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PROJECT RAISE

Immunolabeling of Transporter Proteins on Vaginal Epithelial Cells

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Background

- *Immunocytochemistry*: The use of primary and secondary antibodies to detect specific antigens on cells
- *Antigen*: any substance that triggers an immune response in the body. This can include viruses, allergens, and certain proteins.
- Proteins on the plasma membrane of vaginal epithelial cells, called *transporters*, are of particular interest as the passage of most molecules entering and exiting the cell is mediated by these proteins.
- Little is known about the expression of transporters on vaginal tissues, but by introducing antibodies and fluorescent markers to samples of vaginal cells, it is possible to investigate and study these transporters

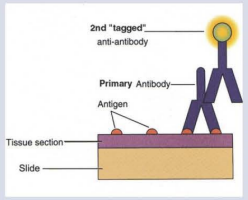

Figure 1. This diagram shows the primary antibody binding with a specific transporter protein (antigen) on the tissue section. The secondary antibody, with a fluorescent marker attached, binds with the primary antibody.

Methods and Materials

- *Sample population*: 58 different vaginal epithelial cell samples, 40 samples from volunteers in the Monrovia community (*VAGOMICS*) & 18 samples from women in South Africa who are HIV positive (*CAPRISA, or* CENTRE FOR THE AIDS PROGRAMME OF RESEARCH IN SOUTH AFRICA)
- *SLC2A1*: Glucose transporter protein; ubiquitous and found on the plasma membrane of cells throughout the human body
- The cells from the samples were embedded in a 2% agarose gel and were subjected to indirect antibody labelling with an anti-SLC2A1 rabbit antibody (*primary Ab*), which recognizes the glucose transporter protein, SLC2A1, embedded within the cellular membranes.
- The samples were then incubated with a goat-anti-rabbit antibody (*secondary Ab*), which is "tagged" with a fluorescent marker and binds to the primary antibody.

Methods and Materials (cont'd)

Each sample was also labeled with other fluorescent cellular markers:

- *CON-A* (*Concanavalin A*) - labels glycoproteins on the plasma membrane
- *DAPI* (*4',6-diamidino-2-phenylindole*) - labels nucleotide-rich portions of a cell.

These markers allow the cells to be imaged with a confocal microscope under specific wavelengths. A set of data can be made by calculating the percentage of cells that label with the secondary Ab in comparison to the total number of cells in a set view of focus.

Additional solutions used for preparation of the specimens are as follows:

- Formaldehyde
- 1x PBS
- 0.12% Glycine
- 0.1% Triton-X 100
- 2% BSA
- Mowiol

Figure 2. This chart shows the steps in order, from embedding samples in 2% Agarose, to imaging samples under a confocal microscope

Results

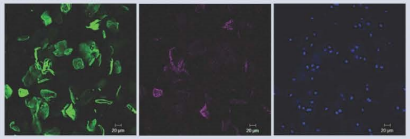

Figure 3. VAGOMICS 011F. CON-A is in green, CY3 is in violet, and DAPI is in blue.

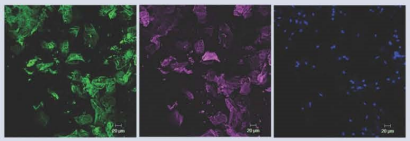

Figure 4. CAPRISA #38

Results (cont'd)

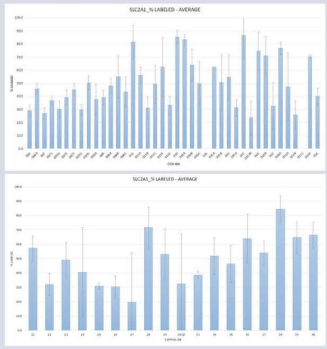

Figure 5. (Top) VAGOMICS samples; (Bottom) CAPRISA samples

Conclusions and Future Implications

- The data indicates that there is an inconsistent pattern of labeling between individuals and the same individuals on separate visits.
- Not all cells label with certain markers, indicating that the transporter protein of interest might not be present on all cells of each sample
- This study can further be expanded by incubating samples with different primary antibodies to compare the rates of labeling between them

References

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2. Reece, J.B., & Campbell, N.A. (2011). *Campbell Biology, 9th Edition*
3. Webster, P., Cortez, J., Webster, S., Gunawardana, M., Pyles, R.B., & Baum, M.M. *Equilibrative nucleoside transporter 1 (SLC29A1) localization on vaginal epithelial cells. J. Histology & Histopathology*. ISSN 2055-091X, Volume 7, Article 5. 2020

Acknowledgments

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At Oak Crest, I imaged vaginal epithelial cells using a confocal laser microscope. I was looking for special proteins on the membranes of these cells, called transporters, and I have collected data on which cells I saw contain these proteins.

Alternate Text:

Alijah Navalta

Quote: "At Oak Crest, I imaged vaginal epithelial cells using a confocal laser microscope. I was looking for proteins on the membranes of these cells, called transporters, and I have collected data on which cells I saw contain these proteins."

Image of Alijah Navalta

Image of text and graphic laden project presentation entitled "Immunolabeling of Transporter Proteins on Vaginal Epithelial Cells. Alijah Navalta"