Chapter I: Overview

1. Introduction

Cell motility governs many aspects of life including embryogenesis, immune response and wound healing. Basic mechanisms of random cell migration are well understood and many signaling pathways associated with motility have been unraveled.[1, 2] However, much like how random movement of molecules does not yield complex activities within a living cell, random motility does not result in the intricate, multicellular processes that govern many biological phenomena.

More specifically, directional migration of cells is a crucial component of cell motility that involves multifaceted regulation, whose precise orchestration is vital for biological development and various responses in the body. For example, neural crest cells must migrate in a highly persistent and ordered fashion during embryogenesis and failure of these cells to do so can result in life threatening, developmental consequences.[3] Furthermore, directed migration plays an important role in pathologies such as chronic inflammatory diseases and tumor metastasis, and inhibitors of directed migration provide a promising venue for treatment.[4, 5]

In directional migration, multiple factors operate at various steps of cell migration to control the stability and direction of lamellipodia. Such factors include topography of the extracellular matrix (ECM),[6-8] receptor signaling and adhesion molecule trafficking,[9, 10] myosin contraction[11, 12] and cell polarity machinery.[5, 13] Many of these

cues converge at the Rho-family GTPases, such as the Rac1 and Cdc42 molecules, to regulate the lamellipodial protrusions that ultimately dictate the bias of directional cell migration.

Although multitudes of gradient-based systems have been used to induce directional cell migration,[14-16] there are many innate limitations that cannot be overcome. Recently, researchers have begun to look into the possibility of a more robust and stable form of directional control using micropatterns that does not require any stimulus gradient or external field. Micropatterning techniques offer precise control over the topography of the ECM and allow for more sophisticated design strategies to enhance, modulate and govern directional cell migration.

2. Directed cell migration

2.1. Mechanism and regulation of polarized cell motility

Net cellular movement in one direction is caused by the asymmetric morphology of a migrating cell with defined leading and trailing edges. Cell motility in the direction of the leading edge is orchestrated by the classic cell motility cycle: polarized intracellular signaling orients protrusions at the lamellipodium of the leading edge, integrins form new adhesions to the substrate in the lamellipodium, and myosin contraction leads to preferential detachment of adhesions in the trailing edge.[1, 2, 17] In many cases, direction of migration is determined by the orientation of the most stable protrusion, and thus cells maintain directionally persistent migration by regulating the

number and orientation of lamellipodia through internal signaling and external cues.[18, 19] Also, new lamellipodial protrusions are often locally generated from pre-existing leading edge instead of randomly across the periphery of a cell and extend laterally from the main longitudinal axis of the cell,[20, 21] and as a result there is usually a gradual sideways shift of lamellipodium when cells do change direction (i.e., cells rarely change direction 180° without external regulation).

Directional cell migration can be caused by intrinsic cell directionality or through various external regulations. Cells with high intrinsic directionality, such as fish epidermal keratocytes, travel less randomly and migrate with high persistence.[22] However, in order to control multiple cells to migrate in the same direction, external regulation must be applied. Such external cues are often gradient-based and include: soluble molecules (chemotaxis),[14, 23] adhesivity to the underlying substrate (haptotaxis),[15, 24] rigidity (durotaxis)[16, 25] and electric field (electrotaxis).[26] However, these gradient-based methods have certain limitations, such as the necessity of the gradient (as a result, cells can only travel a certain distance often in a linear path at a rate proportional to the steepness of the gradient) and inability to control cells individually.

2.2. Key signaling molecules associated with polarized migration

Although the entirety of signaling networks associated with directed migration is complex and still not fully understood, the roles of certain key molecules, namely Par complexes and Rho-family of GTPases, in establishing cell polarity and directed migration are starting to be clear.[13, 27] The Par (partitioning defective) complex

molecules, such as Par3, Par6 and aPKC, are part of the cellular polarity signaling machinery that establish the front-rear (FR) polarity of the cells.[5] Rho-family of GTPases, such as Cdc42, Rac1 and RhoA, are small GTPases that regulate the actin dynamics within the cell.[28] The complex crosstalk among these polarity proteins and small GTPases, as well as other molecules like integrins, Wnt5a and Syndecan 4, largely regulate cell polarization in different cellular contexts across different cell types.[10, 11, 29-33]

In particular, the small GTPase Rac1, which regulates the local actin polymerization at the lamellipodia, has been recently identified as a central determinant for random versus directional motility. High level of Rac1 activity results in the formation of multiple lamellae and lead to non-directional cell movement, while moderate level of Rac1 result in fewer lateral lamellae and lead to directional migration.[34, 35] In addition, mutual inhibition of Rac1-mediated protrusion at the leading edge and RhoA-mediated myosin contraction at the trailing edge has been implicated to aid the stability of FR polarity.[11, 12]

3. Microfabricated systems to control cell motility

Although the concept of contact guidance, the process by which cells are guided by topographical structures, was introduced decades ago,[6, 7, 36] precise physical and geometrical cues for guiding the organization and migration of cells were largely unexplored until recently. Now, the readily available microfabrication techniques have enabled researchers to create well-defined geometrical systems to study how the cells

probe their physical surroundings and acquire mechanical information or signals. Over the past decade, researchers have successfully used microfabrication techniques to control various cell behaviors such as cell shape, survival, differentiation and cell-cell contact.[37-41] However, there has been only limited work on the use of micropatterns to influence cell motility, and thus far, only two main types of micropatterns aimed to geometrically control cell motility exist: line patterns (steps and grooves) and asymmetric patterns (teardrop-shaped).

Line patterns are arrays of straight adhesive tracks; steps and grooves are similar in concept but have an added 3D topography of side walls. Line patterns (steps and grooves) are generally used to polarize and physically limit the movement of cells to one axis and are even effective at nanometer length scales,[36, 42-44] though the direction of movement on the line remains bidirectional (i.e., cells can travel up or down a line). Similarly, arrays of rectangular islands that approximate focal adhesion sizes can be used to control the axis of cell migration, but not the direction of migration.[45] Nonetheless, line patterns at length scales of single cells or below are becoming useful, high-throughput *in vitro* model systems to replicate and study migratory behaviors of cells in natural systems, such as 3D migration through fibrillar matrix[46] and tumor metastasis through blood vessels.[47]

Asymmetric (teardrop-shaped) micropatterns are designed to control the direction of cell movement and are often based on teardrop-shaped islands with a broad, rounded end (‘blunt’ end) and narrow, thin edge (‘tip’ end). Whitesides and colleagues first used such asymmetric geometry to confine and subsequently direct the cell movement after

release from confinement,[48] but this spontaneous bias disappeared shortly after the release of the cells. Recently, Grzybowski and colleagues used a series of asymmetric ratchets to guide cell populations, but due to the very low innate directional bias of the pattern, partitioning and sorting was only partially achieved.[49] All in all, there has been only one successful demonstration of effective and prolonged directed cell motility using micropatterns, and it was by Co and colleagues who utilized four teardrop-shaped adhesive islands set up in a square configuration to induce unidirectional movement of fibroblasts around the islands.[50] These studies suggest that physical interactions of the cells with underlying topography of ECM, independent of chemical factors, can induce responses and signaling to promote directional migration.

4. Unresolved questions on directed cell motility on micropatterns

Because of the limited number and extent of studies with successful directed cell motility using micropatterns, there are many unresolved questions to be addressed. The first and most relevant question is on the mechanism of directed motility on micropatterns, or in other words, why and how do the cells move in a biased fashion? Co and others suggest that the elongated polarization of the cells on the island and the availability of the adjacent islands along the polarized axis cause the directional bias.[50] However, they do not sufficiently address why the cells move to the adjacent island once the lamellipodial extension is made (i.e., why there is a net translocation to the next island). Furthermore, they specify polarization as an important factor for directional bias, but do not explore or alter the degree of polarization in their work. Thus, we still do not

completely understand the cause of directional bias, nor the precise factors contributing to directed cell motility on micropatterns.

The second question is on the generality and robustness of the directed cell motility observed thus far. Co and others have tested their patterns on 3T3 fibroblasts and human microvascular endothelial cells (HMVECs) and confirmed directed movement.[50] However, would other cell lines interpret the underlying geometry differently? In addition, would the bias be in the same direction as previously observed and would the mechanism of directed motility be the same? Although different cell lines are expected to exhibit different motility tendencies and have been observed to do so,[51, 52] it may be worthwhile to explore commonalities between cell types that exhibit similar directional bias and behavior to elucidate the key factors that govern directed cell motility. For example, could it be a specific signaling pathway involved or the morphology they assume under mechanical constraint?

The third question is on strategies to optimize and modulate directed motility, as well as future directions and applications. We have thus far only observed the phenomenon of directed motility through micropatterns, but have not seen any attempt to modulate it through experimental manipulations. Can the directional bias be enhanced or controlled through geometrical alteration of the underlying micropatterns or re-wiring of cell signals and mechanics? Furthermore, as we begin to understand the mechanism of directed cell motility on micropatterns and be able to control it, how can we utilize it for greater applications?

This report aims to answer some of these questions and shed light on this largely unexplored field of directed cell motility using micropatterns.

5. Current results

In this report, we investigate the phenomena of directed cell motility on micropatterns from different angles, explore various factors that affect this phenomena and design strategies based on our observations and hypotheses to enhance or change the directional bias. We also address the concept of single cell versus multicellular manipulation and responses of different cell types on these micropatterns. Overall, the chapters in this report build up in a logical order and may be best followed in sequential order.

Chapter II focuses on the basic single cell analysis of directional bias of cells on teardrop-based micropatterns. Starting from micropatterns proven to be effective in previous studies,[50] we elucidate the fundamental pattern parameters crucial for the directed movement of MCF-10A epithelial cells. Through quantitative analysis of cell motility, we identify highly favored hops and design new patterns to enhance the directional bias. We also closely examine the favored hops and notice the involvement of sideways lamellipodial protrusion. Based on this observation, we hypothesize that altering the Rac1 signal pathway involved in lamellipodial protrusion may change the directional bias and demonstrate that indeed that is the case. In addition, we introduce the splitter motif designed to modulate the flux of cells.

Chapter III focuses on the motility of cells, such as speed and persistence length, and how different motifs of micropatterns can be combined to create a novel, hybrid pattern with enhanced motility. Here, we examine the motility and persistence of MCF-10A cells on line patterns, and find that specific line width optimally enhances the speed and persistence of cells. We proceeded to combine the enhanced motility of line patterns and the directional bias of teardrop-based patterns to create a hybrid pattern, which excels both the original patterns in terms of motility. Through quantitative comparison of cell movement on the classic teardrop pattern and the hybrid pattern, we found that the line component in the hybrid pattern allows the cells to travel longer distances without having to pause at junctions and thus result in enhanced speed and persistence. This chapter also introduces higher-order partition patterns to direct the motility of cell populations. Studies with multicellular systems reveal complexities that were not observed in single cell systems. Nonetheless, the effectiveness of the partition patterns surpass any previously reported pattern-based partitioning.[49]

Chapter IV focuses on the establishment of front-rear (FR) polarity and scaling of micropatterns to enable directed motility for different cell lines. Here, we examine the directional bias of various cell types on the classic teardrop patterns and find that cells with high bias assume a unilamellar morphology with heavily one-sided FR polarity, while the cells with no bias fail to do so. Based on previous studies,[46] we investigate the relationship between the establishment of unilamellar morphology and the physical constraint imposed by the line micropattern, and find that the fraction of cells with unilamellar morphology correlates with the degree of physical constraint (i.e., line width). Thus, in order to increase the directional bias for moderately biased cell line, we design

thin teardrop patterns with decreased width. Indeed, the directional bias increases significantly for the previously moderately biased cell line, but more surprisingly, certain configurations which were not biased with the original teardrop now become biased with the thin teardrop. This suggests that the degree of physical constraint is not only important for the establishment of FR polarity, but is also a crucial factor for dictating the directional tendencies at the ends of the teardrop islands.

Together, these studies deepen our understanding on directed cell motility using micropatterns, offer several strategies to enhance the motility and directional bias for different cell lines, and lay a foundation for wider application using micropatterns such as cell sorting and tissue engineering.

6. References

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