Using LINCS to Identify Novel Drugs for Alcohol Dependence Treatment

Byte Club Meeting
Thursday, June 16

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Microprocessor Transistor Counts 1971-2011 & Moore's Law

The curve shows transistor count doubling every two years.
EROOM’S LAW

The efficiency of research and development of new drugs in the United States halves every nine years or so. Drug developers sometimes call this Eroom’s law — Moore’s law for microprocessors in reverse. Repositioning drugs could help to counter this decline.

FDA tightens regulation post-thalidomide

FDA clears backlog in response to new federal law; also approves several HIV drugs

First wave of biotechnology-derived therapies

Drug discovery 3–6 years  Preclinical testing 3 years  Phase I Phase II 3 years  Phase III 2 years  FDA approval 1–2 years

12–16 years, ~$1 billion to $2 billion

A SHORTER TIMESCALE

Because most repositioned drugs have already passed the early phases of development and clinical testing, they can potentially win approval in less than half the time and at one-quarter of the cost.

Drug repositioning ~6 years, ~$300 million
Can we use genomics to predict drugs that will correct complex disorders (like alcoholism)?


Used gene expression data from IBD patient intestine biopsies to predict drugs to treat IBD– found topiramate which was validated in vivo.

Liu et al., 2015. *Treatment of obesity with celestrol*. *Cell*

Used gene expression data to predict celestrol, which led to a 45% weight loss in diet-induced obese (DIO) mice.
LINCS Dataset

- 9-78 cell types
- 5 neuronal cell types

Chemical & Genetic Perturbations

- Gene over expression:
  - > 3,000 ORFs
- Gene knock down:
  - > 12,000 x3 constructs

- Chemical compounds:
  - > 19,000 small molecules

Includes many FDA-approved Drugs (e.g., naltrexone)
1. Generate a “disease signature,” i.e., a list of genes that have increased or decreased expression in human alcoholics or animal model vs controls.
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2. Identify compounds with signatures that have a strong connectivity to the disease signature.

3. Test the compounds in preclinical models.

Identifying a compound to treat alcoholism

Prioritizing compounds

Disease signature

The Connectivity Map: Using Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease

Justin Lamb, (Eric Lander, Todd Golub), et al.

Harvard/MIT/Broad

Kolmogorov Smirnov non-parametric rank statistic

http://www.broadinstitute.org/cmap/help_topics_linkified.jsp#how connectivity score is calculated
Current Algorithm

Even though the up score is very high
Score = 0
Because the up and down genes are being changed in the same direction overall
Better Algorithm?

Take max between up and down score
2 ways to query LINCS

Query using the web app
http://apps.lincscloud.org/query

Query using C3 – Compute Connectivity on the Cloud
<table>
<thead>
<tr>
<th>Question</th>
<th>Summly (not corrected)</th>
<th>Gutc (corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Which experiments are used for analyses</td>
<td>perturbagens that give reproducible signatures</td>
<td>well-characterized perturbagens that give reproducible signatures ('touchstone' set)</td>
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<tr>
<td>Whether scores include correction</td>
<td>None</td>
<td>provides a measure of how unusual the connectivity score for a given drug is, relative to that drug’s score across all cell lines, times and doses</td>
</tr>
<tr>
<td>How scores are summarized and reported</td>
<td>summarizes results in the 2, 4, and 6 cell lines in which the connections have strongest magnitude</td>
<td>uses the max quantile summarization method, so it produces just a single score</td>
</tr>
<tr>
<td>Parameter</td>
<td>Options</td>
<td></td>
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<tr>
<td>---------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Input gene signature</td>
<td>• All DE genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Top 100 DE genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• L1000 landmark genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• DE genes within modules or pathways of interest</td>
<td></td>
</tr>
<tr>
<td>Genes in LINCS database</td>
<td>• L1000 landmark genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• “bing” (best inferred) genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• All</td>
<td></td>
</tr>
<tr>
<td>Cell types</td>
<td>• Brain cell types</td>
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<tr>
<td></td>
<td>• All</td>
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<tr>
<td>Experiments</td>
<td>• Touchstone</td>
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<tr>
<td></td>
<td>• Gold</td>
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<td></td>
<td>• All</td>
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</tr>
<tr>
<td></td>
<td>• (“touchstone” and “gold” are subsets of experiments that the BROAD has determined are the most reproducible)</td>
<td></td>
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</tbody>
</table>
LINCS API

http://api.lincscloud.org/

Lots of meta-data – you could answer a lot of interesting questions just by querying the LINCS meta-data!

Query examples:
• Lookup expression of a gene across core cell lines

/a2/geneinfo?q={"pr_gene_symbol":"OPRM1"}&l=1

• Return meta-information for terreic-acid

/a2/pertinfo?q={"pert_iname":"terreic-acid"}

• Return only the specified fields – for a drug get signature cell ids, signature strength, and top regulated genes

/a2/siginfo?q={"pert_desc":"terreic-acid"}&f="cell_id":1,"distil_ss":1, "up50_lm":1, "dn50_lm":1}
Identifying a compound to treat alcoholism

1. Generate a "disease signature," i.e., a list of genes that have increased or decreased expression in human alcoholics or animal models vs controls

2. Identify compounds with signatures that have a strong connection to the disease signature

3. Test the compounds in preclinical model

- Existing FDA approval / clinical trial
- Cross BBB – brain access
- Acceptable side effects
- Predicted to be effective in multiple brain areas or across species

Prioritize compounds

Test candidates in vivo
Proof-of-principle

HDID MICE
Genetic model for “binge-drinking”

- Drinking in the Dark
- Selected for high BEC
Identifying a compound to decrease excessive ethanol consumption in HDID mice

1. Generate a “genomic signature,” i.e., a list of genes that have increased or decreased expression in etoh naïve HDID mice vs controls

2. Identify compounds with signatures that have a strong connectivity to the disease signature

3. Test the compounds in HDID mice
Fortney et al., 2015. **Prioritizing Therapeutics for Lung Cancer: An Integrative Meta-analysis of Cancer Gene Signatures and Chemogenomic Data.** *PLOS Comp Bio*

Liu et al., 2015. **Treatment of obesity with celastrol.** *Cell*
Absolute Product Score

Terreic acid

Individual Drugs

Absolute Product Score (CS)
**Proof-of-principle**

**TERREIC ACID**

- B. terreus strain isolated for its potent bioactivity and contribution to ischemic brain injury. (Kawakami, Y., et al., Proc Natl Acad Sci USA, 1999.)


- TA inhibits the transcription of TNFα and IL-2. (Kawakami, Y., et al., Proc Natl Acad Sci USA, 1999.)


- Compound that would not have been picked by traditional approaches in the neuropharmacology of compound... no known function for Btk in alcoholism....etc.

- Aspergillus terreus
Testing terreic acid in vivo

2 hr EtOH
30 mins prior
2 hr
= Drug/Vehicle Treatment
= BEC sample
N=17, 5-6 per dose
Graphs by John Crabbe and colleagues

* P < 0.05
N=5-6 per dose
Day 4 Saccharin Preference

Note that there was a decrease in overall saccharin consumption that is being investigated...

Graph by John Crabbe and colleagues
Summary

• We used gene expression signatures of HDID mouse brain and gene expression signatures of compounds from the LINCS database to predict drugs that might decrease BALs in HDID mice

• Terreic acid emerged as a top hit from our drug prediction analyses

• When tested in vivo, terreic acid decreased BALs by ~100%
Future Directions

• Use qPCR to determine if genes we predicted to be “corrected” by terreic acid are being “corrected”

• Determine if terreic-acid’s effects on a 2-bottle-choice test (20% EtOH vs H2O)

• Select other drugs for HDID mice using different computational strategies

Conclusion

Our successful POC suggests these algorithms could predict other drugs for alcoholism and other psychiatric illnesses
Thank you!

**People**
- Dr. Adron Harris
- Dr. Dayne Mayfield
- Dr. Igor Ponomarev
- Dr. John Crabbe
- Dr. Angela Ozburn
- Ted Natoli
- Lab mates
- Waggoner Center
- INS

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- INS