DNA methylation in prostate cancer

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Overarching theme

• Epigenetic regulation allows the genome to be responsive to the environment
  • Sets the tone for transcriptional response to signals

• Epigenetic derangement provides an exceptional route for cancer cell “evolution” as cancer progresses to advanced phenotypes

  • Environmental stresses drive “evolution” through malignant progression
    • Impaired mitochondrial function – DNA methylation changes associated with loss of mtDNA content
    • Inflammation – Involution of breast ducts post pregnancy; association with breast cancer risk; epigenetic contribution
    • Hormone signaling – Dynamic changes in DNA methylation related to normal AR signaling; distortion in malignancy
    • Hormone signaling – Sudden loss of AR signaling; CpG island methylation and progression to castration recurrence
    • Micronutrients – Folate metabolism and prostate cancer
      • Therapeutic potential; population genetics potential
• Ligand bound AR regulates 100’s of genes
  • Activation and suppression
  • “The AR transcriptome”

• Directs terminal differentiation, blocks growth in normal prostate

• Directs a different transcriptome in primary CaP
  • Promotes growth and survival

• Yet again a different transcriptome in ADT-RCaP

• Promotes growth and survival despite castrate levels of androgens
Onset of Androgen Independence

• “androgen-independent” CaP finds a myriad of ways to ensure there is AR signaling

AR is still interacting with the genome – but transcriptome differs.

Feldman and Feldman (2001), Nat Rev Cancer 1:34-45
The mutational load in prostate cancer is relatively low.

TCGA: Exon Mutation Rates

Argues that epigenetics must play a large role is shaping the prostate cancer phenotype.
Carcinogenesis

• Occurs at between 0% and 10% of CpG islands
  • ~1% to 3% is typical

• CpG island hypermethylation associated with gene silencing

• Why THIS gene, not THAT gene?
  • Selection can only partially explain; susceptibility must play a role
Could epigenetic mechanisms contribute to a different transcriptome being directed by the same nuclear receptor?

Could epigenetic marks reflect a different transcriptome being directed by the same nuclear receptor?

- 15 – benign prostatic hyperplasia (approximate ‘normal’ prostate)
- 13 – androgen stimulated CaP (AS-CaP) – enriched to >70% carcinoma
- 12 – androgen deprivation therapy recurrent CaP (ADT-RCaP)  
  • extremely rare samples – collected by TURP to relieve urinary symptoms during ADT

Perform restriction landmark genomic scanning (RLGS) analysis to measure CpG island methylation

• Assess methylation state of ~1200 CpG islands by methylation sensitive restriction enzyme
Greatly increased methylation phenotype in ADT-RCAP

![Graph showing significantly higher number of methylated CpG islands in AS-CaP compared to BPH, with p < 0.0001.](image-url)
Perspective

Table 1. Survey of RLGS methylation detected in various cancer types.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Samples (n=234 total tumors)</th>
<th>Mean RLGS Methylation (n=11997 spots)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent Prostate</td>
<td>12</td>
<td>7.4%</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>14</td>
<td>3.3%</td>
</tr>
<tr>
<td>Chronic Lymphocytic Leukemia</td>
<td>10</td>
<td>2.9%</td>
</tr>
<tr>
<td>Androgen Stimulated Prostate</td>
<td>13</td>
<td>2.8%</td>
</tr>
<tr>
<td>Acute Myelogenous Leukemia</td>
<td>33</td>
<td>2.0%</td>
</tr>
<tr>
<td>Colon</td>
<td>26</td>
<td>1.9%</td>
</tr>
<tr>
<td>Cervical</td>
<td>17</td>
<td>1.2%</td>
</tr>
<tr>
<td>Non-Small Cell Lung</td>
<td>16</td>
<td>1.2%</td>
</tr>
<tr>
<td>Head and Neck LN Mets</td>
<td>13</td>
<td>0.6%</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>25</td>
<td>0.5%</td>
</tr>
<tr>
<td>Primative Neuroectoderm</td>
<td>8</td>
<td>0.5%</td>
</tr>
<tr>
<td>Nonseminomatous Testicular</td>
<td>9</td>
<td>0.4%</td>
</tr>
<tr>
<td>Breast</td>
<td>14</td>
<td>0.3%</td>
</tr>
<tr>
<td>Head and Neck Primary</td>
<td>17</td>
<td>0.2%</td>
</tr>
<tr>
<td>Seminomatous Testicular</td>
<td>7</td>
<td>0.0%</td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>1.7%</td>
</tr>
</tbody>
</table>

ADT-RCaP has more than double the CpG island hypermethylation on the next most methylating cancer type.
Global confirmation of increased CpG island hypermethylation phenotype in ADT-RCaP
Illumina 450K Bead Array

Probe level analysis

Region level analysis

Pooled T-test

FDR p-value <=0.1  N  Y
Regions hypermethylated specifically in ADT-RCaP are enriched genes found to be downregulated in metastatic CaP from patients who failed ADT
Hypermethylation restricts promoter choice away from differentiation

- ERβ is methylated in early-stage disease – ablates downstream estrogen signaling
- ERβ is re-expressed in late-stage CaP – re-establishing downstream estrogen signaling
  - But a SKEWED estrogen signaling

ERβ targets that are hypermethylated in ADT RCaP enrich for genes involved in differentiation, cell fate decisions, and gland development.

The non-methylated targets do not show any enrichments.
Summary

• CpG island hypermethylation is common in AS-CaP
  • Level of CpG island methylation is similar to that seen in colorectal cancer

• CpG island hypermethylation is dramatically increased in RCaP
  • Level of CpG island methylation is more than double any other cancer

• Dramatically different CpG island methylation landscape in RCaP suggests that the way in which AR interacts with the genome may also be dramatically different.

What is the biological significance of these observations?
More loci are methylated, and also they are methylated more frequently. Suggests there is some level of selection of the targets. Functional? – methylation of the locus helps in acquisition of the phenotype? Locus susceptibility? – loci become preferred targets of broken methylation machinery as the cancer acquires androgen “independence”?
Carcinogenesis
Carcinogenesis

- Meaningful difference, or nitpicking?
  - Nitpicking in terms of gene “on” or “off”
  - Perhaps meaningful in terms of process

- Why should 10% of cells have methylation at a CpG?
  - Do the same cells have methylation in the next CpG, in which 20% of cells are methylated?
  - Does this shed any light on how we get from normal to cancer state?
Stable silencing

Dynamic equilibrium of histone modifications

Deacetylated

Transient silencing

Primed for activity

Strong transcription

Acetylated

RAR\(\alpha\) attracts complexes shifting local equilibrium

RA interaction changes the complexes shifting the equilibrium towards activation

Assembly of transcriptional machinery drives the equilibrium into the red

If RARα is altered, absent, or blocked up with an antagonist:
- perhaps a histone H3K9 de-methylase is lost from the region
- Allows for acquisition of H3K9 methylation
- This signals for DNA methylation

Aberrant RARα function pushes equilibrium towards stable silencing with histone methylation and DNA methylation
Onset of cancer may interfere with regulation, the arrow may be pushed (permanently?) to one extreme.
• Hypothesis: Dynamic, cyclical changes in DNA methylation occur broadly across the genome in response to AR stimulation in non-malignant cells.

  • Such dynamic regions may be susceptible to aberrant DNA methylation and heterochromatinization in malignant cells

• HPr-1AR cell line
  • Immortalized normal prostate epithelial cells (HPV16 E6/E7)
  • Non-malignant
  • Over expresses AR; translocates to nucleus after addition of ligand
    • Without ligand, cells grow well, semi-undifferentiated
    • With ligand, growth arrest; differentiation
Addition of DHT drives a differentiation program towards more luminal like phenotype

Is there a relationship between genes normally regulated by AR and aberrant methylation in CaP?
**Top 10 androgen upregulated genes in HPr-1AR cells are hypermethylated in CaP**

Table 1: Methylation and Expression status of candidate genes in prostate cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Methylated in AS-CaP (n=3)</th>
<th>Methylated in ADT-RCaP (n=3)</th>
<th>Downregulated in CaP; multiple Oncomine data sets</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRT73</td>
<td>80%</td>
<td>61%</td>
<td>X</td>
</tr>
<tr>
<td>TIPARP</td>
<td>76%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>S100P</td>
<td>54%</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td>AQP3</td>
<td>52%</td>
<td>81%</td>
<td>X</td>
</tr>
<tr>
<td>TMEM37</td>
<td>45%</td>
<td>75%</td>
<td>X</td>
</tr>
<tr>
<td>SGK1</td>
<td>43%</td>
<td>75%</td>
<td>X</td>
</tr>
<tr>
<td>SLCO2A1</td>
<td>43%</td>
<td>75%</td>
<td>X</td>
</tr>
<tr>
<td>CXCR7</td>
<td>43%</td>
<td>58%</td>
<td>X</td>
</tr>
<tr>
<td>MGC16121</td>
<td>36%</td>
<td>84%</td>
<td>X</td>
</tr>
<tr>
<td>SEMA3G</td>
<td>N/A</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

• Treat cells with DHT and collect DNA and _nascent_ RNA every 15 minutes
  • Measure transcription _rate_ and DNA methylation at each time point
    • Ask if methylation changes in relation to changes in transcription rate
• Dynamic methylation at SGK1 upstream androgen binding region

• Data represent biological triplicates

• No artificial synchronization other than addition of ligand

• Some CpGs show dynamics in methylation level
Can we apply this concept genome wide?

- Whole Genome Bisulfite Sequencing (WGBS) at 1, 24 and 96 hours
  - Do we see global variation in methylation over time?
  - Do these sites correlate with differential gene expression?
  - Are these sites susceptible to aberrant methylation in prostate cancer?
Whole-Genome Bisulfite Sequencing: Results

Cluster Dendrogram

Cluster Dendrogram

h9

h24

h0

h1
CpG Sites with changes in Methylation (+/- 10% relative to h0)

Gain

- h1: 1,073,546
- h24: 1,143,586
- h96: 1,096,758
- 217,146
- 287,305
- 200,352
- 212,450

Loss

- h1: 1,142,571
- h24: 1,225,292
- h96: 1,317,750
- 183,389
- 185,415
- 198,579
- 213,481
- 1,317,750
CpG Sites with changes in Methylation (+/- 30% relative to h0)

Gain

- h1: 41,676
- h24: 50,136
- h96: 48,958
- h1 and h24: 5,286
- h1 and h96: 5,117
- h24 and h96: 5,478
- h1, h24, and h96: 48,958

Loss

- h1: 44,168
- h24: 59,811
- h96: 73,976
- h1 and h24: 4,758
- h1 and h96: 4,973
- h24 and h96: 7,847
- h1, h24, and h96: 73,976
CpG Sites with progressive changes in Methylation

Gain

\[ h_{96} > h_{24} > h_{1} > h_{0} = 297,311 \]

\[ h_{96} > h_{24} > h_{1} > h_{0} \text{ & } h_{96} - h_{0} > 20 = 54,654 \]

\[ h_{96} - h_{24} > 10 \text{ & } h_{24} - h_{1} > 10 \text{ & } h_{1} - h_{0} > 10 = 711 \]

Loss

\[ h_{96} < h_{24} < h_{1} < h_{0} = 338,636 \]

\[ h_{96} < h_{24} < h_{1} < h_{0} \text{ & } h_{96} - h_{0} < -20 = 74,281 \]

\[ h_{96} - h_{24} < -10 \text{ & } h_{24} - h_{1} < -10 \text{ & } h_{1} - h_{0} < -10 = 1269 \]

- Methylation variable positions (MVPs) defined as showing progressive change over time and totaling >20% methylation change
- 128,935 MVPs out of total of ~11 million CpGs
- ~26,000 associated with transcriptional start sites (TSS)
- ~6,500 TSS have 2 or more MVPs; ~5,700 have 1 MVP; ~14,000 have no MVP
10kb window around TSS of a gene with no MVPs
10kb window around TSS of a gene with many MVPs
Genes containing >2 MVPs are **highly enriched** as targets of methylation in CaP.
Deregulated Promoter Methylation

HPr1AR+10nM DHT
Deregulated Promoter Methylation (1 MVP)

5771

TCGA-PRAD
Deregulated Promoter Methylation

467

p = 0.62

101
Non-MVP containing genes are **DE-ENRICHED** as targets of methylation in CaP.
Whole Genome Bisulfite Sequencing (WGBS) at 1, 24 and 96 hours

- Do we see global variation in methylation over time?
- Do these sites correlate with differential gene expression?
- Are these sites susceptible to aberrant methylation in prostate cancer?

Strong evidence that CpG regions that demonstrate dynamics in methylation levels during “quasi-normal” androgen driven basal to luminal epithelial cell differentiation are hyper-susceptible to aberrant methylation in prostate cancer.

Emphasizes the critical need for understanding normal epigenetic regulation, in order to gain insights into cancer specific epigenetic dysregulation.