Cancer cell proliferation in relation to Clock Proteins

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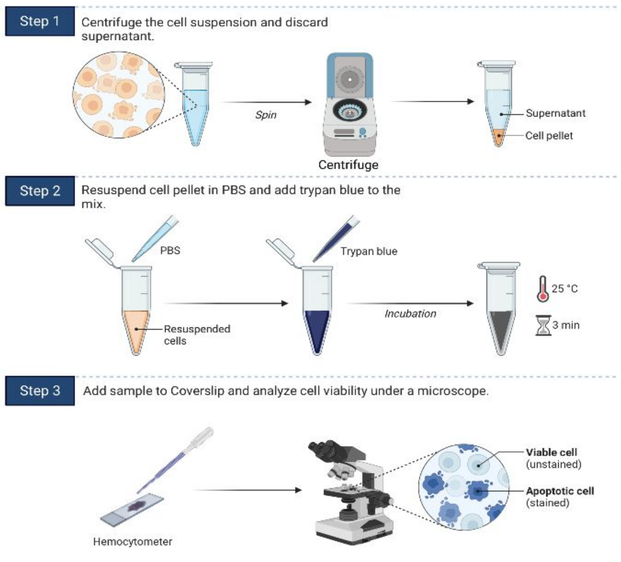
Abstract

Casein Kinase 2 (CK2) is a serine/threonine-protein kinase complex that phosphorylates a large number of proteins containing acidic residues C-terminal to serine or threonine (Keller et al., 2001; Sayed et al., 2002; Shin et al.,2005; St-Denis et al., 2009; Hung et al.,2010; Rabellino et al.,2012; Bae et al.,2015; Kim et al., 2019). CK2 regulates numerous cellular processes such as the cell cycle, cell death and transcription. (Megio and Pinna,2003;Denis and Litchfield,2009; Filhol 2009;) and is therefore an interesting protein to investigate in the context of cancer. Dr. Lili Zhou in the Kay Lab recently found potentially new CK2 targets (unpublished data). These potential targets are well established onco-proteins -C-MYC / TFAP4 / MAX / CHD4. Under the supervision of Dr. Tia Tyrsett Kuo in the Kay lab, we sought out to test this using fluorescence microscopy and western blotting to visualize protein localization and levels of these onco-proteins under CK2 inhibitor (GO289) treatment.

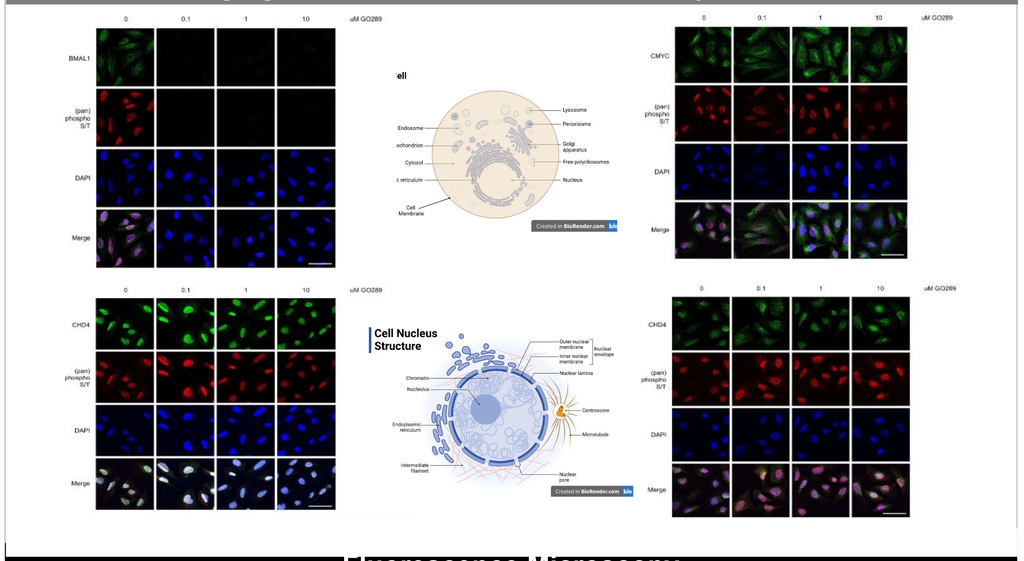
Cell Culturing



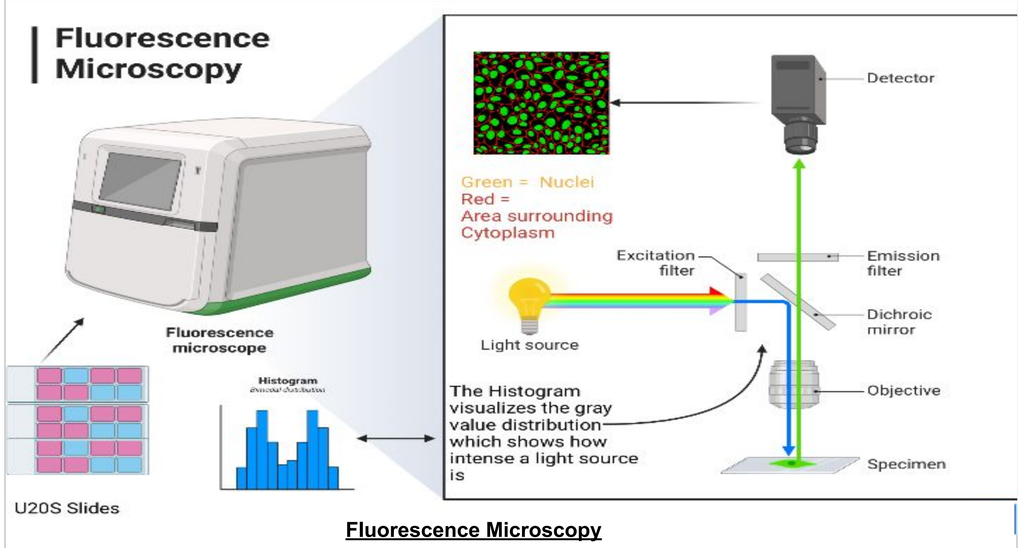
Cell Culturing:

We start by Centrifuging the cell suspension and discard the supernatant. Through this process with the centrifuge, the Supernatant is brought to the top and separated from the cells which sink to the bottom. Then, we resuspend the sunken cells in a median called PBS (Phosphate Buffered saline) and add blue to trypan blue to the mixture. We then incubate the new cell mixture at 25*C for 3 minutes. The final step is to add the sample to the coverslip and analyze the cell viability under the microscope.

Imaging of Nuclei after different Antibody ratios



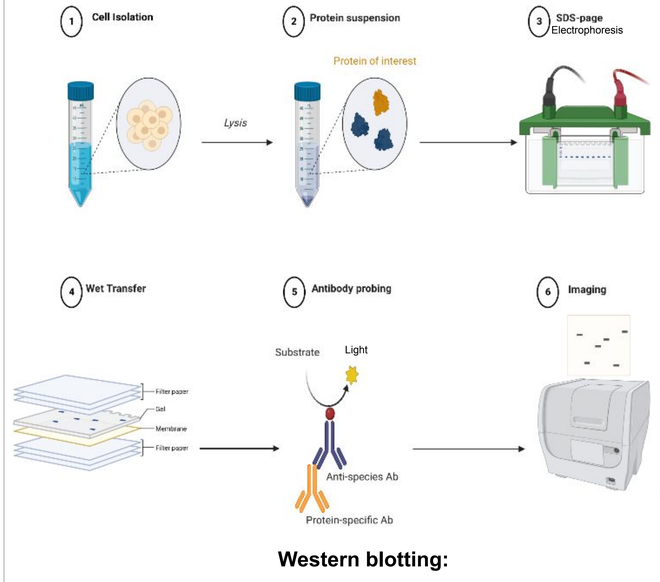
Fluorescence Microscopy



Fluorescence Microscopy

The Leica Microscope can show the slip with the sample through water, dry, oil and affect the way that the slip is recorded through the light patterns.
We are imaging a U20S cell which is an osteosarcoma cell line (bone cancer).
The reason we record using this cell line is because of its large and flat nature.
Even though the U20S cell line is what we are studying, what we are staining for is different.
When a photo is taken and we go into the post processing,
in our case we chose to utilize 3 different Fluorescent dyes.
Using a combination of these fluorescent dyes we are able to observe the localization of DNA, POI (protein of interest), and a protein modification (Phospho - Serine Threonine).

Western Blotting Experiment



Western blotting:

The Western blotting procedure starts by loading the U937 (Leukemia) cell line into the petri dish as well as the drugs.
We then lyse the drugs and the U937 cell line and separate the lucky few cells into a different vial for later extraction. We use a machine called the centrifuge which separates the unwanted cells from the free (soluble) proteins. We take the proteins and transfer them into a SDS Page gel and that creates a ladder that we can photoimage and then study under the microscope.

Summary

We were successful in acquiring fluorescence images and were able to form hypotheses on the effects of CK2 inhibitor on these different onco-proteins. This can be seen as the dosage of CK2 inhibitor increased, there was a significant decrease in fluorescence signal of the protein BMAL1 which means in higher dosage ratios BMAL1 may become unstable. Phosphorylated Serine/Threonine also shared the same characteristic- significant decrease in fluorescence signal as the dosage increases. DAPI, which stains for double strand DNA, serves as a constant representation of the nucleus. DAPI signal remained unchanged as the dosage increased and this is due to the fact that the CK2 treatment should not affect the total DNA content. On the other hand, our western blots were unsuccessful; we were unable to observe distinct protein bands and are currently running optimizations. Once we have fully optimized the technique and antibodies we will be able to run the western blot at maximized potential and in turn giving us clear and optimal results.

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