CCCTC-Binding Factor (CTCF)

- Binding sites universally present in all mammalian differentially methylated regions
- Highly conserved
- Sensitive to methylation,
- Contributes to formation and structure of chromatin
Overview of IDH and its mutations

- IDH proteins involved in Kreb’s cycle, where it catalyzes the production of alpha-ketoglutarate
- Some mutations of IDH lead to the production of abnormal 2-hydroxyglutarate
  - 2-HG is an inhibitor of alpha-ketoglutarate dependent enzymes, including demethylases
Previous work established the prevalence of DNA hypermethylation due to IDH mutations in patients with leukemia.
Survival Rates of patients With GBM IDH1 mutations

Significant because a significant portion of young patients have IDH1 mutations, and were associated with an increase in overall survival

<table>
<thead>
<tr>
<th>Gene</th>
<th>Point mutations*</th>
<th>Amplifications†</th>
<th>Homozygous deletions†</th>
<th>Fraction of tumors with any alteration (%)</th>
<th>Passenger probability‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDKN2A</td>
<td>0/22</td>
<td>0/22</td>
<td>11/22</td>
<td>50</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TP53</td>
<td>37/105</td>
<td>0/22</td>
<td>0/22</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EGFR</td>
<td>15/105</td>
<td>5/22</td>
<td>0/22</td>
<td>23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTEN</td>
<td>27/105</td>
<td>0/22</td>
<td>0/22</td>
<td>26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NF1</td>
<td>16/105</td>
<td>0/22</td>
<td>0/22</td>
<td>15</td>
<td>0.04</td>
</tr>
<tr>
<td>CDK4</td>
<td>0/22</td>
<td>3/22</td>
<td>0/22</td>
<td>14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RB1</td>
<td>8/105</td>
<td>0/22</td>
<td>0/22</td>
<td>8</td>
<td>0.02</td>
</tr>
<tr>
<td>IDH1</td>
<td>12/105</td>
<td>0/22</td>
<td>0/22</td>
<td>11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>10/105</td>
<td>0/22</td>
<td>0/22</td>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>8/105</td>
<td>0/22</td>
<td>0/22</td>
<td>8</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 2. Most frequently altered GBM CAN-genes. All CAN-genes are listed in table S7.
CTCF Binding and Gene Insulation

- Used ChIP-Seq to map CTCF binding for loci in *IDH* mutant (red) vs wild type (black) tumors

- Used WGBS data to determine methylation of loci with reduced CTCF binding
- Used Hi-C to determine TAD boundaries
- Gene correlation for normal brain tissue samples
- *IDH* mutant gliomas exhibit inverse effect (stronger correlation across boundaries)
Topological Domain Boundaries

- Pinpointed boundaries disrupted by $IDH$ mutations - 203
- $IDH$ mutants have boundaries with higher DNA methylation and lower CTCF binding compared to wild-type tumours
- Gliomagenesis: $PDGFRA$ one of the genes in the top scoring domains of overexpression in $IDH$ mutant gliomas
- **PDGFRA**

- HiC data (kb) to investigate topology

- H3K27ac = enhancer-associated (*FIP1L1*)

- Used ChIP-Seq data to confirm boundary contains a CTCF motif with a CpG dinucleotide

- Quantitative ChIP-PCR

- Change in CTCF binding

- DNA methylation at CTCF-motif
**PDGFRA:** Identifying Regulatory Elements

- Enhancer upstream of *FIP1L1* - strong acetylation
- Used Chromosome Conformation Capture (3C)
- Intragenic: ~50kb; *FIP1L1*: ~900kb

![Graphs and data showing the comparison of control and FIP1L1 enhancer intragenic regions in patient specimens and glioblastoma models.](image)
Removing Methylation Causes Effect Reversal
Insulator CRISPR Deletion

e) GSC6 ChIP-seq

f) GSC6 surveyor assay

h) CRISPR deletions

Most common CRISPR deletion sequences

<table>
<thead>
<tr>
<th>Deletion sequence</th>
<th>Deletion size (bp)</th>
<th>Read representation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T G A A C C A G A T A A T G C</td>
<td>4</td>
<td>5.5%</td>
</tr>
<tr>
<td>T G A A C C A C</td>
<td>13</td>
<td>3.9%</td>
</tr>
<tr>
<td>T G A A C C A G A T A A T G C</td>
<td>10</td>
<td>3.7%</td>
</tr>
<tr>
<td>T G A A C C A G A T A T</td>
<td>11</td>
<td>0.91%</td>
</tr>
<tr>
<td>T G A A C C A G A T A A T G C</td>
<td>8</td>
<td>0.30%</td>
</tr>
<tr>
<td>T G A A C C A G A T A T</td>
<td>7</td>
<td>0.01%</td>
</tr>
<tr>
<td>Any other deletion</td>
<td>8.1%</td>
<td></td>
</tr>
</tbody>
</table>

Insulator CRISPR Deletion
Insulator Deletion Results in PDGFRα Expression
CTCF/cohesin-binding sites are frequently mutated in cancer

Riku Katainen\textsuperscript{1,2,6}, Kashyap Dave\textsuperscript{3,6}, Esa Pitkänen\textsuperscript{1,2,6}, Kimmo Palin\textsuperscript{1,2,6}, Teemu Kivioja\textsuperscript{1}, Niko Välimäki\textsuperscript{1,2}, Alexandra E Gylfe\textsuperscript{1,2}, Heikki Ristolainen\textsuperscript{1,2}, Ulrika A Hänninen\textsuperscript{1,2}, Tatiana Cajuso\textsuperscript{1,2}, Johanna Kondelin\textsuperscript{1,2}, Tomas Tanskanen\textsuperscript{1,2}, Jukka-Pekka Mecklin\textsuperscript{4}, Heikki Järvinen\textsuperscript{5}, Laura Renkonen-Sinisalo\textsuperscript{1,5}, Anna Lepistö\textsuperscript{5}, Eevi Kaasinen\textsuperscript{1,2}, Outi Kilpivaara\textsuperscript{1,2}, Sari Tuupanen\textsuperscript{1,2}, Martin Enge\textsuperscript{3}, Jussi Taipale\textsuperscript{1,3} & Lauri A Aaltonen\textsuperscript{1,2}
Mutations at CTCF/cohesin sites

Mainly mutations to C and G

Occurs most at sites bound by CTCF and cohesin

Not due to POLε proofreader
Mutations at CTCF/cohesin sites

Mainly mutations to C and G

Occurs most at sites bound by CTCF and cohesin

Not due to POLε proofreader
Example site

Somatic mutations
Reference
CTCF Motif + flanking region

GERP score
Observed in other cancer types
Questions?