Using Gene Chips to Study the Genetics of Lung Cancer (A DNA Microarray Lab)

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Carolina Biological
DNA Chips: Genes to Disease (#211520 $49-$82)

No significant materials are needed prior, except a hot water bath or microwave to keep the agarose reagents liquefied; most schools have this readily available.

Per the *Teacher Instructions*, the primary purposes of this investigation are to teach the following:

1. gene chips (DNA microarrays) are a powerful new technology that scientists use to measure the activity (transcription) of thousands of genes at one time;
2. microarrays highlight important connections between genetics, cell biology, genes, DNA, chromosomes, gene expressions, transcription, cancer biology, proteins, technology and bioethics;
3. genes are differentially regulated: all cells in an organism contain the same genes, but different genes are expressed (transcribed) in different tissues under different conditions which is what gives different tissues their different phenotypes;
4. and even genes that are not highly expressed (transcribed) may play an important role in the cell and that the lack of expression of a gene may also play an important role in the cell.

Microarray analysis is a powerful new research tool that enables technicians to view and interpret at one time, on one small surface, the extent to which thousands of genes have been expressed in cells. Researchers developed and continued to refine the technology by merging strides in genomics, computer science, and nanotechnology.

Detecting patterns or changes in transcription in cells is a way to understand both normal and abnormal aspects of cell function. A researcher who wanted to look for changes in transcription in a specific cancer gene could use microarray analysis. As the first step in the process, a gene chip would be created. DNA chip, microarray, gene chip, and genome chip are all terms that describe a solid matrix, such as a glass slide, that is imprinted with a precisely arranged pattern of spots, each made up of many copies of a specific nucleotide representing part of genome.

As the next step, the DNA chip would be used to analyze complementary DNAs (cDNA) that were made from mRNA isolated form cancerous and noncancerous parts of the same tissue. The cancerous and noncancerous DNA samples are flagged with dyes and applied to the prepared chip. The extent to which each flagged gene adheres to its complement on the chip directly indicates the extent to which transcription occurred. Computer

analysis of the DNA chip reveals which genes were transcribed in the cancerous tissue and which in the normal tissue, and thus indicates which genes might be important in the development of the cancer. The use of a microarray in this application allows suspect genes to be identified years sooner than would have been possible with previous technologies that were unable to analyze so many genes so precisely at one time.

A single microarray can contain more than 30 000 spots of DNA, each representing a different gene in an organism. In this investigation, the concept of a DNA microarray is used to study the expression of six different genes in normal lung cells and lung cancer cells. These results will show how these six genes are transcribed in normal versus cancerous lung cells. Students will use reagents that simulate the process, composed of various combinations of basic indicators in an agarose solution.

Scientists have found that some genes are not transcribed as much in cancer cells as in normal cells. These repressed genes may play an important role in allowing the cancer cells to spread and grow. Other genes are transcribed more in cancer cells than in normal cells. These genes may also play an important role in making the cells cancerous. There are also many genes that are transcribed at the same level in both cell lines. These genes probably do not play a significant role in causing cells to become cancerous. There are also some genes that may not be expressed at all in normal or cancerous lung cells. It is important to have a discussion with students prior to the lab that cancerous effects can be caused by both over- and under-expression of certain genes, and that some genes on being tested may fall into either "housekeeping" or specific to cell type, in which case they would express differently.

Indiana Academic Standards addressed by this investigation include:
B.1.1.
Recognize that and explain how the many cells in an individual can be very different from one another, even though they are all descended from a single cell and thus have essentially identical genetic instructions. Understand that different parts of the genetic instructions are used in different types of cells and are influenced by the cell's environment and past history.

B.1.4.
Understand and describe that the work of the cell is carried out by the many different types of molecules it assembles, such as proteins, lipids, carbohydrates, and nucleic acids.

B.1.5.
Demonstrate that most cells function best within a narrow range of temperature and acidity. Note that extreme changes may harm cells, modifying the structure of their protein molecules and therefore, some possible functions.

B.1.8.
Understand and describe that all growth and development is a consequence of an increase in cell number, cell size, and/or cell products. Explain that cellular differentiation results from gene expression and/or environmental influence. Differentiate between mitosis and meiosis.

B.1.21.
Understand and explain that the information passed from parents to offspring is transmitted by means of genes which are coded in DNA molecules.

B.1.24.
Explain that gene mutations can be caused by such things as radiation and chemicals. Understand that when they occur in sex cells, the mutations can be passed onto offspring; if they occur in other cells, they can be passed on to descendant cells only.

B.1.25.
Explain how gene mutations in a cell can result in uncontrolled cell division, called cancer. Also know that exposure of cells to certain chemicals and radiation increases mutations and thus increases the chance of cancer.

B.1.26.
Demonstrate how the genetic information in DNA molecules provides instructions for assembling protein molecules and that this is virtually the same mechanisms for all life forms.

Limitations

The primary limitation was that the protocol, as followed, provided the same results to each student group. As a result, student understanding and execution of the protocol is difficult to evaluate, as is their overall understanding of the underlying concepts. Changes considered for the next run included simply changing the gene numbers on the droppers. However, after a brief online search, the reagent combinations were located and it was decided that new stock solutions would be produced with new combinations of the two primary indicators (phenolphthalein, thymolphthalein), agarose and water (and/or student groups would be given different combinations). This then insures that each unique student group will have a unique set of results, known only to the instructor, and making evaluation of student understanding more authentic. With this change, the door is open to bring in scenarios other than cancerous versus normal lung cell genes, and students can then further investigate the function of those genes to determine whether or not they would have a role as a disease agent. OR—with unique student group results, each group would be given the task of testing for activity within a specific group of genes for a possible diagnosis. This investigation shows students practical application of current work, and allows further investigation of how using gene activity information could potentially lead to a treatment. Students would need to be provided information regarding gene expression in different diseases in order to make such determinations.

When this kit was modeled with peer teachers, the general impression was such that while the results were good, the concept would be more appropriate for advanced level classes rather than a regular level biology class. My experience with a regular (freshman) level biology class was such that most students did have a basic grasp of the idea and were able to read the results accurately, more time needs to be dedicated to the discussion of gene expression and how they can impact phenotype.

I would introduce/supplement this lab investigation with the following NIH link. The link uses both historical scenarios of various cancers, and animations of the cell cycle that include the action of protooncogenes and tumor suppressor genes; it also includes a discussion of the impact of protooncogene→oncogene consequences. Teacher resources and student handouts are available at the site as well.

http://science.education.nih.gov/supplements/nih1/Cancer/default.htm

Possible Alternative Diseases

High Grade Astrocytoma:
Nine genes are known to be dysregulated: 4 ribosomal proteins are upregulated, 5 other genes were downregulated. One, APOD, was the most differentially expressed and has been shown to inhibit tumor cell and smooth muscle cell proliferation, suggesting that APOD might be critical for malignancy, and therefore a possible source for therapy.

MacDonald TJ, Pollack IF, Okada H, Bhattacharya S, Lyons-Weiler J. "Progression-Associated Genes in Astrocytoma Identified by Novel Microarray Gene Expression Data Reanalysis." *Microarray Data Analysis: Methods and Applications*. (5/2007): 203-221. http://www.springerlink.com/content/n220510v2u5706p3/

Retinoblastoma:
RB1 Gene is a tumor suppressor gene and is typically downregulated in retinoblastoma, and therefore allowing retinal cells to divide uncontrollably.

(http://ghr.nlm.nih.gov/condition=retinoblastoma)