Gene Therapy for Breast Cancer

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Objective

The objective of this project is to use gene therapy to suppress the growth of breast cancer cells

Introduction

A cancer is an uncontrolled proliferation of cells. Cancer cells contain several mutated genes. These almost always include mutations in genes that are involved in mitosis; that is, in genes that control the cell cycle, such as tumor suppressor genes. Tumor suppressor genes normally inhibit mitosis. **P53** gene is one of the tumor suppressor genes in cells. Its product, p53 protein, prevents a cell from completing the cell cycle if its DNA is damaged or the cell has suffered other types of damage. It does this by binding to a transcription factor called E2F. This prevents E2F from binding to the promoters of such proto-oncogenes as c-myc and c-fos. Transcription of c-myc and c-fos is needed for mitosis. Blocking the transcription factor needed to turn on these genes prevents cell division. If the cell's damage is minor, p53 halts the cell cycle until the damage is repaired; if the damage is major and cannot be repaired, p53 triggers the cell to commit suicide by **apoptosis.** These functions make p53 a key player in protecting us from cancer; that is, an important tumor suppressor gene. However, these are also the reasons that more that half of all human cancers do, in fact, harbor p53 mutations and have no functioning p53 protein.

Gene therapy is a technique for correcting defective genes responsible for disease development. In most gene therapy studies, a "normal" gene is inserted into the genome to replace a disease-causing gene. A vector must be used to deliver the therapeutic gene to the target cells. The most common vector is a virus that has been genetically altered to carry normal human DNA.

Experimental Design

<u>Construction of retrovirus vector for gene therapy</u>

- Seed the cells on plates on the day before experiment. Prepare and clone recombinant retrovirus vector plasmid containing p53.
- Co-transfect the recombinant retrovirus vector plasmids into packaging cells
- Packaging cells are treated so they express:
  - An RNA copy of the human p53 gene along with: [1]a **packaging signal (Ψ)** needed for the assembly of fresh virus particles; [2] **long terminal repeats** (**LTR)** at each end, which aids insertion of the DNA copies into the DNA of the target cell.
  - An RNA copy of the retroviral **gag**, **pol**, and **env** genes but with **no packaging signal** (so these genes cannot be incorporated in fresh viral particles). The env gene is modified by replacing a portion of the gene with ~150 codons from the HER2 protein.

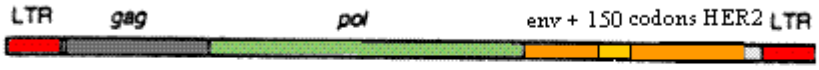
Treated with these two genomes, the packaging cell produced a crop of **retroviruses** with:

- The envelope protein needed to infect the cells with HER2 receptor at the membrane.
- an RNA copy of the human p53 gene, complete with LTR sequences at each end
- reverse transcriptase, needed to make a DNA copy of the p53 gene that can be inserted into the DNA of the target cell
- None of the genes (gag, pol, env) that would enable the virus to replicate in its new host.

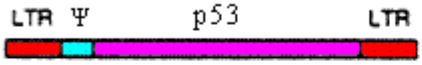
Once the virus has infected the target cells, the p53 RNA are reverse transcribed into DNA and inserted into the chromosomal DNA of the host.

Appendix:

Genomes used in packaging cells:

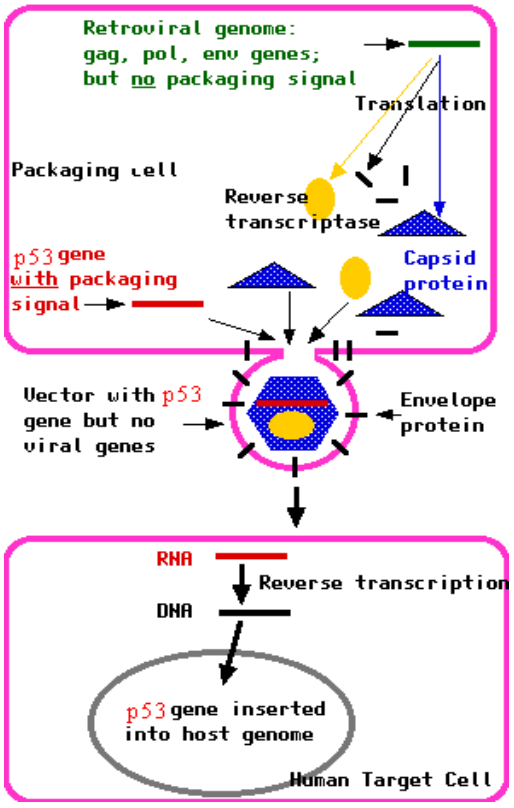


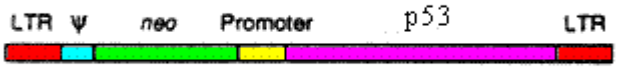
RNA copy of the retroviral gag, pol, and env genes but with no packaging signal



An RNA copy of the human p53 gene along with a packaging signal (Ψ)

Construction of retrovirus vector





Viral Vector Gene

Results and Interpretation:
The ideal result to this experiment is that the most of the breast cancer cells stop dividing and die after they are infected with the retrovirus containing p53 gene, but the healthy cells survive.

Relevant Application:
Gene therapy is a fast developing technology that is being frequently used in various areas. This project itself is an application of using gene therapy to treat diseases, such as cancer. If the result of the experiment turns out to be what is expected, then this therapy can potentially be used in clinical trial. On the other hand, the virus vector can carry different gene for different treatment or dealing with different diseases. The protein coat of the virus vector can also be altered for target delivery. Gene therapy is going to become the most effective way to treat life-threatening diseases, like HIV, immunodeficiency and other genetic diseases.

Materials and Methods
Gene cloning using polymerase chain reaction (PCR):

- Firstly identify a portion of the sequence of p53 DNA molecule.
- Synthesize primers: short oligonucleotides (containing about two dozen nucleotides) that are precisely complementary to the sequence at the 3' end of each strand of the p53 gene.
- P53 DNA molecule is heated to separate its strands and mixed with the primers.
- If the primers find their complementary sequences in the DNA, they bind to them.
- Synthesis begins (as always 5' -> 3') using the original strand as the template.
- The reaction mixture contain:
    - all four deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP)
    - A DNA polymerase from Thermus aquaticus is used so that it is not denatured by the high temperature needed to separate the DNA strands.
- Polymerization continues until each newly-synthesized strand has proceeded far enough to contain the site recognized by the other primer.
- Now you have two DNA molecules identical to the original molecule.
- You take these two molecules, heat them to separate their strands, and repeat the process.
- Each cycle doubles the number of p53 DNA molecules.

Construct recombinant retrovirus vector plasmid:

- Use restriction enzymes to cut the p53 DNA molecule and give it specific single stranded ends
- Use DNA ligase to glue the DNA molecule with packaging signal (Ψ), long terminal repeats (LTR), bacterial plasmid sequences and antibiotic resistance gene (eg. Ampicillin) that will allow you to replicate (multiply) your construct in bacteria.
- Put plasmids into bacteria by transformation.

Transforming E. coli

Treatment of E. coli with the mixture of religated molecules will produce some colonies that are able to grow in the presence of both ampicillin and kanamycin.

- A suspension of E. coli is treated with the mixture of religated DNA molecules.
- The suspension is spread on the surface of agar containing ampicillin. The next day, a few cells — resistant to the antibiotics — will have grown into visible colonies containing billions of transformed cells.
- Each colony represents a **clone** of transformed cells.
- Identify cells containing the recombinant plasmid by their ability to grow in presence of ampicillin but not tetracycline; clone these selected bacteria.

(Standard DNA Cloning Technique)

Tumor cell culture:

In this lab, two cell cultures from a breast cancer cell line MDA MB-231 will be used. One serves as a cancer model (control) and the other cell culture will be infected with retrovirus vectors. Both of these cell lines will contain the same number of breast cancer cells and healthy cells.

Measuring cell growth or cell death

The simplest assay for cell death measurement is measuring plasma membrane integrity. This can be assessed in two ways: the ability of a cell to prevent a fluorescent dye from entering it and the ability of a cell to retain a fluorescent dye within it.

In this lab, I will use both **propidium iodide** and **fluorescein diacetate** (FDA). **Propidium iodide** is a non-permeant dye that can penetrate the membranes of dying/dead cells, because as a cell dies, its plasma membrane becomes permeable allowing fluorescent dyes present outside the cell to enter it and fluoresce. It intercalates into the major groove of the DNA and produces a highly fluorescent adduct so **non-viable** cells can be identified by positive **red** fluorescence. **Fluorescein diacetate (FDA)** is a non-polar, non-fluorescent fluorescein analogue which can pass through the cell membrane whereupon intracellular esterases cleave off the diacetate group producing the highly fluorescent product fluorescein. The fluorescein will accumulate in cells which possess intact membranes so the **green** fluorescence can be used as a marker of cell viability. Cells which do not possess an intact cell membrane or an active metabolism may not accumulate the fluorescent product and therefore do not exhibit green fluorescence.

When using combination of PI and FDA, the non-viable cells will take up the PI and stain **red** whereas viable cells will not take up the PI and should only stain **green**. This 2-colour separation of non-viable and viable cells provides for a more accurate quantization of cell viability than single color analysis.

Timeline of project

December: Design retrovirus construction and gene cloning strategy

January and February: amplify p53 gene; construct retrovirus genome and packaging cells;

March and April: create retrovirus vector using packaging cells; infect cells and analyze p53 expression and viability.