DYNAMICS OF MULTICELLULAR AGGREGATION AND DISAGGREGATION:

IMPLICATIONS FOR TISSUE ENGINEERING AND CANCER METASTASIS

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ABSTRACT

DYNAMICS OF MULTICELLULAR AGGREGATION AND DISAGGREGATION:

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Epithelial tissues play an important physiological role. Tightly cohesive epithelial sheets form protective barriers that line organs, and in addition, fold into a wide variety of complex 3D architectures with specialized functions. A key facet of tissue morphogenesis involves the aggregation of similar cells into cohesive groups. Here, we have analyzed the dynamics of aggregation using quantitative imaging techniques (Chapter II). We show that multicellular aggregation dynamics adhere to a transport-reaction model that is broadly appreciated for physicochemical systems. This model of aggregation dynamics differs from the classical equilibrium paradigm of cell aggregation based on differential adhesivity of cells to neighboring cells versus the underlying substratum. Our findings reveal a previously unrecognized role for cell motility during developmental aggregation processes and provide design principles for promoting cell aggregation dynamics in contexts such as tissue engineering that are distinct from the currently accepted paradigm.

Multicellular aggregation is reversible. In fact, the break-up of multicellular clusters ("cell scatter") is not only important for developmental processes, but also contributes to metastasis. However, current molecular genetics studies of cell scatter are predominantly qualitative and do not provide a quantitative assessment of the relative strengths of molecular signals in inducing cell scatter. By developing and implementing an automated image processing algorithm, we quantify two aspects of cell scatter – the breakdown of cell-cell adhesions and the dispersion of detached cells – in mammary epithelial cells treated with different combinations of biochemical cues (Chapter III). We demonstrate that our metrics of cell scatter identify the effects of individual cues and detect synergies between them. We envision that this approach will be useful for mapping the relative potencies of regulators of cell scatter and may guide therapeutic strategies.

Multicellular processes such as aggregation and scatter involve molecular-level changes within cell-cell adhesions. To complement imaging-based strategies at the cellular and multicellular levels, we developed a quantitative microtiter assay for examining the expression of cell-cell adhesion proteins and associations between them (Chapter IV). Using two case studies related to cancer biology, we demonstrate that our assay provides a more detailed quantitative picture of molecular changes within epithelial adhesive structures, which can provide added insight into the regulation of morphogenetic events.

TABLE OF CONTENTS

Chapter III. Automated Quantitative Analysis of Epithelial Cell Scatter

Chapter IV. A microtiter assay for quantifying protein-protein interactions associated with cell-cell adhesion

LIST OF TABLES

LIST OF FIGURES

Chapter I. INTRODUCTION

1. Introduction

The assembly of migratory cells into a cohesive group is a key facet of embryonic tissue development. Multicellular condensation occurs in the initial stages of chondrogenesis (cartilage formation), for example, and is believed to be a prerequisite for chondrogenic differentiation.[1] In addition, migrating neural crest cells are observed to condense into sub-populations en route to distant embryonic sites. This is thought to facilitate the immobilization of these cells to form solid tissues.[2]

In addition to multicellular aggregation, the dissociation of single cells from condensed tissue can also play a key role in tissue development. Kidney development, for example, is characterized by repeated cycles of condensation and dissociation.[3-5] In addition, delamination of endothelial cells from the embryonic atrioventricular canal gives rise to a cell population that eventually forms the heart valves.[6] Cellular dissociation also plays a role in tumorigenesis. In this context, metastatic cells delaminate from the tumor mass and migrate to secondary sites, thereby initiating the lethal phase of cancer progression.[7]

At the molecular level, condensation and dissociation events are often associated with a mesenchymal-epithelial transition or an epithelial-mesenchymal transition (EMT), respectively.[8] These transitions are triggered by extracellular activators and

characterized by the gain (MET) or loss (EMT) of epithelial characteristics such as the expression of cell-cell adhesion receptors. Condensation and dissociation also involve cellular processes, such as cell-cell adhesion, cell-matrix adhesion and cell migration on the extracellular matrix.

2. Adherens junctions mediate strong cell-cell adhesion

In epithelial tissues, E-cadherin-containing adherens junctions are important mediators of cell-cell adhesion.[9] E-cadherin is a transmembrane protein, the extracellular domain of which homotypically binds E-cadherin molecules on adjacent cells.[10] The intracellular tail connects to the actin cytoskeleton via protein:protein interactions involving α-catenin, β-catenin, vinculin and many other proteins. In this manner, adherens junctions link the actin cytoskeleton of contacting cells, creating an "actin belt" that imparts structural strength to a multicellular aggregate (Figure I-1).

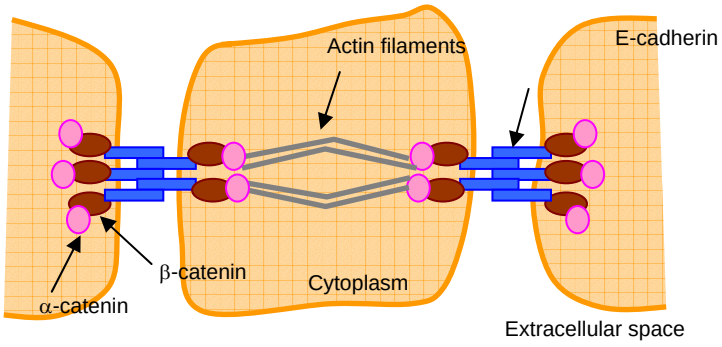

Figure I-1. Generalized structure of adherens junctions.

E-cadherin molecules span the plasma membrane to bind E-cadherin molecules on neighboring cells. E-cadherin's cytoplasmic tail interacts with β-catenin and α-catenin, forming a connection to the actin cytoskeleton.

3. Focal adhesions assemble at sites of cell-matrix adhesion

Cells use many adhesion receptors to attach to the extracellular matrix, the most prominent being the integrin family of transmembrane receptors. Integrin extracellular domains recognize a protein component of the extracellular matrix, while the intracellular domain interacts with numerous anchor proteins to form signaling-rich complexes called focal adhesions.[11] These anchor proteins, which include talin, α-actinin and vinculin, tether integrins to the actin cytoskeleton and enable cell-generated contractile forces to be transmitted onto the underlying substratum, generating the driving force needed for cell migration (Figure I-2).[12]

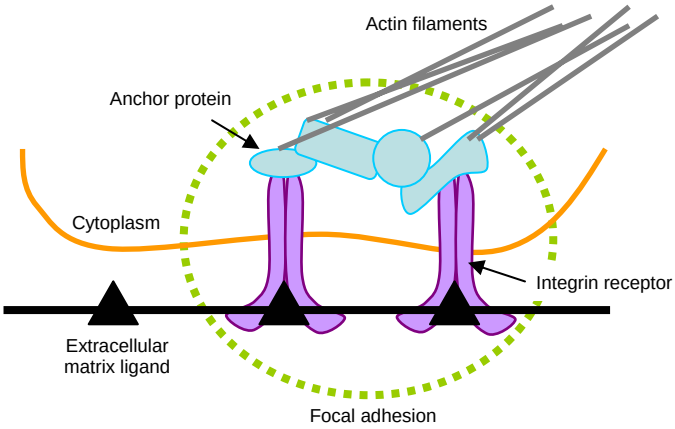

Figure I-2. Generalized structure of focal adhesions.
Integrin transmembrane receptors bind to extracellular matrix ligands. Integrin cytoplasmic tails bind to multiple anchor proteins, which tether integrins to the actin cytoskeleton to mediate strong cell-substratum adhesion.

4. Cell migration on the extracellular matrix

Cell migration is a cyclic process. A cell first extends membrane protrusions – spike-like filopodia and broad lamellopodia – in the direction of movement. These

protrusions are driven by actin polymerization and stabilized by the assembly of focal adhesions beneath them. Focal adhesions provide the traction necessary to translocate the cell body forward, and are subsequently disassembled at the cell rear to allow the trailing edge to detach from the substratum (Figure I-3).[12] Because migrating cells must be able to exert traction yet readily detach, cell speed exhibits a biphasic dependence on substratum adhesivity – a weakly adhesive substratum facilitates little traction while a strongly adhesive substratum inhibits detachment.[13]

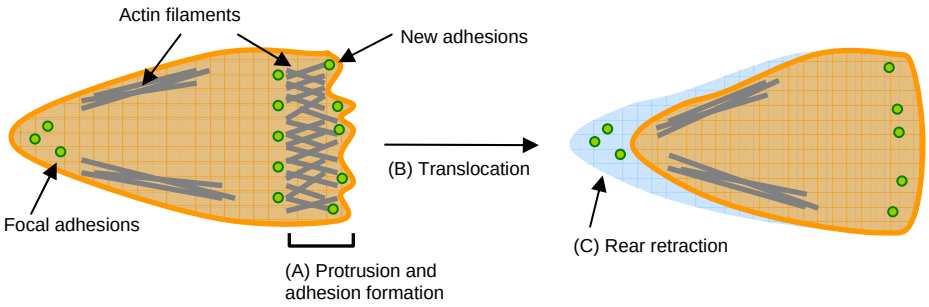

Figure I-3. Steps in cell migration.

(A) Actin polymerization drives membrane protrusions at the leading end of the cell, where new cell-substratum adhesions are formed. (B) These newly-formed adhesions provide the traction necessary to translocate the cell body forward. (C) Adhesions are disassembled at the trailing end of the cell to allow cell detachment from the substratum. Adapted from Ridley et al. *Science* (2003).

5. Current results

To better understand the roles of cell-cell interactions, cell-matrix adhesions and cell migration in condensation and dissociation, we utilize *in vitro* aggregation and scatter assays that mimic these *in vivo* phenomena. Epithelial cells cultured on adhesive substrata self-assemble into 2D multicellular clusters ("aggregation"). In response to

extracellular cues, these cell clusters dissociate and individual cells disperse ("scatter"). Aggregation and scatter are widely used model systems in which to study condensation and dissociation events. In fact, the current understanding of the complex biochemical network regulating EMT comes largely from studies of cell scatter.[8]

The classical paradigm describing multicellular aggregation asserts that the equilibrium state of aggregation is determined by differential cell adhesivity to neighboring cells versus the underlying substratum.[14-16] Because organ formation is a multi-step process, *in vivo* aggregation events must occur within specific time constraints. Therefore, aggregation dynamics are likely to be important. We assert that because cells must first "find" a neighbor (a transport step) before forming cell-cell contacts, aggregation dynamics may follow a transport-reaction model ascribed widely to physiochemical systems.[17, 18] In Chapter II, we present quantitative studies of aggregation dynamics that probe this possibility. Using timelapse microscopy, we tracked collisions between migrating cell pairs and quantified the lifetimes of cell-cell interactions ($t_{adhesion}$). Based on the initial density of seeded cells and quantitative measurements of cell speed, we also determined the mean time between cell-cell collisions ($t_{motility}$). We find that $t_{motility}$ is greater than $t_{adhesion}$ across a range of adhesive substrata, suggesting aggregate assembly to be transport-limited. Quantitative measurements of aggregate size confirm this to be the case: aggregate size exhibits a biphasic dependence on substratum adhesivity, which mimics the biphasic trend observed for cell speed. These results are consistent with a two-step physiochemical model for multicellular aggregation dynamics and highlight a previously unrecognized role for

cellular motility in aggregation dynamics. Our study provides insight into a developmental phenomenon and design principles useful for facilitating multicellular aggregation in tissue engineering contexts.

Because of the physiological and clinical significance of dissociation events, much effort has been made to identify the molecular signals that control cell scatter. Advancements are being made in cataloging the signaling pathways involved; however because current studies of cell scatter are largely qualitative, an emerging challenge is to understand the quantitative contributions of these signals and any coupling between them. In Chapter III, we have therefore developed novel quantitative metrics to systematically characterize cell scatter. Our metrics capture two aspects of scatter – the break-down of cell-cell contacts and cellular dispersion. In mammary epithelial cells treated with stimulatory cocktails containing epidermal growth factor (EGF), we demonstrate that our metrics delineate the effects of individual molecular signals and detect synergies between them. To facilitate the rapid extraction of our metrics from fluorescence images of scattering cells, we have also developed automated image processing techniques using MATLAB. We envision that these metrics coupled with our automated image processing techniques will facilitate quantitative mapping of the biochemical cues governing scatter as well as high throughput screening of cancer therapeutics.

Multicellular processes such as aggregation and scatter involve changes to the expression levels of and interactions between cell-cell adhesion proteins. The association of E-cadherin with intracellular binding partner β-catenin, for example, is essential for

the proper function of epithelial adherens junctions and is an attractive molecular readout of their integrity. To complement our imaging-based studies at the cellular and multicellular levels, we have developed a quantitative microtiter assay for determining the amount β-catenin bound to E-cadherin in cultured cells (Chapter IV). In two case studies closely related to cancer cell biology, we demonstrate that our assay can provide a more detailed picture of molecular dynamics within adherens junctions, which will provide added insight into aggregation and scatter.

Taken together, these studies provide novel insights into the regulation of aggregation and scatter as well as methodologies useful for both future studies and biomedical applications.

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