CS4220: Knowledge Discovery Methods for Bioinformatics
Unit 7: Pathway Perturbations in a Disease Context

Niranjan Nagarajan
Identifying causal genes for a disease

- Genetic polymorphism and mutation calling
- Basic association analysis
- Causal genes in Cancer
- Challenges in identifying causal genes in Cancer
- Pathways and Integrated Analysis

Genetic Polymorphism and Mutation Calling
Genomics

- DNA – Sequence of As, Cs, Gs and Ts (~3 billion in human genome)
- Reference Genome – Reference sequence for a species
Polymorphism

Vs

Somatic Mutation

Individuals

Heterozygous vs Homozygous

Human genome is Diploid

Cells

Cancer
Classes of Genomic Variations (GVs)

- Single Nucleotide Variations (SNVs)
- Short Insertions and Deletions (Indels) <100bp
- Copy Number Variations (CNVs) >100bp
- Structural Variations (SVs)
Ways to detect GVs

- **Microarrays**
  - Hybridization Based
  - SNP array, CGH array
  - Probes for common polymorphisms, >1 million
  - Distributed “evenly” over the genome

- **Sequencing**
  - Directly “read” DNA sequence
  - Compare to reference genome
  - Or Reconstruct sample genome *de novo*
Affymetrix GeneChip Array

Source: Affymetrix
Using Arrays to calls SNPs

- **SNPs** = Single Nucleotide Polymorphisms i.e. common variant positions with two “alleles”
- **Information**: A allele and B allele intensity
- **Normalize Data**
- **Assign to Cluster**

Image Source: Lamy et al, NAR 2006
Using arrays to call CNVs

- **BAF** = “B Allele Frequency” i.e. normalized measure of relative signal of the B and A alleles
- **RR** = “Log R Ratio” i.e. total intensity (normalized)

Sequencing Technologies

<table>
<thead>
<tr>
<th></th>
<th>Read Length</th>
<th>Time/run</th>
<th>Cost/Mbp</th>
<th>Sequence (Mbp)</th>
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<tbody>
<tr>
<td>Sanger</td>
<td>~700 bp</td>
<td>&lt;1 day</td>
<td>$1000</td>
<td>2</td>
</tr>
<tr>
<td>454</td>
<td>~500 bp</td>
<td>&lt;1 day</td>
<td>&lt;$100</td>
<td>~500</td>
</tr>
<tr>
<td>Illumina</td>
<td>~ 100 bp</td>
<td>~1 week</td>
<td>&lt;$3</td>
<td>&gt;100,000</td>
</tr>
<tr>
<td>SOLiD</td>
<td>~ 50 bp</td>
<td>~1 week</td>
<td>&lt;$5</td>
<td>&gt;100,000</td>
</tr>
</tbody>
</table>

Sanger Sequencing (1977)  
Gel based, Chain Termination

454 Sequencing (2004)  
Chip, Pyrosequencing

Reversible Terminator, Ligation, Nanopores, …
State of the art in Sequencing

http://www.youtube.com/watch?v=v8p4ph2MAvI
Workflow for calling SNVs

1. Read Mapping
2. Date Cleaning - Read Alignment, Removing Duplicates, Recalibration
3. SNV Calling

Refer to the diagram for the flowchart.
SNV Calling (General Idea)

Bayes Rule: Genotype = AA|AB|BB

\[
P(\text{Genotype} | \text{Data}) = P(\text{Data} | \text{Genotype}) P(\text{Genotype})
\]

Maximize Posterior  
Likelihood  
Prior

Base Quality \rightarrow Probability of sequencing error

Q_{20} \rightarrow \text{prob. of error} = 10^{(-20/10)} = 10^{-2} = 0.01

Q_{30} \rightarrow \text{prob. of error} = ??

Prior = SNV every \sim1000 bases for humans
Rare SNV calling

• What if the variant has allele frequency $<< 0.5$?

If average error-rate is 1% can variants at 1% frequency be discovered?
Ruling out Sequencing Error

• Null Hypothesis – variant bases are from sequencing errors

• Null Model
  – If all base qualities are the same?
  – With different base qualities?
    • Binomial(n, p) -\(\rightarrow\) Poisson-Binomial(n, \(<p_1, \ldots, p_n>\))

• \(P\)-value = probability \(k\) variant bases or more under null model

\[
P_n(X=k) = P_{n-1}(X=k)(1-p_n) + P_{n-1}(X=k-1)p_n
\]

Sensitivity is only limited by Quality and Coverage
Performance improves for high frequency SNVs as well

Real data, mixed *in silico*
SNVer and Breseq do not fully exploit base quality values
Rare variants can be experimentally validated

Fluidigm Digital PCR

LoFreq – 9/9
Breseq – 7/9
SNVer – 2/9
Calling Somatic Mutations

- Ad hoc
- Joint
- Rare mutations

Cancer

Normal

\[ \text{A} = \frac{7}{10}, \text{C} = \frac{1}{10} \]

\[ \text{A} = \frac{7}{10}, \text{C} = \frac{3}{10} \]
Ad hoc

1. Call SNVs in Cancer
2. Call SNVs in Normal
3. Filter Cancer list using Normal list
4. Remove SNVs where Normal has >1 base of that kind

Software: JointSNVMix

- Hypothesis: simultaneous analysis will result in better detection of shared signals (SNPs or technical noise) and weak signals for somatic mutations

<table>
<thead>
<tr>
<th>$g_N \backslash g_T$</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>AA</td>
<td>Wild-type</td>
<td>Somatic</td>
</tr>
<tr>
<td>AB</td>
<td>LOH</td>
<td>Germline</td>
<td>LOH</td>
</tr>
<tr>
<td>BB</td>
<td>Error</td>
<td>Error</td>
<td>Germline</td>
</tr>
</tbody>
</table>

LOH = Loss of Heterozygosity
Joint SNVmix Model

Model parameters and latent variables trained using EM

## Results

<table>
<thead>
<tr>
<th>Caller</th>
<th>TP</th>
<th>FP</th>
<th>TN</th>
<th>FN</th>
<th>F-meas</th>
<th>MCC</th>
<th>FP Germlines</th>
<th>FP Wild-types</th>
</tr>
</thead>
<tbody>
<tr>
<td>JointSNVMix1 (Trained)</td>
<td>140</td>
<td>13</td>
<td>999788</td>
<td>59</td>
<td>0.795</td>
<td>0.802</td>
<td>8</td>
<td>2</td>
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<tr>
<td>JointSNVMix1</td>
<td>153</td>
<td>50</td>
<td>999751</td>
<td>46</td>
<td>0.761</td>
<td>0.761</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>SNVMix1 (Trained)</td>
<td>190</td>
<td>823</td>
<td>998978</td>
<td>9</td>
<td>0.314</td>
<td>0.423</td>
<td>743</td>
<td>70</td>
</tr>
<tr>
<td>SNVMix1</td>
<td>178</td>
<td>1653</td>
<td>998148</td>
<td>21</td>
<td>0.175</td>
<td>0.295</td>
<td>1632</td>
<td>0</td>
</tr>
</tbody>
</table>
1. Cancers can have a heterogeneous mixture of cells

2. Sample might also have normal cells

=> Mutations need not have 50% frequency if they are heterozygous
MuTect Algorithm

1. Call SNVs in Cancer aggressively
2. Filter artifacts
3. Filter potential germline SNVs aggressively

\[ L(M^m_f) = \text{Likelihood of having a mutation at frequency } f \]
\[ L(M_0) = \text{Same as above with } f=0 \text{ i.e. no mutation} \]
\[ P(m,f) = \text{Probability of a mutation} \]
• **Call SNVs in Cancer aggressively**

\[
L(M^m_f) = P(\{b_i\} | \{e_i\}, r, m, f) = \prod_{i=1}^{d} P(b_i | e_i, r, m, f)
\]

\[
P(b_i | e_i, r, m, f) = \begin{cases} 
  f^{e_i/3} + (1-f)(1-e_i) & \text{if } b_i = r \\
  f(1-e_i) + (1-f)^{e_i/3} & \text{if } b_i = m \\
  e_i/3 & \text{otherwise}
\end{cases}
\]

Really checking if \( \log(\frac{L(M^m_f)}{L(M_0)}) \geq 6.3 \)

• **Filter potential germline SNVs aggressively**

Remove positions with bases in normal having \( \geq 2 \) observations of the alternate allele or \( \geq 3\% \) of the reads and sum of their quality scores being \( > 20 \)
High Sensitivity

Low False Positive Rate?
Comparison to other Methods

- MuTect is more sensitive for rare mutations
Summary

• GVs vary in size and impact on the genome

• Microarrays and Sequencing can be used to detect GVs with corresponding tradeoffs

• Model-based approaches are extremely effective at calling SNPs and somatic mutations from sequencing data

• Rare somatic mutations can be called without sacrificing precision
Must Read


Good to Read


Basic Association Analysis
Basic Idea behind Association Analysis

Odds Ratio = \( \frac{\text{Frequency in Case}}{\text{Frequency in Control}} \) (e.g. \( \frac{52.6}{44.6} = 1.17 \))

Statistical Test

\( \chi^2 \) test (1-degree of freedom)

Correct for multiple-hypothesis testing
Genome-wide Association Study (GWAS)

WTCCC, Nature 2007

Manhattan Plot

Nitric oxide

K+ Channel Q

Na+ Channel

K+ Channel H

K+ Channel E
**Search studies using keywords**

Enter study ID, authors, pubmed ID, other terms

- **p-value threshold**
- **Markers per Study**

- e.g. HGVST107, Todd JA, 21738484, metabolic

---

**1,008 Studies in the database containing data with -log p ≥ 0 (showing 1:20)**

Pages: 1 2 3 4 5 6 7 8 9 10 >>

No. per page: 20

Export these results as:

Related data:

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<th>Name</th>
<th>Phenotype(s)</th>
<th>Total p-values</th>
<th>Related citations</th>
<th>Add data sets to Browser</th>
<th>Related data</th>
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<tbody>
<tr>
<td>HGVST1012</td>
<td>GWAS of bipolar disorder</td>
<td>Bipolar disorder (dominant)</td>
<td>11</td>
<td>Jiang Y et al. Hindorff LA et al.</td>
<td><img src="link" alt="Add" /></td>
<td>Results Chromosomes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bipolar disorder (recessive)</td>
<td></td>
<td></td>
<td><img src="link" alt="Add" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bipolar disorder (additive)</td>
<td></td>
<td></td>
<td><img src="link" alt="Add" /></td>
<td></td>
</tr>
<tr>
<td>HGVST1013</td>
<td>GWAS of prion diseases</td>
<td>Prion diseases</td>
<td>11</td>
<td>Mead S et al. Hindorff LA et al.</td>
<td><img src="link" alt="Add" /></td>
<td>Results Chromosomes</td>
</tr>
<tr>
<td>HGVST1014</td>
<td>GWAS of atopic dermatitis</td>
<td>Atopic dermatitis</td>
<td>6</td>
<td>Paternoster L et al. Hindorff LA et al.</td>
<td><img src="link" alt="Add" /></td>
<td>Results Chromosomes</td>
</tr>
<tr>
<td>HGVST1003</td>
<td>GWAS of ankle-brachial index</td>
<td>Ankle-brachial index</td>
<td>2</td>
<td>Murabito JM et al. Hindorff LA et al.</td>
<td><img src="link" alt="Add" /></td>
<td>Results Chromosomes</td>
</tr>
<tr>
<td>HGVST1004</td>
<td>GWAS of mean platelet volume</td>
<td>Platelet counts</td>
<td>36</td>
<td>Gieger C et al. Hindorff LA et al.</td>
<td><img src="link" alt="Add" /></td>
<td>Results Chromosomes</td>
</tr>
<tr>
<td>HGVST1005</td>
<td>GWAS of IgA nephropathy</td>
<td>IgA nephropathy</td>
<td>9</td>
<td>Yu XQ et al. Hindorff LA et al.</td>
<td><img src="link" alt="Add" /></td>
<td>Results Chromosomes</td>
</tr>
</tbody>
</table>

>1000 studies!
Challenges

1. What if the SNVs are not “common”? 
2. What if the association is not to a SNV? 
3. What if the impact of the SNV (“effect size”, odds ratio) is small? 
4. Are the controls appropriate?
Frequency and Effect Size

- SNP is not on array?
  - Typically in “linkage” with a SNP that is
Are the controls appropriate?

Do cases and controls have some obvious differences that could explain things?

Alternately: Is the SNP associated with other confounding factors?

- Sex
- Age
- Geographical or historical populations (Population Stratification)
Genes mirror Geography

Software: Eigenstrat

Based on PCA

1. Properties?
2. Adjust for variation
   \[ g_{ij} \leftarrow g_{ij} - \gamma_i a_j \]
   \[ \gamma_i = \sum_j a_j g_{ij} \]
3. Do association analysis with adjusted data

Example for Genotype Adjustment with Eigenstrat

- Suppose principal component 1 (PC1) is perfectly correlated with $g_i$ s.t. $g_{ij} = 0$ if $a_j = 0.1$ and $g_{ij} = 1$ if $a_j = -0.1$

- Let $a_j = 0.1$ for 50 out of 100 samples and -0.1 otherwise

- Then $\gamma_i = 50 \times 0.1 \times 0 + 50 \times -0.1 \times 1 = -5$

- For $a_j = 0.1$, adjusted $g_{ij} = 0 - 0.1 \times -5 = 0.5$

- For $a_j = -0.1$, adjusted $g_{ij} = 1 - (-0.1 \times -5) = 0.5$

Thus the impact of ancestry is cancelled out from the genotypes values!
Pathways and GWAS

- Same idea as expression analysis
  - Pathways can help identify meaningful collections of genes

- [Wang et al 2007] Modified GSEA algorithm based on using $\chi^2$ scores with a Kolmogorov-Smirnov statistic

Table 1. The Most-Significant Gene Sets or Pathways in the Fung et al.12 GWA Study of PD Identified by a Modified GSEA Algorithm

<table>
<thead>
<tr>
<th>Gene Set or Pathway</th>
<th>Rank of Most Significant</th>
<th>Nominal P</th>
<th>FDR</th>
<th>FWER P</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-glycotransferase activity</td>
<td>89</td>
<td>59 41</td>
<td>&lt;.001</td>
<td>.006</td>
</tr>
<tr>
<td>O-glycan biosynthesis</td>
<td>23</td>
<td>69 49</td>
<td>&lt;.001</td>
<td>.011</td>
</tr>
</tbody>
</table>

NOTE.—Two pathways (database identifiers: GO accession number 0008194 and KEGG accession number hsa00512) demonstrate statistical significance after adjustment for multiple testing, but the SNPs and genes within these pathways cannot be detected by the most-significant SNPs/genes approach.

PD = Parkinson’s Disease
Good To Read


Glossary

Allele – An alternative form of the gene
Diploid – Carries 2 copies of each chromosome
Germline – In cells that can give rise to offspring
Heterozygous – Alleles are different
Homozygous – Alleles are same
Mutation – Change in nucleotide sequence
Polymorphism – Common variant of a gene
SNV – Single-nucleotide Variant
Somatic – Not in germline cells
Variant – Differs from the reference genome
Causal Genes in Cancer
Cancer is not a Single Disease

- Classification
  - Typically by the type of cells and the presumed origin of the cancer
    - Lung (small-cell, non-small-cell)
    - Breast (ductal, lobular)
    - Leukemia (acute, chronic, lymphoblastic, mylogenous)
  - Perturbed Pathways

- Staging
  - I, II, III, IV …
Scale of Genomic Changes in Cancer

- >10,000 point mutations and indels
- 100s of CNVs
- Merging, splitting of chromosomes

Image Source: Nagarajan et al, Genome Biology (2012)
Oncogenes and Tumor Suppressor Genes

**Oncogenes** – potential to cause cancer when “activated” (e.g. WNT, MYC, RAS)

**Tumor Suppressor Genes (TSGs)** – “protects” a cell from cancer s.t. inactivation leads to cancer (e.g. TP53, PTEN, APC)

Image Credit: www.cancer.gov
Many different normal processes are hijacked and altered in Cancer

Complex Interactions

Image Source: KEGG
Experimental Approaches

1. **Transfect** a gene into over-express it
2. **Knock** a gene **down**

**Drawbacks**
- Artificial cell-line specific information
- Time-consuming

Image Source: www.genegnews.com
## Frequently Mutated Genes (Gastric Cancer)

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Gene Name</th>
<th>Length</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>cellular tumor antigen p53 isoform b</td>
<td>1182</td>
<td>50%</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatidylinositol-3,4,5-trisphosphate</td>
<td>1212</td>
<td>18%</td>
</tr>
<tr>
<td>AQP7</td>
<td>aquaporin-7</td>
<td>1029</td>
<td>10%</td>
</tr>
<tr>
<td>ACVR2A</td>
<td>activin receptor type-2A precursor</td>
<td>1542</td>
<td>10%</td>
</tr>
<tr>
<td>STAU2</td>
<td>double-stranded RNA-binding protein Staufen</td>
<td>1713</td>
<td>10%</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>catenin beta-1</td>
<td>2346</td>
<td>10%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>phosphatidylinositol-4,5-bisphosphate 3-kinase</td>
<td>3207</td>
<td>13%</td>
</tr>
<tr>
<td>TTK</td>
<td>dual specificity protein kinase TTK isoform 1</td>
<td>2574</td>
<td>10%</td>
</tr>
<tr>
<td>COPB2</td>
<td>coatomer subunit beta'</td>
<td>2721</td>
<td>10%</td>
</tr>
<tr>
<td>DHX36</td>
<td>probable ATP dependent RNA helicase DHX36</td>
<td>3027</td>
<td>10%</td>
</tr>
</tbody>
</table>

Nagarajan et al, Genome Biology (2012)
Challenges in Identifying Driver Genes in Cancer
Mutations are not Unbiased

- Mutations by different “mutagens” have different biases
- For e.g. in Gastric Cancer, C>T mutations are common in genes …
- … and specifically in CpG, GpC motifs

Nagarajan et al, Genome Biology (2012)
Cancer Subtypes

- Different drivers for each subtype
- Expression clustering to define subtypes

Breast Cancer

Patient-specific Drivers?

• Every patient has a unique complement of mutations

• Even a single tumor may have several different sub-populations …
Integrated Analysis
What have we learned?

• Cancers are heterogeneous in terms of mechanism of origin

• Driver changes often hide in a sea of “passenger” mutations

• Frequently mutated genes can provide hints for potential drivers

• Integration of genomic and transcriptional information is needed
Pathways and Integrated Analysis
Integration Approaches

1. Mutations in Network (HOTNET)
2. CNVs + Expression (CONEXIC)
3. Mutations/CNVs + Expression in Network (DriverNet)
4. Patient-specific drivers (OncoIMPACT)
HOTNET Idea

Find sub-networks that are frequently mutated

• Hubs affect connectivity of graph

• Compute “influence” between all pairs of nodes
  – A influences B if there are few and short paths between them
  – Modelled as a “diffusion process”

Finding a Sub-Network

Method I (Combinatorial)
  i. Threshold on Influence
  ii. Find sub-network of size K that maximizes # of mutated samples

Method II (Enhanced Influence)
  i. Weight edges by Influence and # of mutated samples
  ii. Threshold on weight and reported connected components
Statistical Significance

Null Model: Permute mutations or gene labels

Statistical Testing:

Problem - Large search-space of sub-networks \( \Rightarrow \)
Correction for multiple hypothesis testing might be too stringent

Solution - Two-step procedure

Step I: What is probability of getting \( \geq r \) sub-networks of size \( K \) by chance?

Step II: If all \( r \) sub-networks are reported is the FDR low?

**Theorem 3.** Fix \( \beta_1, \beta_2, \ldots, \beta_K \) such that \( \sum_{i=1}^{K} \beta_i = \beta \). Let \( s^* \) be the first \( s \) such that \( \bar{r}_{s} \geq \frac{E[\bar{r}_{s}]}{\beta} \). If we return as significant all connected components of size \( \geq s^* \), then the FDR of the test is bounded by \( \beta \).
Alternate Proof for Theorem 3

- If \( \tilde{r}_s \geq \frac{E[r_s]}{\beta_s}, \beta_s \geq \frac{E[r_s]}{\tilde{r}_s} \geq FDR \) for component of size \( s \).

- So overall FDR is bounded by \( \sum_s \beta_s = \beta \).
## Results I

### Model I – Combinatorial

<table>
<thead>
<tr>
<th>dataset</th>
<th>$k$</th>
<th>samples</th>
<th>$H_0^{\text{sample}}$</th>
<th>$H_0^{\text{gene}}$</th>
<th>$H_0^{\text{all}}$</th>
<th>$H_0^{\text{RTK/RAS/PI(3)K}}$</th>
<th>$H_0^{\text{p53}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBM</td>
<td>10</td>
<td>67</td>
<td>$&lt; 10^{-10}$</td>
<td>$4 \times 10^{-3}$</td>
<td>$3 \times 10^{-4}$</td>
<td>$8 \times 10^{-4}$</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>78</td>
<td>$&lt; 10^{-10}$</td>
<td>$&lt; 10^{-3}$</td>
<td>$10^{-5}$</td>
<td>$8 \times 10^{-5}$</td>
<td>0.05</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td>140</td>
<td>$&lt; 10^{-10}$</td>
<td>0.02</td>
<td>$8 \times 10^{-6}$</td>
<td>/</td>
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<tr>
<td></td>
<td>20</td>
<td>151</td>
<td>$&lt; 10^{-10}$</td>
<td>0.03</td>
<td>$3 \times 10^{-3}$</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

### Model II – Enhanced Influence

<table>
<thead>
<tr>
<th>$s$</th>
<th>$# \text{ c.c.} \geq s$</th>
<th>$H_0^{\text{sample}}$</th>
<th>$H_0^{\text{gene}}$</th>
<th>$\text{enrichment p-val}$</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>$\mu$</td>
<td>$\mu$</td>
<td>RTK/RAS/PI(3)K</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>22.18</td>
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<tr>
<td>3</td>
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<td>6.37</td>
<td>4.38</td>
<td>/</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>$&lt; 10^{-3}$</td>
<td>0.07</td>
<td>0.9</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>$&lt; 10^{-3}$</td>
<td>0.05</td>
<td>$4 \times 10^{-6}$</td>
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</tbody>
</table>
Results II

Combination of Network and Frequency Identifies Rare Pathways

MAPK pathway

Notch signaling pathway
CONEXIC Idea


The Genomic Signature of a Driver

Assumptions on driver mutations:

A. A driver mutation should occur in multiple tumors more often than would be expected by chance

B. A driver mutation may be associated with the expression of a group of genes that form a ‘module’

C. Copy number variations often influence the expression of genes in the module via changes in expression of the driver

Slide: Anja Kiesel
Algorithm I

1. **Selection of Candidate Drivers:**
   - GISTIC algorithm to identify genes that overlap CNV regions often
   - 513 peak genes in 27 amplified regions
   - 384 peak genes in 23 deleted regions

2. **Expression Filtering:**
   - Remove genes that are expressed at constant level or not expressed
   - Final set of 428 genes

3. **Single Modulator Step (Initial Model):**
   - Correlation between CNV and expression
   - 347 candidate drivers left
   - **Associating target genes with driver gene**
   - 78 modulators explaining behavior of 4018 genes (min. 20 genes per module)

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Algorithm II

4. Network Learning Step (Iteratively alternating 2 tasks):
   a) Learn regulation program by choosing candidate driver, that best splits gene expression of the module genes into 2 distinct behavior
   b) Re-assign each gene into the module the best models its behavior

64 modulators explaining behavior of 7896 genes (of 7981 genes in total)

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CONEXIC Results

Many Modulators are Involved in Pathways Related to Melanoma

LitVAn = Literature Vector Analysis

Searches for overrepresented terms in papers associated with genes in a gene set (manually curated database - NCBI Gene)

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Pathway</th>
<th>Band</th>
<th>Genes in Region</th>
<th>Validation p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MITF</td>
<td>Melanoma</td>
<td>3p14.2-p14.1</td>
<td>1</td>
<td>&lt;10^-6</td>
</tr>
<tr>
<td>TBC1D16</td>
<td>Vesicular Trafficking</td>
<td>17q25.3</td>
<td>24</td>
<td>&lt;10^-5</td>
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<tr>
<td>ZFP106</td>
<td>Insulin/Ras</td>
<td>15q15.1</td>
<td>7</td>
<td>&lt;10^-5</td>
</tr>
<tr>
<td>DIXDC1</td>
<td>Wnt/JNK/PI3K</td>
<td>11q23.1</td>
<td>17</td>
<td>0.0001</td>
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<tr>
<td>OIP5</td>
<td>Cell Cycle</td>
<td>15q15.1</td>
<td>13</td>
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<tr>
<td>TTBK2</td>
<td></td>
<td>15q15.2</td>
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<td>0.0383</td>
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<tr>
<td>TRAF3</td>
<td>NFkappaB/JNK</td>
<td>14q32.32</td>
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<tr>
<td>RAB27A</td>
<td>Vesicular Trafficking</td>
<td>15q15-q21.1</td>
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<tr>
<td>C12orf35</td>
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<td>12p11.21</td>
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</tr>
<tr>
<td>WBP2</td>
<td></td>
<td>17q25</td>
<td>92</td>
<td>0.0275</td>
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<tr>
<td>MOCS3</td>
<td></td>
<td>20q13.13</td>
<td>16</td>
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</tr>
<tr>
<td>NDUFB2</td>
<td></td>
<td>7q34</td>
<td>10</td>
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<tr>
<td>ST6GALNAC2</td>
<td></td>
<td>17q25.1</td>
<td>92</td>
<td>&lt;10^-5</td>
</tr>
<tr>
<td>GRB2</td>
<td>EGFR/Ras</td>
<td>17q24-q25</td>
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<td>0.1373</td>
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<tr>
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<td>KCNC1</td>
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<td>0.202</td>
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<tr>
<td>DPM1</td>
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<td>20q13.13</td>
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<td>0.097</td>
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<td>PFKP</td>
<td>Metabolism</td>
<td>10p15.3-p15.2</td>
<td>3</td>
<td>0.0801</td>
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<tr>
<td>KLF6</td>
<td>Cell cycle, c-JUN (JNK)</td>
<td>10p15</td>
<td>3</td>
<td>&lt;10^-5</td>
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<tr>
<td>TIMM8B</td>
<td>Mitochondria</td>
<td>11q23.1-q23.2</td>
<td>17</td>
<td>0.7622</td>
</tr>
</tbody>
</table>

Slide: Anja Kiesel
Results II

A known driver, **MITF**, is correctly associated with target genes

- MITF expression correlates with targets better than copy number (A,B)
- MITF correctly annotated with its known role in melanoma (C)
  - 2 types of melanoma: **high** MITF expression => proliferation
  - **low** MITF expression => invasion

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DriverNet Idea


Rare drivers can be discovered by their “influence” on genes with outlying expression levels

Overrepresented pathways of genes exhibiting outlying expression associated with EGFR high level amplification

PI3K signaling: FOX1, FOX3, AKT1, AKT3, GNA10, SOS1, PIK3R5, PIK3R1, GSK3B, JAK1
MAPK signaling: PDGFA, FGF10, AKT1, MAPK7, AKT3, EGFR, BRAF, FLNA, JUN, PPM1A, KRAS, ELK4, SOS1, JUND, NFATC4, RASA2, TAO1, RPS6KA2, RAP1A, GCN4
ErbB signaling pathway: BTC, STAT5B, AKT1, MAP2K7, AKT3, EGFR, BRAF, JUN, SRC, KRAS, SOS1, CAMK2D, PIK3R5, PIK3R1, PLCG1, GSK3B
Algorithm

Min. number of mutated genes that explain the max. number of differentially expressed genes
Statistical Significance

Null Model

Permute entries of the mutation and differential expression matrices (not gene or sample based)

Statistical Testing

Is the number of covered genes observed rarely in 500 random instances?

Benjamini-Hochberg correction for multiple-hypothesis testing
Results I

Higher Concordance to Known Drivers

A, B, C, D, E, F diagrams showing concordance with Cancer Gene Census and COSMIC for different approaches.
# Results II

Rare Drivers are Predicted and Meaningful

<table>
<thead>
<tr>
<th>Table 2 The predicted rare drivers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dataset</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>METABRIC</td>
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<tr>
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<tr>
<td>METABRIC</td>
</tr>
</tbody>
</table>
Driver genes must be associated with the deregulation of key “phenotype” genes
Step 1: Constrained Association

Parameter Selection

- Permutation-based Null Model
- JS Divergence
  - Observed vs Random Phenotype gene frequencies
Step 2: Back-seat Drivers and Parsimony

Back-seat Drivers:
False-association due to proximity to large deregulated module

Matching Drivers with Phenotypes

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential Drivers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation Frequency</td>
<td>0.75</td>
<td>0.50</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td>Samples with Mutation</td>
<td>S1, S2, S3</td>
<td>S2, S3</td>
<td>S2, S3</td>
<td>S4</td>
</tr>
</tbody>
</table>

Diagram showing the association between potential drivers and samples with mutation.
Step 3: Modules and IMPACT

- **IMPACT** = average fold-change of genes in module
- Sort drivers by IMPACT
- Modules enable functional annotation
Results I: *In silico* validation

**Glioblastoma**

**Ovarian Cancer**

- **OncoIMPACT**
- **DriverNET**
- **Frequency**
Results II: Robustness

- **Stability** = Fraction of positives in full-set
- **Recovery** = Fraction of full-set in positives
Results III: Cell-line Analysis

Why?

- Robustness to noise
- Important *in vitro* models

### Glioma cell line

![Glioma cell line graph](image)

### Ovarian cancer cell line

![Ovarian cancer cell line graph](image)
Patient-specific Drivers

Low FPR at patient-specific level

shRNA-based validation on 23 ovarian cancer cell-lines

$p$-value $= 9.5 \times 10^{-4}$
A template for personalized-target identification

Tumor Omics Profiling

Mutations (SNVs, CNVs, SVs, …)

Expression Changes

Driver Predictions

OncoIMPACT

Validation

Patient-derived Cell-line

Candidate Therapeutic Targets

**BRAF** – well-known driver in Melanoma
**TRIM24** – novel candidate (ubiquitinates TP53 in breast cancer)
Tumor Stratification

Consensus NMF Clustering

Glioblastoma

CDKN2A  EGFR  GLI1

Ovarian Cancer

PIK3CA  MYC  CCNE1

Consensus NMF Clustering
Prognostic Mutation Signatures

- **Glioblastoma**
  - Mean Survival: 7.3 months vs 18.4 months (Glioblastoma Cluster 1 vs Cluster 2)

- **Ovarian Cancer**
Good To Read


- **[CONEXIC]** Akavia et al. “An Integrated Approach to Uncover Drivers of Cancer” *Cell 143*:1005-17, 2010


Acknowledgements

Denis Bertrand

Anja Kiesel

- Slides on association analysis were adapted from slides by Dr. Chiea Chuen Khor (Senior Research Scientist, Genome Institute of Singapore)