

# 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 decreases over the course of treatment regimens. This is especially true for cancers of the pancreas, brain, and others. In some cases, ineffective treatment options can be fatal when cancers are not eligible for surgery. One approach to better understand why these therapies are ineffective is to study how cells globally respond to chemotherapy.

Carcinogenesis, which is the initiation of cancer formation, is a multipart process. It involves the activation of proto-oncogenes to oncogenes and deactivation of tumor suppressor genes.<sup>14</sup> 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.<sup>14</sup> 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. As this process continues, it can lead to the loss of genes or to the modification of genes. If this process of copying damaged DNA continues, eventually a proto-oncogene can become modified and cancer may develop.<sup>14</sup>

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.<sup>2,14</sup> 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.<sup>2</sup> It is possible to be exposed to carcinogens in a number of ways, such as through 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.<sup>2</sup>

Apoptosis is a natural cellular function in which the cell detects DNA damage and then, through the activation of tumor suppressor genes, triggers programmed cell death. Most chemotherapeutic drugs cause DNA damage; this damage is often sensed by p53.<sup>4</sup> p53 is a tumor suppressor gene product encoded by the gene *TP53*.<sup>4</sup> When the DNA damage is excessive or non-repairable, p53 induces apoptosis. When cells lose p53, referred to as p53-null, 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 pathways controlled by p53 (including mutated p53), there has been little research focused on the global profile of protein interactions in cancer cells that have lost p53 and are being treated with chemotherapeutic drugs.

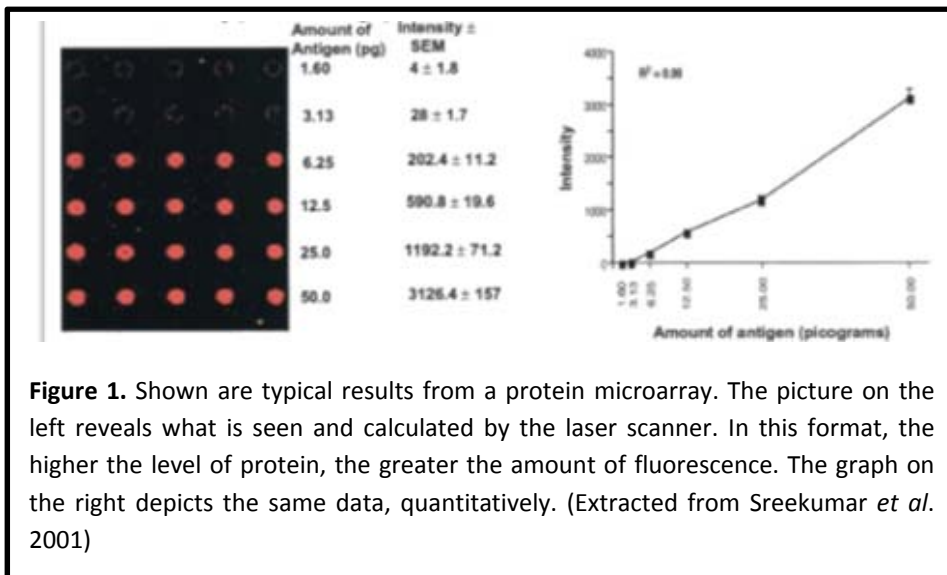
The process of drug discovery and design will require ongoing experiments and research. Our goal here in this first step is to identify proteins that are present in different amounts in p53-wild type versus p53-null cells. We are particularly interested in proteins that are involved in apoptosis, cell cycle progression or stress response. These proteins will be candidates for future research and may eventually lead to the discovery of an alternative means of fighting p53-null cancer cell types.

## Experiment

If we can better understand the ways that protein networks function and change, on a global level, when p53 is lost from the cell, we may be able to improve current cancer treatments or to develop new treatments. The goal of this experiment is to identify potential proteins and genes that may be linked to a p53-null phenotype and contribute to chemotherapy resistance.

To begin, we will culture cells of breast, lung, pancreas, and brain cancers. We will select cells with known p53 status and responsiveness (e.g., p53-wild type and p53-null). We will treat both of the p53 groups in each different cancer types with common chemotherapeutic drugs. Docetaxel will be a good agent to start with, as it is a widely utilized therapy, is relatively inexpensive, and is readily available in the US. Docetaxel is known to damage structures involved in cell division, so cells cannot proliferate and the tumor growth is inhibited. We will then prepare our control by treating a different set of each of these types of cells with DMSO, the diluent that will be used to prepare the docetaxel treatments. By doing this, will ensure that the control is prepared in the same fashion as the treatment group.

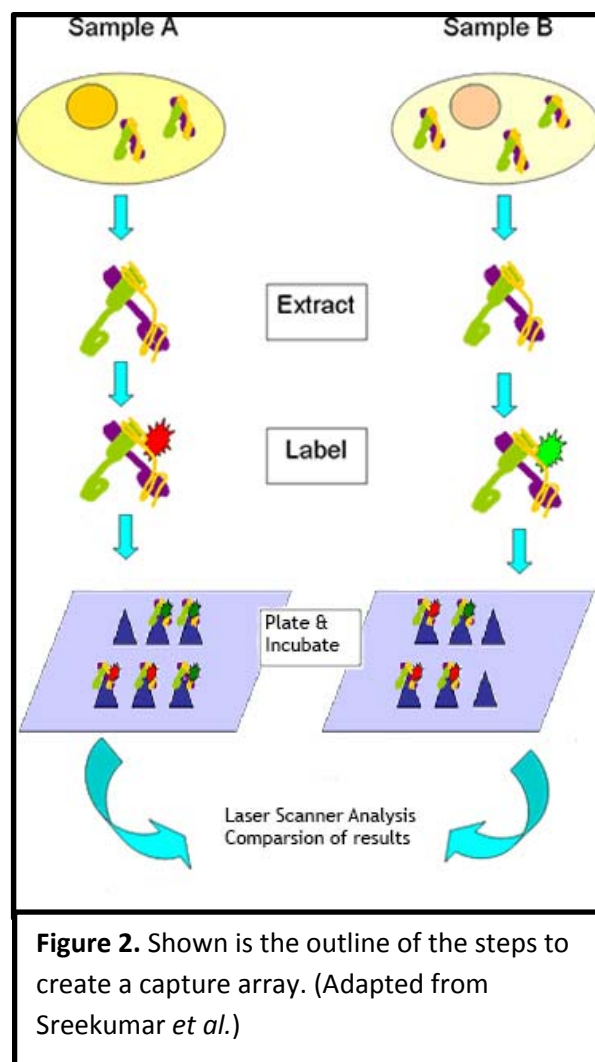
Once we have treated the cells with these chemotherapeutic agents, we will then isolate cellular protein from each treatment group and prepare a protein microarray, otherwise known as a capture or antibody microarray. A protein microarray is a method of simultaneously analyzing the quantity of a large number of proteins that are present in a cell.<sup>18</sup> A capture or antibody microarray depends on the use of commercially available antibodies that are selected based on their ability to bind to the proteins of interest.<sup>19</sup> Detection is then made using a fluorescent dye that reacts with bound antibodies. This process is quantifiable and high read-through.



**Figure 1.** Shown are typical results from a protein microarray. The picture on the left reveals what is seen and calculated by the laser scanner. In this format, the higher the level of protein, the greater the amount of fluorescence. The graph on the right depicts the same data, quantitatively. (Extracted from Sreekumar *et al.* 2001)

There are a number of different machines that can analyze the positive fluorescent signal of a protein or gene microarray. We choose to use a laser scanner, due to its high level of resolution.<sup>18</sup> Figure 1 is an image from Sreekumar *et al.* and shows an example of what results from a protein microarray look like.

To prepare the proteome microarray for this experiment, the cells will be lysed and have their proteins extracted. This will be done by RIPA buffer (to cause lysis) and Halt protease inhibitor (a commercially available product designed to prevent the proteins from being degraded). Isolated proteins will then be adhered on to a specially-designed glass slide, known as a protein chip. This protein chip will contain affinity agents, in this case commercially available antibodies that will be selected to identify proteins that are involved with cell cycle progression, the stress response, and apoptosis. Next buffers and fluorescent dyes are added to each sample separately, and then each is placed on to a separate protein chip. Once this occurs an adhesive film is applied and this is placed in an incubation chamber for at least an hour. While the array is in the incubator the antibodies that were on the custom designed array react with the different proteins and separate them out. From there the protein chip is placed in to the laser scanner the levels of labeled protein antibodies are counted. Then we will compare the results of each cancer cell's p53-wild type against its p53-null type and see if there are any differences noted in the protein expression levels. Figure 2 outlines this process.



**Figure 2.** Shown is the outline of the steps to create a capture array. (Adapted from Sreekumar *et al.*)

## Discussion

In this particular experiment outlined here it may be worth considering the levels of expressed apoptosis in between the two treatment groups. This may be relevant because it would give us a quantifiable piece of data to confirm how effective the selected chemotherapeutic agent is in fact the p53-null group versus the p53-wild type. This could probably be easily added in to the experiment at some point. We also have no method built-in presently to count the actual number of cells being lysed and put into the protein array. It is possible that the data may need to be normalized to account for differences in the number of cells in the sample.

After the protein levels have been determined if differences are noted between the groups tested then we can identify which proteins are down or up regulated between the two different cell types. For example if we find a protein in column A, row 1 is downregulated in p53-null cells and based on the type of protein chip ordered we know that A1 was where the BaX antibody was placed then we have an important clue in designing a second experiment. BaX or Bcl-2-associated X protein, is a protein that promotes apoptosis in cells. So this next experiment could explore increasing levels of BaX, or any other protein found to be out of range, then treating the cells with chemotherapeutic agents and measuring the levels of apoptosis. This could involve altering genetic codes to remove or add genes to throttle the expression of genes and then repeating the chemotherapeutic treatments and gauging the apoptotic response of cells to the therapy.

The main source of results for these continued experiments will be protein microarrays. Since there are other types of protein microarrays, such as category called functional protein microarrays, which can tell us about protein-protein and protein-drug interactions. These tools will be useful in these future experiments.

Then through continued experiments we may find conclusive evidence that certain proteins that are involved with the functions related to cancer development then we may be able to develop a treatment to increase an apoptotic response in p53-null cells after chemotherapy, then we may be able to design a specific therapy to target that “Achilles heel” of cancer. The specific therapy (e.g., small molecule, antibody, etc.) would then be used in combination with the indicated chemotherapeutic to improve treatment efficacy and, potentially, bring the therapeutic range down to a dose that would cause less damage to normal tissues.

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