Investigation of The Role of KrsB in Rap1-Mediated Adhesion of *Dictyostelium discoideum*

Kelsey Roberts and Dr. Yulia Artemenko

Department of Biological Sciences, SUNY Oswego

Introduction

- *Dictyostelium discoideum* is a soil-dwelling amoeba.
- *D. discoideum* is a good model organism for cell migration studies because its movement is like other amoeboid cells, such as neutrophils and metastatic cancer cells.
- Cell adhesion to substrate is an essential component of migration, yet little is known about its mechanisms in *D. discoideum.*
- Rap1 is a small GTPase. Its expression has been previously shown to increase cell adhesion, possibly by regulating Talin and Myosin II.[1,2]
- Kinase responsive to stress B (KrsB) is a negative regulator of cell adhesion, so activation of KrsB leads to decreased cell adhesion. This could be because it is negatively regulating Rap1.
- Previous studies have shown that expression of KrsB is not required for Rap1 to function, as its absence does not appear to change effect of Rap1 on adhesion.

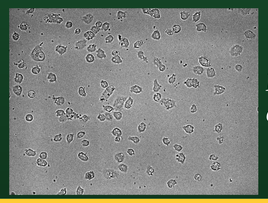


Image of D. discoideum cells in brightfield view

Research Question and Hypothesis

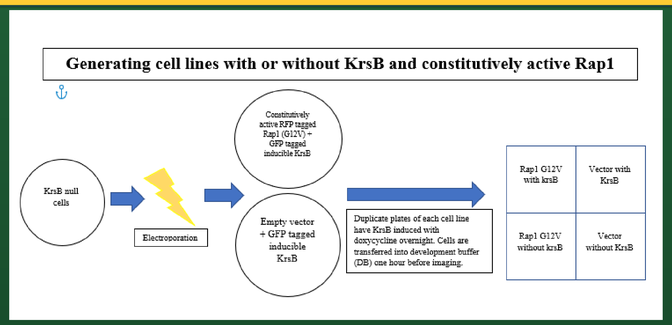
Question:

Does the expression of KrsB in *D. discoideum* reduce cell adhesion by negatively regulating Rap1?

Hypothesis:

- KrsB is a negative regulator of Rap1. If this hypothesis is true, the overexpression of KrsB should reduce the ability of Rap1 to increase spreading.

Methods



- Random migration and cell spreading was imaged at 400X magnification on an LSM700 Zeiss confocal microscope.
- Three different positions were imaged per well in brightfield. Images were acquired every 15 sec for 45 frames.
- Images from the last frame were used in measuring cell spreading.
- After random migration, GFP and RFP images were taken for each ending position using epifluorescence.

Methods

Quantification:

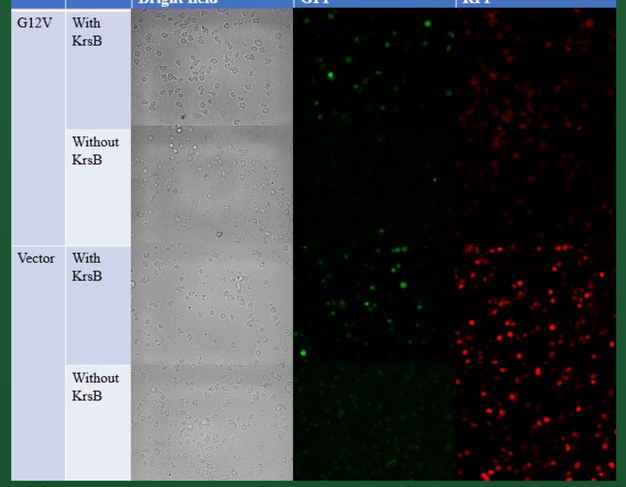
- Cell velocity was measured using Tracking tool pro. Area, perimeter, and GFP brightness were measured using FIJI (Image J).
- For KrsB-induced cells, only cells with high KrsB expression (measured by GFP brightness values) were quantified.

Statistical Analysis:

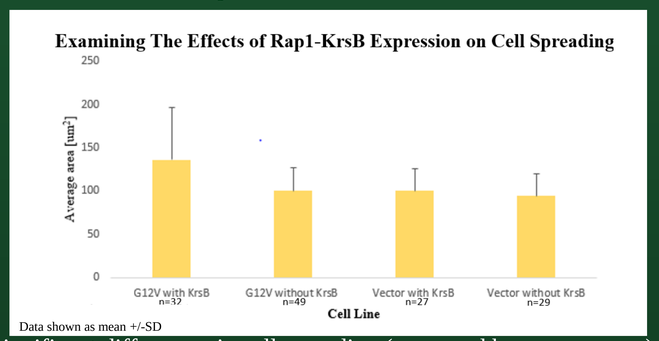
- A one-way ANOVA was conducted to test for a relationship between KrsB expression/ mutant type and cell velocity, area and perimeter.

Results

Testing expression of RFP-Rap1 G12V or RFP alone (vector) and KrsB-GFP following doxycycline induction in KrsB-null cells

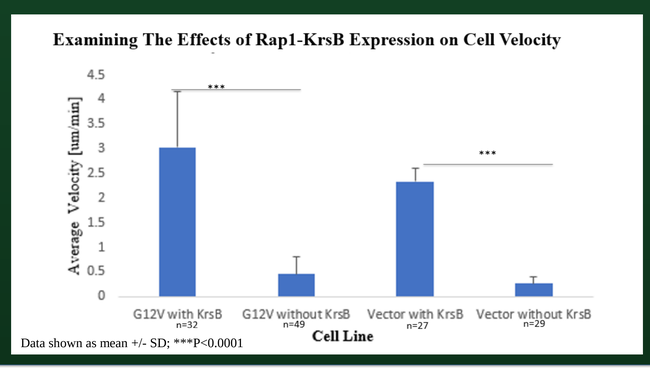


- Brightfield images were acquired every 15 sec for 10 min and quantified to determine velocity. The last frame, which was used to quantify cell spreading, is shown. RFP and GFP images were captured for each position immediately after the last frame. One representative field is shown for each cell line.



Data shown as mean +/-SD

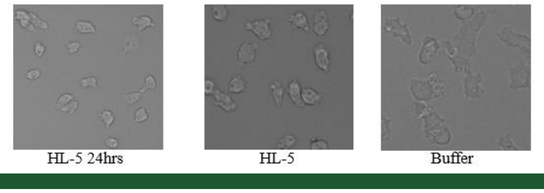
- No significant differences in cell spreading (measured by average area) were found between cell lines.

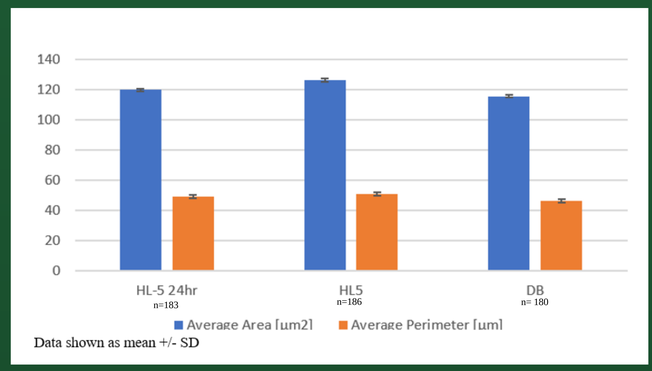


Data shown as mean +/- SD; ***P<0.0001

Results

Evaluating Spreading in Cells Imaged in HL-5 and Buffer





Data shown as mean +/- SD

- No significant difference was found between cell spreading in cells imaged in either buffer or HL-5 media.

Discussion and Conclusions

- Results of the random migration show that KrsB expression in KrsB-null cells with or without constitutively active Rap1 improves migration. Interestingly, this is not accompanied by changes in cell spreading.
- Since Rap1 G12V did not show the expected increase in spreading, we tested the imaging conditions using Wild-Type cells to see if there was a difference in spreading when cells are imaged in buffer.
- There was no difference in cell spreading found between imaging conditions.

Set-Backs and Future Directions:

- Rap1 expression was not considered during quantification, meaning that some of the cells that were measured may have lacked Rap1.
  - Rap1 expression will be looked at, and only cells with Rap1 will be measured
- Transformed Rap1 G12V cells tended to lose KrsB expression as they aged, at a much faster rate compared to the other cell lines.
  - More recent zaps will be used in future experiments
- Throughout the experiment we had frequent problems with contamination, leading to a smaller sample size.
  - Future experiments will ensure a more sterile environment when handling cells.

References

[1]Artemenko et al. Assessment of development and chemotaxis in Dictyostelium discoideum mutants. *Methods Mol Biol*. 2011;769:287-309. doi:10.1007/978-1-61779-207-6_20

[2]Artemenko Y, Devreotes PN. Assessment of Dictyostelium discoideum Response to Acute Mechanical Stimulation. *J Vis Exp*. 2017;(129):56411. Published 2017 Nov 9. doi:10.3791/56411

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