

Chemotherapy Drugs: Less is More

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Background

Since chemotherapy was first recognized as a proven treatment for cancer, it has been the subject to intense scientific scrutiny. Chemotherapy refers to the treatment of tumours through the introduction of chemicals designed to kill rapidly growing (cancerous) cells. Often, one major downside to chemotherapy treatments is the ever-present side effects, and the possible return of the cancer. One of the most devastating forms of cancer, and the one that most affects people worldwide is breast cancer. Breast cancer is usually caused by defective inherited genes such as BAX, BRCA 2 and p53. The BAX gene, induces apoptosis by traveling to the surface of the mitochondria of cells where it triggers the release of cytochrome c; ultimately binding to Apaf-1 protein (Apoptotic Protease Activating Factor-1). Genes such as BAX are referred to as tumour suppressors as they help control tumour growth. We explored the idea of using BAX as a chemosensitizer, which is a drug that makes tumours more sensitive to the effects of chemotherapy. We also explored the use of a virotherapy, specifically, adenovirus Δ -4EGFP as a chemosensitizer.

Purpose

The objective of our experiment was to reduce the quantity of drug generally given to patients by making the cells more chemosensitive and prone to apoptosis through their treatment with Δ -4EGFP adenovirus or pEGFP-BAX plasmid. With a reduction in the amount of chemotherapy drug used, there would be a reduction of side effects to chemotherapy experienced by patients (due to lower dosage). We used two colon cancer cell lines: one with only one mutant allele, BAX (+/-), and another with two mutant, BAX (-/-), to assess the impact to both. BAX (+/-) has one portion of the functional BAX gene, whereas BAX (-/-) lacks the entire functional BAX genes.

Hypothesis

The quantity of Chemotherapy Drug administered to patients can be reduced by making cancerous cells more prone to apoptosis with the use of Δ -4EGFP adenovirus and pEGFP-BAX plasmid.

Procedure

Our experiment was conducted with the usage of two different genotypes of the colon cancer cell cultures HCT 116, BAX (+/-) and BAX (-/-). Both cell lines were infected with the chemotherapy drugs at different concentrations (100uM, 200uM and 375uM). Along with the drug, an MOI (Multiplicity of Infection): 50 was used for the adenovirus. We used the BAX gene carried by a plasmid, as it functions in a key role in inhibiting the growth of tumour cells, and is lacking in our 2 cell lines. We had an untreated control present along with all of our cell samples of drug and drug with plasmid in an effort to compare cell viability between the different samples. We performed the same procedure for our adenovirus. In order to keep our cancerous cells alive for the duration of our experiment, we passed the cells every 2 days following the cell passage protocol below.

Cell Passage Protocol:

1. From a donor flask, remove the old media and wash cells with PBS.
2. Add 1X Trypsin, pH 7.4 and incubate at 37°C for 2 mins.
3. Add DMEM (media with 10% FBS) directly to the cells and pipette gently.
4. Pass the cells to new flasks or plates (35 mm plates).

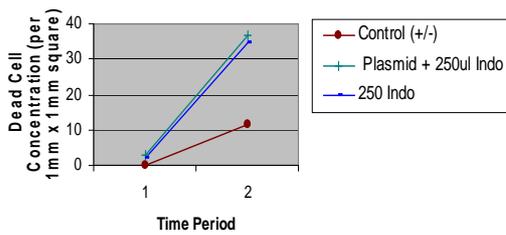
Results

Preliminary results were mainly qualitative as we assessed cell structure before performing cell counts. Initially, after testing all three chemotherapy drugs (Indomethacin, Cyclohexamide, Sulindac), we concluded that Indomethacin would be the most effective chemotherapy drug to be used in our experiments as it was the fastest operating. In our untreated control we observed healthy, medium confluency cells, rapidly growing and as we increased the concentration of Indomethacin we began to see far fewer, apoptotic cells bearing a cloud like formation.

pEGFP- BAX Plasmid Transfection Results:
(Counted at 0, 24, 48 Hour Periods)

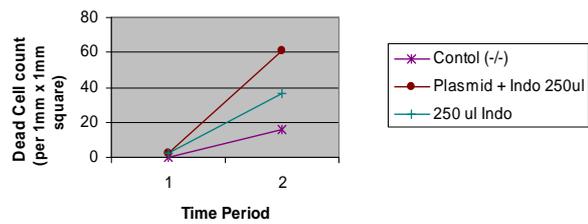
0, 48 Hours Post Transfection: **BAX (+/-)** Genotype 0, 48 Hours Post Transfection: **BAX (-/-)** Genotype

Figure 1a) Cell Death BAX (+/-)



As shown in the graph, there was minimal difference in cell death due to the addition of the BAX plasmid in the BAX (+/-) genotype

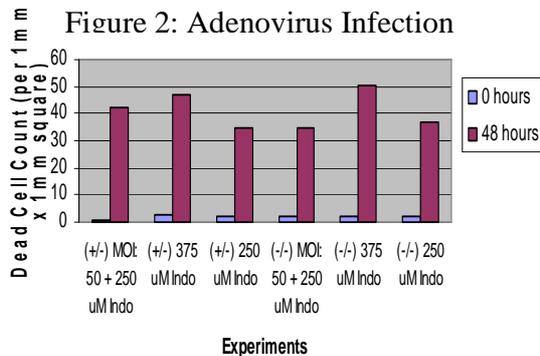
Figure 1b) Cell Death BAX (-/-)



As shown in the graph above, there was **substantial difference** in cell death due to the addition of the BAX plasmid in the BAX (-/-) genotype. Thus Plasmid transfection was effective.

As seen in the graphs above, as well as microscopic pictures of cell structure, we were able to conclude that the addition of a functional BAX gene along with a medium (250 uM) concentration of Indomethacin proved to be more effective in killing cancerous cells than just the drug alone. Overall, we noticed a 22% increase in cell death in the BAX (-/-) due to the addition of the plasmid, thus proving this form of treatment more effective in the BAX (-/-) genotype.

Δ4EGFP Adenovirus Infection Results:
(Counted at 0, 48 Hours)



Upon deciding Indomethacin would be used we performed repeated tests with the combination of Adenovirus (at MOI: 50) and Indomethacin at concentrations 100uM, 250uM, and 375uM. After 3 trials, the adenovirus proved to consistently assist in the apoptosis among these concentrations. The adenovirus was particularly effective in the BAX (+/-) genotype and had a significant 8% increase in the amount of dead cells from just 250 uM Indomethacin to 250uM and adenovirus.

Conclusions

After many experiments, pictures, and counts, we concluded that our hypothesis was correct. The combination of adenovirus and Indomethacin drug treatment showed increased cell death compared to drug alone. Similarly, a combination of BAX expressing plasmid transfection showed higher levels of cell death compared to drug alone. Our results demonstrated that a lower chemotherapy drug amount can be used as long as it is co-administered with a plasmid or adenovirus. From our preliminary experiments, the BAX plasmid was most effective in cell killing of the BAX (-/-) genotype cells, and the adenovirus was most effective in the BAX (+/-). All of our experiments were performed with three trials, however, to attain more accurate results this experiment should be repeated more times. Based on these preliminary experiments using chicken adenovirus and a BAX plasmid to treat cancer cells, we feel that through our project we have discovered new ground for scientific research to further develop this concept and perhaps provide a novel approach in which lower levels of chemotherapeutic drugs would be needed to control a cancer.

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