Keratin and actin dynamics during epithelial cell migration

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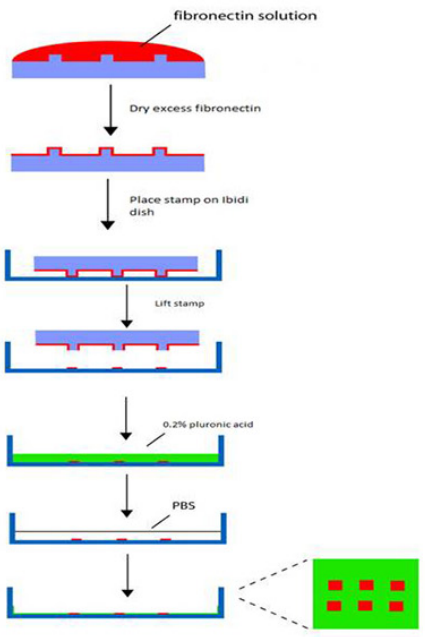
Abstract

Cytoskeleton dynamics are highly regulated and important for proper cell migration. The most abundant cytoskeleton components in epithelial cells are the keratin intermediate filaments. And yet, the involvement of keratin intermediate filaments in cell migration is mostly unknown. Combining confocal imaging in human primary keratinocytes with drug treatments and micro-patterning, we aim to dissect the interdependence of keratin intermediate filaments and actin during cell migration. Within these migratory epithelial cells there is a clear border between the keratin and actin filaments, where these filaments seem to interact. The interactions might be mechanically, but can also occur though crosslinkers such as myosin or plectin. Further research is needed to elucidate how the keratin-actin border is established and how this border and the interactions there are involved in cell migration.

Materials and methods

Cells: primary neonatal human epithelial keratinocytes (nHEK). Cells were transiently transfected 1 day prior to imaging with either Keratin5-YFP and LifeAct-RFP or Keratin14-YFP with LifeAct-mRuby or tdTomato-MyosinIIA. Cells were seeded on fibronectin coated (2,5µg/cm^2) glass bottom dishes. nHEKs were treated 10min with 5µM Latrunculin-B, before washing twice with medium.

Microcontact printing: Ibidi glass bottom dishes were patterned using PDMS stamps with 1800µm^2 circular pattern according to the method described by Théry and Piel [1]:



Microscopes: Zeiss LSM710 inverted microscope with Airyscan detector, PerkinElmer Ultraview VoX on an Olympus IX81 inverted microscope and Nikon N-SIM.

Results

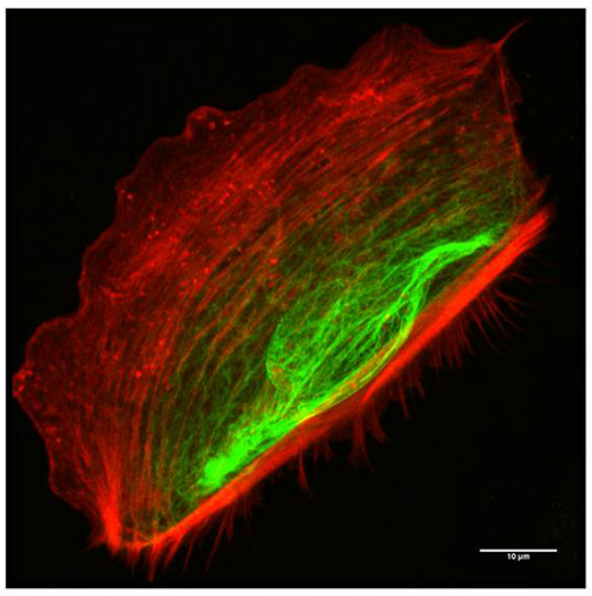


Figure 1: Maximum projection of a migrating nHEK with actin (red) and keratin (green), showing a clear separation of the two cytoskeleton networks.

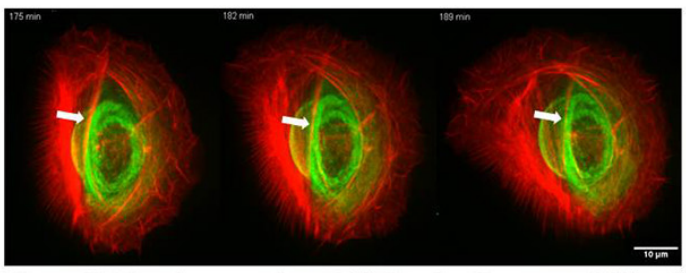


Figure 2: Time lapse series of nHEK migrating on an 1800µm^2 circular Fn pattern demonstrating the inward movement of both actin (red) and keratin (green) filaments, where the keratin filaments seem to be pushed by the actin (arrows.)

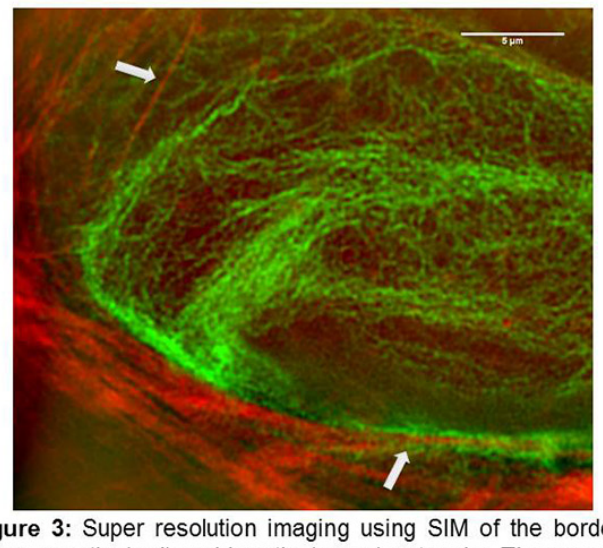


Figure 3: Super resolution imaging using SIM of the border between actin (red) and keratin (green) networks. The arrows point towards actin filaments that are surrounded by keratin filaments, moving simultaneously during cell migration.

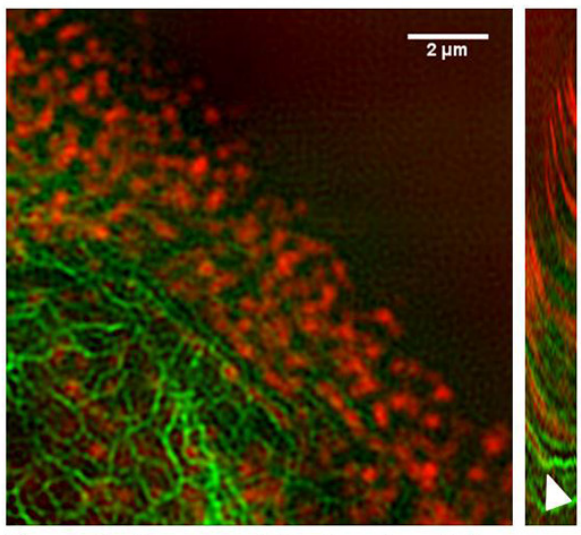


Figure 4: Super resolution imaging of the same border region, looking at keratin (green) and myosin-IIA (red.) As for actin, myosin-IIA shows a clear border with keratin. The kymo-graph shows myosin-IIA decelerating upon contact with keratin, sometimes even disappearing from the imaging plane (arrowhead.)

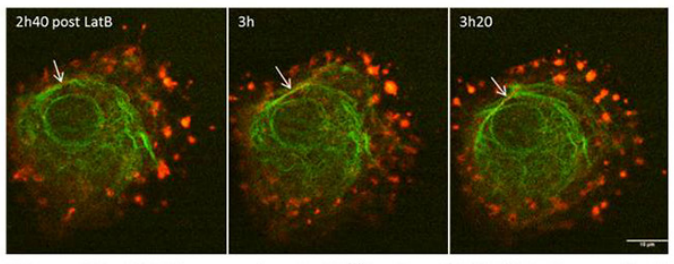


Figure 5: nHEKs were treated 10 min with 5µM Latrunculin-B to abolish the actin network. The keratin network (green) quickly recovered without an actin network. However, thicker keratin filaments only appeared together with actin fibers (red) and disappeared together with these fibers as well (arrow).

Conclusions and outlook

- During cell migration, actin and keratin inward movement is somehow linked.
  - It remains to be elucidated what the exact link between the two networks is
  - Candidates are myosin and crosslinkers such as plectin
- The inward movement of actin via the actomyosin system is stopped at the border with keratin.
- Although thicker keratin filaments seem to depend on actin for their movement, the keratin network does not depend on actin for its continued cycling

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Literature cited

[1] M. Théry and M. Piel, "Adhesive micropatterns for cells: A microcontact printing protocol," *Cold Spring Harb. Protoc.*, vol. 4, no. 7, Jul. 2009.