Name _____

Checking Up on Genes

You are oncologists specializing in breast cancer and will be conducting a microarray analysis on one of two newly diagnosed breast cancer patients, Mrs. Jones and Mrs. Brown. You will be adding a solution to each spot on the array that represents the complementary DNA (cDNA) of your patient to determine her gene expression profile. After you complete your microanalysis, you will decide on her course of treatment.

Procedure

1. Read your "How DNA Microarrays Work" student handout to learn what microarrays are used for and how they work.
2. The plastic grid your teacher will give you represents the microarray for your patient. Each spot represents one gene. The solution represents the cDNA of a cancer patient. Using the solution your teacher has given you, use a pipette to add three drops in each spot on the microarray for your patient.
3. Once all the spots have been treated, use the key on your "Gene Locations on Array" handout to interpret your results. Then record the result for your patient under each gene name on the same handout.
4. After you have interpreted the results, use your "Cancer Therapy Options" handout, which describes several treatments for breast cancer. Use the results of your microarray analysis to determine which therapies might be indicated for your patient. Then answer the questions on this page.

Analysis Questions

1. Which treatment or treatments would you recommend for your patient?

_____
_____
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_____
_____
_____

2. Some genes, such as ERB-B2 and ESR1, have been found to be associated with particular diseases or conditions such as cancer. Other genes, such as the ABC-B2 gene, are not associated with a disease but are involved in resistance to certain drugs or treatments. Why would it be useful to test for the expressions of genes like the ABC-B2 gene on a microarray?

_____
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How DNA Microarrays Work

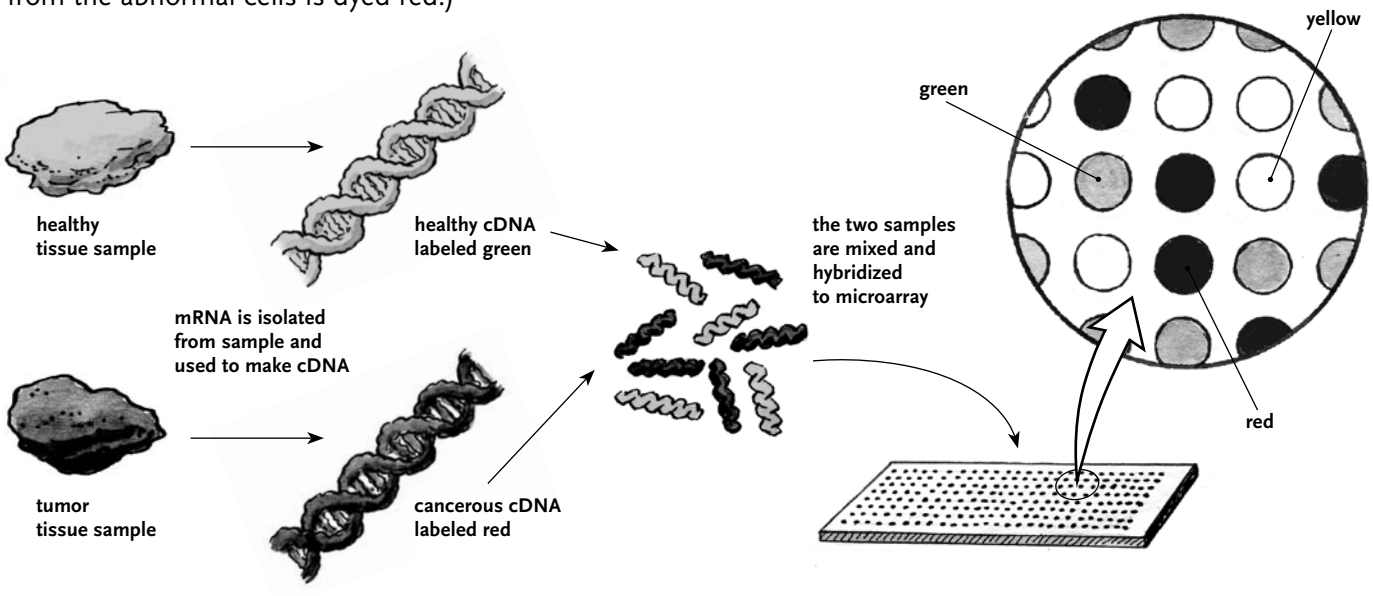
In each type of cell, like a muscle cell or a skin cell, different genes are expressed (turned on) or silenced (turned off). If the cells that are turned on mutate, they could—depending on what role they play in the cell—trigger the cell to become abnormal and divide uncontrollably, causing cancer.

By identifying which genes in the cancer cells are working abnormally, doctors can better diagnose and treat cancer. One way they do this is to use a DNA microarray to determine the expression levels of genes. When a gene is expressed in a cell, it generates messenger RNA (mRNA). Overexpressed genes generate more mRNA than underexpressed genes. This can be detected on the microarray.

The first step in using a microarray is to collect healthy and cancerous tissue samples from the patient. This way, doctors can look at what genes are turned on and off in the healthy cells compared to the cancerous cells. Once the tissues samples are obtained, the messenger RNA (mRNA) is isolated from the samples. The mRNA is color-coded with fluorescent tags and used to make a DNA copy (the mRNA from the healthy cells is dyed green; the mRNA from the abnormal cells is dyed red.)

The DNA copy that is made, called complementary DNA (cDNA), is then applied to the microarray. The cDNA binds to complementary base pairs in each of the spots on the array, a process known as hybridization. Based on how the DNA binds together, each spot will appear red, green, or yellow (a combination of red and green) when scanned with a laser.

- A red spot indicates that that gene was strongly expressed in cancer cells. (In your experiment these spots will be dark pink.)
- A green spot indicates that that gene was strongly repressed in cancer cells. (In your experiment these spots will be light pink.)
- If a spot turns yellow, it means that that gene was neither strongly expressed nor strongly repressed in cancer cells. (In your experiment these spots will be clear.)
- A black spot indicates that none of the patient's cDNA has bonded to the DNA in the gene located in that spot. This indicates that the gene is inactive. (All of the genes in your experiment are active.)



A microarray is an orderly arrangement of rows and columns on a surface like a glass slide. Each of the spots on an array contains single-stranded DNA molecules that correspond to a single gene. An array can contain a few, or thousands, of genes.

Gene Locations on Array

The following charts show which genes are represented by each spot on your array. Use the key on this page to determine the expression level of each gene for your patient. Then record whether each gene was strongly expressed (+), strongly repressed (–), or neither strongly expressed nor repressed (0) underneath the name of each gene.

Patient 1 Profile

	1	2	3	4	5	6
A	ESR1	ABC-C6	BCL2	DPYD	TOP2A	GSTP1
B	DHFR	EGFR	ERB-B2	ABC-B2	MT1	TGFB3

Patient 2 Profile

	1	2	3	4	5	6
A	ESR1	ABC-C6	BCL2	DPYD	TOP2A	GSTP1
B	DHFR	EGFR	ERB-B2	ABC-B2	MT1	TGFB3

Key

+ = strongly expressed (dark pink)
– = strongly repressed (light pink)
0 = neither strongly expressed nor repressed (clear)

Cancer Therapies

Cyclophosphamide

Brand Names: Cytoxan, Neosar

What it is: chemotherapy drug

How it works: Cyclophosphamide acts by transferring one or more saturated carbon atoms to cellular macromolecules. This damages the cancer cell DNA, and slows or stops the growth of the cancer cells.

Do not use if one or more is true: ABC-B2 = +
GSTP1 = +
MT1 = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Doxorubicin

Brand Names: Adriamycin, Rubex

What it is: chemotherapy drug

How it works: Doxorubicin inhibits RNA synthesis and causes DNA strand breakage. This slows or stops the growth of the cancer cells.

Do not use if one or more is true: EGFR = +
ABC-C6 = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Fluorouracil (5-FU)

Brand Name: Adrucil

What it is: chemotherapy drug

How it works: Fluorouracil binds with and deactivates a key enzyme (thymidylate synthetase) in thymidine biosynthesis. This slows or stops the growth of the cancer cells.

Do not use if one or more is true: EGFR = +
BCL2 = +
DPYD = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Methotrexate

Brand Names: Mexate, Folex

What it is: chemotherapy drug

How it works: Methotrexate binds to and inactivates the enzyme dihydrofolate reductase (DHFR), and inhibits the synthesis of purine and pyrimidine. This prevents the growth of cancer cells.

Do not use if one or more is true: BCL2 = +
DHFR = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Paclitaxel

Brand Name: Taxol

What it is: chemotherapy drug

How it works: Paclitaxel binds to tubulin and blocks cell division. This slows or stops the growth of cancer cells.

Do not use if one or more is true: BCL2 = +
ERB-B2 = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Tamoxifen

Brand Name: Nolvadex

What it is: hormone (antiestrogen)

How it works: Tamoxifen binds to the estrogen receptor, preventing cell growth. It also affects the cycling of the cell in the natural cell cycle.

Do not use if one or more is true: ESR1 = 0 or –
ERB-B2 = +

Safe to use for: **Patient 1** yes no **Patient 2** yes no

Trastuzumab

Brand Name: Herceptin

What it is: monoclonal antibody

How it works: Herceptin binds to the ERB-B2 growth factor receptor and prevents the cell from dividing.

Do not use if one or more is true: ERB-B2 = 0 or –

Safe to use for: **Patient 1** yes no **Patient 2** yes no