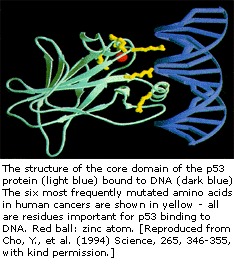
IS P53 MORE LIKELY TO TRIGGER APOPTOSIS OR CELLULAR SENESCENCE AS A RESPONSE TO A TUMOR OR DNA DAMAGE?

1. Introduction:

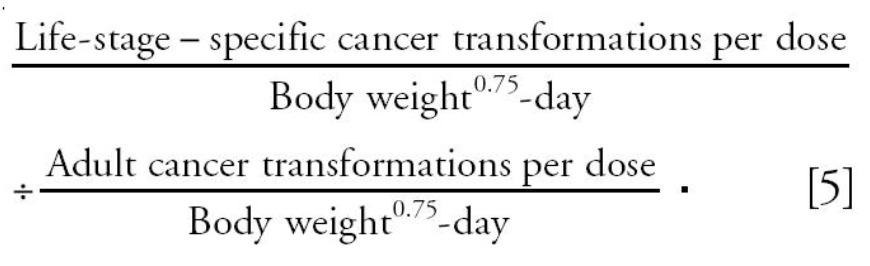
**** TP53, otherwise known as p53, codes for the regulation of the cell cycle thus, preventing the progression of a tumor (Brooks and Gu). Understanding the role of p53 in the genome is crucial to explaining cancer and tumor suppression. Fifty percent of all human tumor growth is due to the mutations or deletion of the p53 gene, this then severely reduces a cells ability to suppress tumors, and can lead to early stages of tumor development (Rivilin et al). Cells with this p53 mutation often undergo uncontrollable growth leading to “immortal” cancer cells. P53 protects the integrity of the cell by regulating several cellular processes when triggered by various stress signals determining the fate of the cell; this includes cell cycle arrest, DNA repair, apoptosis, and cellular senescence (Brooks and Gu). Here we will focus on two of these processes, apoptosis and cellular senescence. Cellular Senescence and apoptosis are both mediated by the p53 gene, but take different approaches in suppressing the tumor.

Apoptosis on one hand is programmed cell suicide. This occurs in three stages, stage one activation of initiator caspases, stage two execution and engulfment, and lastly stage three activation of effector caspases/degradation (Gerl and Vaux). The activation of apoptosis occurs through death receptor mediated extrinsic pathways, triggered by ligand binding plasma membrane death receptor. After receiving a triggered death signal the process of eliminating the cell is well controlled and organized. Apoptosis is often considered a major goal in cancer therapies, eliminating the dysfunctional cell rapidly, and in an organized manner where other cells are not disturbed (Gerl and Vaux).

Cellular senescence suppresses cancer by irreversibly arresting cell proliferation (Kahlem et al). The response irreversibly loses the capacity of proliferation within the cell, changing the expression of many genes. Hayflick and Moorhead originally described cellular senescence in the 1960s, discovering that insufficient telomere after multiple cell divisions exposed chromosome ends trigger DNA damage signals lead to senescence (Dimiri). Cellular senescence, happening in G1, is qualitatively equal to apoptosis in that it prevents cells from progressing through the cell cycle, but is a more natural response to DNA damage.

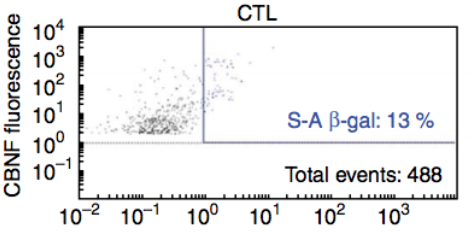
Using Hirao et al (2000) experimenting with activation of p53 we understand how to activate p53 for our advantage. Rather than just understand how to use ionizing radiation to damage DNA inducing p53, we want to go a step further, and see what p53 is more likely to do to suppress the rise of a tumor. According to research done by Childs et al (2014), they hypothesized that cellular senescence may be a much more stable, viable, and more effective process in comparison to apoptosis, because it provides an effective defense mechanism in its own right. This observation comes from years of study, but is still not a hard line for identifying cellular senescence as a better course of treatment in cancer patients. The theory is remains obscure and hypothetical, but this also builds to the following proposed experiment. Through the following experiment, we attempt to discover what is more likely to occur in a cancerous cell, apoptosis or cellular senescence.

II. Experiment:

 The goal of the following experiment is take the results of Hirao et al (2000) activating p53 and determining what tumor suppression mechanism is occurring more frequently with the cell. In order to perform the following experiment human cancer cells need to be collected. To allow the experiment to work at its fullest potential these cells must be in the earlier stages of tumor development. This meaning the primary tumor is growing at a constant rate invading surrounding tissue but has not yet spread. Susceptibility and age play a larger factor in the collection of these cells that we intend to use, others we need to take into consideration include body weight, tumor site, species, age, gender, etc. Dale Hattis et al (2004) they come to understand the “generic parameters” when determining the susceptibility of one cell in relation to others. This equation is a fitted to take in raw observations and estimates of possible dosing, and tests confidence levels of the cells. This will only be necessary if all cells are not taken from the same host.

Using ionizing radiation we will damage the DNA inside of the cells using UV irradiation removing electrons from an atom, causing the DNA to be damaged. Ionizing radiation is measured by the amount of energy absorbed per unit weight of tissue, and so the dose in Gy of radiation we need must be enough that DNA is damaged without destroying the nucleus, this would lead the cell to be dysfunctional or may not even work at all. Levels of possible dosing can be seen through research provided by Hattis et al as well as their work with age and susceptibility. Determining a proper dose for all the cells being tested is important, because uniformity of tumor size, cell susceptibly, etc. are needed when running multiple trails to get a cohesive answer. The UV light passes through the tissue and cleaves electrons off of the atom causing disruption, which then induces DNA damage to the genome and its surrounding, this will the hopefully activates p53 due to the cellular stress. Activation of p53 occurs as a response to the DNA damage via checkpoint pathways, these pathways are in place to monitor the integrity of our chromosomes. According to Hirao et al results serine/threonine kinases make it possible for the stress signals to be detected, and phosphorylation to occur. These cells must be in culture, in order for them to continue growing but also so that signals and triggers can be easily visible.

After administering the dose and the activation of p53, the cell either begins to undergo p53s tumor-suppression or anti-proliferation properties, this occurs through the induction of key downstream regulatory factors. If all goes well the tumor will be suppressed by either apoptosis or cellular senescence and we will be able to use molecular biomarkers to understand which process the cell underwent. Biomarkers are measurable indicators of a biological state or condition. They play a valuable role in this experiment and in identifying disease and possible treatments. Using biomarkers SA-β-gal and the release of cytochrome c we can determine what mechanism was used by p53 in order to achieved tumor suppression. These exposed cells will undergo two sets of treatments in order to determine the existence of either biomarker.

 At a pH level of 6.0, meaning slightly acidic, senescence-associated beta-galactosidase, SA-β-gal, can be easily detected by a cytochemical assay-using X-gal as a substrate. X-gal is used in molecular biology to easily identify enzymes. SA-β-gal is a hydrolase enzyme found only in senescent cells. The cytochemical assay uses a specific substrate, in our case X-gal, to identify biochemical contents in a cell. X-gal is a colorless, analog to lactose that tests for the presence of [β-galactosidase](https://en.wikipedia.org/wiki/Beta-galactosidase). According to Chainiaux et al and his work experimenting on specific protocol in which to test SA-ß-Gal as a biomarker we come to an understanding of how to detect senescence biomarkers. First the fixed cells need to be incubated with a buffer with a X-gal substrate, and left to incubate one more time. The cells must be rinsed with a phosphate-buffered saline solution, which is used to maintain the pH of 6.0. The colorless X-gal will have bound to any traces of [β-galactosidase](https://en.wikipedia.org/wiki/Beta-galactosidase) and react as a stain producing a blue coloring on the surface of the plate. These blue stains can then be counted and quantified under a bright field microscope, if the presences of these blue stains exist than we can deduce that cellular senescence has occurred. Our results should look something like the figure to the right from where Chainiaux et al experiment, we determine can conclude a total number of events/occurrences of [β-galactosidase](https://en.wikipedia.org/wiki/Beta-galactosidase) and also a percent of how much SA-ß-Gal was found in the entire cell.

The biomarker for apoptosis on the other hand is the release of cytochrome c from the mitochondria to the cytosol (Adu-Quare et al). Cytochrome c is a highly soluble trans-membrane protein complex, primarily known to function in the mitochondria. When a cell undergoes apoptosis, and receives an apoptotic stimulus, cytochrome c is released into the cytosol and triggers programmed cell death, apoptosis (Ward et al). This release is not only known for being a trigger but if found in the cytosol considered a biomarker. There have been many speculations of other biomarkers for apoptosis but none are as efficient and reliable as the release of cytochrome c. This biomarker is a key enzyme to the execution stage of the apoptotic pathway. To identify cytochrome c as a biomarker, we are going to use centrifugation and standard Western Blotting technique. We begin by taking the cells and placing them in a tissue grinder, this crush up the cells releasing the organelles of the cell. They are then taken and centrifuge which will enable us to see the solution separating into the supernatant and pellets, where the supernatant is then removed, and the process will be repeated two more times, increase the force of the centrifuge and for longer periods of time. Taking the final supernatant of the third centrifugation we perform Western blotting. Using a phosphate buffer saline solution, we fill the electrophoresis gel wells with our final product, after running the electrophoresis we will see bans that run throughout the gel. This gel is then taken and placed on a membrane where we will use cytochrome c antibodies. These antibodies will attach to appearances of cytochrome c, then in order to visualize the possible attachments we will stains. Using a standard western blotting technique we will correctly identify where the protein is found in the sample we provide it. After identifying which biomarker is present, we can understand which process the cell underwent in order to suppress the tumor. This needs to be run a number of times until an answer is reached.

III. Discussion:

If all goes according to the outline of the proposed experiment, p53 will mediate either apoptosis or cellular senescence, and we would have identified these outcomes through detecting each process’s biomarkers, SA-ß-Gal and the release of Cytochrome c. Through testing these outcomes of the tumor suppressor gene, p53, we are hoping to see a higher frequency in cellular senescence, because senescence is a much more protective mechanism. If we were to see that apoptosis is a more predisposed outcome, we would need to discover a new form of cancer therapy in which cellular senescence is more likely to occur. Since the study of cellular senescence is quiet limited, constructing new forms of cancer therapies that are surely to induce cellular senescence would make a relatively large breakthroughs in tumor suppression research.

P53 has been known to be a crucial mediator for tumor suppression, but if p53 doesn’t activate it could potentially affect the furtherance of the experiment. Since this experiment is heavily based on p53 functioning, if it fails to activate or cannot in fact suppress the tumor effectively the use of another tumor suppressor genes, Rb, or VHL or ARF, will be necessary. Another pitfall may be a flaw in the technique used, in line with the results of Jiang et al, ionizing radiation may lead to damage that is strictly in need of apoptotic mechanisms. This would prevent p53 from ever mediating cellular senescence, effectively skewing the potential results. Though this goes without all cells are different and will act differently to different signals. The intention is to develop better ways of treating cancer. Understanding what is done more frequently but also if it’s working can help us further determine how to go about treatments. Using the understand how cells are going about suppression cancerous tumors, and understanding if it is the best possible way to treat tumors.

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