Boolean Implications Identify Wilms’ Tumor 1 Mutation as a Driver of DNA Hypermethylation in Acute Myeloid Leukemia

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Aberrant Methylation in Acute Myeloid Leukemia

- Acute Myeloid Leukemia (AML) is a disease characterised by the accumulation of myeloid precursor cells in the bone marrow that are blocked in their ability to differentiate into mature blood cells.
- AML is associated with widespread deregulation of DNA methylation.

1. Identify genetic drivers of aberrant methylation.
2. Find leads for a mutation-specific therapy.
Boolean Implications (IF – THEN Rules)

- Four different implications:
  - HIHI: IF A high, THEN B high
  - HILO: IF A high, THEN B low
  - LOHI: IF A low, THEN B high
  - LOLO: if A low, THEN B low
Computational Pipeline

TCGA AML samples

Mutation Data

17 Recurrent mutations

CpG site filtering = 285, 320 probes

Discretize me values

Generate Boolean Implications

Count number of methylation HIHI and HILO

Boolean implications for each mutation

DNMT3A mutation

IDH2 mutation
WT1 mutation AML is linked to hypermethylation

<table>
<thead>
<tr>
<th>Gene</th>
<th>No. of hypermethylation implications</th>
<th>No. of hypomethylation implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH2</td>
<td>12950</td>
<td>36</td>
</tr>
<tr>
<td>WT-1</td>
<td>2028</td>
<td>13</td>
</tr>
<tr>
<td>CEBPA</td>
<td>7839</td>
<td>42</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>325</td>
<td>3469</td>
</tr>
<tr>
<td>Cohesin</td>
<td>185</td>
<td>852</td>
</tr>
</tbody>
</table>

**Hyper-**

**Hypo-**

**Mixed**

**Very few (<500)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>No. of hypermethylation implications</th>
<th>No. of hypomethylation implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUNX1</td>
<td>4384</td>
<td>399</td>
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<tr>
<td>IDH1</td>
<td>4074</td>
<td>1345</td>
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<tr>
<td>TET2</td>
<td>1314</td>
<td>894</td>
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<tr>
<td>FLT3</td>
<td>614</td>
<td>1350</td>
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<tr>
<td>NPM1</td>
<td>2145</td>
<td>3683</td>
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<tr>
<td>TP53</td>
<td>4175</td>
<td>4870</td>
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<tr>
<td>KIT</td>
<td>54</td>
<td>281</td>
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<tr>
<td>KRAS</td>
<td>9</td>
<td>23</td>
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<tr>
<td>MT-CO2</td>
<td>105</td>
<td>15</td>
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<tr>
<td>NRAS</td>
<td>182</td>
<td>53</td>
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<tr>
<td>PTPN11</td>
<td>107</td>
<td>8</td>
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<tr>
<td>U2AF1</td>
<td>60</td>
<td>108</td>
</tr>
</tbody>
</table>

**Hypermethylation Index**

- Hypermethylation / Hypomethylation
- Scale: 0, 50, 100, 150, 200, 250, 300, 350, 400
Distinct CpG sites and associated genes linked to hypermethylating mutations
WT1 mutation induces hypermethylation in AML cells

COSMIC
ins/dels

WT1 NH₂ P/Q rich T2A GFP COOH
ZF ZF ZF ZF

WT1mut NH₂ P/Q rich COOH

transduced THP-1 stable cell-lines
10 passages
450K Beadchip array

CpG me (β value)
0.0 to 0.2
0.2 to 0.4
0.4 to 0.6
0.6 to 0.8
0.8 to 1.0

Overlap with patients: 8.5E-34
Fisher's exact test
Mutant WT1 methylation signature is enriched for PRC2 target genes

<table>
<thead>
<tr>
<th>Patient samples with WT1 mut</th>
<th>Gene Sets</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Benporath ES with H3K27ME3</td>
<td>1.6E-87</td>
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<tr>
<td>Benporath EED targets</td>
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<tr>
<td>Benporath Suz12 targets</td>
<td>1.65E-81</td>
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<tr>
<td>Benporath PRC2 targets</td>
<td>8.13E-63</td>
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<td>Mikkelsen MEF HCP with H3K27ME3</td>
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<tr>
<td>Mikkelsen Brain HCP with H3K27ME3</td>
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<td>H3K4ME3 and H3K27ME3</td>
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<tr>
<td>H3K27ME3</td>
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<td>Meissner Brain HCP with H3K27ME3</td>
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<tr>
<td>Mikkelsen NPC HCP with H3K27ME3</td>
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<td>Meissner NPC HCP with H3K27ME3</td>
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</tbody>
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<table>
<thead>
<tr>
<th>THP1 cell-line with WT1mut</th>
<th>Gene Sets</th>
<th>P-value</th>
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<tbody>
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<tr>
<td>Mikkelsen MEF HCP with H3K27ME3</td>
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<tr>
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<tr>
<td>Benporath SUZ12 targets</td>
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<tr>
<td>Benporath PRC2 targets</td>
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<td>Mikkelsen Brain HCP with H3K27ME3</td>
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<tr>
<td>Mikkelsen NPC HCP with H3K27ME3</td>
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<tr>
<td>Mikkelsen MCV6 HCP with H3K27ME3</td>
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<tr>
<td>Meissner NPC HCP with H3K27ME3</td>
<td>1.65E-30</td>
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<tr>
<td>Mikkelsen MEF HCP with H3K27ME3</td>
<td>9.65E-21</td>
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</tbody>
</table>

Enrichment score (ES) NES: 2.47

-log (p-value)
WT1 mutant AML shows aberrant repression of Polycomb repressor complex 2 targets.

K562 cells

H3K27me3 CHIP (ENCODE)

PRC2 marked genes in adult hematopoiesis

Does WT1 mutation block myeloid differentiation?
Inhibition of PRC2 promotes differentiation in AML with WT1 mutation

TF1+ GM
TF1+ EPO
Isotype control
WT1mut + GM
WT1mut + EPO

Intracellular Fetal Hb (anti-human HbF-PE)

WT1mut+ NK AML, SU359

TF1+ GM
70%
TF1+ EPO
0%
Isotype control
0.2%
WT1mut + GM
9%
WT1mut + EPO

* p<0.01

* * p<0.01

in vitro differentiation
Conclusions

- Mutation in WT1 is strongly linked to DNA hypermethylation in AML
- Introduction of mutant WT1 into wildtype cells induced the same pattern of DNA hypermethylation
- The pattern of methylation and gene expression is consistent with a differentiation block caused by WT1mut through dysregulated silencing of PRC2 targets
- Differentiation block in WT1mut AML can be overcome by EZH2 inhibition
- **EZH2 inhibitors have activity in WT1mut AML**
- **Boolean implications are a useful data mining tool for large, heterogeneous cancer data sets**
Acknowledgements

Dan Thomas

David Dill
Stanford Centre for Cancer Systems Biology (CCB, NCI)
Progenitor Cell Biology Consortium (PCBC, NHLBI)

Ravindra Majeti
NHMRC CJ Martin Fellowship
Hem/Oncology HSANZ Targetted Therapy Fellowship

Sylvia Plevritis

Andrew Gentles

Andrew Feinberg

Namyoung Jung