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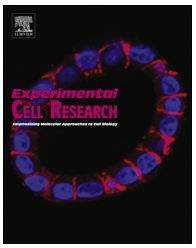

Research Article

Collective cell migration of primary zebrafish keratocytes

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ABSTRACT

Fish keratocytes are an established model in single cell motility but little is known about their collective migration. Initially, sheets migrate from the scale at ~145 μm/h but over the course of 24 h the rate of leading edge advance decreases to ~23 μm/h. During this period, leader cells retain their ability to migrate rapidly when released from the sheet and follower cell area increases. After the addition of RGD peptide, leader cell lamellae are lost, altering migratory forces within the sheet, resulting in rapid retraction. Leader and follower cell states interconvert within minutes with changes in cell–cell adhesions. Leader cells migrate as single cells when they detach from the leading edge and single cells appear to become leader cells if they rejoin the sheet. Follower cells rapidly establish leader cell morphology during closing of holes formed during sheet expansion and revert to follower cell morphology after hole-closure. Inhibition of Rho associated kinase releases leader cells and halts advancement of the leading edge suggesting an important role for the intercellular actomyosin cable at the leading edge. In addition, the presence of the stationary scale orients direction of sheet migration which is characterized by a more uniform advance of the leading edge than in some cell line systems. These data establish fish keratocyte explant cultures as a collective cell migration system and suggest that cell–cell interactions determine the role of keratocytes within the migrating sheet.

Introduction

Zebrafish keratocytes rapidly and collectively migrate when established in explant cultures. Although the mechanisms of motility of keratocytes as single cells have been extensively studied [1–6], little is known about the mechanisms which direct the migration of keratocytes as cell sheets. This experimental system is particularly compelling in vitro model for collective cell migration during reepithelialization in response to wounding for several reasons. First, establishment of explant cultures promptly initiates an epithelial to mesenchymal transition (EMT), which rapidly progresses over the first seven days of explant culture as assessed by changes in gene expression including a switch from E-cadherin to N-cadherin expression, morphological changes, and cytoskeletal rearrangement [7,8]. As changes in gene expression consistent with inflammation and wound healing occur concurrently with EMT, this system has been characterized as a wound healing model [7]. Second, as explant cultures, keratocytes are not transformed and have not undergone the changes in gene expression associated with transformation or passage of primary cultures [9–13]. Thus, the

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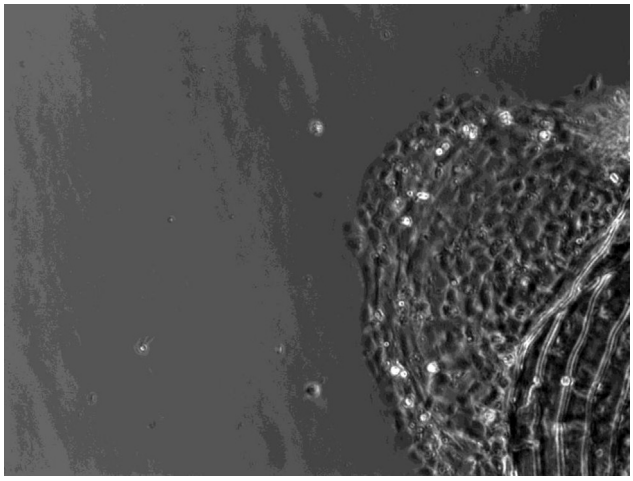

Video 1 Migration of keratocytes from underneath a stationary scale in an explant established 8 h prior to filming. The elapsed time is 5 h with frames taken 5 min apart. A video clip is available online. Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.yexcr.2014.06.011.

model allows rapid collective cell motility assays (complete within 24 h of initial treatment) in untransformed cells in the context of a defined wound healing process.

During the re-epithelialization stage of wound healing, large sheets of epithelial cells collectively migrate to reestablish the intact epidermis. Key to this process are the alterations in quiescent, pre-wound epithelial cells to form a polarized sheet with the formation of leader cells at the wound edge (reviewed in [14]) and an epithelial to mesenchymal transition which typically promotes motility. Although the common hallmarks of collective cell migration have been established, including the presence of leader and follower cells and intact cell junctions, many of the mechanisms guiding collective cell migration are yet to be determined. Due to its compelling characteristics, including rapid motility assays, the zebrafish keratocyte system has promise to shed light on mechanisms of collective cell migration.

The data presented here establish the migration of zebrafish keratocyte cell sheets as a collective cell migration system through 1) characterization of distinct leader and follower cells in 24 h sheets and 2) demonstration of the presence of cell–cell junctions that coordinate an actomyosin cable between adjacent

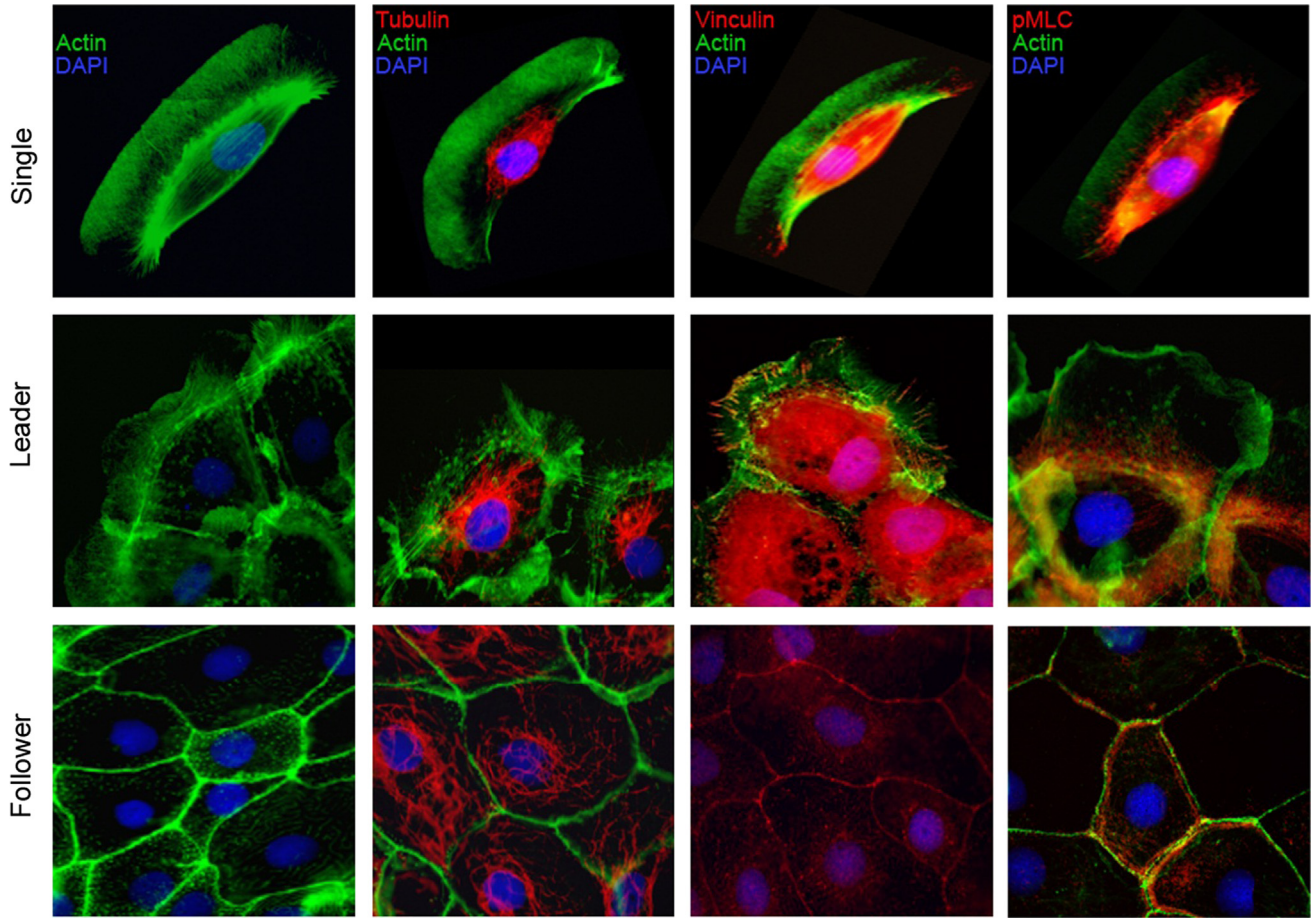

Fig. 1 – Actin, tubulin, vinculin, and phosphomyosin light chain in single, leader, and follower keratocytes. Untreated 24 h explant cultures were fixed and stained with primary antibody to tubulin, vinculin, or phosphorylated myosin light chain (red) and counterstained with phalloidin (green), and DAPI (blue). Vinculin localization in follower cells is presented without phalloidin counterstain as display of actin obscures vinculin localization. Single cells resemble cells at the leading edge while follower cells (photographed in regions with a single cell layer) are not polarized. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)