Simultaneous Identification of Multiple Driver Pathways in Cancer

Max Leiserson
ISMB 2014
Clonal theory of cancer

Clonal Theory (Nowell 1976)

Passenger mutations

Founder cell

Driver mutation

Time (cell divisions)

Cell population

“typical tumor”: ~10 driver mutations

100’s – 1000’s of passenger mutations

sequence genome
Clonal theory of cancer

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Types of Variation in Tumor Genomes

<table>
<thead>
<tr>
<th>Single Nucleotide Variants</th>
<th>Copy Number Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy</strong> ..ACGTCATCATGA..</td>
<td><img src="image" alt="Healthy Variants" /></td>
</tr>
<tr>
<td><strong>Tumor</strong> ..ACGTCAGCATGA..</td>
<td><img src="image" alt="Tumor Variants" /></td>
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- **deletion**
- **amplification**
Clonal theory of cancer

Clonal Theory (Nowell 1976)

Passenger mutations

Founder cell

Driver mutation

Time (cell divisions)

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Types of Variation in Tumor Genomes

Single Nucleotide Variants

Copy Number Variants

Healthy . .ACGTCATCAGTGA . .

Tumor . .ACGTCAGCAGTGA . .

amplification

deletion

Compare variation across tumors

= gene = SNV / CNA
Significantly mutated genes in cancer

**Significance Score**
Mutations weighted by:
- Gene length
- Mutation context
- Expression level
- Replication timing
- ...

![Graph showing the distribution of significance scores across genes with a 'long tail' of low-frequency mutations in 200-500 tumor samples. The graph highlights the significance scores for 10-35 genes compared to the broader 'long tail' of less significant genes.](graph.png)
“Long tail” of mutated genes complicates finding driver mutations

**Significance Score**
Mutations weighted by:
- Gene length
- Mutation context
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- …

**Comparison of significantly mutated genes**
*TCGA Pan-Cancer Dataset* [TCGA Research Network, 2013]
>3000 tumor samples of twelve cancer types
“Long tail” of mutated genes complicates finding driver mutations

Significance Score
Mutations weighted by:
- Gene length
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Comparison of significantly mutated genes
TCGA Pan-Cancer Dataset [TCGA Research Network, 2013]
>3000 tumor samples of twelve cancer types

50 genes agreed upon by all methods
Hundreds found by only one method
Mutations target pathways

Significance Score
Mutations weighted by:
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Comparison of significantly mutated genes
TCGA Pan-Cancer Dataset [TCGA Research Network, 2013]
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Identifying significantly mutated pathways

TCGA Colorectal (Nature 2012)

TCGA Ovarian (Nature 2011)
Identifying significantly mutated pathways

TCGA Colorectal (Nature 2012)

- Novel pathways
- Crosstalk
- Origin specific

TCGA Ovarian (Nature 2011)

Significance Score

10-35 genes

A interacts with B “long tail”

Gene

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
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<tr>
<td>CDKN2A 32% Downregulated 50%, deleted 2%</td>
<td>CCNE1 20% Amplified</td>
</tr>
<tr>
<td>CCND1 4% Amplified</td>
<td>CCND2 15% Upregulated</td>
</tr>
<tr>
<td>Relative abundance: 10%</td>
<td>RAS 4%</td>
</tr>
<tr>
<td>RB1 10% Deleted 8%, mutated 2%</td>
<td>Cell cycle progression</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PTEN 7% Deleted 7%, mut. &lt;1%</th>
<th>NF1 12% Deleted 8%, mut. 4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA 18% Amplified</td>
<td>KRAS 11% Amplified, mut. &lt;1%</td>
</tr>
<tr>
<td>AKT1 3%</td>
<td>BRAF 0.5% Mutated</td>
</tr>
<tr>
<td>AKT2 0% Amplified</td>
<td>Proliferation/survival</td>
</tr>
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Cancer pathways often harbor **mutually exclusive** mutations

Few driver mutations distributed across *multiple* pathways

→ Approximately one driver mutation per pathway per patient
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1. Exclusivity

[Thomas, et al. (2007)]
Cancer pathways often harbor *mutually exclusive* mutations

*Few* driver mutations distributed across *multiple* pathways

→ Approximately one driver mutation per pathway per patient

1. **Exclusivity**

→ Many patients have a mutation in important cancer pathway

2. **High Coverage**
De novo driver exclusivity (Dendrix)

de novo: without prior biological information (pathways, interactions, etc.)

Goal: Find sets $M$ of genes with:
- High coverage: many patients with $\geq 1$ mutation in $M$
- Approximate exclusivity: most patients have $\leq 1$ mutation in $M$

Finding optimal set is NP-Hard.
MCMC algorithm samples sets in proportion to weight.

Iter-Dendrix: multiple pathways

Cancer requires mutations to more than one pathway

Find multiple pathways greedily.

$m$ genes
$n$ patients

= gene $i$ is mutated in patient $j$,  
= otherwise.

Iter-Dendrix: multiple pathways

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Greedy can fail

Cancer requires mutations to more than one pathway

Find multiple pathways greedily.

Groups most frequently mutated genes even without exclusivity.

- = gene $i$ is mutated in patient $j$,
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= otherwise.
Multi-Dendrix

Cancer requires mutations to more than one pathway

Find **multiple pathways simultaneously**

- ILP rapidly finds optimal solution
- Searches wide range of parameters to find stable collections of gene sets

Multi-Dendrix

Cancer requires mutations to more than one pathway

Most samples have approximately one mutation in each of $t$ pathways.

\[
W'_\alpha(M) = \sum_{M \in M} |\Gamma(M)| - \alpha \omega(M)
\]

Multi-Dendrix

Cancer requires mutations to more than one pathway

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$maximize\ W'_\alpha(M) = \sum_{M \in \mathcal{M}} |\Gamma(M)| - \alpha \omega(M)\$

parameter to control “weight” of exclusivity

coverage overlap

Integer Linear Program

Multi-Dendrix

Cancer requires mutations to more than one pathway

Most samples have approximately one mutation in each of $t$ pathways.

\[ |M_i| \in [k_{\min}, k_{\max}] \]

\[
\text{maximize } W'_\alpha(M) = \sum_{M \in M} |\Gamma(M)| - \alpha \omega(M)
\]

parameter to control “weight” of exclusivity

coverage overlap

Integer Linear Program

Contributions

A new algorithm, **Multi-Dendrix**, for identifying driver pathways *de novo*:

1. Outperforms previous methods on simulated data in speed and accuracy

2. Identifies gene sets that overlap known pathways in TCGA datasets

3. Ongoing work extending Multi-Dendrix to large datasets and overlapping pathways
**Multi-Dendrix is significantly better on simulated data**

<table>
<thead>
<tr>
<th>$q$</th>
<th>Multi-Dendrix</th>
<th>Iter-RME*</th>
<th>Iter-Dendrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.02 ± 0.19</td>
<td><strong>0.01 ± 0.12</strong></td>
<td>0.30 ± 0.86</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.02 ± 0.18</td>
<td><strong>0.01 ± 0.16</strong></td>
<td>0.30 ± 0.86</td>
</tr>
<tr>
<td>0.0005</td>
<td><strong>0.04 ± 0.23</strong></td>
<td>0.10 ± 0.40</td>
<td>0.35 ± 0.89</td>
</tr>
<tr>
<td>0.001</td>
<td><strong>0.10 ± 0.35</strong></td>
<td>0.32 ± 0.60</td>
<td><strong>0.44 ± 1.01</strong></td>
</tr>
<tr>
<td>0.005</td>
<td><strong>0.44 ± 0.71</strong></td>
<td>–</td>
<td>0.75 ± 1.07</td>
</tr>
<tr>
<td>0.01</td>
<td><strong>1.03 ± 1.00</strong></td>
<td>–</td>
<td>1.20 ± 1.15</td>
</tr>
<tr>
<td>0.015</td>
<td><strong>1.68 ± 1.16</strong></td>
<td>–</td>
<td>1.78 ± 1.26</td>
</tr>
<tr>
<td>0.02</td>
<td><strong>2.17 ± 1.24</strong></td>
<td>–</td>
<td>2.21 ± 1.29</td>
</tr>
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</table>


### Example planted pathway

- High coverage and mutually exclusive
- Add passenger mutations (noise) to all genes

360 mutated genes in 160 patients
Multi-Dendrix is significantly faster on simulated data. Multi-Dendrix completes in fewer than 5 seconds for each dataset.

Multi-Dendrix pipeline for identifying mutated cancer pathways

Multi-Dendrix pipeline for identifying mutated cancer pathways

0. Data preprocessing

- Gistic widepeaks
  - CNV filtering
  - Consistent amp/del
  - Remove artifacts

- nsSNVs indels
  - SNV filtering
  - Mutation frequency
  - Expected no. of SNVs

Multi-Dendrix pipeline for identifying mutated cancer pathways

0. Data preprocessing

1. Input mutation matrix

Gistic widepeaks → CNV filtering
- Consistent amp/del
- Remove artifacts

nsSNVs indels → SNV filtering
- Mutation frequency
- Expected no. of SNVs

Mutation Matrix A
- m patients
- n genes and mutation classes

Multi-Dendrix pipeline for identifying mutated cancer pathways

0. Data preprocessing

1. Input mutation matrix

2. Analysis and annotation

Multi-Dendrix pipeline for identifying mutated cancer pathways

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- CNV filtering
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- SNV filtering
  - nsSNVs, indels
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1. Input mutation matrix

- Mutation Matrix A
  - \( n \) genes and mutation classes
  - \( m \) patients

2. Analysis and annotation

- Multi-Dendrix
  - Rapidly identifies sets of driver pathways
  - Max. gene set size
  - Min. gene set size
  - No. gene sets

- Subtype-specific mutations
- Stability measures
- Permutation test
- Compute enrichment stats
- iREFIndex

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Alternative explanation for exclusive mutations

Multi-Dendrix pipeline for identifying mutated cancer pathways

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**Direct Interactions Test**
- Measures enrichment of PPI interactions within individual gene sets or in a collection of gene sets

Multi-Dendrix pipeline for identifying mutated cancer pathways

0. Data preprocessing

1. Input mutation matrix

2. Analysis and annotation

3. Output

Direct Interactions Test

- Measures enrichment of PPI interactions within individual gene sets or in a collection of gene sets

Consensus modules

- Run Multi-Dendrix across a range of parameters.
- Identify the stable “modules” of genes that appear together multiple times.

Contributions

A new algorithm, **Multi-Dendrix**, for identifying driver pathways *de novo*:

1. Outperforms previous methods on simulated data in speed and accuracy

2. Identifies gene sets that overlap known pathways in TCGA datasets

3. Ongoing work extending Multi-Dendrix to large datasets and overlapping pathways
Results: Glioblastoma

*Mutation data: 398 genes (events) in 261 patients

Direct interactions test: $P=0.002$

Results: Glioblastoma

Comprehensive genomic characterization defines human glioblastoma genes and core pathways

Results: Glioblastoma

Comprehensive genomic characterization defines human glioblastoma genes and core pathways

The Cancer Genome Atlas Research Network

RTK/RAS/PI(3)K signaling

Rb signaling

p53 Signaling

**Results: TCGA breast cancer**

**Multi-Dendrix (α=2.5)**

*Mutation data:* 375 genes (events) in 507 patients

\[ W'_\alpha(M) = \sum_{M \in M'} \Gamma(M) - \alpha \omega(M) \]

higher \( \alpha \) \( \Rightarrow \) more exclusivity

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Multi-Dendrix MCMC

Sample collections of gene sets in proportion to their weight

- Finds a distribution — optimal and suboptimal — of solutions
- Newest version on GitHub, requirements are all open-source

---

**Distribution of collections of gene sets**

<table>
<thead>
<tr>
<th>Geneset</th>
<th>Weight</th>
<th>Sampling Frequency</th>
</tr>
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<tbody>
<tr>
<td>G1, G2, G3, G4, G5, G6, G7, G8</td>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>G9, G10, G11, G12, G13, G14, G15, G16</td>
<td>170</td>
<td>30</td>
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<tr>
<td>G1, G2, G3, G4, G5, G7, G15, G16</td>
<td>130</td>
<td>9</td>
</tr>
<tr>
<td>G17, G18, G19, G20, G13, G14, G15, G16</td>
<td>80</td>
<td>1</td>
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http://github.com/raphael-group/multi-dendrix
Marginal probability graph

- Marginal probability graph defines consensus subnetworks

- Edges \((u, v)\) are weighted by how often gene \(u\) is sampled in the same gene set as gene \(v\)

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<td>(G_1, G_2, G_3, G_4)</td>
<td>200</td>
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<td>(G_5, G_6, G_7, G_8)</td>
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Complete, weighted marginal probability graph

→ unconstrained size and number of gene sets
→ gene sets can overlap
Marginal probability graph

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Hsin-Ta Wu

\[ \text{Complete, weighted marginal probability graph} \]

- unconstrained size and number of gene sets
- gene sets can overlap

Dendrix++ results: GBM

Comprehensive genomic characterization defines human glioblastoma genes and core pathways

The Cancer Genome Atlas Research Network*

CDKN2A(D) (176)
MDM2(A) (23)
CDK4(A) (53)
TP53 (76)
RB1

Recent genome-wide profiling studies have also shown inactivation of the p53 and retinoblastoma tumour suppressor pathways and of the phosphatidylinositol-3-OH kinase (PI(3)K) pathway; and (3) regulation of growth factor signalling via amplification and mutational events in human glioblastomas, including the following: (1) dysre-
Recent genome-wide profiling studies have also shown inactivation of the p53 and retinoblastoma tumour suppressor pathways, activation of receptor tyrosine kinase (RTK) genes; (2) activation of growth factor signalling via amplification and mutational events in human glioblastomas, including the following: (1) dysregulation of Rb signaling.

Two decades of molecular studies have identified important genetic alterations in glioblastoma, which comprise more than 90% of primary glioblastomas, which arise de novo in adults. Primary glioblastoma, which comprises more than 90% of adult glioblastoma, arises de novo in adults. Patients with newly diagnosed glioblastoma have a median survival of approximately 1 year with generally poor responses to all therapeutic modalities. Albeit fragmentary, there is now overwhelming evidence for the existence of molecular subclasses within glioblastoma that may, when fully defined, allow stratification of treatment.

With the complete sequencing of the human genome and continuing improvement of high-throughput genomic technologies, it is now feasible to contemplate integrated multi-dimensional analyses. The Cancer Genome Atlas (TCGA) pilot project aims to assess the value of large-scale multi-dimensional analysis of these molecular characteristics in human cancer and to provide the data rapidly to the research community. Here we report the interim integrative analysis of DNA copy number, gene expression and DNA methylation aberrations in 206 glioblastomas—the most common type of primary adult brain cancer—and nucleotide sequence aberrations in 91 of the 206 glioblastomas. This analysis provides new insights into the roles of Rb, p53 and other key pathways in glioblastoma.

**Rb signaling**
- CDKN2A(D) (176) mutation in 52%
- CDKN2C (Homozygous deletion in 47%)
- CDKN2B (Homozygous deletion in 2%)
- Amplification in 18%
- Amplification in 2%
- Amplification in 1%
- CDK4 (Amplification)
- CCND2 (Amplification)
- CDK6 (Amplification)
- RB1 (Homozygous deletion, mutation in 11%)
- RB signaling altered in 78%
- G1/S progression

**p53 signaling**
- CDKN2A (HIF)
- MDM2
- TP53
- MDM4
- p53 signaling altered in 67%

**CDK4(A)** (53)
**RB1 (19)**
**TP53 (76)**
**MDM2 (23)**

The Cancer Genome Atlas Research Network* defines human glioblastoma genes and comprehensive surveys of human cancer genomes. The Cancer Genome Atlas aims to catalogue and discover major cancer-causing mechanisms and pathways. Through high-throughput genomic technologies, it is now feasible to contemplate integrated multi-dimensional analyses. The Cancer Genome Atlas (TCGA) pilot project aims to assess the value of large-scale multi-dimensional analysis of these molecular characteristics in human cancer and to provide the data rapidly to the research community. Here we report the interim integrative analysis of DNA copy number, gene expression and DNA methylation aberrations in 206 glioblastomas—the most common type of primary adult brain cancer—and nucleotide sequence aberrations in 91 of the 206 glioblastomas. This analysis provides new insights into the roles of Rb, p53 and other key pathways in glioblastoma.
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**RTK/RAS/PI(3)K signaling**

- **PIK3R1**
- **PTEN**
- **PDPN, PRDM2(A)**

**Rb signaling**

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  - mutation in 52%
  - homozygous deletion in 47%
  - amplification in 18%
- **CDKN2B**
  - homozygous deletion in 2%
- **CDKN2C**
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  - G1/S progression
- **RB1**
  - RB signaling altered in 78%

**p53 signaling**

- **CDKN2A(AHF)**
- **MDM2**
- **MDM4**

**CDK4(A)**

**TP53**

**PIK3CA**

**PTEN(D)**

**RB1**

**CDKN2A(D)**

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**Dendrix++ results: GBM**

**Comprehensive genomic characterization defines human glioblastoma genes and core pathways**

The Cancer Genome Atlas Research Network®

Summary

• Multi-Dendrix: Fast, exact ILP for identifying collections of gene sets with exclusive mutations

• Identifies modules that overlap multiple cancer pathways in glioblastoma and breast cancer

• Dendrix++: New algorithm that can identify more complex pathways (in preparation)
Acknowledgements

Research group
Ben Raphael
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