two-phase working hypothesis (8, 12). The first phase entails the conversion of single cells into a polarized monolayer, regulated primarily by an intrinsic genetically determined program. This is initiated in filter-supported systems by the establishment of cell-cell contact, which serves as a cue for the recruitment of adherens junction molecules (such as E-cadherin) and proteins of the tight junction (such as ZO-1). The ensuing cytoskeletal rearrangements (10), and selective sorting of plasma membrane proteins to the apical or basolateral domains as demarcated by tight junctions (9), complete the generation of a polarized epithelium. In three-dimensional systems, the first phase begins with several rounds of cell division. Daughter cells display lateral membrane domains containing adherens and tight junctions at sites of cell-cell contact and basal domains containing integrin-receptor complexes at sites of cell-ECM contact. Elaboration and assembly of laminin at the basal surface provide a cue for lumen formation and generation of apical domains. Cells devoid of ECM contact undergo programmed cell death, resulting in luminal expansion and generation of a mature cyst. The second phase of epithelial morphogenesis results in remodeling of the polarized monolayer and includes processes such as branching morphogenesis (23), tubulogenesis (16), and pseudostratification (14). This phase is regulated by extrinsic factors, the most prominent being hepatocyte growth factor (HGF), which is the focus of this review.

HGF was originally identified as a mitogen for hepatocytes and subsequently shown to be identical to scatter factor (SF), a fibroblast-derived ligand that possesses a distinct property of inducing epithelial cell dissociation. Increasing evidence now suggests that HGF is a multifunctional molecule that exerts a plethora of effects on epithelial cells, including mitogenesis, motogenesis, morphogenesis, tubulogenesis, induction of cell polarity, inhibition of apoptosis, and promotion of invasiveness (6, 16, 23). HGF is a heterodimeric molecule that is biosynthesized and secreted as an inactive precursor by cells of mesodermal origin in mature (fibroblasts, monocytes, platelets) and developing (placenta, liver, kidney) humans. Conversion to the active form requires proteolytic processing by extracellular activators such as urokinase-type plasminogen activator and HGF-specific serine protease. The high-affinity receptor for HGF is the receptor tyrosine kinase c-Met, and all of the pleiotropic cellular consequences of HGF activation are thought to be transduced via Met (3, 16, 23). Met is expressed in virtually all epithelial cells and is markedly induced in a variety of human epithelial tumor cells (17). The essential role of HGF and Met during mammalian development has been documented by targeted deletion of either of these genes in mice, which resulted in embryonic lethality due to impaired organogenesis of the placenta and liver. Binding of HGF to Met induces receptor dimerization and autophosphorylation at a conserved two-tyrosine motif within the receptor docking site (3). The significance of this is underscored by the finding that mice harboring mutations at the tyrosine residues display the same phenotype as the HGF or Met knockouts. Met activation results in the recruitment of a growing array of intertwined downstream signal transduction molecules that are the subjects of intense current research (3, 16, 17, 23). Mounting evidence suggests an intriguing specificity of phenotypic readout depending on the intracellular pathway that is activated (3).

Madin-Darby canine kidney (MDCK) cell culture models have been instrumental in elucidating the role of HGF in epithelial morphogenesis. First, single cells or small colonies grown on impermeant supports become motile and scatter away when exposed to HGF, providing a useful model for HGF-induced tumor invasion and metastasis. Second, cells grown as cysts within ECM systems respond to HGF by forming complex branching tubules. This system has been studied extensively as a model of branching morphogenesis that occurs during normal development of epithelial organs such as the kidneys and lungs. Third, MDCK cells grown to confluence on permeable filter supports form fully polarized monolayers with morphologically and functionally distinct apical and basolateral membrane domains separated by a continuous tight junction belt. Met is localized to the basolateral cell surface, and cells respond to basolateral but not apical...
HGF. This system has proved particularly relevant to the study of alterations in cell polarity commonly associated with acute injuries of epithelial organs. HGF induces confluent monolayers of MDCK cells to undergo pseudostratification, a response that requires an increase in monolayer thickness, an increase in tortuosity of lateral membranes, and the extension and crawling of cells over each other (2). The mechanisms and functional consequences of this peculiar morphogenetic response to HGF have been elegantly examined in the current article in focus by Pollack et al. (Ref. 13; see p. C482 in this issue).

Using fully confluent MDCK cell monolayers containing a low percentage of cells expressing the polymeric immunoglobulin receptor (plgR), Pollack et al. (13) show that 20 h of exposure to HGF causes individual cells to crawl over each other while maintaining cell-cell borders as assayed by E-cadherin staining. Although ZO-1 staining was localized to multiple levels at sites of cell-cell contact through the pseudostratified layer, projections obtained by summing up all sections revealed the presence of complete rings of ZO-1 around cells in the pseudostratified layer, indicating morphologically intact tight junction belts. The functional integrity of the tight junctions was established by ruthenium red staining and by direct measurements of transepithelial resistance (TER) in multiple subclones. In dose-response studies, HGF in low concentrations (2.5 ng/ml) elicited a full scattering response with no significant change in TER, whereas higher doses of HGF (100 ng/ml) resulted in pseudostratification and a surprising transient increase in TER. This property of inducing motility and cellular rearrangements within a confluent monolayer without compromising the paracellular barrier function may be particularly pertinent to processes such as wound healing in tissues. For example, it is well known that acute noxious stimuli in several epithelial organs such as kidney (19), liver (7), lung (21), and intestines (11) can induce the local production and activation of HGF and Met. It is intriguing to speculate that the local increase in HGF concentration at sites of tissue injury may contribute to recovery by inducing cell crawling without jeopardizing the tight junction, although direct evidence for this is lacking.

Pollack et al. (13) have made another highly significant observation. To examine the role of Met activation, they utilized two independent approaches. First, MDCK cells were stimulated with an agonistic monoclonal antibody to c-met (DO24) that has been shown to induce several of the biological responses to HGF (15). This resulted in the expected dose-dependent scattering response but, surprisingly, only a minimal increase in TER even at concentrations far exceeding those required to induce scattering. Second, MDCK cells expressing a trk/met chimeric receptor were stimulated with NGF, which reproduces all known biological effects of HGF/Met activation in epithelial cells (20). Once again, although NGF was able to activate the scattering of the trk/met cells, even high doses did not induce pseudostratification or increased TER. These data provide compelling novel evidence for a Met-independent epithelial morphogenesis induced by HGF.

These intriguing findings beg the question, Has HGF met another match? It is worthwhile noting that the concentrations of HGF required to induce pseudostratification and increased TER are >10-fold that which stimulates scattering and are at saturating levels for the high-affinity Met receptor. It has been known for some time now that epithelial cells also possess higher-capacity HGF binding sites that display an affinity that is 10-fold lower than Met (22). Their interactions with HGF are heparin sensitive, suggesting a composition of heparan sulfate proteoglycans (1). Recent reports indicate that the presence of heparin and related oligosaccharides can markedly potentiate the biological effects of HGF and Met (4, 5). The prevailing notion, therefore, is that these low-affinity HGF binding sites may act as cofactors in Met signaling pathways. Another fascinating possibility raised by the findings of Pollack et al. (13) is that they constitute an independent HGF receptor pathway that is selectively activated by the higher concentrations of HGF, which may contribute to wound healing. Identification and characterization of these low-affinity binding sites for HGF will be important endeavors for the future, and current candidates include syndecan, perlecan, and fibroplacan (1, 4, 5). The potential use of HGF as a therapeutic agent is a matter of considerable contemporary enthusiasm and promise. Because of its organotrophic effects, clinical trials of HGF are either under way or planned in acute and chronic renal failure, liver cirrhosis, myocardial infarction, lung injury, and gastric mucosal injury, to name a few. A better understanding of the downstream signaling pathways that mediate each of the myriad effects of HGF will be important to optimally tailor therapy to the underlying pathophysiological condition while minimizing potential side effects. The lessons learned from transgenic mice overexpressing HGF that develop diverse tumors, polycystic kidney disease, and inflammatory bowel disease (18) serve to caution us against leaping from the proverbial frying pan into the fire when contemplating HGF therapy.

REFERENCES


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