Using Microarrays to Study Leukemia – teacher instructions/answers

Introduction
This activity is divided up into many pieces. The introduction piece is the background information on leukemia necessary to do any of the additional activities.

Diagnosis of ALL vs AML using microarrays
An easy activity that high school students of all levels should be able to do
Can be done alone without the other pieces

ALL Subtypes
This activity is harder. However, solving the activity is dependent more on pattern recognition than on science knowledge

Microarray data to identify the type of cancer cell.
This is an advanced activity.
If you do this activity without doing the activity ALL Subtypes, you will need to give the students the answers from that activity.

Identification of possible treatments
This is an advanced activity.
If you do this activity without doing the activity ALL Subtypes, you will need to give the students the answers from that activity.

Diagnosis ALL vs AML using microarrays

The activity
A printable teachers guide and student activity can be found at http://science-education.nih.gov/newsnapsshots/TOC_Chips/Chips_RITN/chips_ritn.html

The name of the package of activities is “DNA Chips: A Genetics Lab in the Palm of Your Hand”
Note – the exact URL for this activity changes frequently, so the provided address may be outdated. You can use the title to search for the activity.

The activity package also contains information on how microarrays work.
How microarrays can be used to sequence DNA
And some ethical questions related to identification by DNA fingerprinting

Answers are found on page 6 of the activity packet.
**ALL Subtypes**
In this activity, you will explore whether gene expression patterns can be used to identify different subtypes of ALL. You will begin this portion of the activity by looking at microarray results from 12 different patients.

**Questions**
1. Which patients are found in each of your two groups (assume patient 1 is in group 1)?
   - **Group 1**: 1, 2, 6, 9, 10, 12
   - **Group 2**: 3, 4, 5, 7, 8, 11

2. Next, in general which genes are overexpressed in group 1 and underexpressed in group 2?
   - **Overexpressed in group 1**: APP, CD22, CD24, CD79B, DNTT, Lig4, MME
   - **Underexpressed in group 1**: CCNA1, CD44, FLT3, HOXA9, LGALS1, PROML1, SPN

**Two subtypes of ALL**
The markers expressed on the surface of lymphoid and B-cell precursors are described and outlined below (Table 1 and Figure 2)

![Diagram of cell lineage]

**Figure 2**: Marker expression in various B-cell precursors.
Table 1: Markers expressed in ALL vs MLL

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Cells that normally express this marker</th>
<th>Function (if known)</th>
<th>Expression in ALL and MLL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD19</strong></td>
<td>B-cell specific, indicates a cell that will become a B-cell</td>
<td>B-cell development?</td>
<td>CD19 is expressed in equal amounts on cells from both ALL and MLL patients.</td>
</tr>
<tr>
<td><strong>CD24</strong></td>
<td>B-cells and myeloid cells. Expression of CD24 increases as B-cells mature.</td>
<td>Its function may be to help activate T cells.</td>
<td>Higher in ALL</td>
</tr>
<tr>
<td><strong>CD44</strong></td>
<td>WBCs and RBCs. In B-cells, its expression decreases as B-cells mature.</td>
<td>Receptor for extracellular matrix components</td>
<td>Higher in MLL</td>
</tr>
<tr>
<td><strong>CD79B</strong></td>
<td>Found on the surface of B-cells and expression increases as B-cells mature. Presence of this marker indicates commitment to the B-cell lineage.</td>
<td>This marker is an antibody. It functions as the antigen receptor for the B-cells and determines the specificity of the antigen that will be recognized by the B-cell.</td>
<td>Higher in ALL</td>
</tr>
<tr>
<td><strong>SPN</strong></td>
<td>Expression of CD43 decreases as B-cells mature</td>
<td></td>
<td>Higher in MLL</td>
</tr>
</tbody>
</table>

Questions
1. See table

2. Cells from patients with ALL are arrested at the Pre B-Cell stage. Looking at the gene expression profile data from patients with ALL and MLL, at which stage do you think cells from MLL are stopped? (Refer to figure 2 as necessary).

*MLL cells are stopped earlier than ALL cells at the pro B cell stage.*

3. Explain your answer by describing the levels of expression of the different markers.

*You know MLL are stopped earlier due to the higher expression of CD44 and SPN and the lower expression of CD24 and CD79B.*
You know MLL are not in the lymphoid precursor stage because they express CD19 (upper right hand box of table 1) which is not expressed on lymphoid precursor cells (figure 1)

Potential Drug Targets for Treatment of MLL
Your next goal will be to use the gene expression profiles you generated to identify potential drug targets for treatment of MLL. For this it will be useful to know some other specific drugs used to treat different types of leukemia.

- Chronic myelogenous leukemia (CML) – This type of leukemia is caused by a mutation that leads to production of a new tyrosine kinase. A chemotherapeutic agent called Gleevec is a new and exciting treatment for CML. Gleevec is a tyrosine kinase inhibitor designed specifically to inhibit the tyrosine kinase created by the CML mutation.
- Acute promyelocytic leukemia (APL) – This type of leukemia has a specific mutation that results in expression of a retinoic acid receptor. APL is treated with retinoic acid (in conjunction with other treatments). Retinoic acid acts by causing the cancerous cells to rapidly divide, differentiate, and then undergo apoptosis.

Answer the following questions about each of the three proteins.

**CCNA-1**
What are other names for CCNA1?

*Cylin A1*

What is the function of CCNA1 (what does it do)?

*Regulator of CDK kinases and plays a role in cell cycle control
Expressed in testis and brain and leukemia cell lines*

What diseases are associated with mutations in the CCNA1 gene?

*Disorders in spermatogenesis*

Does CCNA1 have anything in common with other drug targets that have been successfully used to treat other types of leukemias?

*Nothing obvious*

Do you think CCNA1 is a good molecular target for treating MLL?

*It could potentially be a target, but not high on the list*

**FLT3**
What are other names for FLT3?
Fms related tyrosine kinase, FLK2, STK1, CD135

What is the function of FLT3 (what does it do)?

Marker for hematopoetic stem cell differentiation
Tyrosine kinase

What diseases are associated with mutations in the FLT3 gene?

leukemia

Does FLT3 have anything in common with other drug targets that have been successfully used to treat other types of leukemias?

Yes, Gleevec is a drug that targets a tyrosine kinase found in CML

Do you think FLT3 is a good molecular target for treating MLL?

Yes

PROML1
What are other names for PROML1?

Prominin 1, CD133, AC133

What is the function of PROML1 (what does it do)?

Transmembrane glycoprotein

What diseases are associated with mutations in the PROML1 gene?

Autosomal recessive retinal degeneration

Does PROML1 have anything in common with other drug targets that have been successfully used to treat other types of leukemias?

No

Do you think your PROML1 is a good molecular target for treating MLL?

Not really

Summary
In this activity you explored how microarray technology can rapidly allow researchers to identify gene expression profiles of diseases. These gene expression
profiles allow researchers to develop diagnostic tests and treatments, as well as leading to a better understanding of the disease.

In this case, the disease identified was a subtype of ALL that was previously difficult to identify, and which did not respond to typical treatment for ALL. Because ALL is rapidly fatal, it is critical to quickly and correctly diagnose ALL so that patients can receive appropriate treatment and increase their chances of survival. With the advent of microarray technology researchers can now see all the genes that are differentially expressed allowing them to design an accurate test for this previously difficult to identify subtype of ALL.

The gene expression profile also provides a better understanding of the disease process and most importantly, more targets for development of chemotherapeutic agents for a previously unresponsive type of leukemia. Researchers have already tested a FLT3 inhibitor and found it active against MLL cells in a Petri dish. Thus, this inhibitor holds promise for treatment of MLL.