Discovering Small Molecule Modulators of Apoptotic Gene Expression

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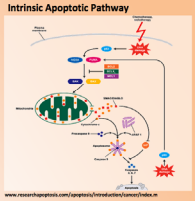
INTRODUCTION

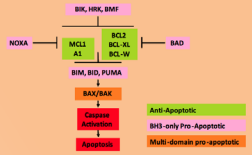
APOPTOSIS

Apoptosis is a morphologically distinct form of programmed cell death that is essential for normal development and tissue homeostasis in multicellular organisms. Studies indicate that defects in apoptosis can play a major part in tumor development and drug resistance. By targeting the apoptosis machinery with small molecules that modulate this pathway, we may have the potential to treat many types of cancer.

Intrinsic Apoptotic Pathway



BCL2 Protein Family



THE BCL2 PROTEIN FAMILY

The BCL2 family of proteins regulates a critical control point in apoptosis. BCL2 family members are located upstream in the apoptotic pathway of the point after which the cell death decision is irreversible. Family members can be either pro- or anti-apoptotic. Pro-apoptotic members of this family activate a cascade of events in the cell and lead to caspase activation, a "point of no return" in the apoptosis process. Anti-apoptotic proteins regulate this process by binding to pro-apoptotic proteins and inhibiting them.

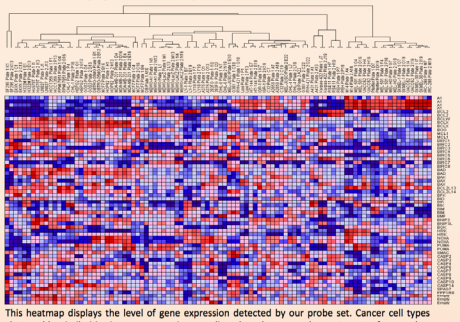
THE BCL2 FAMILY MEMBER A1

A1, also known as BCL2A1 or BFL-1, is an anti apoptotic protein that is preferentially expressed in normal hematopoietic and endothelial cells, and certain types of cancer. For example, A1 was found to have high levels of expression in a certain subgroup of Diffuse Large B-cell Lymphoma samples. High expression levels of A1 suggest the cell's dependency on A1 for survival, and the contribution to an apoptosis resistant phenotype. Despite the increased awareness of the role of A1 in cancer, no direct inhibitor for A1 has been developed.

Our goal is to discover small molecule inhibitors of A1 that may lead to the development of a targeted cancer therapy.

RESULTS

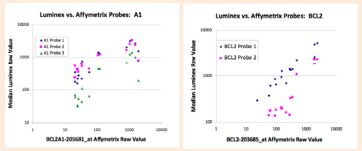
Apoptotic Gene Expression in 50 Cancer Cell Lines



This heatmap displays the level of gene expression detected by our probe set. Cancer cell types clustered by similarities in gene expression are aligned on the top, and gene targets of our probes line the right hand side. Note the consistency across different probes and sample batches.

PROBE VALIDATION

Experimental Goal: To validate our gene-specific probe set by comparing Luminex results to Affymetrix data



Median values from the Luminex assay (samples were run in triplicate) were compared to raw Affymetrix data across 15 cell lines.

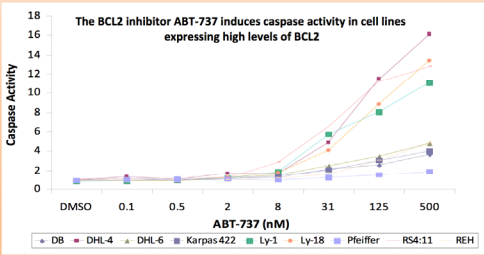
Conclusions:

- Most probes were determined to accurately measure gene expression, including A1 and BCL2. However, a few other probes will need improvement.

Future Directions:

- Redesign suboptimal probes and continue validation.
- Design probes for more genes of interest.

PHARMACOGENOMICS



Cell lines expressing high levels of BCL2 were selected from the above heat map and tested in a separate assay for sensitivity to ABT-737, a BCL2 inhibitor. Caspase activity is a measure of apoptosis.

Experimental Goal: To test the BCL2 inhibitor, ABT 737, against potential BCL2 dependent cell lines whose profiles were identified in the above heatmap.

Conclusions:

- Expression patterns of apoptotic genes are unique to each cell line.
- ABT 737 is able to induce caspase activity in cell lines that have high levels of BCL2 expression.
- ABT-737 can be used as a negative control in the A1 target assay.

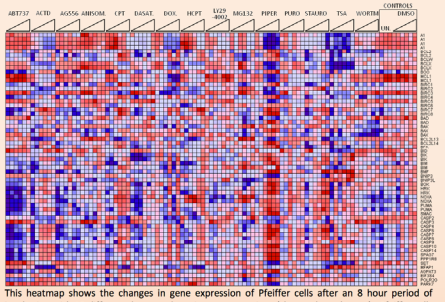
Future Directions:

- Profile patient samples and more cell lines, to ascertain genetic similarities and differences across cancer types.
- Test connections between over expression of apoptotic genes and drug sensitivity.

TARGETING A1 WITH SMALL MOLECULES

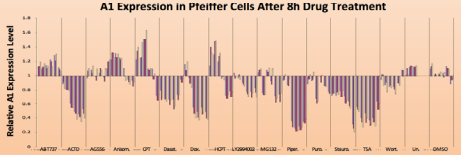
Experimental Goal: Identify small molecules that specifically target A1.

Apoptotic Gene Expression in Pfeiffer Cells Treated with Small Molecules



This heatmap shows the changes in gene expression of Pfeiffer cells after an 8 hour period of incubation with 15 different drugs, and varying concentrations (shown along the top). Pfeiffer cells were chosen for their high levels of A1 expression.

A1 Expression in Pfeiffer Cells After 8h Drug Treatment



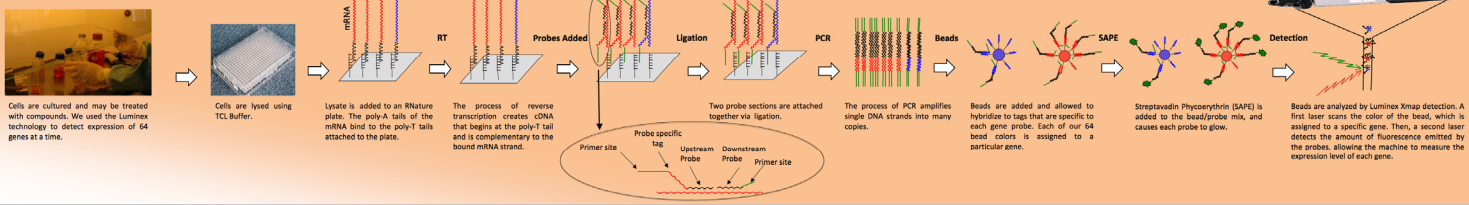
Conclusions:

- The Pfeiffer cell line expresses high levels of A1 and is a good model for discovering small molecules that target A1. Preliminary results indicate that molecules can be identified that decrease levels of A1 expression.

Future Directions:

- Screen for A1 inhibitors using Broad compound libraries.
- Perform follow up caspase assays using hits from this screen on Pfeiffer cells and other A1 dependent cell lines.

METHODS: THE LUMINEX PROCESS



Cells are cultured and may be treated with compounds. We used the Luminex technology to detect expression of 64 genes at a time.

Cells are lysed using TCL Buffer.

Lysate is added to an RNature plate. The poly-A tails of the mRNA bind to the poly-T tails attached to the plate.

The process of reverse transcription creates cDNA that begins at the poly-T tail and is complementary to the bound mRNA strand.

Two probe sections are attached together via ligation.

The process of PCR amplifies single DNA strands into many copies.

Beads are added and allowed to hybridize to tags that are specific to each gene probe. Each of our 64 bead colors is assigned to a particular gene.

Streptavadin Phycoerythrin (SAPE) is added to the bead/probe mix, and causes each probe to glow.

Beads are analyzed by Luminex Xmap detection. A first laser scans the color of the bead, which is assigned to a specific gene. Then, a second laser detects the amount of fluorescence emitted by the probes, allowing the machine to measure the expression level of each gene.

REFERENCES

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