Cancer Epigenetics

Peter W. Laird

USC Epigenome Center
USC/Norris Comprehensive Cancer Center
Keck School of Medicine
University of Southern California
DNA Methylation Alterations in Cancer

NORMAL CELL

• Methylated CpG Island promoters are transcriptionally silenced in cancer
• Areas of low-CpG density may lose DNA methylation in cancer

GLOBAL HYPOMETHYLATION

FOCAL HYPERMETHYLATION

CANCER CELL

• CpG Islands may acquire abnormal hypermethylation in cancer
• Methylated CpG Island promoters are transcriptionally silenced in cancer
• Areas of low-CpG density may lose DNA methylation in cancer
Epigenetic Silencing of \textit{BRCA1} in Serous Ovarian Cancer

- Red: Fallopian Tubes
- Purple – Somatic Mutation
- Green – Germline Mutation
- Blue – Epigenetic Silencing
  - Hollow – Not Sequenced

Outline

• CpG Island Methylator Phenotypes - Glioblastoma
G-CIMP Is a Subset of Proneural Glioblastomas with Better Survival

Noushmehr et al. 2010 Cancer Cell 17, 510
Glioma-CpG Island Methylator Phenotype (G-CIMP) (TCGA)

G-CIMP is Tightly Linked to IDH1 Mutation

Noushmehr et al. 2010 Cancer Cell 17, 510
Model for G-CIMP

*IDH1* Mutation Causes Aberrant CpG Island Methylation

…the does not explain G-CIMP *IDH1*\(^{wt}\) cases
Outline

- CpG Island Methylator Phenotypes - Glioblastoma
- Cross-tumor Comparisons
Comparison of 2,275 TCGA Cancer Samples and 409 Normal Tissues

3,450 Probes

409 Normal

2,275 Cancer Samples
Outline

• CpG Island Methylator Phenotypes - Glioblastoma

• Cross-tumor Comparisons

• Bisulfite Sequencing - Epigenetic Origins of Cancer
## TCGA Whole Genome Bisulfite Sequencing (WGBS)

### Table: TCGA Sample Type Description

<table>
<thead>
<tr>
<th>TCGA Sample</th>
<th>Type</th>
<th>Description</th>
<th>Bisulfite non-conversion</th>
<th>Mean cvg</th>
<th># CpGs</th>
<th>1x cvg (% CpGs)</th>
<th>5x cvg (% CpGs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-3518-01A</td>
<td>COAD</td>
<td>MSI-H</td>
<td>0.92%</td>
<td>23x</td>
<td>51.8M</td>
<td>92%</td>
<td>90%</td>
</tr>
<tr>
<td>AA-3518-11A</td>
<td>COAD - N</td>
<td></td>
<td>0.86%</td>
<td>22x</td>
<td>51.5M</td>
<td>91%</td>
<td>90%</td>
</tr>
<tr>
<td>A7-A0CE-01A</td>
<td>BRCA</td>
<td>Basal-like subtype</td>
<td>0.31%</td>
<td>19x</td>
<td>50.7M</td>
<td>90%</td>
<td>86%</td>
</tr>
<tr>
<td>A7-A0CE-11A</td>
<td>BRCA - N</td>
<td></td>
<td>0.36%</td>
<td>19x</td>
<td>50.3M</td>
<td>89%</td>
<td>85%</td>
</tr>
<tr>
<td>AA-3518-01A</td>
<td>UCEC</td>
<td>Grade 1 endometrioid</td>
<td>0.31%</td>
<td>19x</td>
<td>52.1M</td>
<td>92%</td>
<td>90%</td>
</tr>
<tr>
<td>AA-3518-11A</td>
<td>UCEC - N</td>
<td></td>
<td>0.31%</td>
<td>18x</td>
<td>51.8M</td>
<td>92%</td>
<td>89%</td>
</tr>
<tr>
<td>60-2722-01A</td>
<td>LUSC</td>
<td>Classical subtype</td>
<td>0.30%</td>
<td>21x</td>
<td>51.8M</td>
<td>92%</td>
<td>89%</td>
</tr>
<tr>
<td>60-2722-11A</td>
<td>LUSC - N</td>
<td></td>
<td>0.61%</td>
<td>5x</td>
<td>39.3M</td>
<td>69%</td>
<td>33%</td>
</tr>
</tbody>
</table>

- In Production: 3 Lung squamous Tumors, 3 Breast Tumors
- In Sample Selection: 2 GBM Tumors, 3 Renal Cell Kidney Pairs
Whole Genome Bisulfite Sequencing of TCGA Tumors and Normal Tissues

MP: Methylation Prone Regions

COAD
LOW METHYLATION
HIGH
COLON NORMAL
LOW

BRCA
LOW METHYLATION
HIGH
BREAST NORMAL
LOW

UCEC
LOW METHYLATION
HIGH
ENDOMETRIUM
LOW

LUSC
LOW METHYLATION
HIGH
ENDOMETRIUM
LOW
Methylation-Prone Elements are Enriched for Stem-Cell Polycomb Marks

ENCODE chromatin types from J. Ernst et al. Nature 2011
- Active promoter: K4me3, K9ac, K27ac
- Weak promoter: K4me3, K9ac
- Poised promoter: K4me1/2, K27me3

- Strong enhancer: K4me1/2, K9ac, K27ac
- Weak enhancer: K4me1/2
- CTCF Insulator: CTCF
Transcriptional Potential Associated with Histone H3 Methylation

ARTKQTARKSTG ⋯ ⋯ RKSAP — H3

MLL, SET7/9 (TRITHORAX ACTIVATION MARK)

EZH2 (POLYCOMB REPRESSIVE MARK - PRC2)

Polycomb Target Genes in Embryonic Stem Cells:
• Master regulators of differentiation and development
• Poised to be turned on during differentiation
• Bivalent epigenetic state: Active (H3K4me3) and Repressive Marks (H3K27Me3)
Polycomb Target DNA Methylation Starts in Normal Tissues

16,846 CpG Probes
1,000 ES-Cell Polycomb Targets
Model: Polycomb Crosstalk Leads to Cumulative Stochastic Methylation

Abnormal DNA Methylation at ES-Cell Polycomb Targets Even though Polycomb Repressors no Longer Occupy these Promoters

Widschwendter at al. (2007) Nature Genetics 39, 157
This Model....

• Would explain the DNA methylation behavior for about half of cancer-specifically methylated genes

• Is consistent with the observation of epigenetic field effects adjacent to tumors

• Is consistent with the stem-cell like behavior of cancer cells and with the evidence for tumor-initiating cells

• Suggests that therapeutic cloning strategies using human ES cells or IPS cells should incorporate screening for PRC2 DNA methylation abnormalities

• Suggests that the first steps of oncogenesis may be epigenetic

Widschwendter at al. (2007) Nature Genetics 39, 157
Hinoue et al. (2011) Genome Research, In Press
Outline

• CpG Island Methylator Phenotypes - Glioblastoma

• Cross-tumor Comparisons

• Bisulfite Sequencing - Epigenetic Origins of Cancer

• Bisulfite Sequencing – Long Range Instability
Methylation-Prone CpG Islands

Berman et al. 2011 Nature Genetics 43, In Press
Regions of Focal Hypermethylation and Long-Range Hypomethylation Coincide

Part of Chromosome 3q
Genes
CpG Islands

ES-Cell Methylation
Normal Colon Methylation
Colon Tumor Methylation
Hypermethylated in Cancer
Hypomethylated in Cancer

Berman et al. 2011 Nature Genetics 43, In Press
A Subset of the Cancer Epigenome Has Partially Lost Methylation

20-kb Windows

Berman et al. 2011 Nature Genetics 43, In Press
Regions of Focal Hypermethylation and Long-Range Hypomethylation Coincide

Berman et al. 2011 Nature Genetics 43, In Press
Outline

• CpG Island Methylator Phenotypes - Glioblastoma

• Cross-tumor Comparisons

• Bisulfite Sequencing - Epigenetic Origins of Cancer

• Bisulfite Sequencing – Long Range Instability

• Bisulfite Sequencing – Nuclear Architecture
Hypomethylated “Oceans” Correspond to Lamin Attachment Domains

Hypomethylated in Cancer
Hypermethylated in Cancer

Part of Chromosome 3q
Genes
CpG Islands

ES-Cell Methylation
Normal Colon Methylation
Colon Tumor Methylation
Hypermethylated in Cancer
Hypomethylated in Cancer

non-TCGA Colon PMD
TCGA COAD PMD
TCGA UCEC PMD
TCGA BRCA PMD
TCGA LUSC PMD
IMR90 PMD
Nuclear Lamina-Associated
Non-Lamina-Associated

Berman et al. 2011 Nature Genetics 43, In Press
Spatial Organization of the Epigenome

- Lamin Attachment
- Late Replication
- Epigenetic Instability in Cancer

- Active Transcription
- Epigenetically Stable in Cancer

Bas Van Steensel, *Curr Opin Cell Biol* 2010
SUMMARY

Epigenetic Subtypes
- CpG Island Methylator Phenotype in Glioblastoma – *IDH1* Mutation

Epigenetic Origins of Cancer
- Polycomb Repressor Binding in ES-Cells Predisposes to Aberrant DNA Methylation in Cancer
- Polycomb Repressor Predisposition Seen Across Cancer Types

The Role of Nuclear Architecture in Epigenetic Instability
- Focal Hypermethylation and Long-Range Hypomethylation Coincide in Partially Methylated Domains (PMDs)
- Epigenetically Unstable PMDs are Associated with Nuclear Lamina Attachment and Late-Replicating Regions
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