Epigenetics in Cancer Pathogenesis

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**Epigenetics**

Modification of the genome without alteration of the nucleotide sequence

**Histone Modifications**
(PRCE2, KDMs, MLLs, JARIDs)

**DNA Methylation**
(DNMTs, TETs)

**“Active” Gene**
H3K4me3
Open chromatin
DNA hypomethylation

**“Repressed” Gene**
H3K27me3
Closed chromatin
DNA hypermethylation
Genetics contains all the information, Epigenetics are the instructions
Histone Modifications

$\text{H3K9me3} = \text{constitutively repressed genes}$

$\text{H3K9ac} = \text{transcriptionally active promoters}$

$\text{H3K36me3} = \text{actively transcribed gene bodies}$

$\text{H3K4me3} = \text{transcriptionally active promoters}$

$\text{H3K27me3} = \text{facultatively repressed genes}$

$\text{H3K4me3 + H3K27me3} = \text{“bivalent promoters”}$

Transcriptionally silent but poised for activations
Enhancer Regulation of Transcription

Open chromatin = DNAse hypersensitivity sites, ATAC-seq peaks

H3K4me1 = enhancers

H3K27ac = active enhancers

Transcription Factors = active enhancers
“Super” Enhancers

- Long genomic stretches of H3K27ac (Enhancers = kBs, Super-Enhancers = MBs)
- Important for cell-type identity
- Really just tissue-specific enhancers

Hnisz et al., 2013, Cell
An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element

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Your genome is more than the DNA sequence!!!

Epigenetic dysregulation is a major contributing factor in the pathology of a wide range of cancers.
Cancer = Genetics + Epigenetics

Figueroa et al. (2010)
Cancer Cell
Unlike genetic mutations, epigenetic modifications are malleable and can be (relatively) easily reversed.

**ClinicalTrials.gov**
A service of the U.S. National Institutes of Health

A Phase 1 Dose Escalation and Expanded Cohort Study of EPZ-5676 in the Treatment of Pediatric Patients With Relapsed/Refractory Leukemias Bearing a Rearrangement of the MLL Gene

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
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<tbody>
<tr>
<td>Leukemia</td>
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<td>Acute Lymphocytic Leukemia</td>
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<td>Acute Leukemias</td>
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**DOT1L inhibitor**

A Study Evaluating CPI-1205 in Patients With B-Cell Lymphomas

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<td>Phase 1</td>
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**EZH2 inhibitor**
Epigenetic Therapies in Cancer

DNA hypomethylating agents (HMAs) such as Decitabine are effective in a subset of high-risk myelodysplastic syndrome (MDS) patients.

Randomized Controlled Trial of Azacitidine in Patients With the Myelodysplastic Syndrome: A Study of the Cancer and Leukemia Group B

Low-Dose 5-Aza-2'-Deoxycytidine, a DNA Hypomethylating Agent, for the Treatment of High-Risk Myelodysplastic Syndrome: A Multicenter Phase II Study in Elderly Patients

Decitabine Improves Patient Outcomes in Myelodysplastic Syndromes

Results of a Phase III Randomized Study

- Current therapies cause global epigenomic changes
- Few specific changes likely important for pathogenesis
- Identification of targets, specific epigenomic remodelling

Silverman et al. (2002) J Clin Onc

Wijermans et al. (2000) J Clin Onc

Kantarjian et al. (2006) Cancer
DNA methylation

- **DNMT1** = DNA methylation maintenance
- **DNMT3A/B** = de novo DNA methylation
- **TET1/2/3** = demethylation
DNA methylation

Biological Functions of DNA Methylation
- Genomic imprinting
- X-chromosome inactivation
- Silencing of repetitive elements
- Regulation of gene transcription

Precise regulatory role of DNA methylation in different genomic contexts still relatively undefined
### Mutations in Epigenetic Modifiers in Hematopoietic Disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>Epigenetic Modification</th>
<th>HSC Mutant Phenotype</th>
<th>Hematopoietic Disease</th>
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<tbody>
<tr>
<td>DNMT3A</td>
<td>DNA methylation</td>
<td>Self-renewal advantage</td>
<td>AML, MDS, MPN, T-ALL, T-lymphoma</td>
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<tr>
<td>TET2</td>
<td>5-hydroxymethylcytosine</td>
<td>Self-renewal advantage</td>
<td>AML, MDS, MPN, T-lymphoma</td>
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<tr>
<td>IDH1/2</td>
<td>5-hydroxymethylcytosine*</td>
<td>Self-renewal advantage</td>
<td>AML</td>
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<td>ASXL1</td>
<td>Chromatin</td>
<td>Reduced self-renewal</td>
<td>AML, MDS, MPN</td>
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<td>EZH2</td>
<td>H3K27me3</td>
<td>Increased differentiation and apoptosis*</td>
<td>AML, MDS, B-lymphoma, T-ALL</td>
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<td>SUZ12</td>
<td>H3K27me3</td>
<td>Enhanced HSC activity</td>
<td>MPN, T-ALL</td>
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<tr>
<td>MLL1</td>
<td>H3K4me3</td>
<td>Increased proliferation and differentiation</td>
<td>MLL-fusions</td>
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<td>DOT1L</td>
<td>H3K79me3</td>
<td>HSC exhaustion</td>
<td>MLL-fusions</td>
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<tr>
<td>DNMT3B</td>
<td>DNA methylation</td>
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<td>ICF syndrome</td>
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**Chen et al. (2013)** Nature Genetics

**Patel et al. (2012)** NEJM
DNMT3A and TET2 Regulate DNA Methylation

- DNMT3A catalyzes DNA methylation at CpG dinucleotides
- TET2 converts 5-methylcytosine to 5-hydroxymethylcytosine

Shih et al., Nature Reviews Cancer, 2012

**Biochemically antagonistic but the mutations produce similar functional consequences for HSCs**
AML, MDS

Papaemmanuil et al., 2013, Blood
**DNMT3A and TET2 Mutations in AML**

- Founding clone / initiating event
- Poor clinical outcomes
- Resistant to conventional chemotherapy / mediate relapse

**Ley et al., 2010, NEJM**

**Klco et al., 2015, JAMA**
Modelling DNMT3A and TET2 Mutations in Mice

• Genetic inactivation of *Dnmt3a* and *Tet2* in mouse hematopoietic system leads to MDS-like disease
  - *Tet2* inactivation in mouse hematopoietic system leads to CMML.
  - Non-competitive transplantation of *Dnmt3a*-null HSCs / BM leads to cytopenias and bone marrow failure resembling MDS.

  Moran-Crusio *et al.*, Cancer Cell, 2011

  Celik *et al.*, Blood, 2015

• Competitive transplantation of *Dnmt3a*- and *Tet2*-null HSCs leads to a clonal expansion in the bone marrow.
  - Higher self-renewal on a per-HSC basis.

  Moran-Crusio *et al.*, Cancer Cell, 2011

  Challen *et al.*, Nature Genetics, 2012
Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Genovese et al., 2014, NEJM
Hematopoietic Stem Cells (HSCs)

- Regenerate the blood
- Long-term self-renewal
- Multi-lineage differentiation
Loss of *Dnmt3a* Affects HSC Fate Decisions *in vivo*

**Mx1-cre:Dnmt3a**

- **1° Tx**
  - 250 purified HSCs
  - 200,000 whole bone marrow cells
  - CD45.1 wild-type competitors
  - Loss of *Dnmt3a* Affects HSC Fate Decisions

**Graphs:**
- **% Donor-derived peripheral blood cells**
  - Weeks Post-Transplant
  - Control vs. Dnmt3a-KO
- **# of Donor-derived HSCs / mouse**
  - Control vs. Dnmt3a-KO
Dnmt3a Regulates the Balance Between HSC Self-Renewal and Differentiation

**Differentiation**

Differentiation per HSC

= WBC cells generated / donor HSCs

**Self-Renewal**

Self-Renewal per HSC

= number of HSCs generated / input HSC

Dnmt3a Regulates the Balance Between HSC Self-Renewal and Differentiation
DNA Methylation Changes do not Predict Gene Expression Differences in \textit{Dnmt3a}-null HSCs

\textbf{DNA Methylation (RRBS, WGBS)}

\textbf{Gene Expression (Microarray, RNA-SEQ)}

\textbf{No global correlations between changes in DNA methylation and altered gene expression}

\textbf{Able to find cause-and-effect for a select subset of genes > HSC self-renewal network}
Differentiated Progeny of *Dnmt3a*-null HSCs Show Incomplete Repression of “HSC Stemness” Genes

**MS-HPLC**

- **Runx1**
  - Avg. meth = 92.4%
  - Avg. meth = 55.5%
- **Vasn**
  - Avg. meth = 86.1%
  - Avg. meth = 22.9%

**DREAM**

- Hypomethylation and expression of HSC genes in differentiated cells
- **Dnmt3a** represses the “stem cell program” in HSCs to permit lineage differentiation

**Control B-cell DNA Methylation**

- **Dnmt3a-KO B-cell DNA Methylation**

**Vasn, Runx1**

- B-cells
Function of Dnmt3a in Normal and Malignant Hematopoiesis

Signal for differentiation

LT-HSCs

Self-renewal gene
DNA methylation - HSCs

Self-renewal gene
DNA methylation – differentiated cells

Pathogenesis

Control

Dnmt3a

Dnmt3a

Dnmt3a-KO

Mechanisms of DNMT3A mutant transformation?

• Altered DNA methylation?
• Something else

Additional genetic and/or epigenetic lesions
CHIP

AML, MDS

T-ALL

Jaiswal et al., 2014, NEJM

Papaemmanuil et al., 2013, Blood

Odejide et al., 2014, Blood
DNMT3A Mutations in T-ALL

Grossman et al., 2013, Genes Chrom Cancer

Poitras et al., 2016, Oncotarget
T-ALL DNMT3A Mutations are Different than AML

- Almost always heterozygous.
- Enriched for R882 dominant negative variant.
- Founding mutation, resistant to conventional chemotherapy, often mediates relapse.

- Can be heterozygous, homozygous, or compound heterozygous.
- Not enriched for R882 mutations.
- Unclear if founding / co-operating / passenger mutation, role in relapse not defined.
**Dnmt3a** loss-of-function Co-operates with **Notch1** gain-of-function to accelerate T-ALL

Control mice: Mx1-Cre+:Dnmt3a^{+/+}
Test mice: Mx1-Cre+:Dnmt3a^{fl/fl}

4-weeks

5-FU injection

6-days

Harvest BM, purify c-Kit+ cells, transduce

MIG-Notch1 Intracellular domain (NICD)

Transplant

Lethally irradiated CD45.1 recipients

Control NICD T-ALL

Dnmt3a^{K0} NICD T-ALL

**Survival (%)**

Days post-transplant

Control

Dnmt3a^{HET}

Dnmt3a^{KO}
Molecular Profiling to Identify Therapeutic Targets

**eRRBS (DNA methylation)**

- Dnmt3a-null T-ALL characterized by hypomethylation.
- DNA methylation does not predict gene expression.
Summary and Significance

• Epigenetic modifications provide alternative pathways to oncogenesis *in lieu* of or in collaboration with genetic mutations.

• Genetic mutations in epigenetic modifiers are common in hematopoietic malignancies.

• *DNMT3A* and *TET2* loss-of-function mutations predispose HSCs to transformation and a wide range of diseases.
Clinical Implications and Open Questions

• *DNMT3A* and *TET2* mutations almost always occur in the founding disease clone, and are almost never cleared by conventional chemotherapy.

• What other epigenetic or molecular dependencies do these mutations induce in the HSCs that might be amenable to therapeutic intervention?

• How do these mutations give HSCs a clonal advantage and cause disease?
  
  ❖ Necessary but not sufficient
  ❖ Cell intrinsic versus cell extrinsic contributions
  ❖ If not altered DNA methylation, then what else?
DNMT3A mutations promote anthracycline resistance in acute myeloid leukemia via impaired nucleosome remodeling

Olga A Guryanova¹, Kaitlyn Shank¹, Barbara Spitzer², Luisa Luciani³, Richard P Koche⁴,⁵, Francine E Garrett-Bakelman⁶, Chezi Ganzel⁷, Benjamin H Durham¹, Abhinita Mohanty⁸, Gregor Hoermann⁹, Sharon A Rivera¹⁰, Alan G Chramiec⁴, Elodie Pronier¹, Lennart Bastian¹, Matthew D Keller¹, Daniel Tovbin¹, Evangelia Loizou⁵, Abby R Weinstein¹, Adriana Rodriguez Gonzalez¹, Yen K Lieu¹⁰, Jacob M Rowe⁷, Friederike Pastore¹, Anna Sophia McKenney¹, Andrei V Krivtsov⁴, Wolfgang R Sperr¹¹, Justin R Cross¹², Christopher E Mason¹³, Martin S Tallman¹⁴, Maria E Arcila⁸, Omar Abdel-Wahab¹,¹⁴,¹⁵, Scott A Armstrong²,⁴,⁵, Stefan Kubicek¹⁶, Philipp B Staber¹¹, Mithat Gönen¹⁷, Elisabeth M Paitetta¹⁸, Ari M Melnick⁶, Stephen D Nimer³, Siddhartha Mukherjee¹⁰ & Ross L Levine¹,⁴,¹⁴,¹⁵
Figure 1 – $Dnmt3a^{R878H}$ mutations augments HSC function and cooperates with co-occurring AML disease alleles in vivo.

**Dnmt3a**
Supplementary Figure 1 – Characterization of the steady-state hematopoietic phenotype in aged Dnmt3a\textsuperscript{mut} mice
Figure 2 – Expression of mutant *DNMT3A* leads to anthracycline resistance
Figure 2 – Expression of mutant DNMT3A leads to anthracycline resistance
Figure 3 – Cells with mutant DNMT3A have a DNA damage signaling defect in response to anthracyclines.
Supplemental Figure 5 – Cells with mutant DNMT3A have attenuated response to anthracycline-induced DNA damage
Figure 4 – Expression of mutant DNMT3A impairs chromatin remodeling in response to DNA distortion

(a) Viability, relative to untreated, vs. Aclarubicin concentration (μM).
(b) Viability, relative to untreated, vs. Etoposide concentration (μM).
(c) Western blot analysis showing changes in soluble nuclear extract and chromatin proteins after treatment with DnR (50 ng/ml).
(d) Western blot analysis showing specific binding of Dnmt3a after IP with DnR (250 ng/ml).
(e) Western blot analysis showing changes in DNMT3A expression in 32D cells treated with DnR (250 ng/ml).
(f) Western blot analysis showing changes in SPT-16 and TFIIH expression in U2OS cells with shSPT-16.
(g) Western blot analysis showing changes in histone H3 expression in U2OS cells treated with DnR (100 ng/ml, 4 h) and mutant DNMT3A.
Supplemental Figure 7 – Direct protein-protein interaction between Dnmt3a and Spt-16

A

<table>
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<th>10% input</th>
<th>IP: DNMT3A</th>
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293T

Vector
DNMT3A wt
DNMT3A mut

DNMT3A

SPT-16

IgG light chain

H3K36me3

B

MEF

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Dnmt3a
Spt-16
H3K36me3

C

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MOLEM-13

DnR
100 ng/ml, 4h

SPT-16
DNMT3A
Supplemental Figure 7 – Direct protein-protein interaction between Dnmt3a and Spt-16