Client Depletion Assay Comparison of Paclitaxel to Hsp90 Inhibitors

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Abstract: Many researchers have claimed they have found a successful inhibitor of Hsp90; however, we suspect they are only successful when the inhibitor is used in large quantities. On top of being one of the easiest ways to show an effective inhibitor, researchers are claiming they have found an inhibitor when only meeting as few as two hallmarks. Due to the researchers' inhibitors only meeting two of the criteria, we are trying to show whether these limited expectations are a valid way in identifying Hsp90 inhibitors. We think the client depletion assay test is not specific to Hsp90 inhibitors but instead is a generalized cell response to cell death. At this point in our research, we know a lot about client depletion assays and Hsp90s; however, we do not know if the claims these researchers are making are valid claims because of the amount of Hsp90 inhibitor they are using.

Keywords: Hsp90s, Client Depletion Assay, Apoptosis, Inhibitors

Introduction

Heat shock protein 90 (Hsp90s), being one of the most abundant heat shock proteins (Neckers 2002), target individual proteins that have folded onto themselves incorrectly. They work to unfold these proteins and refold them the correct way. If the protein continues to fold incorrectly, the cell could go into a state of programmed cell death - apoptosis. The cell will eventually go into apoptosis if enough proteins are folded incorrectly or if enough other things go wrong within the cell. The process of apoptosis is highly regulated in the body, because it is hard to stop once the process has begun. Because Hsp90s monitor the folding of proteins and help to make sure the proteins are in order, Hsp90s are called molecular chaperones. Hsp90s have been researched for many years because they are more sensitive in cancer cells. Cancer cells require more Hsp90 than normal cells to survive, suggesting that they play a huge role in the growth of cancer tumors (Tsutsumi et al. 2009). Hsp90s help enhance telomerase, which causes cancer progression (Neckers 2002).

Inhibitors, or compounds found to restrict the number of Hsp90s' functions, work by competing with ATP binding, causing the chaperone cycle to slow or completely stop. This process decreases the amount of client proteins able to be unfolded, eventually causing more cells to go into apoptosis (Tsutsumi et al. 2009). Hsp90s' client proteins play a big role in the establishment of cancer and the hallmarks, or changes, they make to the cell that helps them to continuously keep growing. Some of these hallmarks include: self-sufficient growth signals, the abilities to avoid signals from other proteins, stop apoptosis, replicate, and invade tissues. In a client depletion assay, the experimenter administers a candidate inhibitor to see whether it stops Hsp90 from functioning in the cell. The inhibitors indirectly target proteins that Hsp90 unfold, and the inhibitors cut the Hsp90 off from helping the protein. The protein not being able to fold correctly causes the client protein to slowly destabilize and eventually degrade (Neckers 2002). In our experiment, we were working with paclitaxel, a chemotherapy drug, which is proven to not be an Hsp90 inhibitor. As discussed, Hsp90 inhibitors cause client depletion because they slow the process of correcting proteins folded incorrectly. This causes more cells to go into apoptosis. However, if paclitaxel causes client depletion, we can conclude client

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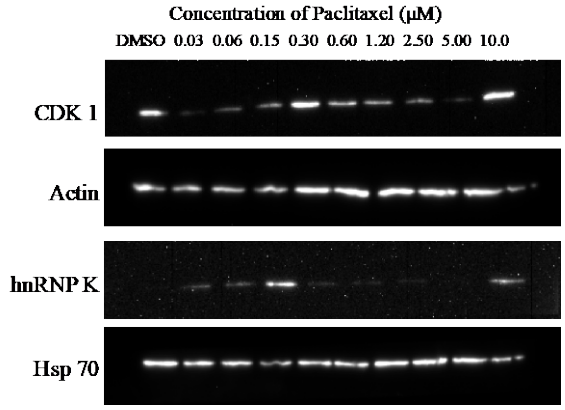

Figure 1: These are the results from our Western blot. Based off of these results as well as results from previous experiments, we were able to conclude Hsp90s are not a cause of cell apoptosis. Photo courtesy of SuperSignal West Pico PLUS Chemiluminescent Substrate.

depletion is not specific to Hsp90 inhibitors and is rather a generalized cell response.

Methods

In our lab, we did our testing on Jurkat cells, a type of leukemia cell line. We grew 25 milliliters of the cancer cells for each drug concentration we used in a 25 milliliter flask. This was done by making cell cultures. In our research, we did suspension cultures - when the cells are free floating in Roswell Park Memorial Institute media, which contains fetal bovine serum (Pierce 89986) 200 mM L-glutamine, 500 nM/ml streptomycin, and 100 U/ml penicillin (Voruganti et. al). Once the media and cells were mixed, they were cultured overnight statically in a humidified CO_2 incubator at 37℃ (Voruganti et. al). After incubating overnight, we put paclitaxel, a chemotherapy drug, into one of the flasks. We did a drug dose curve by a multiple of 10 nM increments, so we could measure if the client depletion assay decreased, stayed the same, or increased, further showing if it was a Hsp90 inhibitor or not.

We compared the cultures' client depletion assays using a Western blot test (Figure 1). After the gel was ran, the membrane of the gel was taken and put into an antibody that was specific to our protein. Then, another antibody that had an enzyme attached was added to the membrane to induce staining, radioactivity, etc. The membrane was then put into a substrate which made the antibody with the enzyme glow, helping us to be able to identify the protein by observing the proteins the antibody latches onto. The Western Blot test we used was the SuperSignal West Pico PLUS Chemiluminescent Substrate. We were also able to tell by the color intensity of the band on the gel and were also able to estimate the size of the protein.

We also performed another test to compare the samples net protein concentration called the BCA assay - an abbreviation for bicinchoninic acid assay. We compared the colors of the net proteins after inserting the BCA compound (Figure 2). The BCA compound is green before binding to the proteins. After binding, the compound turns purple. We were able to determine the concentration of protein by visually comparing the color intensity of each sample. The more protein there are in the lysate, the darker the solution will look. We used a plate reader to obtain a specific concentration of proteins within each of our samples.

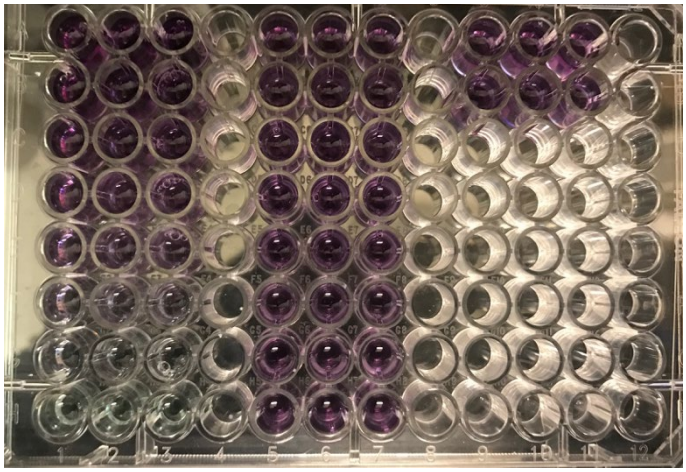

Figure 2: This figure is the results of our BCA assay. Visually, we were able to tell which samples had more protein concentration based off the darker purple color. Photo courtesy of Taylor Cartmell.

Results and Discussion

In conclusion, our lab still has quite a few questions we want answered. Our Hsp70 did not induce; therefore, it is probably not an Hsp90 inhibitor. Also, there was no evidence for the dose dependent depletion of CDK1 or hnRNP, meaning it is most likely not an Hsp90 inhibitor. In our experiment, there was also an error in line loading. There was also an error in our PARP, which leaves us with a lot of questions about dose efficiency.

Literature Cited

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