Exploration of a Lung Cancer Cell Line: Resistance and Sensitivity to Taxol

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Through the years, we have become increasingly aware of the risk and prevalence of cancer in various forms. Although the causes attributed to these cancers can range from genetic makeup to lifestyle choices, one form that has received much media attention is lung cancer. It is supposed to be the most preventable form of cancer, but, out of all deaths due to cancer, the deaths of 32% of men and 25% of women are attributed to lung cancer. With new technologies available to help treat and survive cancer, this is a staggering number of people. One of the ways to treat cancer is with the use of a drug called taxol, which holds great promise for cancer sufferers.

Cancer occurs when a cell does not function properly or begins to divide uncontrollably. Normally, cells have a predetermined lifespan. When they are too old or need to be replaced, the cell will undergo apoptosis, which is programmed cell death. After the death of the old cell, neighboring cells can undergo mitosis, which is a type of cell division, to maintain the number of healthy cells in the organism. The life cycle of cells involves a complex self-checking system that ensures the proper function of a cell. Most of the time, a cell can repair itself during the replication process. If a mutation occurs or if a cell is not the correct size, and the cell cannot repair itself, a cascade of events will take place that initiates apoptosis of the cell. If an error occurs in one of the self-checking systems, the cell may divide instead of dying. The continuous replication of this faulty cell is what leads to cancer.

It is known that only 5% of our DNA actually code for proteins that are transcribed and translated. The remaining 95% of our DNA used to be thought of as junk DNA. Part of this junk DNA included sections called Alu sequences. Alu sequences are an example of middle repetitive DNA, which, in humans, encompasses more than 5% of the entire genome. It has been found that the Alu sequences make RNA transcripts, but they are not translated into proteins. When certain cells are stressed by heat shock, increased amounts of Alu transcripts have been found to be produced along with the expected mRNA from genes that respond to stress conditions. This may indicate that the Alu sequences, along with other stretches of DNA that do not code for proteins, actually regulate or influence the expression of other genes.

Currently, a popular method of treating cancer is by way of surgical removal of any cancerous growth and then treatment with chemotherapeutic agents. One such chemotherapeutic agent is a drug called taxol, also known as paclitaxel. Taxol interferes with the normal function of microtubule growth in cancerous cells. It binds to the tubulin protein of microtubules, locking them into place. This microtubule/taxol complex is not able to disassemble, which is detrimental to the functioning of the cell because the disassembly is necessary for cell growth and division. Taxol is also able to induce programmed cell death in cancer cells by binding to Bcl-2, which is an apoptosis stopping protein, serving to hinder their function. Taxol is able to selectively target cancerous cells by interfering with the functioning of both rapidly dividing cells and cells that contain the apoptosis stopping protein.

The lung cancer cells to be studied are called adenocarcinoma cells, which were isolated in 1972 from a 58-year old Caucasian male. The A549 parent cell line is sensitive to Taxol, meaning that Taxol is effective in controlling the survival and replication of these cancer cells. The A549-T24 cells are the resistant to Taxol, meaning that these cells can tolerate the presence of Taxol even at relatively high concentrations. Since the resistant A549-T24 cells were derived from the sensitive A549 cells, any altered characteristics between the cell lines are probably due to this developed resistance, which is also a common problem in vivo. Insight about the mechanism of this developed resistance may assist future treatment of such cancers.

The mutated A549-T24 cell line has already been found to produce increased amounts of a protein necessary for cell division called tubulin. It is not known whether the increased amounts of tubulin are

due to an increased number of copies of that gene in the mutated cell line, or to an over expression of the gene that codes for the tubulin.

One of my key hypotheses is that the expression of Alu sequences in the DNA of the cells I am studying will differ for cells drawn from cell lines sensitive to Taxol and those that are resistant to it. If in fact I find a difference in the expression of Alu sequences in the A549 versus the A549-T24 cells, this could provide some clues regarding the functions of these sections of DNA in *all* cells. I will examine this by determining the methylation of these Alu sequences in each cell line. Methylation of a gene, or of a specific region on the DNA, indicates deactivation of that gene in the cell. If there is a difference in the methylation between the A549 and the A549-T24 cells, this could indicate a certain mechanism in the development of resistance to taxol.

To test for the differential methylation between two cell lines, I need to extract the DNA from each cell line. This is done by a series of centrifugations in different buffer solutions to break open the cells while keeping the DNA intact, followed by wither phenol/chloroform or ethyl alcohol washings to remove the chemicals remaining in solution. Pure samples of each kind of DNA are cut using restriction enzymes, including isoschizomers. Restriction enzymes cleave to the DNA backbone at very specific base pair sequences, and isoschizomers are pairs of restriction enzymes that cleave at the same base pair site—one will cleave regardless of methylation, while the other cannot cleave to the site if methylation is present.

A sample of DNA cut with restriction enzymes from each cell line is then used to run a gel electrophoresis. The agarose gel provides a medium for the DNA to travel through, and the electrical current helps to separate the DNA pieces on the gel by size. DNA is negatively charged and all the fragmented pieces have the same charge to mass ratio. Since they all have the same charge to mass ratio, all the DNA fragments would move the same distance in the presence of an electrical current. But by using the agarose gel, the DNA fragments move different distances based on size with the smaller fragments moving faster through the gel. The final product of electrophoresis is the presence of DNA bands along with DNA markers to show the base pair size of each DNA band.

The bands are visualized by staining with ethidium bromide so that a picture can be taken of the gel with visible DNA bands. After de-staining the gel, a Southern Blot analysis of the DNA is performed to transfer the separated DNA bands onto a nylon membrane, which can then be treated with DIG DNA probes to verify the results of the experiment. The DNA bands seen on the agarose gel using ethidium bromide are not sensitive enough to verify the outcome of the experiment, so the DNA must be transferred to a nylon membrane and visualized using another more sensitive label.

The final product of this experiment will provide the information necessary to tell whether or not the methylation patterns differ between the Alu sequences of the taxol-resistant and taxol-sensitive cell lines. Since the resistant line was developed from the sensitive line, the results may provide insight on the evolution of the cancerous cells.