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How Does Caspase-3 Affect Apoptosis and Cell Death in Cancer Cell line SW480?

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ABSTRACT

Colon cancer is the second leading cause of death from cancer in the world. There are multiple causes of colon cancer. Colon cancer can be caused by an altered β-catenin expression in the APC gene. An immense amount of research is being conducted to understand prevention and treatment of colon cancer. These include TRAIL, chemotherapy, and antibodies, which can lead to a process known as apoptosis, ultimately denaturing the cell and causing tumor death. The goal of this experiment is to test whether increasing concentrations of an anti-body (Caspase-3) would increase apoptosis by increasing the number of dead tumor cells in colon cancer cell-line SW480. In order to address this question, the experiment followed three procedures: cell culturing, hemocytometry, and DNA analysis to confirm a correlation between concentration of antibody concentration and the number of dead cells. The data collected provides evidence that increased antibody increases the amount of dead tumor cells. There was a strong positive correlation between antibody concentration and the number of dead tumor cells. In addition, DNA analysis showed lower than standard absorption values in the treated samples compared to control. This data therefore provides evidence that antibodies may help in colon cancer treatment by causing apoptosis through affecting DNA structure.

Keywords: *Colon Cancer, SW480, Caspase-3.*

INTRODUCTION

Cancer cells are abnormal cells that are changed due to many aspects in life that cause mutations. Such aspects include: viruses, the immune system, environment, genetics, or even age. Cancer cells are able to divide without control due to high levels of telomerase that helps with cell growth. Cancer cells can spread to other parts of the body, which is called metastasis. There are many different types of cancer but we are going to focus on the carcinoma colon cancer. A specific kind of cancer cell mutation is a mutation that affects the APC gene. This mutation can be used “in vitro” to generate a specific cell-line called SW480. SW480 cells can be used in laboratory studies of cancer cell properties. Colon cancer is the second most death causing cancer type (O’Brien, et al 2007).

SW480 can be caused by altered β-catenin expression, mutations of the APC gene (Adenomatous polyposis coli) that helps with tumor formation, or the mutations in the CTNNB1 (β-catenin). When there is an accumulation of β-catenin, it activates a T cell Factor that leads to transcription. Transcription then leads to activation of cyclin D1 that causes cancer cells to grow (Nath, et al 2003) Research done on SW480 cells were maintained in DMEM media and 10% Fetal Bovine Serum (FBS) and antibiotics. Incubated under 4-10% CO_2 conditions (Muller, et al 1993).

A leading cause of tumor death is the TRAIL or tumor necrosis factor-related apoptosis-inducing ligand. This ligand is a membrane protein that is part of a tumor necrosis factor family. These families are responsible for inducing apoptosis. This ligand is unique in the fact that it is potent when inducing cancer cells but when it comes to normal cells, it has no cytotoxic effect. TRAIL has some advantageous effects in the medical treatments of cancer cells because of the non-cytotoxic effect on normal cells. TRAIL is responsible for trimer formation and so has been shown to induce apoptosis when binding to their receptors. TRAIL binds to two receptors, TRAIL-R1 and TRAIL-R2 that result in trimerization. That leads to the apoptosis induced signaling complex. TRAIL signaling recruits activity of caspase 8 (Burns and El-Deiry, 2001).

Another important ligand to consider is Caspase, a monoclonal antibody, which is a natural protein with modular structure. Antibodies are produced against antigens. Antigens are substances that diffuse in the presence of antibodies. Treatment of antibody bevacizumab showed an increase in free survival and overall survival of patients with breast and colon cancers (Guilleminault, et al 2012). Activating caspases allow for many recognizable features of apoptosis. Some of these features include DNA fragmentation, cytoplasmic shrinkage by cleaving cellular proteins (Ahmad and Shi, 2000). Activation of Caspase involves activation of a cascade of other caspase domains. Caspase 8, 9, and 10 are the initiator caspases that are at the beginning of the caspase cascade. This cascade results in apoptotic signaling and cleaves proteins that end up denatures the cell. With the help of caspase regulation, scientists can manipulate apoptosis for medical treatments (Thornberry and Lazebnik, 1998).

Scientists have found the pathway, which Caspase-

3 activates and thus leads to apoptosis. Molecules that are known as inflammasomes recruit caspase-3. Activation of Caspase-3 leads to cleavage of pro-IL-1β (pro-inflammatory cytokine pro-interleukin). There are a variety of caspase substrates that lead to a rapid degradation of the cell and result in cell death. Caspase activation can also be used to secrete IL-1β (interleukin-1 beta). The interleukin is a converting enzyme that mediates and brings about apoptosis. So Caspase-3 can lead to cell death directly or activate enzymes that result in apoptosis (Denes, et al 2012).

To help us understand more about the role of caspase in cancer cell growth, we cultured SW480 cancer cell lines with different amounts of Caspase. We used a hemocytometer to determine the number of dead and alive cells we obtained from culturing. DNA analysis was conducted to determine DNA purity.

MATERIALS AND METHODS

We used three methods to conduct the experiment: cell culturing, hemocytometry, and DNA analysis. Cell culturing involves the process of growing cell under certain conditions and maintaining them so they can be used later on. Using a hemocytometer and trypan blue staining we can estimate the number of dead and alive cells. DNA analysis is conducted via UV spectrometry at 260nm and 280nm.

Cell Culturing:

Cell culturing consists of using Leibovitz's L-15 medium because of its special properties of maintaining CO_2 levels in cells. Cells were cultured in $25cm^2$ flask with 10% fetal bovine serum and 1% penicillin. Then the flasks are incubated at 37℃ for 72 hours. One control group has no antibody, while four flasks have (1, 5, 10 µg of Caspase-3) and 10 µg of DMSO, respectively. Having a control and treatment groups help test for apoptosis and number of cells in the cell cycle. After the 72 hour growth stage, cells are harvested with 1% PBS and a scraper (Nahta, et al 2004).

Hemocytometer:

After cells were harvested trypan blue staining was done by taking 20 µl of cell/PBS mixture and 4 µl of trypan blue and was then mixed. The cells were inserted into hemocytometer. Then using a microscope to count the cells in one square, you can estimate the amount of cells in your overall hemocytometer. Cells that are dead with be colored blue because of the loss of the cell membrane integrity while the living cells will have no color (Tran, et al 2011).

DNA Analysis:

Using 160 microliters of PBS/cell mixture, DNA extraction was conducted for each concentration by mixing TES buffer, lysozyme mix, and phenol chloroform and then centrifuging the mixture at 12,000rpm for 10 minutes. After centrifuging the supernatant was added to ethanol and centrifuged for one minute at the same rpm. A DNA pellet was formed and the supernatant was removed. The DNA pellet was then mixed with TE buffer and inserted into a disposable $1cm^2$ cuvette. Absorption was then measured on a UV Spectronic Genesys 2 at 260nm and 280nm wavelengths. Due to limitations on the disposable cuvettes, a blank cuvette without anything in it was also measured at 260nm and 280nm to reduce limitation. Then subtracting the extracted DNA absorbances from the blank cuvette gave us more accurate readings. Then the ratio of 260/280nm was calculated to examine how pure was the extracted DNA (Cunningham, et al 2013).

RESULTS

We counted dead and alive cells and generated different graphics as shown below. Figure 1 is the correlation between increasing Caspase-3 concentration and the number of dead cells. We found an R-value of .94212. The graph is a positive correlation and thus as one side of the graph increases, the other side does as well. The number of dead cells increases once past 1 µg of Caspase-3.

Figure 2 is the percent of control of living cells. The percentage of control is set as the base. That means the control has the maximum number of living cells possible. As we increase concentration of Caspase-3, the percent of control decreases and the number of living cells decreases.

Figure 3 represents the percent of control of the number of dead cells. Again the control is a percentage and is set at 100 percent. So as the concentrations increase, the percent of control goes past 100%. Looking at the graph we see that once past 1 µg of Caspase-3, the percent of control increases. Reaching 10 µg of Caspase-3, the percent of control of dead cells increases by about 200 percent.

Figure 4 represents the absorbance ratios of 260nm/280nm to test for DNA purity. The range of values from control to DMSO is 1.803, 0.9512, 0.9231, 0.8428, and 0.8563 respectively. Standard for pure DNA absorbance is 1.8 at 260nm and pure protein absorbance is 2.0 at 280nm (Leite, et al 2013).

DISCUSSION

We accept our hypothesis that we increased concentration of Caspase-3 and the number of dead cells increased once the concentration was greater than 1µg of Caspase-3. There was a dose-dependent increase in percentage of dead cells with increasing concentration of Caspase-3. The results of Figure 1 come to the conclusion that an increase in apoptosis

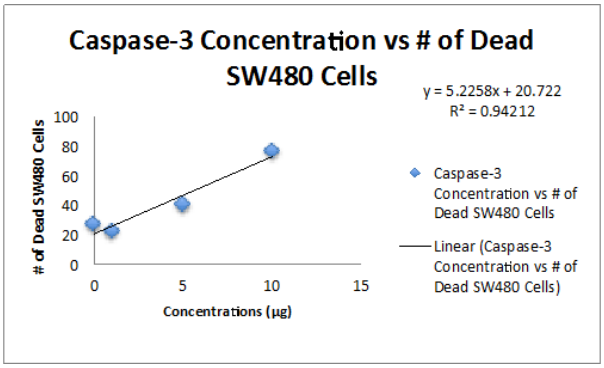

Figure 1. Correlation Between Concentration of Caspase-3 and Dead SW480 Cells

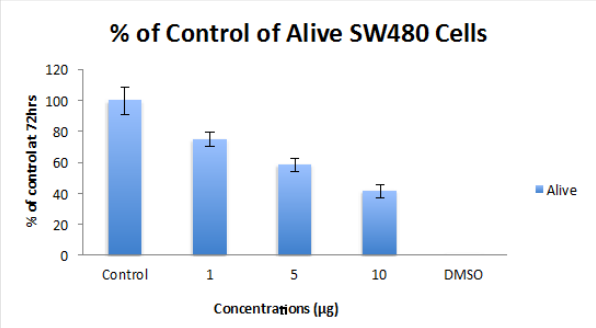

Figure 2. Percent of Control for Alive SW480 Cells

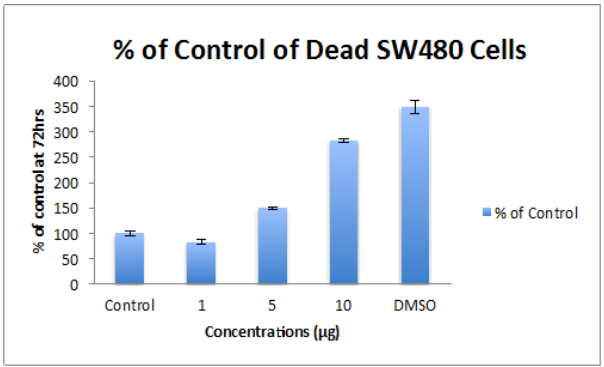

Figure 3. Percent of Control for Dead SW480 Cells

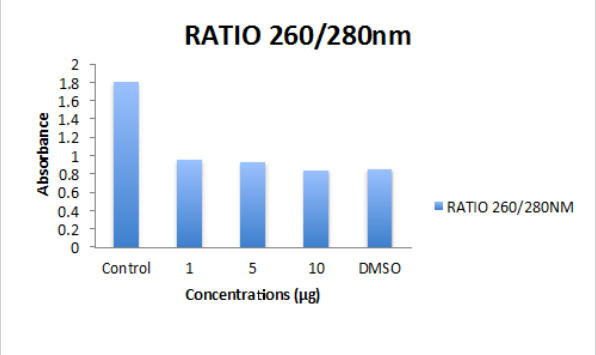

Figure 4. DNA Absorption Ratio of 260/280nm

in cells is related to the increase concentration of Caspase-3 added. The graph shows a correlation between the two and the R-value= .94212. This high R-value tells us that there is a very strong relationship with increased concentration and increased dead cells. The number of dead cells increased by about 60 dead cells at 10 µg of Caspase-3 compared to the control.

Combining results of Figure 2 and 3, we can conclude that with increased concentration of Caspase-3, the percent of control for living cells decreases and the percent of control for dead cells increases once past 1 µg of Caspase-3. Caspase-3 does induce some form of cell death because the number of living cells decreased and the number of dead cells increased when treatment was introduced compared to the control. Comparing the data to (Yue, et al 1997) with his interferon FN-γ antibody compared to the Caspase-3 we used, the data showed that an increased concentration of antibody does cause increased number of dead cells. Caspase-3 showed that the percentage of dead cells increased by about 200% compared to the control group. We can acknowledge that increased concentration of Caspase-3 induces apoptosis and increases number of dead cells in cancer cell line SW480.

From Figure 4, the respective absorbance ratio at 260nm vs. 280nm of supposedly collected “DNA”. Based off the results, the control had an absorbance at 1.8 that indicates that we extracted pure DNA. The treatments of Caspase-3 reduced the absorbance values tremendously and thus lower than the standards for pure DNA and protein. From these lower absorbance values, we can suggest that apoptosis does occur with treatment of Caspase-3 because we had a control of pure DNA and the remaining treatments were lower in value. Apoptosis causes the cell to break apart and so the lower than standard absorbance values of Caspase-3 treatment could’ve been caused from apoptosis.

In conclusion, the experiment was to determine the effects of Caspase-3 on cell death and apoptosis in cancer cell line SW480. The data collected and results suggest that Caspase-3 as an antibody to cancer cells does cause increased apoptosis and cell death in SW480. To help increase cell death in cancer, one must look at the pathways in which antibodies have an effect on increased cell death. An increase in concentration of Caspase-3 leads to a 3-fold increase in percentage of dead cells when compared to the control. Antibodies have a variety of functions, but an important function is cell death and should be further looked into when researching cell death in cancer cells.

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