Chapter VI. Epithelial cell patterning by soluble ligands

Abstract

In metazoans, the epithelial-mesenchymal transition (EMT) is a fundamental process governing embryonic morphogenesis and metastatic cancer. In non-tumorigenic epithelial cells, we have investigated how soluble ligands regulate an EMT-like phenomenon, the aggregation and dissociation of cell-cell contacts. We demonstrate that deprivation of the soluble factors contained in growth medium induces cell aggregation, a phenomenon that is reversible upon restoration of growth medium. Among the soluble factors constituting growth medium, only epidermal growth factor and cholera toxin contribute to cell scattering; notably, these factors can synergize to induce greater scattering than either factor alone. Using pharmacological inhibitors, we begin to parse the mechanisms that control dissociation of intercellular junctions and cell migration. Taken together, these studies provide insight into the control of epithelial cell aggregation and dissociation by soluble factors, which may prove relevant to understanding EMT and metastasis.

1. Introduction

In general, epithelial cells are highly polarized, with distinct apical and basal domains, and characterized by strong intercellular adhesions. In contrast, mesenchymal cells are migratory, non-polarized cells embedded in an extracellular matrix. The epithelial-mesenchymal transition (EMT) is a process by which epithelial cells lose many of their epithelial characteristics and acquire properties associated with mesenchymal cells (Thiery and Sleeman, 2006). During embryonic development, metazoans require the precise control of EMT to form many multicellular structures, including the parietal endoderm, the mesoderm, and the primitive streak during gastrulation (Vincent-Salomon and Thiery, 2003).

Notably, EMT is also prominent in the development of metastatic cancer. The majority of human cancers arise in epithelial tissues, which undergo an EMT to become migratory and invasive. EMT is characterized by several criteria, including the loss of epithelial polarity, separation into individual cells, and subsequent dispersion after the acquisition of cell motility (Vincent-Salomon and Thiery, 2003). Additionally, EMT is typically accompanied by loss of E-cadherin expression and a shift from expression of epithelial markers such as cytokeratins to mesenchymal markers like vimentin (Christofori, 2006).

Most studies in EMT involve *in vitro* assays where epithelial cell lines can be converted into fibroblast-like cells. Studies into the molecular basis of EMT have demonstrated that these phenomena can be controlled by soluble ligands in the cellular

microenvironment or by mutations that mimic ligand-mediating signaling. The ligands most prominently associated with EMT are hepatocyte growth factor (HGF) and transforming growth factor β (TGF-β). HGF was originally discovered as a protein secreted by stromal fibroblasts that could induce scattering of normal epithelial cells (Stoker and Perryman, 1985). Subsequent studies revealed that HGF binds to and activates the c-Met receptor tyrosine kinase, recruiting a number of adaptor molecules that activate downstream signaling pathways (Birchmeier et al., 2003). Similarly, TGF-β ligands mediate the assembly and activation of receptor complexes that activate the Smad family of transcription factors (Massague et al., 2000). For full induction of EMT, both HGF and TGF-β crucially require several common downstream signaling molecules, including small GTPases and signaling cascades such as ERK (Thiery and Sleeman, 2006). Together with cues from the extracellular matrix and cell-cell interactions, these extracellular signals then execute the complex genetic program involved in EMT.

Although several of the molecular pathways and cellular mechanisms controlling EMT have been identified, little is known about EMT-like phenomena in non-cancerous cells. In particular, what aspects of EMT can be recapitulated by non-tumorigenic epithelial cells *in vitro*? Using a non-tranformed, epithelial cell line (MCF-10A), we investigate how soluble factors such as the epidermal growth factor (EGF) and cholera toxin (ChT) influence epithelial cell plasticity. Notably, we demonstrate a synergistic interaction between EGF and ChT to induce dissociation of intercellular contacts, a phenomenon similar to the early stages of EMT. Quantitative studies of the mechanisms

controlling this EMT-like phenomenon should lead to a better understanding of EMT in development and metastasis.

2. Materials and Methods

2.1. Cell Culture

MCF-10A cells were cultured in Dulbecco's modified Eagle's medium/Ham's F-12 containing HEPES and L-glutamine (Invitrogen) supplemented with 5% (v/v) horse serum (Invitrogen), 20 ng/ml EGF (Peprotech), 0.5 μg/ml hydrocortisone (Sigma), 0.1 μg/ml cholera toxin (Sigma), 10 μg/ml insulin (Sigma), and 1% penicillin/streptomycin. For serum starvation, the cells were washed twice in PBS and then cultured in Dulbecco's modified Eagle's medium/Ham's F-12 supplemented with 1% (v/v) penicillin/streptomycin and 0.1% bovine serum albumin (Sigma) for 24 h.

2.2. Phase Contrast Microscopy

Phase contrast images were captured at 10X magnification using a Zeiss Axiovert 200M inverted microscope.

2.3. Pharmacological Inhibition

LY 294002 and PD 98059 were purchased from Calbiochem and reconstituted in DMSO. For experiments, cells were pre-treated with LY 294002 and PD 98059 for 15 or 60 min, respectively, before stimulation in the continued presence of these inhibitors.

3. Results and Discussion

3.1. Growth Medium controls the reversible formation of cell colonies.

To test whether normal epithelial cells can exhibit EMT-like behavior, we investigated whether MCF-10A cells, a non-tumorigenic epithelial cell line (Soule et al., 1990; Tait et al., 1990), exhibited EMT-like characteristics in tissue culture. When cultured at a subconfluent density in growth medium (GM), MCF-10A cells exhibit random scattering and minimal intercellular contacts (Figure VI-1A). However, when MCF-10A cells were starved of GM, we noted the formation of cell colonies, whereby cells formed "islands" with substantial cell-cell contacts with neighbors. In this starved state, cells were non-motile, although perimeter cells occasionally exhibited membrane ruffling or membrane protrusions (data not shown). Notably, the formation of cell colonies was reversible, as dissociation of cell colonies was induced by re-stimulation with growth medium (Figure VI-1B).

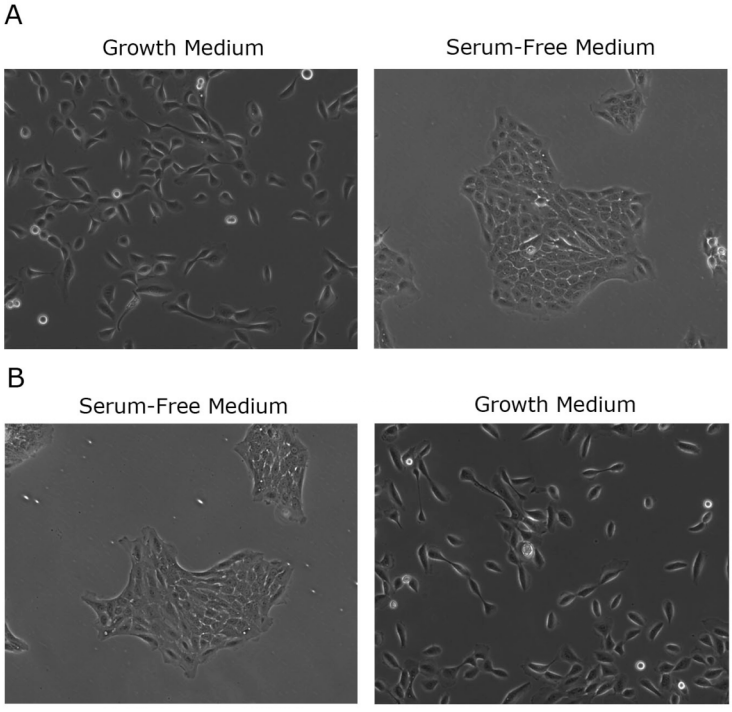

Figure VI-1. Reversible formation of epithelial cell colonies by growth medium starvation or stimulation (A) Subconfluent MCF-10A cells were either serum-starved or maintained in growth medium, and phase contrast images were captured 24 h later. (B) Serum-starved MCF-10A were stimulated with serum-free medium or growth medium, and phase contrast images were captured 24 h later.

3.2. EGF, but not serum, can prevent cell aggregation.

MCF-10A GM contains multiple soluble factors, including EGF, ChT, insulin, hydrocortisone, and serum factors, and the scattered phenotype of MCF-10A maintained in GM could be due to one or all of these factors. To parse the requirements for maintenance of the scattered phenotype, subconfluent cells that were initially scattered were deprived of various soluble factors. Surprisingly, in cells maintained in serum factors, formation of cell islands was robust and exhibited virtually no difference compared to fully-starved MCF-10A (Figure VI-2). In contrast, cells stimulated with EGF showed only partial aggregation into islands, with many EGF-stimulated cells remaining outside of cell islands and exhibiting migratory phenotypes. Additionally, cells treated with GM minus EGF (i.e., supplemented with ChT, hydrocortisone, insulin,

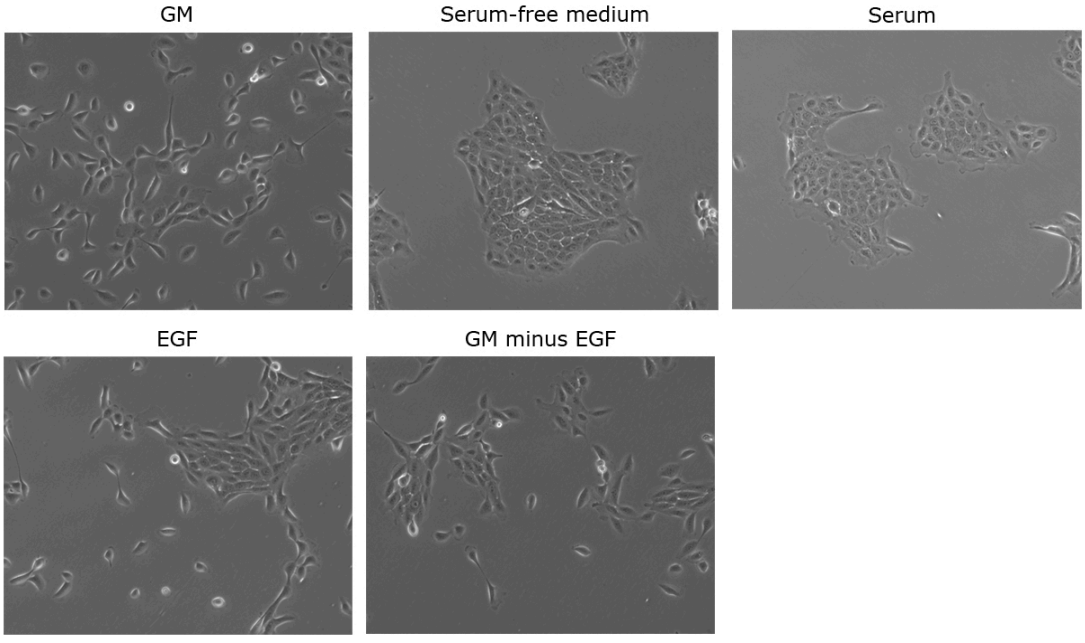

Figure VI-2. EGF, but not serum, prevents cell island aggregation.
Subconfluent MCF-10A cells maintained were either maintained in full GM or transferred to serum-free medium, serum-containing medium, EGF (20 ng/ml), or GM containing all factors except for EGF. Phase contrast images were captured 24 h later.

and serum factors) became partially aggregated, with many cells remaining outside of cell colonies.

3.3. EGF and ChT induce mild dissociation of cell colonies.

Having demonstrated that MCF-10A could partially maintain the scattered phenotype in the presence of either EGF or GM minus EGF, we asked whether these same factors could induce dissociation of cell islands. To test this question, we stimulated starved cell colonies with each factor individually (ChT, EGF, hydrocortisone, insulin, or serum factors) and assessed the degree of cell dissociation. Interestingly, EGF and ChT were the only components of growth medium to induce mild cell dissociation (Figure VI-3). However, even upon treatment with EGF or ChT, the majority of cells

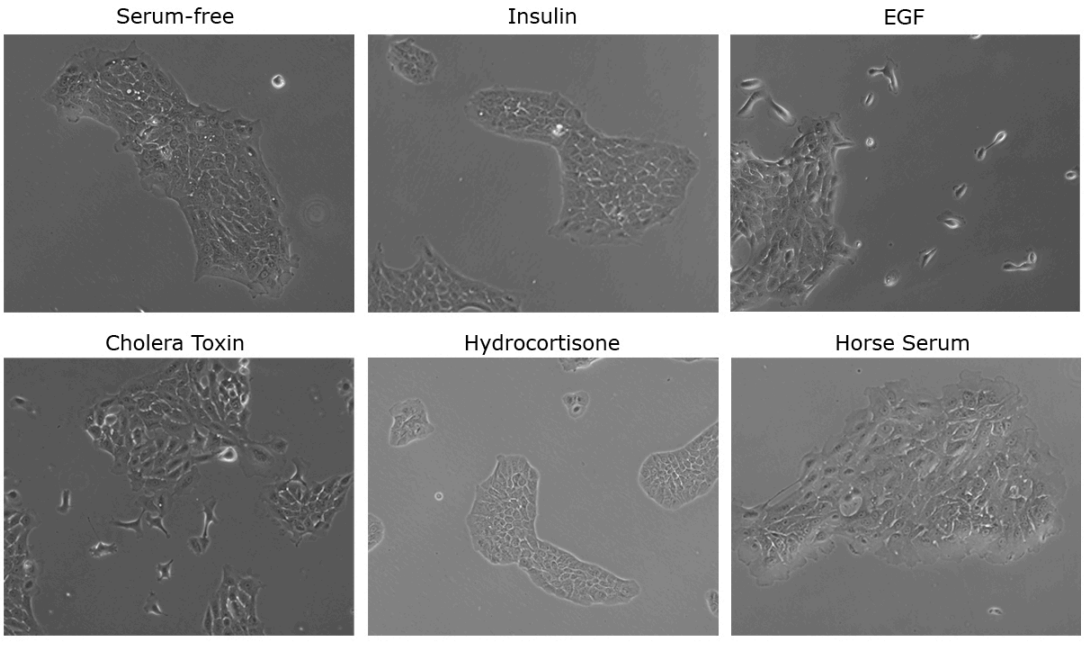

Figure VI-3. EGF and ChT are the only components of growth medium that induce cell colony dissociation.
Serum-starved MCF-10A cells were stimulated with serum-free medium, insulin, EGF, cholera toxin, hydrocortisone, or horse serum at concentrations identical to those of full GM. Phase contrast images were captured 24 h later.

remained in islands, with only a minority of cells "escaping." In contrast, hydrocortisone caused perimeter cells to form a smooth, rounded exterior, suggesting that hydrocortisone may inhibit basal membrane ruffling.

3.4. EGF and ChT cooperate to induce synergistic cell scattering.

Since neither EGF nor ChT alone reproduced the highly-scattered phenotype of GM-treated cells, we asked whether co-stimulation with these two factors could induce greater scattering than either factor alone. Indeed, co-stimulation of MCF-10A cell colonies with EGF and ChT demonstrated a synergistic response (Figure VI-4). Notably, co-stimulation with EGF and ChT still did not reproduce the fully-scattered phenotype of GM-treated cells, suggesting that there may exist further synergy between EGF, ChT, and another of the factors present in GM.

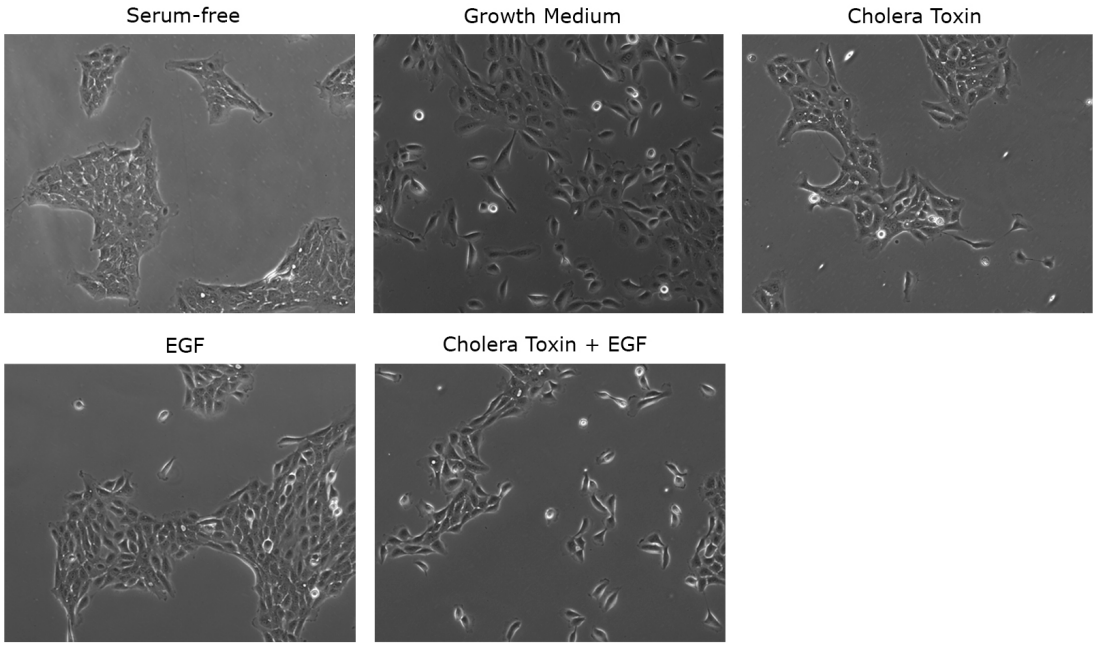

Figure VI-4. EGF and ChT can synergize to induce cell island dissociation.
Serum-starved MCF-10A cells were stimulated with either serum-free medium, full growth medium, cholera toxin (0.1 μg/ml), EGF (20 ng/ml), or cholera toxin plus EGF. Phase contrast images were captured 24 h later.

Among the components of GM, it is not surprising that EGF is a pro-scatter factor, since previous reports have demonstrated that EGF induces scatter in both non-tumorigenic epithelial cells (Matthay et al., 1993) and some carcinoma systems (Boyer et al., 1997; Edme et al., 2002; Lu et al., 2003). Additional support for the scatter capacity of EGF comes from the observation that overexpression of an active mutant of the EGFR family member ErbB-2 induced cell scattering in MDCK normal epithelial cells (Khoury et al., 2001).

In contrast to EGF and other RTK-binding ligands, ChT has not been associated with dissociation of intercellular junctions or induction of cell migration. *In vivo*, ChT exists as a hexamer, with one A subunit and five B subunits. The B subunits bind to cell surface gangliosides and mediate entry of the hexamer into the cell. Once inside the cell, the A subunit activates the intracellular G-protein $G_{s\alpha}$, inducing dissociation of $G_{s\alpha}$ from $G_{s\beta\gamma}$. This in turn activates adenylate cyclase, increasing in intracellular cAMP concentrations and activating protein kinase A (PKA) (Salmond et al., 2002). Because PKA has been shown to be active in leading-edge, protrusive structures and essential for chemotaxis in fibroblasts (Howe et al., 2005), PKA may be responsible for the pro-migratory effects of ChT MCF-10A.

In addition to its effects on intracellular cAMP, ChT stimulation has been reported to induce secretion of a variety of cytokines, including TNFα (Viana et al., 2002; Yan et al., 1999) and a variety of interleukin family members (Braun et al., 1999; Bromander et al., 1991; Cong et al., 2001; McGee et al., 1993). These cytokines,

however, are not known to stimulate dissociation of intercellular junctions or cell migration. In addition to TNFα and interleukins, ChT has also been reported to cause a 2-fold increase in TGF-β1 mRNA levels (Kim et al., 1998), which is intriguing because TGF-β signaling plays a significant role in EMT (Christofori, 2006; Thiery, 2003). In support of the connection between ChT and TGF-β, Lewis rats which were administered a conjugate of ChT subunit B and myelin basic protein were found to have increased levels of TGF-β in the spinal cord (Sun et al., 2000). Because TGF-β can induce scattering of pancreatic cancer cells (Ellenrieder et al., 2001), ChT-induced scattering of MCF-10A cells might be an indirect result of ChT-induced TGF-β secretion.

3.5. MAPK and PI3K control various aspects of cell scattering.

Because studies have demonstrated that phosphatidylinositol 3-kinase (PI3K) is required for EMT in several contexts (Larue and Bellacosa, 2005), we investigated the role of PI3K in MCF-10A cell scattering. In cells treated with LY 294002, a pharmacological inhibitor of PI3K kinase activity, GM-induced scattering was minimal (Figure VI-5A). However, the intercellular contacts did appear to loosen, with some cells dissociating from their neighbors but unable to migrate away from cell islands. This "loosening" of cell junctions could be a result of incomplete inhibition of PI3K activity by LY 294002. Alternatively, PI3K may not be involved in the breakdown of cell-cell junctions, a necessary step for dissociation of cell colonies. Similarly, inhibition of PI3K in MDCK transformed with active ErbB-2 did not affect breakdown of cell-cell junctions (Khoury et al., 2001).

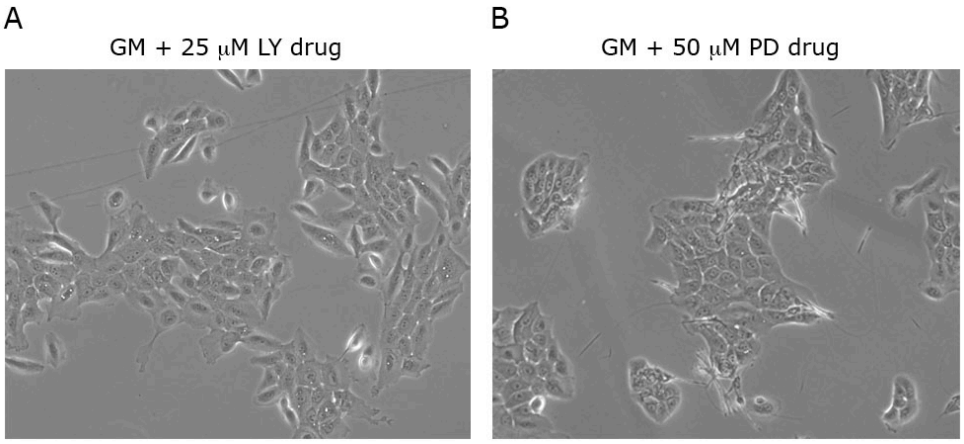

Figure VI-5. PI3K and MAPK are required for different aspects of GM-induced cell scattering. Serum-starved MCF-10A cells were pre-treated with the indicated concentrations of (A) the PI3K inhibitor LY 294002 or (B) the MAPK inhibitor PD 98059 and then stimulated with GM in the continued presence of the pharmacological inhibitors. Phase contrast images were captured 24 h later.

In other epithelial cell models, the ERK signaling pathway is required for HGF-mediated breakdown of cell junctions (Potempa and Ridley, 1998). To test the role of ERK in scatter of MCF-10A, we inhibited MEK, the kinase upstream of ERK, with the pharmacological agent PD 98059. In contrast to PI3K, blocking activation of ERK completely ablated both dissociation of cell junctions and cell scattering in MCF-10A stimulated with GM (Figure VI-5B). This confirms other reports that have demonstrated an absolute requirement for ERK activity in EGF-induced cell scattering (Boyer et al., 1997; Edme et al., 2002; Khoury et al., 2001). Also, given the possible link to TGF-β signaling through ChT, it is noteworthy that ERK is required for TGF-β mediated disruption of adherens junctions, cell scattering, and EMT (Ellenrieder et al., 2001; Zavadil et al., 2001).

3.6. Relevance of cell scattering to EMT

Two of the criteria for an EMT are separation of epithelial cells into individual cells and subsequent dispersion after acquisition of cell motility (Vincent-Salomon and

Thiery, 2003). As such, the aggregation and scatter phenomena exhibited by MCF-10A cells recapitulate a small portion of the EMT. Other criteria for a full EMT include the loss of epithelial polarity, expression of mesenchymal proteins such as vimentin, and the loss of E-cadherin expression. While we have not explicitly tested for these characteristics, it is doubtful that MCF-10A cells undergo a full EMT. For example, the scattering phenomena observed in MCF-10A are fully reversible on a short time scale (~ 24 h) (Figure VI-1). In a similar but transformed mammary epithelial cell system (EpRas), FGF and HGF induced an EMT-like, spindle migratory phenotype, but neither growth factor was able to induce a full mesenchymal phenotype (Janda et al., 2002). Given the similarity between our system and growth factor-stimulated EpRas cells, MCF-10A probably do not exhibit additional EMT characteristics such as lasting loss of E-cadherin expression or mesenchymal gene expression patterns. In addition, when MCF-10A reach confluency, cells become non-motile and express high levels of E-cadherin, even in the presence of GM (data not shown). Thus, the EMT-like phenomena in MCF-10A likely do not represent a full EMT.

The inability of *in vitro* culture assays to recapitulate the full extent of EMT is well-known (Vincent-Salomon and Thiery, 2003); however, phenomena such as cell scattering still can provide some insight into the mechanisms regulating EMT *in vivo*. For example, native MCF-10A cells are considered non-tumorigenic, as evidenced by the fact that MCF-10A do not form tumors in nude mice (Soule et al., 1990). Thus, the ability of MCF-10A to scatter in response to soluble factors such as EGF and ChT demonstrates the degree of plasticity exhibited by normal epithelial cells. By comparing

the EMT-like capabilities of normal epithelial cell systems to other systems that exhibit metastatic phenotypes *in vivo*, it may be possible to delineate the acquired capabilities of cancer cells that permit EMT, invasion, and metastasis (Hanahan and Weinberg, 2000).

4. Future Work

The results discussed here describe a system for the study of cell aggregation and scattering in non-tumorigenic epithelial cells. These preliminary studies suggest several additional lines of experimentation needed to gain a more thorough understanding of the molecular mechanisms underlying these EMT-like phenomena.

4.1. Further elucidation of the mediators of EGF- and ChT-induced cell scatter

Although preliminary examination of GM-mediated cell scatter has pinpointed specific roles for PI3K and ERK, the contribution of other signaling molecules remains unexplored. Since EGF and ChT can synergize to induce cell scattering (Figure VI-4), a first test should be to probe the role of several other prominent signaling molecules using pharmacological inhibitors. In particular, because ChT upregulates intracellular cAMP concentrations, it would be interesting to test whether inhibition of PKA affects cell scattering. Additionally, since the Src kinase has been implicated in EGF-induced scattering of NBT-II epithelial cells (Boyer et al., 1997), it would be interesting to test whether the Src inhibitor PP2 affects cell scattering. To strengthen conclusions made with pharmacological agents, retroviral expression of dominant-negative proteins or siRNA-mediated protein knockdown could be used to test the role of MAPK, PI3K, Src,

and/or PKA in cell scattering. Finally, because ChT may induce autocrine production of migratory ligands, particularly TGF-β, it would be interesting to probe the existence of autocrine signaling loops using either neutralizing antibodies or soluble decoy receptors.

4.2. The role of cadherins and other adhesion proteins in cell scattering

In epithelial cells, E-cadherin regulates intercellular adhesion and has been implicated in both EMT and cancer progression (Thiery, 2002). E-cadherin is a single-pass transmembrane protein whose extracellular domain homotypically binds E-cadherin molecules on neighboring cells. The intracellular domain of E-cadherin binds β-catenin, which in turn binds α-catenin and bridges E-cadherin to the actin cytoskeleton. Notably, it has been suggested that the reorganization of cells into colonies, such as that observed upon growth factor deprivation of MCF-10A cells (Figure VI-1A), is mediated by E-cadherin and the subsequent re-organization of the actin cytoskeleton (Adams et al., 1998).

The loss of E-cadherin function has also been implicated in EMT, since blocking E-cadherin interactions with inhibitory antibodies can induce mesenchymal phenotypes (Imhof et al., 1983). Some scatter-inducing growth factors have also been linked to downregulation of E-cadherin. For example, HGF has been shown to induce cell scattering through MAPK/Egr-1-mediated upregulation of Snail, a transcriptional repressor of E-cadherin (Grotegut et al., 2006). As such, it would be interesting to test whether E-cadherin is functionally involved in both the cell aggregation (Figure VI-1A) and cell scattering (Figure VI-1B) phenomena in MCF-10A. One method for testing the

role of E-cadherin in these processes would be to selectively deplete E-cadherin by siRNA. Alternatively, one could inhibit E-cadherin function via anti-E-cadherin antibodies. Using these tools, one could ask, do MCF-10A deprived of soluble factors aggregate in absence of cadherin-mediated adhesion? Alternatively, if E-cadherin-mediated cell contacts are required for cell colony formation, does E-cadherin overexpression reduce cell scattering? In addition to classical overexpression techniques, it might be possible to increase E-cadherin expression using RNA duplexes that target the endogenous E-cadherin promoter (Janowski et al., 2007).

Although the adhesive function of E-cadherin may play a role in EMT-like phenomena, the E-cadherin-binding partner β-catenin may also promote cell scatter, since EGF-mediated β-catenin:Tcf/Lef transcription can contribute to processes resembling EMT (Lu et al., 2003; Muller et al., 2002). Other growth factors such as IGF-II also induce β-catenin signaling during EMT (Morali et al., 2001). Although these reports did not demonstrate a functional requirement for β-catenin in EMT, siRNA knockdown of β-catenin levels ablated HGF-induced cell scattering (Rasola et al., 2007). Additionally, Rasola et al. demonstrated that cell scattering was induced by expression of a constitutively active, non-degradable β-catenin mutant, even in the absence of HGF. Thus, β-catenin may promote EMT though gene transcription, particularly because Tcf/Lef target genes include matrix metalloproteinases (Brabletz et al., 1999; Takahashi et al., 2002), which can degrade the basement matrix during metastasis, and Slug (Vallin et al., 2001), a transcriptional repressor of E-cadherin.

In MCF-10A, we have previously demonstrated that EGF induces β-catenin:Tcf/Lef signaling (Graham and Asthagiri, 2004). As such, does β-catenin signaling play a role in EGF- and ChT-induced cell scattering? As a first test of this question, does siRNA-mediated depletion of β-catenin inhibit cell scattering? Since β-catenin regulates expression of Tcf/Lef gene targets in MCF-10A, would expression of dominant-negative Tcf4, which ablates Tcf/Lef transcription, affect cell scattering? If Tcf/Lef gene targets are functionally involved in EGF-mediated cell scatter, would inhibition of individual Tcf/Lef target genes block cell scatter? Methods to test this last question include pharmacological inhibition of matrix metalloproteinases or siRNA directed against individual Tcf/Lef target gene products. Answers to these questions would provide meaningful insight into the cellular mechanisms regulating cell scattering by soluble factors.

4.3. Quantitative metrics of cell scatter

The observations of cell aggregation and scatter in this report are highly qualitative. While qualitative analysis is informative, quantitative metrics might permit more rigorous dissection of the mechanisms underlying cell scattering. One semi-quantitative metric for characterization of cell scattering is the percentage of cells expressing membrane-localized desmoplakin (Boyer et al., 1997; Edme et al., 2002), since desmoplakin is indicative of desmosomes, which mediate intercellular contact. However, quantification of desmoplakin membrane localization requires fixation and immunostaining of cells, as well as subjective judgments about what constitutes membrane localization.

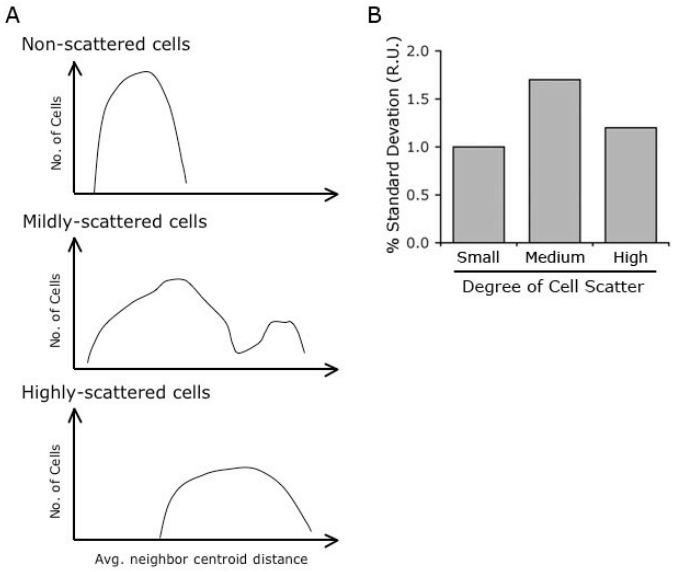

Figure VI-6. Average neighbor centroid distance as a metric for quantification of cell scattering
A representative image of the proposed metrics for quantification of cell scattering: (A) the average distance between neighboring cells and (B) the percent standard deviation of the average neighbor distance, expressed in Relative Units (R.U.)

Ideally, a quantitative metric of cell scattering would be objective and derived from live cell imaging, so that one could track the temporal evolution of scatter. One technique that has been used for quantification of cell movement in collagen gels is the annular grayscale method (Vernon and Gooden, 2002), which measures how far cells migrate away from an initially circular colony. Although MCF-10A cell colonies are not strictly circular (Figure VI-1), adaptation of this method for quantification of irregularly-shaped colonies is theoretically possible. Other simple metrics for quantification of cell colony dispersal include the percent of colonies that are dissociating, as well as the percentage of single cells migrating away from colonies (Jourquin et al., 2006).

Another possible metric for cell scatter is the average distance between a cell's centroid and the centroids of its nearest-neighbors (i.e., the ten closest cells). Highly

scattered cells (e.g., GM-treated cells) would exhibit average neighbor distances much greater than one cell diameter (Figure VI-6A), whereas cells organized in colonies (e.g., fully-starved cells) would have average neighbor distances of approximately one cell diameter (Table VI-1). One drawback of this metric is that distinguishing mildly-scattered cells (e.g., EGF-treated cells) from non-scattered cells would be difficult because the small percentage of mildly-scattered cells would only slightly increase the average neighbor distance above one cell diameter. To distinguish these relatively similar cell patterns, a useful metric might be the percent standard deviation of the average neighbor distances (Figure VI-6B). Because mildly-scattered cells will have a few cells that are highly detached from neighbors, the percent standard deviation of the average neighbor centroid distances should be much higher for mildly-scattered cells than for non-scattered cells.

Table VI-1. Potential Scatter Metric: Average and percent standard deviation of neighbor distance

	Low Scatter	Medium Scatter	High Scatter
Average distance of 10 nearest neighbors	Low (~1 cell diameter)	Fairly low (<2 cell diameters)	High (>2 cell diameters)
% Standard Deviation of Average Nearest Neighbor Distance	Low	High	Low (?)

5. Conclusions

In this report, we demonstrate a system that recapitulates one of the earliest hallmarks of EMT, the detachment of epithelial cells from their neighbors and the acquisition of a migratory phenotype. Using the non-tumorigenic epithelial cell line MCF-10A, we show that subconfluent cells aggregate into islands when deprived of GM;

this aggregation phenomenon is reversible, as restoration of GM re-establishes cell scattering. By individually assessing the contributions of each of the soluble factors in GM, we find that cell scattering is controlled by both EGF, a ligand known to regulate EMT, and ChT, a ligand not known to be involved in epithelial cell plasticity. Interestingly, these ligands can function synergistically to induce greater cell scatter than either ligand alone. Finally, pharmacological inhibition of the PI3K and MAPK signaling pathways demonstrates that PI3K may be involved in cell-cell junction disassembly, but not migration, whereas MAPK may be essential for both processes. Notably, this report leaves several questions unanswered. In particular, the mechanisms connecting EGF and ChT to dissolution of cell-cell junctions remain to be elucidated. Some intriguing possibilities, including the involvement of the cell-cell adhesion protein E-cadherin, intracellular kinases such as Src and PKA, and autocrine production of TGF-β, also remain to be explored. As such, the MCF-10A system may prove ideal for investigating the mechanisms underlying the plasticity of non-tumorigenic epithelial phenotypes, EMT, and metastasis.

6. References

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