**Pharmaceutical Re-activation of Pathways in non-p53 Type Cancer Cells to Improve Chemotherapeutic Efficacy**

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| **Introduction** |

Cancer is a leading cause of death worldwide. Cancer treatments are often ineffective or their effectiveness decrease over the course of treatment regimens. This is especially true for cancers such as pancreas, brain, and others. These ineffective treatments often lead to fatal outcomes as these cancers are often not eligible for surgery. One approach to better understand why these therapies are ineffective is to study how cells globally respond to treatments.

Carcinogenesis, which is the initiation of cancer formation, is a multipart process. It involves activation of proto-oncogenes to oncogenes and deactivation of tumor suppressor genes.13 Proto-oncogenes are genes that are present in normal healthy cells and they are used to stimulate cell growth. When these proto-oncogenes become mutated or altered they become oncogenes and can promote tumor formation or unregulated cell growth.13 Tumor suppressor genes are also present in normal cells and they control the process of cell death. A mutation in a tumor suppressor gene can allow cells who have damaged DNA to replicate and as this process continues it can lead to the loss of genes or modifications of genes. If this process of copying damaged DNA continues eventually a proto-oncogene can become modified and cancer may develop.13

Mutations to proto-oncogenes or tumor suppressor genes can occur in a number of ways. Sometimes the mutations are inherited but more commonly they are acquired.2,13 These acquired mutations can be caused by a multitude of factors such as through the aging process or through environmental exposures to damaging substances called carcinogens. A carcinogen is any substance or radiation that is directly involved in causing cancer.2 It is possible to be exposed to carcinogens in a number of ways such as life style choices, natural exposures, medical treatments, workplace or household exposures, and pollution. Common human carcinogens include tobacco, pollution, asbestos, ultraviolet light, radon gas, chemotherapy, x-rays, and immunosuppressant drugs.2

Apoptosis is a natural cellular function in which the cell detects DNA damage and then through the use of tumor suppressor genes triggers programmed cell death. Most chemotherapeutic drugs cause DNA damage which is sensed by p53*.4* P53 is a tumor suppressor gene product encoded by the gene *TP53.4* P53 has been identified to have this DNA damage detection and pro-apoptotic function. When cells lose p53 they are notoriously more difficult to fight with current chemotherapy treatments. While there has been a lot of research about the biochemical and biological functions of the protein produced by p53 and even mutated p53 there has been little research focused on looking at the global profile of cancer cells that have lost p53and are being treated with chemotherapeutic drugs.

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| **Experiment** |

 If we can better understand these cellular functions and how they change when p53 is lost from the cell we may be able to improve current cancer treatments or develop new treatments. The goal of this experiment is to identify potential proteins and genes that may be linked to p53-null cell types’ resistance to chemotherapy.

To begin we will culture cells of breast, lung, pancreas and brain cancers there will be two groups of each one that is p53-wild type and one that is p53-null. Once these cells are cultured we’ll setup the control group and obtain our baseline data for comparison. To do this we will set aside a small amount of untreated cells as our control.

Then we would treat both of the p53 groups in each different cancer type with common chemotherapeutic drugs. Group 1 will receive docetaxel. Docetaxel is known to damage structures involved in cell division and so cells cannot proliferate and the tumor growth is inhibited. Group 2 will receive Tamoxifen, which is a drug designed to treat breast cancer and it is known to produce cytostatic effects by binding to estrogen receptors causing cells to decrease DNA synthesis. Group 3 will receive Paclitaxel, which is a mitotic inhibitor and works by interrupting the final processes of cellular division. Group 4 will receive 5-fluorouracil, which inhibits DNA synthesis by blocking the thmidilate synthetase. This stops the conversion of deoxyuridilic acid to deoxythmidlyic acid so thymine is not available on the newly formed DNA strand. Group 5 will receive gemcitabine. Gemcitabine is an antimetabolite that prevents cells from making DNA or RNA by interfering with the production of nucleic acids. Group 6 will receive cyclophosphamide. Cyclophosphamide binds to the DNA strands inside of cancer cells and blocks DNA polymerase. Group 7 will be treated with procarbazine, Group 8 lomustine Group 9 vincristine. These last three drugs are known as PCV and make a common treatment for brain cancer. Procarbazine stops the cell from producing proteins and DNA through a mechanism that is not well understood but when it is metabolized it produces products that break DNA strands. Lomustine is an alkylating agent that damage DNA by adding alkyl groups to DNA molecules which causes proteins to be unable to bond as they should. Vincristine is a mitotic inhibitor that interferes with microtubules which causes mitosis to halt during metaphase.

Once we have treated the cells with these chemotherapeutic agents we will then run them and the control group through SDS-Page analysis. SDS-Page analysis is a technique of using gel electrophoresis that separates proteins based on their charge on and size. Protein material will be collected by precipitation using methanol/chloroform extraction.6 Next using protein fractionation then microcapillary reversed phase liquid chromatography (mcRP-LC) and electrospray ionization mass spectro (ESI-MS) we can identify quantity and proteins present in each of the cell types.6 mRPH-LC is a high performance method of chromatography that allows us to thoroughly separate out the products in each sample. Then ESI-MS allows us to produce ions and utilize mass spectrometry of these products. The mcRP-LC and ESI-MS approach will allow us to study the composition and folding of the proteins collected from the samples

After the proteins have been identified we will apply a bioinformatics analysis and utilize tools like GOFact, Reactome and Protein Identifier Cross-Reference service (PICR).6 GOFact can be utilized to classify where proteins are in the gene ontology (GO). Reactome can be utilized to identify biological pathways and PICR will help to identify proteins’ functions.

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| **Discussion** |

Now, once we have collected this data and identified the proteins we can then compare the results between the p53-wild type and p53-null cells to identify if there are any pathways that are deactivated, proteins that are altered, absent or in excess. This will hopefully increase our understanding of what is lost or gained in cellular function when *p53* is no longer present.

For example, if we discover that p53-null has a shortage or excess of a particular protein after treatment we can examine that protein’s function. If the identified protein involves proto-oncogene, tumor suppression, DNA repair or other similar functions it will be a good candidate to study further in future experiments.

After we have identified all the proteins that are present in altered amounts and are involved with some phase of carcinogenesis, mitosis or cellular maintenance we can then take this data and design a second experiment to explore the functions of each of the identified proteins. An experiment this would likely involve altering genetic codes to remove or add genes to throttle expression of genes, then repeating the chemotherapeutic treatments and gauging the apoptotic function.

From here if we identify a few genes that can increase apoptosis in p53 null cells then we may be able design a treatment to increase or decrease these gene products then we may be able to increase chemotherapeutic treatments effectiveness in p53-null type cells. This would lead us to further in vitro studies to test the therapy and finally on to in vivo studies in mice.

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