The Role of the Nucleus in Collective Cell Migration

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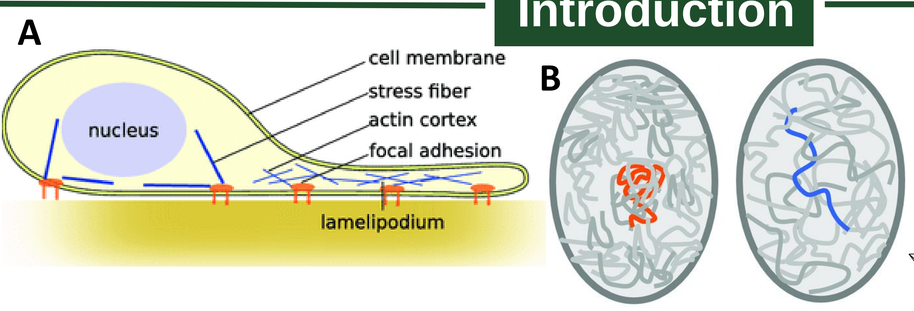
Introduction



- Collective cell migration is crucial for many biological processes including development, wound healing, and tissue engineering applications.
- The cell nucleus is emerging as a key player in this process as it's the largest and stiffest organelle[4,5].
- We hypothesize that chromatin remodeling, inhibited by GSK126, and nuclear stiffness, inhibited by Trichostatin A (TSA), are key mediators of cell migration.

Figure 1. (A) Visualization of single cell crawling in 2D[1]. (B) Depiction of condensed chromatin (left) and decondensed chromatin (right)[2] illustrating GSK126 and TSA treatments respectfully. (C) Visualization of chromosome structure showing a chromosome, chromatin fiber, histones, nucleosomes, and DNA from top to bottom[3].

Goal

To investigate how nuclear mechanics and chromatin remodeling affect collective cell migration using a model scratch wound assay.

Materials and Methods

Scratch Wound Assay:

- A layer of murine fibroblast cells (3T3) is cultured in an 8-well plate.
- A scratch is applied, and time lapse imaging of the healing process provides migration data.
- Automated cell tracking is done on stained nuclei to collect quantitative data using TrackMate[6] for Fiji[7].
- All imaging done using Zeiss LSM 980 microscope.

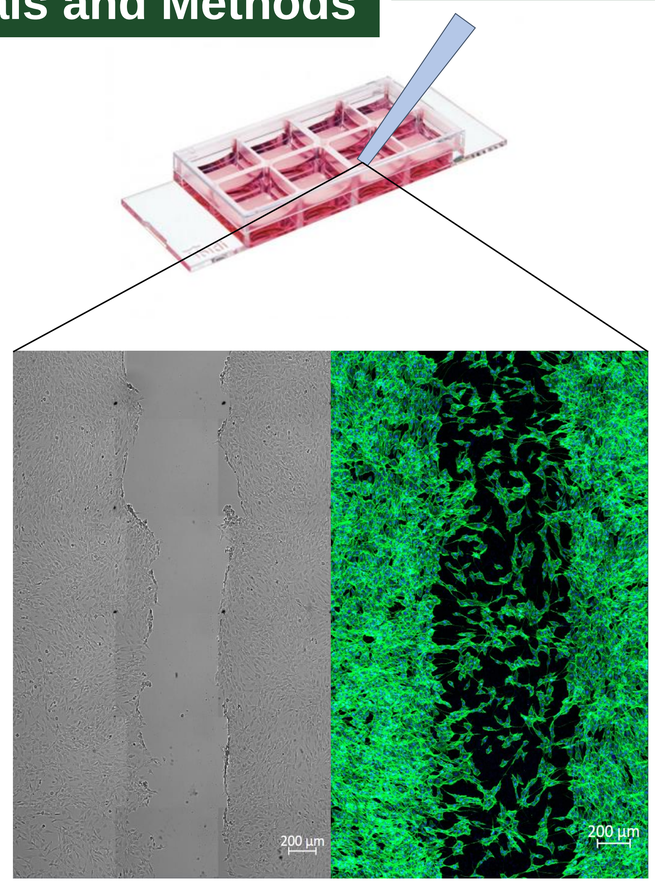
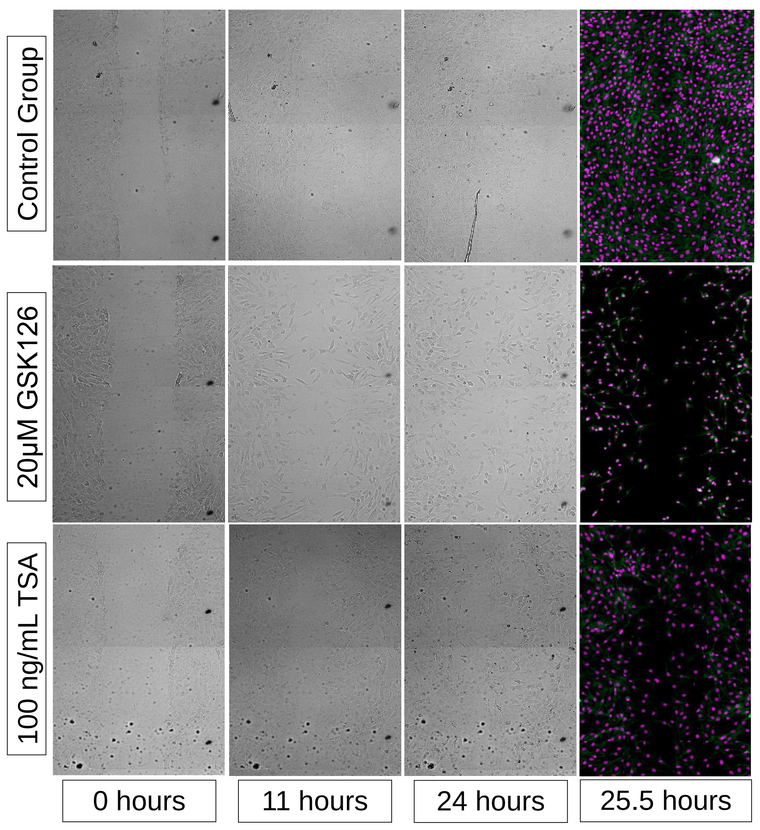


Figure 2. A cartoon illustrating the initiation of the scratch wound assay (top), how this scratch looks under the microscope (bottom-left), and what migration into the wound looks like with cells stained for the nucleus and actin (bottom-right).

Results and Discussion



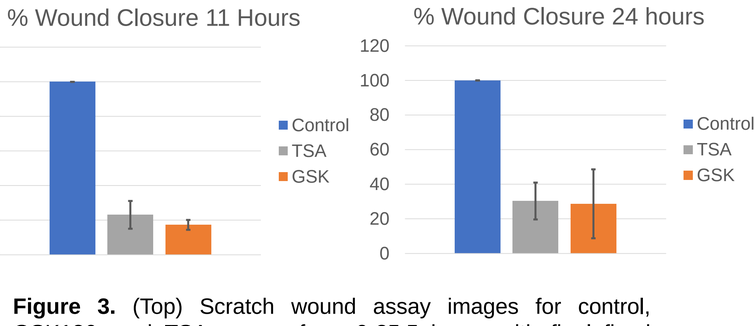


Figure 3. (Top) Scratch wound assay images for control, GSK126, and TSA groups from 0-25.5 hours with final fixed images stained for nuclei in purple and actin in green. Scale bar is 100 µm (Bottom) Wound closure percentage graphs of groups at 11 and 24 hours showing a significant decrease for the TSA and GSK126 treatments. Error bars show standard deviation about the mean with (n = 3) samples.

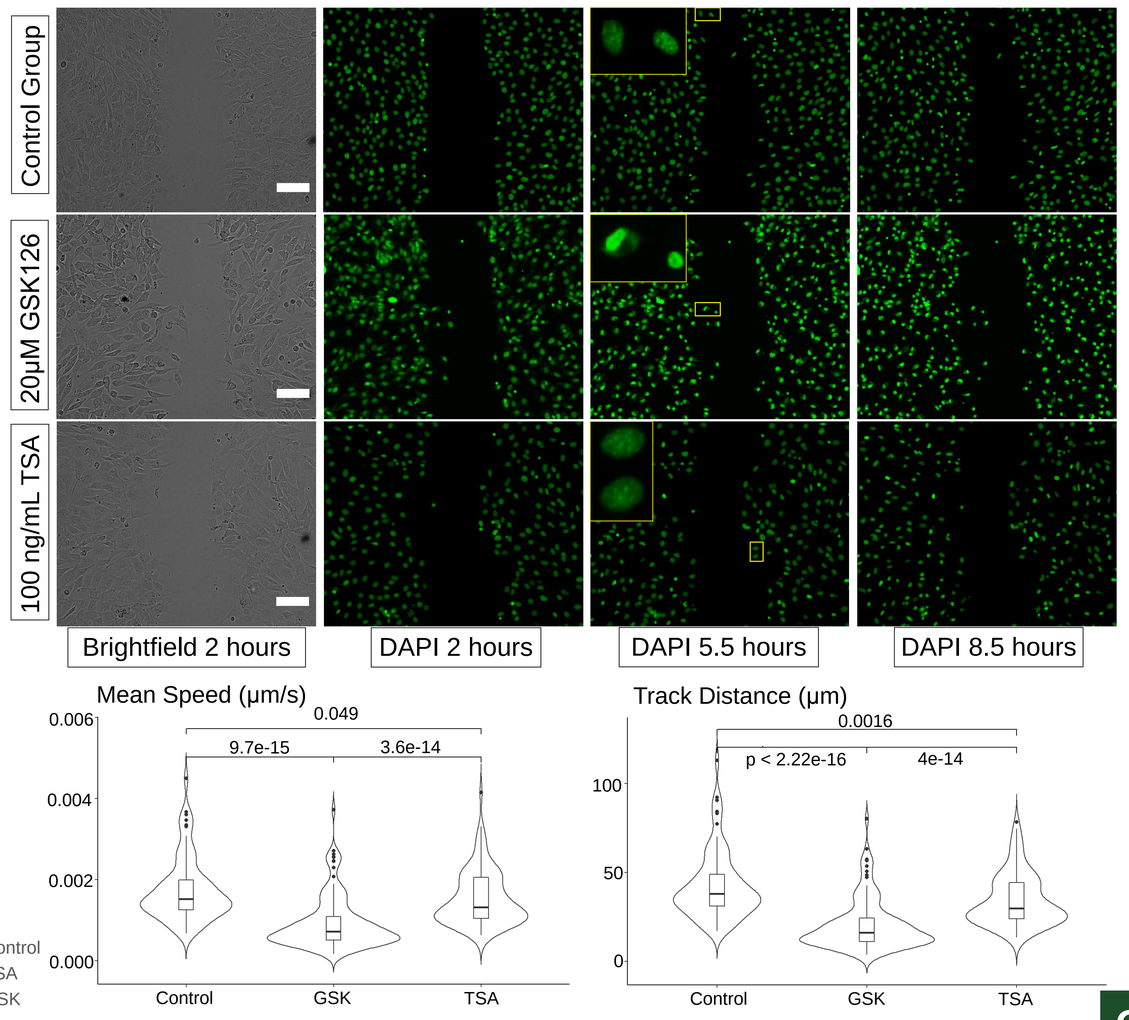


Figure 4. (Top) Scratch wound assay images live stained for the nucleus with DAPI. Detailed chromatin architecture is highlighted in the 5.5-hour images. Scale bar is 100 µm (Bottom) Violin plots for the mean speed and track distance of migrating cells at the wound edge. TSA shows a small decrease in speed and distance while GSK126 drastically decreased speed and distance. (n > 73) cells tracked for each group.

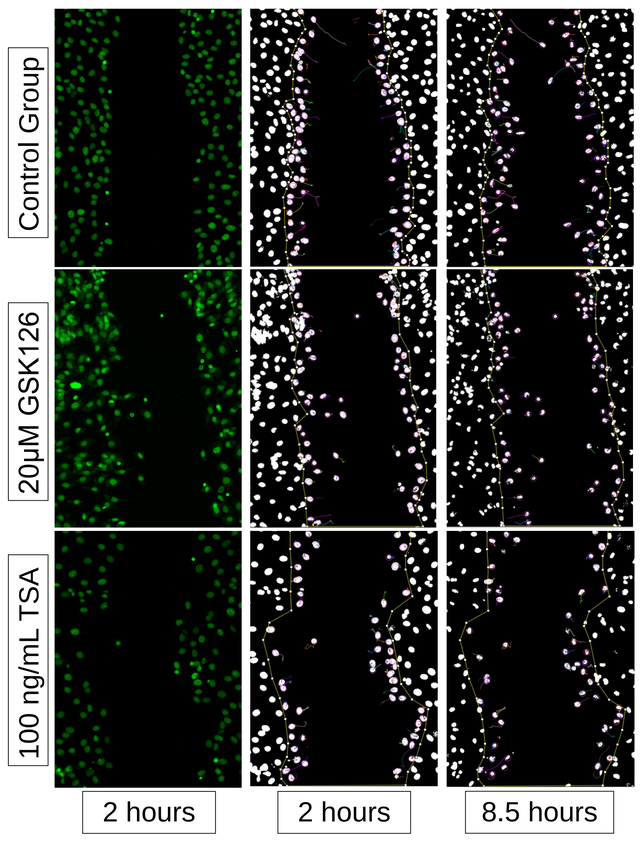


Figure 5. TrackMate path visualization for cells migrating in figure 4. Cell nuclei are shown as white dots with spot detection shown as purple rings and migration tracks shown as colored lines for cells on the wound edge encompassed by a yellow polygon.

Conclusion

→ Trichostatin A decreases migration speed and wound closure efficiency

→ GSK126 decreases wound closure efficiency and drastically reduces migration speed

Future Work

Next Steps:

- Investigate angular components of migration to determine if chromatin remodeling affects the ability to follow collective migration gradients.
- Development of a predictive biophysical model to determine migration parameters from the nuclear mechanics/chromatin remodeling parameters.
- Adapting the methods created in this study to other cell types for fundamental biophysical research and applied translational research.

References and Acknowledgments

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