Effects of Microtubule-Targeting Agents on Glioma Cell Traction

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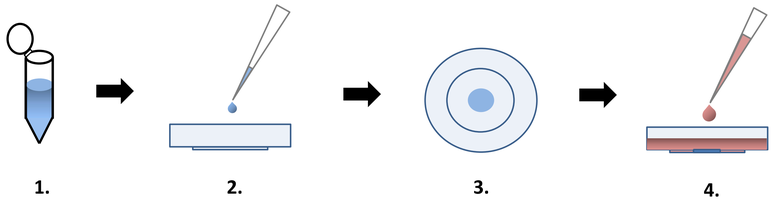
Abstract

Glioblastoma multiforme, also known as Grade IV Glioma, is a malignant brain tumor in humans that is characterized by highly migratory and invasive cells that spread throughout the brain. Standard treatment of many invasive cancers employs the use of microtubule-targeting agents, which disrupt microtubule dynamics inside cells. Microtubules play a role in regulating the traction forces necessary for cell migration, but the mechanism by which they do this is not yet known. If more is learned about microtubules' role in cell migration, there will be potential for improvements on current treatment methods. Here we show that vinblastine, a drug that promotes microtubule depolymerization, increases the traction forces exerted by glioma cells on the extracellular environment *in vitro*. We also show that cells treated with blebbistatin (a drug that decreases cell traction force by inhibiting myosin II motor proteins) experience a recovery of traction force when treated with vinblastine. Based on these results, we conclude that increased depolymerization of microtubules in glioma cells causes an increase in cell traction force by increasing myosin II activity.

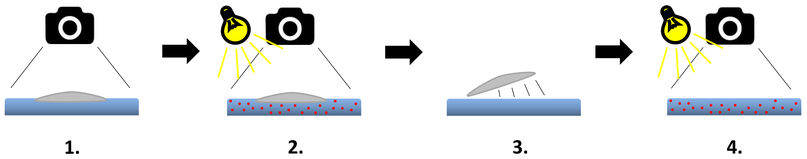
Methods

Creation of Polyacrylamide Substrates and Cell Plating



1. **A polyacrylamide gel (PAG) solution is mixed with fluorescent nanobeads in a microcentrifuge tube.**
2. **PAG is pipetted into a 35 mm glass-bottom MatTek dish.**
3. **PAG is allowed to polymerize and is then coated with collagen.**
4. **U251 glioma cells suspended in media (pink) are allowed to adhere to collagen-coated PAG substrate.**

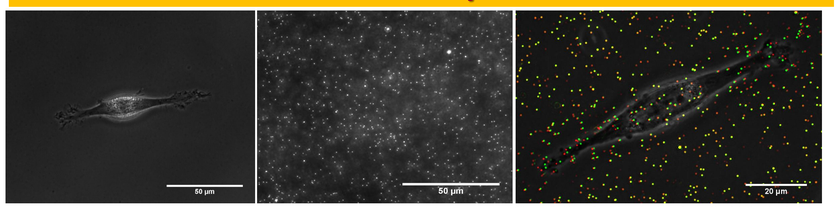
Traction Force Microscopy



1. **A cell is imaged with transmitted light. The cell is exerting a traction force on the substrate.**
2. **Nanobeads in the substrate directly under cell are imaged. The beads fluoresce under yellow light.**
3. **The cell is chemically detached from the substrate with Trypsin, a compound that targets integrins.**
4. **The nanobeads are imaged again with no force being exerted on the substrate.**

If a drug is to be used for a data set, the desired concentration of the drug is added to the cell media in the dish 30 minutes prior to imaging.

Data Analysis

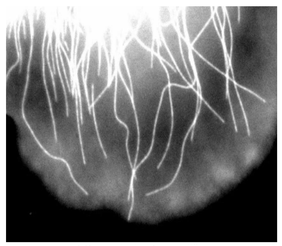


A glioma cell on a PAG substrate. Fluorescent nanobeads beneath the cell. Glioma cell with nanobeads (green) and superimposed beads in the relaxed substrate.

The image of the cell, the image of the beads with an exerted force, and the image of the beads with the cell detached are combined into a stack. A MATLAB script is used to match and align the bead images. Because the traction force of the cell causes the beads to move between the two images, only those beads further from the cell are used in the alignment. The distance the beads around the cell move is now apparent, and because the Young's modulus of the substrate is known, another MATLAB script is then used to determine the force that was being exerted by the cell.

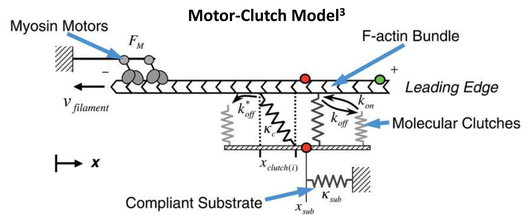
Background

In glioma cells, as well as all animal cells, microtubules inside the cells are continually polymerizing and depolymerizing, extending from and retreating back to the centrosome. This is known as dynamic instability. It has been observed that dynamic instability plays a role in regulating cell traction, as well as movement.[1] Vinblastine is a microtubule-targeting agent that promotes microtubule depolymerization, which causes microtubules to extend toward the edges of the cell but never retreat, disrupting dynamic instability.



Microtubules in a cell[2]

It is hypothesized that the mechanism by which cells exert forces on their surroundings is a motor-clutch system.[3] In this system, actin fibers polymerize at the leading edge of the cell and bond to integrins, or "clutches," which the cell uses adhere itself to its substrate. Myosin II motors pull the actin fibers retrogradely, and thus the cell exerts a traction force through the clutches.



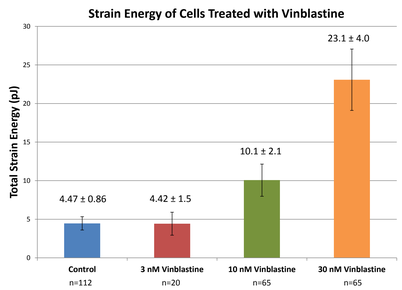
Since the disruption of dynamic instability affects cell traction, it can be concluded that microtubules have an influence on the function of the motor-clutch system. Many tools and methods can be used to investigate the details of this influence, one of which is the drug blebbistatin. Blebbistatin decreases myosin motor activity, so it can be used in conjunction with other drugs, such as vinblastine, to gain insight into the influence microtubules have on the motor-clutch system.

Results

Increasing Dose of Vinblastine

Cells were treated with 3, 10, or 30 nM vinblastine. Cells treated with higher concentrations of vinblastine experienced a greater rise in traction force. These averages include only those cells which were fully adhered to the substrate.

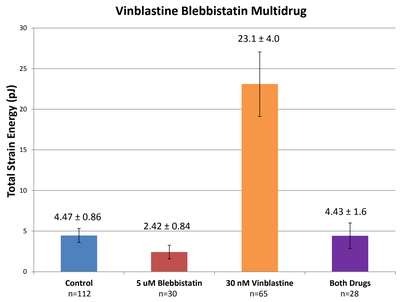
Additionally, it was observed that in dishes treated with higher concentrations of vinblastine, a greater fraction of cells were rounded and barely adherent to the substrate.



Multidrug – Vinblastine and Blebbistatin

Cells were treated with 5 uM blebbistatin, 30 nM vinblastine, or a combination of both drugs. The resulting traction force data shows that cells treated with both drugs experience a recovery of traction force when compared to cells treated with blebbistatin only.

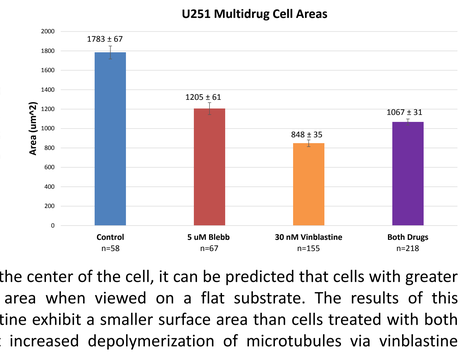
Because blebbistatin decreases traction force by inhibiting myosin II motors, this data suggests that the increase in traction force of vinblastine-treated cells can be explained by increased myosin II activity.



Results (continued)

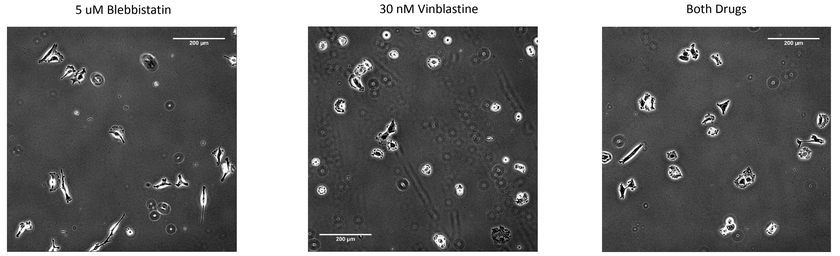
Multidrug Cell Areas

Cells were treated with 5 uM blebbistatin, 30 nM vinblastine, or a combination of both drugs. A large image of a PAG substrate with cells was taken, after which a MATLAB script was used to determine the areas of each cell.



Because myosin pulls actin and clutches toward the center of the cell, it can be predicted that cells with greater myosin activity will exhibit a smaller surface area when viewed on a flat substrate. The results of this experiment show that cells treated with vinblastine exhibit a smaller surface area than cells treated with both drugs. This data supports the hypothesis that increased depolymerization of microtubules via vinblastine results in greater myosin activity.

5 uM Blebbistatin | 30 nM Vinblastine | Both Drugs



It is evident that cells treated with vinblastine have a smaller surface area than cells treated with blebbistatin or both drugs

Conclusions

The results of the experiments suggest that increased depolymerization of microtubules in glioma cells increases myosin II activity. When cells are treated with vinblastine, which promotes depolymerization, an increase in cell traction is observed. When cells are treated with blebbistatin and vinblastine, they experience a recovery of traction force when compared to cells treated with blebbistatin only. Additionally, cells treated with vinblastine exhibit smaller surface areas than cells treated with both blebbistatin and vinblastine. These results are all supportive of the hypothesis that microtubules influence myosin motor activity, and that increased depolymerization of microtubules increases motor activity.

References

[1]Danowski BA (1989) Fibroblast contractility and actin organization are stimulated by microtubule inhibitors. Journal of Cell Science 93:255–266.

[2]"David Odde Laboratory." *University of Minnesota*. University of Minnesota, n.d. Web. 13 Apr. 2015.

[3]Bangasser, Benjamin L., Steven S. Rosenfeld, and David J. Odde. "Determinants of Maximal Force Transmission in a Motor-Clutch Model of Cell Traction in a Compliant Microenvironment." Biophysical Journal 105.3 (2013): 581-92. Web.