**Effects of Specific Protein Suicide Inhibitors on Cancer Cells**

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**Abstract**

Melanoma, a popular cancer found in humans, is a base for modern research. Strands of this cancer can be isolated and cultured for study using different inhibitors of proteins related to the production of proteins and cell defense systems. Targeted gene expression could be regulated from an external source by treatment of inhibitors on proteins associated with these functions. Cultures treaded with various combinations of suicide inhibitors were examined to better understand the role that these proteins have in melanoma tumor growth and development. Targeted proteins were: 1. Heat Shock Protein (Hsp90) and it’s relationship with protein folding and function within the signal cascade of many cells. This relationship allows for the regulation of specific proteins that are weakened or over taken by cancer cells. 2. Heme-Oxygenase (Hsp32) is an enzyme that in recent years has been found in abnormal concentrations in active tumor cells. It plays a vital role in Oxidative stress and Heme degradation in functioning cells, but it seems to be upregulated in cancer cells. This experiment coupled Hsp90 inhibitors and Hsp32 inhibitors on A375 melanoma cells.

**Introduction**

Our body operates on a highly complex and sensitive regulatory cycle. Protein production is highly regulated by histone modification, allosteric inhibitors, and other co-factors. The use of proteins for our bodily functions is detrimental to life. Inhibiting or increasing production or function of a specific protein can lead to a cascade of events. This type of research is currently being used to understand how cancer affects cells and how it can be treated. Modified DNA polymerase may be able to correct the mutation that caused the cell to become cancerous using its inherited 5’ to 3’ proofreading capabilities. Possibly, the DNA helicase that unwinds DNA to be read so that cells can propitiate into daughter cells can be inhibited specifically in cancer cells. This type of technology may have seemed far-fetched in the beginning; however, the time is upon us to implement such resources in the war on cancer. This paper will discuss the work of Ignazio Barbagallo, Rosalba, et. al. and their research on modified organelles and their effects on tumors.

**Recent Progress**

The role of Hsp90 protein is the functionality of proteins formed in the Endoplasmic Reticulum. According to a peer reviewed journal entry, Hsp90 contains an ATPase at its active site meaning it cleaves a phosphate from ATP (ATP ADP + Pi). This is needed to fuel the energy of many endergonic reactions within the cell. When active, Hsp90 dimerizes and is responsible for the activation energy and maturation of proteins (Armstrong, Wolmarans, Mercier, Mai, and LaPointe). NMS E973 has a very high affinity and is very selective for kinases meaning it tightly binds and inhibits Hsp90 at its active sight. It is proven to have a Km at subnanomolar concentration (Fogliatto, et.al.), making it a great candidate for inhibition of Hsp90.

 The role of Hsp32 protein is to catalyze the isomer specific oxidation of heme molecules. It is responsible for the tight binding of oxygen radicals at its heme regulatory motif and is a suicide inhibitor at translation and transcription levels ( Maines, Panahain). This means it can bind and inhibit genes at the RNA level, and also at the protein level before it has been folded or after it has been folded.

 Objectives to be measured in this study were stress induced on the endoplasmic reticulum, production of oxygen radicals, expression of Hsp (90 & 32), and cell apoptosis with in the cancer cells treated with Hsp90 inhibitor, Hsp32 inhibitor, and treated with both inhibitors. Several ways were utilized to obtain quantum evidence of these objectives. Immunocytochemistry, 20,70 dichorofluorescein diacetate, Real-time PCR, and annexin V were all used as a means of detection.

**Roles of Targeted Organelles**.

The Endoplasmic Reticulum (ER) is responsible for the synthesis of proteins, fatty acids, and lipids within healthy cells. In cancer cells, the over production of certain proteins is essentially the cause of the over expression of growth hormones/proteins leading to the growth of tumors. Increasing stress on the ER should result in cell death. The formation of oxygen radicals (ROS) such as Peroxide radicals, was a positive correlation to the percentage of cell apoptosis (Barbagallo, et.al).

**Methods used in Recent Progress**

 Measuring ER stress was accomplished by immunocytochemistry. Once anti-calnexin specific antibody bound to the ER surface, a secondary antibody was used and incubated for a period of time. After incubation, cells were washed thoroughly and counterstained with DAPI. After being washed, the specimens were mounted on a medium of polyvinyl alcohol and DABCO. These were then viewed with a fluorescence microscope. It was found that the coupled treatment of both inhibitors leads to a higher rate of ER stress and the over production/upregulation of BIP and CHOP proteins. It should be noted that treatment with a single inhibitor also lead to protein upregulation, just not as significant as when used together.

 Measuring reactive oxygen species formation was done by the use of fluorescence technology once again. A fluorescent probe was used to gather intensity of fluorescents per mg of protein within the cell. It was found that treatment with both inhibitors increased the amount of oxygen radicals within the treated cells. Either inhibitor alone did not increase reactive oxygen concentration.

Measuring expression of Hsp90 and Hsp32 proteins was accomplished using Western Blot and real time PCR. Samples of human cells were taken from a culture stored in subzero temperatures and using TaqMan gene expression assay, were subjected to Real Time PCR. Samples were rinsed with cold DPBS, lysed to expose contents of the cell, and RNA was isolated and treated. Reverse Translation kits were used to create and use the template strand of cDNA as the PCR primer.

Measuring cell apoptosis was accomplished by annexin V. Cells were collected, twice washed with cold PBS, and re-suspended in a binding buffer. Then, annexin V was added and cells were vortexed and incubated for 15 min at ambient temperature in the absence of light.

**Results**

Results from the study showed that treatment with both Hsp90 and Hsp32 inhibitors, NMS E973 and SnMP respectively, made much higher Endoplasmic Reticulum stress and produced harmful reactive oxygen species that were detrimental to the cell. Ultimately, the cells underwent apoptosis and proved to be a possible effective treatment for melanoma.

**Discussion**

One major problem that still looms over this experiment is the targeting of cancer cells. Much like the use of chemotherapy to combat cancer, it is not specific. All cells, healthy or not, must be able to defend against ROS and properly regulate protein expression. These inhibitors are not yet capable of specifically targeting desired cells for treatment. Exposing healthy cells to these treatments may prove to be deadlier than cancer itself, leading to the possible death of the patient. The future of this type of research is to use bioinformatics to fully understand the cascading events of protein inhibition in human cells. Understanding how a pathway is regulated is much more important that knowing if specific genes are upregulated or down regulated (Minghetti, 2018). Modern advances in molecular technology will hopefully lead humanity to a cure for all types of cancer, restoring the quality of life for many people.

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