



Filipin Staining: Detecting Neuropathic Pain in a Cell

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Introduction:

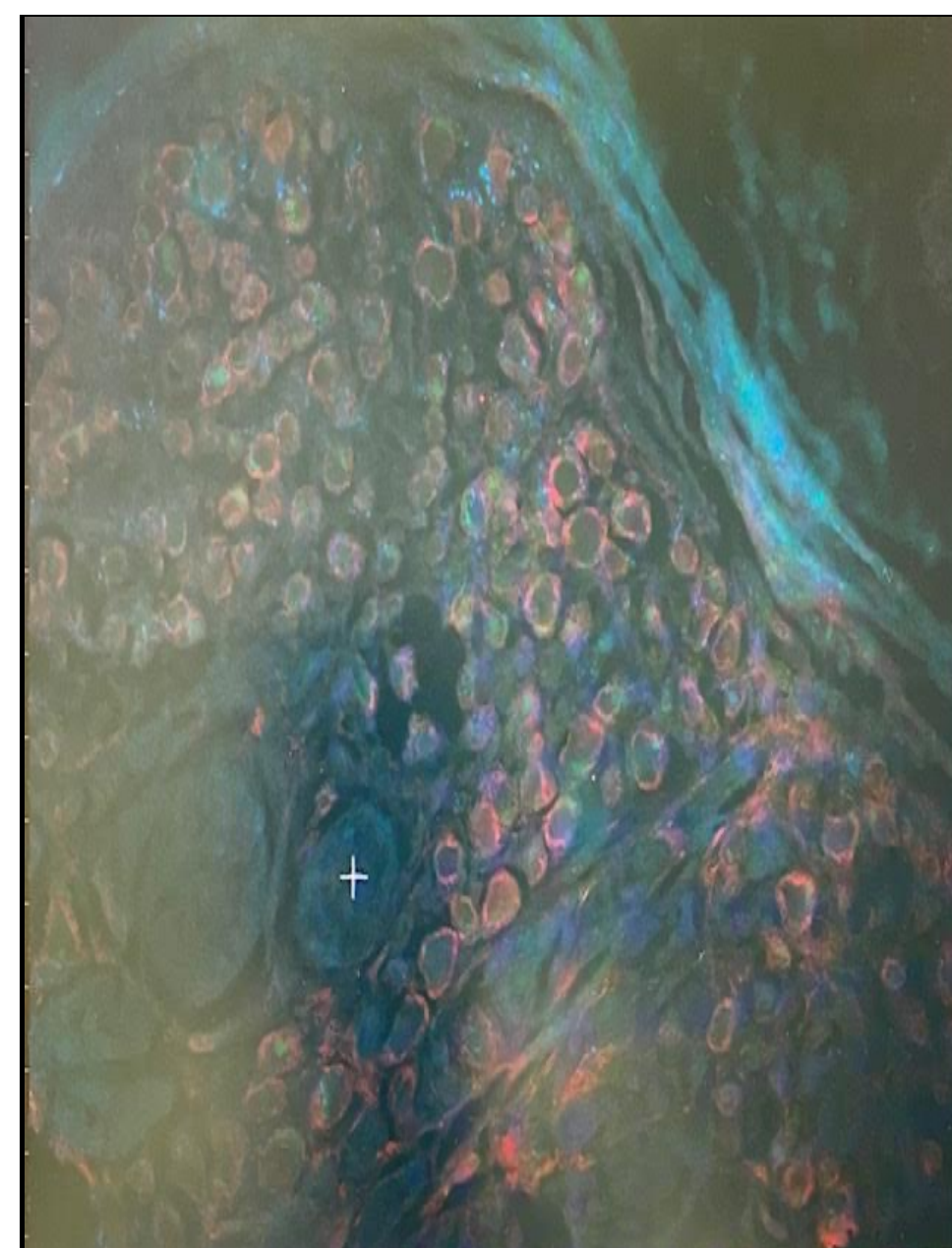
Cancer victims often suffer from neuropathic pain associated with nerve compression. My research consisted of becoming familiar with TLR Pathways, which play a critical role in immune responses. Significant receptor channels that directly affect pain within the body consist of: *TLR4*, *Nav1.7*, *Cav3.2*, *TRPV1*, *HCN1*, and *HCN2*. Despite channel activity being suppressed by an increase in membrane cholesterol, Na channels and TRP channels which pain receptors travel through are inhibited by cholesterol depletion. Lipid rafts, which are partially composed of cholesterol are vital to the research since they play a role in regulation trafficking and act as activation signals to other cells. Based on cholesterol's drastic effect on ion channel signaling, I formulated a proposal on how this phenomenon could potentially combat neuropathic pain. My hypothesis states since paclitaxel induces an uptake in cholesterol, this activity indirectly causes Filipin clusters to be visible under UV fluorescence due to presence of excited neurons, which induces neuropathic pain in the ganglia of cancer patients.

Methods:

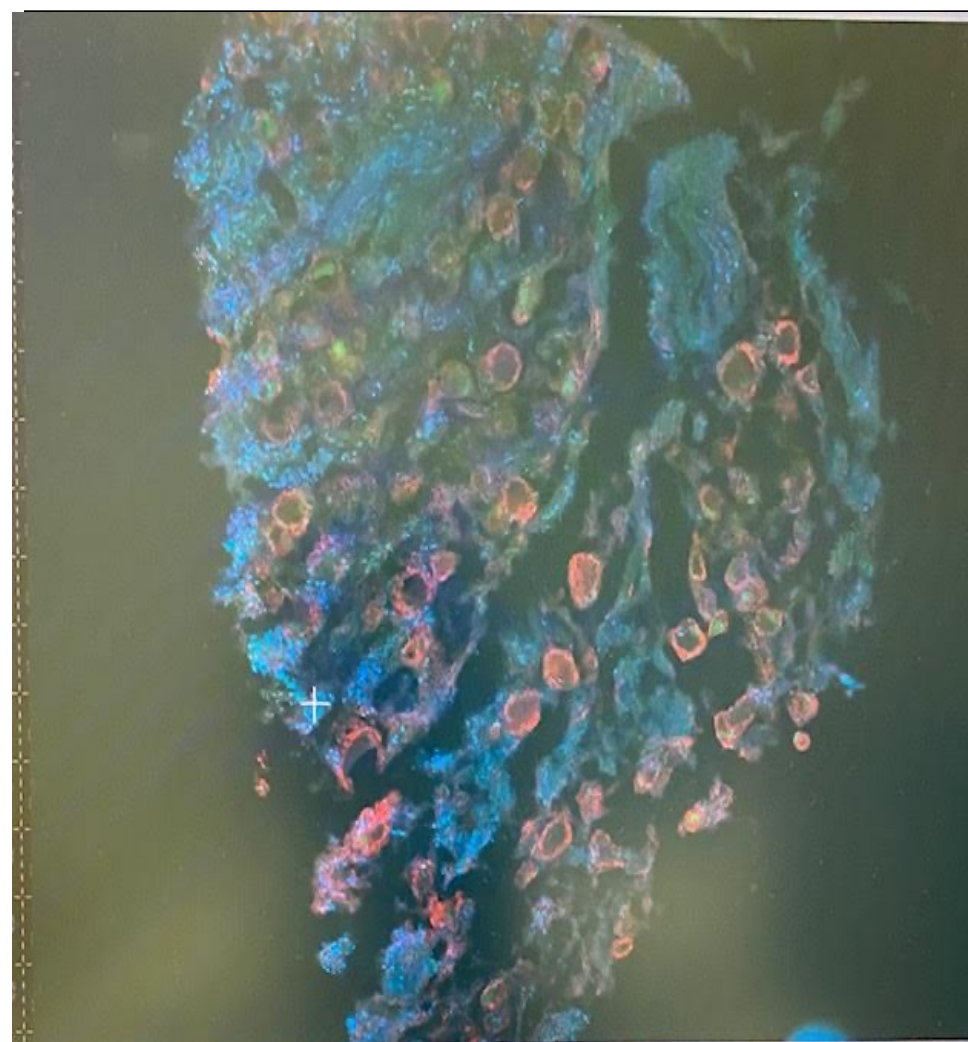
Filipin slides were conducted under consecutive PBS "phage buffer washes". Primary solution 1° was formed while HCN1 was extracted from Human DRG samples who had cancer and exhibited either normal or pain tissue. After slide application, filipin stains were mounted and prepared for microscopy and imaging under FITC, TRITC, UV, and CY5 fluorescence. Roughly four images per slide were captured to give proper magnitude of neuron activity, tissue presence, as well as fat. Blinded clinical experiment to some degree because I was oblivious to some DRG samples being absent of pain receptor sensitivity, thus meaning no neuropathic pain present.

Images/Graphs

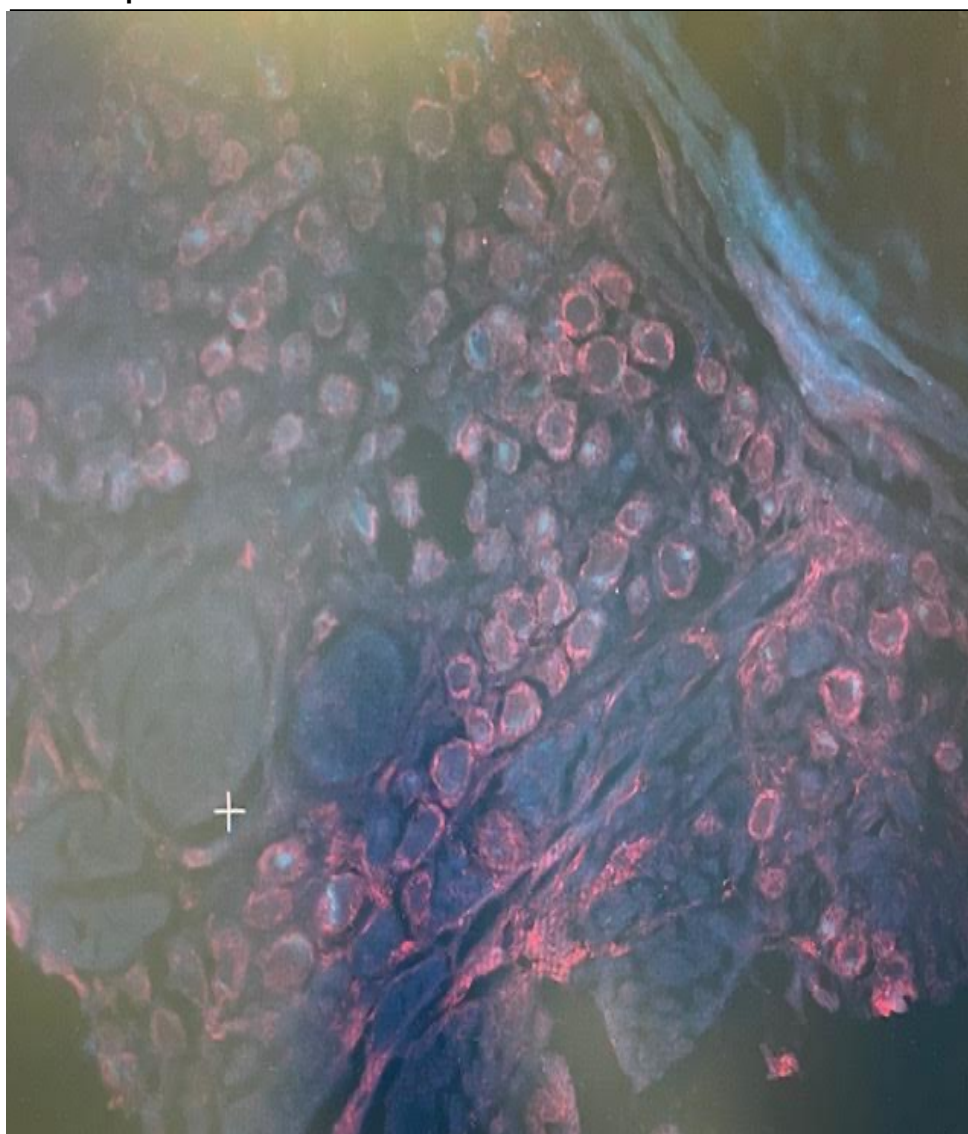
The images displayed exhibit illumination under UV, FITC, TRITC, & CY5 microscopy fluorescence. While each patient had multiple images of their neuron activity captured, the ones with most significant correlation to my hypothesis, whether supportive or unsupportive were chosen for this presentation. Imaged are Filipin stains from Patient numbers: 60, 64, 72, and 91



Filipin is evident with blue fluorescence present around the cells in both samples.



This particular image captures ample Filipin presence which indicates highly sensitive pain receptors.



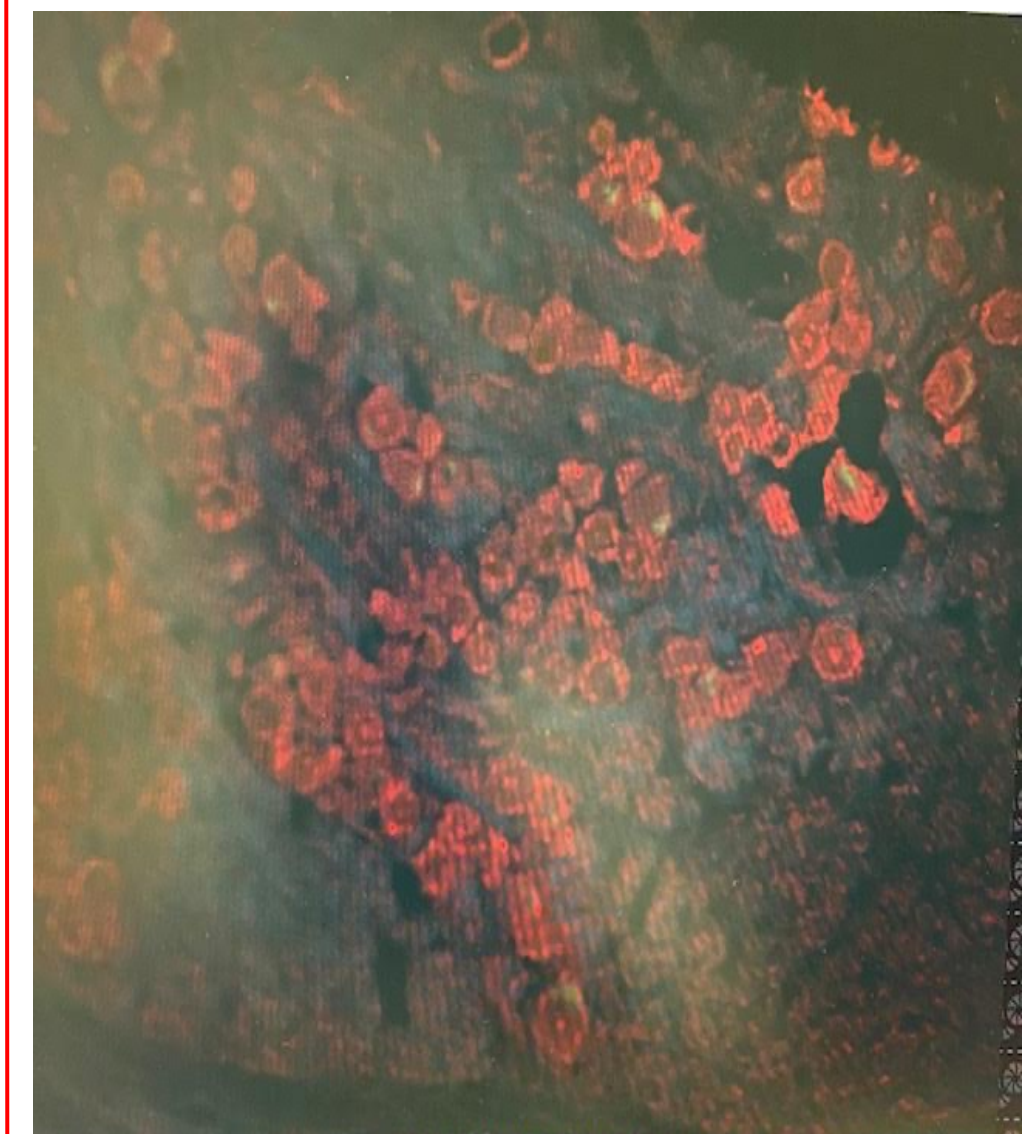
Excel Charts:

There was no method available within our lab to collectively and accurately quantify the Filipin staining. Thus, there was no need for an Excel chart to be constructed and the only numerical symbols used in the experiment were that of each patient sample.

- Patient 60
- Patient 64
- Patient 72
- Patient 91



Much less Filipin present in these samples, as it is actually Tissue and not to be mistaken for actual Filipin presence. Such particular images indicate little to no neuropathic pain.



Results:

Of the four cancerous Human DRG stained for Filipin: normal tissue could be readily identified by sparse neuron activity, while pain tissue exhibited an abundance of illuminated neurons under UV fluorescence. A good indicator of neuropathic pain cells were large amounts of neurons in Patient 60, with minimal fat present. Additionally, there were three entire columns of neurons in Patient 72 despite low FITC fluorescence—who likely was victim to extreme neuropathic pain. Patient 64 appeared to exhibit little to no neuropathic pain as there was scarce activity and merely tissue presence which was not to be mistaken for neuron activation. Lastly, patient 91 exhibited a left side dominance of neurons only, which can be deduced to some neuropathic discomfort. Thus, the results of this experiment explicitly distinguished the image appearance of excited neuron activity—which indicates chemotherapy induced peripheral neuropathy, or normal tissue neuron activity—indicating no pain.

Conclusions

Based on the results achieved from the Filipin staining, bright illumination under UV imaging indicates that *Paclitaxel* induces an uptake in cholesterol activity, which directly supports the hypothesis stated. One error that persisted within the experimentation was the lack of correlation and regard to sexual dimorphism and ethnic influence—two factors that could influence the results. Because we are only seven weeks into a ten-week laboratory, this remains an open conclusion and require further tests and lab work to increase scientific validity.

References

- 1) Longhou, Fang. Regulation of Lipid Rafts; 2019
- 2) Fand, Yun. Cholesterol & Ion Channels; NIH 2010
- 3) Li, Yan. DRG Neuron Excitability; PAIN 2017
- 4)Woller, Sarah. Jung An, Eun. Yaksh, Tony, Inhibition of Neuroinflammation; 2018