Aberrant epigenetic programming in cancer

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Genetics and Epigenetics

EPIgenetic information

DNA - genetic information

Inherited across generations

Transmitted by mother to daughter cells

Required for life
Comparing genetic and epigenetic codes

DNA - genetic code
4 nucleotides

EPIgenetic code
Hundreds of proteins and chemical modifications

DNA Methylation  Histones  Polycomb proteins

.... A T C G ....
Epigenomic programming is at least in part encoded through DNA methylation and histone modifications.

CpG methylation

Histone modifications

Transcription factors

EVI1
CEBPA
PU1
AMLETO
PMLRAR
BCL6
Epigenetic vs Genetic information

- **EPIgenetic information has plasticity**
- **Genetic information is constant**

Conception ➔ Death
Epigenetic plasticity is required for complexity
Epigenetic programming can be erased

EPigenetic code - Can be reprogrammed

Genetic code - Cannot be reprogrammed

Initiation  Death
Cloned melanoma cells contribute to normal development

Horschlinger et al, Genes and Development
Terminally differentially cells can be reprogrammed into stem cells

Amabile and Meissner et al, Trends in Molecular Medicine
De novo changes in epigenetic patterning

Thus, depending on which way the ball happened to roll (i.e., depending on the cell's particular developmental history and current signaling events), the cell's fate and its opportunities for reprogramming would change. Similarly, the cell could affect the landscape of other cells that are fellow travelers by its own influence on the environment within the developing tissue through such mechanisms as nonautonomous cell signaling.

Note that it was recently shown that increasing signaling variance in a cell population increases (group) information transduction capacity as much as 4-fold during tumor necrosis factor signaling (Cheong et al., 2011). What we are describing can be seen, in cartoon form, as a revision of Waddington's landscape (Figure 2, right); here, the changing depth of the hills and valleys are governed, in part, by changes in nuclear structure, which could include LADs, LOCKs, hypomethylated blocks, and 3D structural variations of the nuclear lamina. Such structures are continually responding to cues and signals, both intra- and extracellular.

We call attention to the fact that neither we nor Waddington intend for the rolling ball analogy to represent an inexorable pathway from stem cell to end stage differentiation. Waddington himself says, anticipating our own travails as biologists in constructing our model, “A multidimensional phase space is not very easy for the simple-minded biologist to imagine or to think about,” (Waddington, 1957), but he is interested in “the course by which [developmental change] gets there,” as are we. We are proposing that the epigenome contributes not only to the mean levels of gene expression (as others have discussed), but also to altering variability and how it is affected by stochastic noise; thus, the epigenome facilitates noise-induced phase transitions and the promotion or resolution of pluripotency.

Waddington represented his idea as a system of ordinary differential equations. In reading his “The Strategy of the Genes,” one can appreciate that these mathematical constructs were important to how Waddington conceptualized embryology and development, with numerous mentions of phase spaces and steady states. We thus find it appropriate to attempt to extend the mathematical formalism as we attempt to extend the proposed biological mechanisms underpinning the landscape.

We can adopt mathematical language from modern statistical mechanics. The idea that phase transitions (such as states of matter, oscillating chemical reactions, and optical bistability) can be induced by noise has been used in the study of many physical processes, which can be modeled by a stochastic differential equation of the following form:

\[
dX_t = f(X_t) \, dt + s \, dW_t
\]

in which \(W_t\) describes a Wiener process, or continuous stochastic process. The transitions between the available states occur across the overall space described by the coordinate \(X\), and \(X_t\) reflects a developmental state that may be affected by environmental signals. Thus, the change in state depends on...

Figure 2. Regulated Noise in a Dynamic Epigenetic Landscape

On the left is a depiction of the classical Waddington representation of canalization, in which the ball rolling down the hill is directed into one of multiple valleys as a consistent endpoint, despite perturbation that might occur on the way. Waddington suggested a deterministic model with genes (small black circles below) pulling on the landscape from below to direct these endpoints. Changes in the landscape would arise by mutations in the genes. On the right, we suggest that modulation of the effects of noise is regulated during development and in response to external cues, which affects the contour of the epigenetic landscape itself.

During differentiation, as the ball rolls down the hill, nuclear structure changes in a metastable manner through, for example, structures such as LOCKs and methylated blocks, thus changing the steepness of the valleys. At the same time, new chromosomal interactions could increase localized variability in ways that were not possible at the ground state—in this case, changing the landscape to open an alternative pathway to diversity (new bifurcation shown below the ball). The other shapes represent chromatin modifications (red circles), lamin proteins (green), and chromosome interactome mediators (red pentagon).
Stress induced epigenetic reprogramming as a trigger for malignant transformation?

Johnstone and Baylin, Nature Reviews Genetics
Stress induced epigenetic reprogramming as a trigger for malignant transformation?

![Graph showing protein levels](image)

O'Hagan et al, Cancer Cell 2011
Genetic profiling identifies extensive mutation in epigenetic modifiers

Gene Expression Potential

DNA METHYLATION

NUCLEOSOME (Remodeling & Positioning)

HISTONE (Modifications & Variants)

DNMT1
DNMT3A
TET1/2
IDH1/2

SNF5
BRG1
BRM
ARID1A
ARID2
PBRM1
ATRX

MLL1/2/3
SETD2
EZH2
JARID1C
UTX
BMI1
LSD1

PA Jones, Cancer Cell 2012
Hypothesis: Epigenetic programming encodes tissue specification including neoplasia
Aberrant epigenetic programming is hallmark of leukemia, and can be used to classify the disease into biologically relevant subtypes.
Aberrant epigenetic programming is a hallmark of AML

DNA methylation, Gene expression, Histone Modification, Mutation Profiling

344 AML patients from HOVON clinical trials

383 AML patients from ECOG E1900 phase III trial

Unsupervised analysis = 16 epigenetically defined clusters

Some linked to genetic background, others are unique

Some are hypomethylated, others hypermethylated

Much of the variation is outside of CpG islands

Figueroa et al, Cancer Cell, Jan 2010, Figueroa et al, Cancer Cell, Dec 2010
Genetically defined B-ALL subtypes display distinct epigenetic profiles

**DNA methylation, Gene expression, Mutation Profiling**

Figueroa et al, J Clin Invest (under review)

167 B-ALL patients seen at St Jude Childrens Hospital

Figueroa, et al; in preparation

178 independent Child B-ALLs

167 B-ALL patients seen at St Jude Childrens Hospital

Figueroa, et al; in preparation

215 adult B-ALL patients enrolled in E2993 phase III clinical trial

Geng et al Cancer Discovery 2012

Geng et al unpublished
Cytosine methylation profiles classify CLL into three distinct disease subtypes

DNA methylation, Gene expression, Mutation Profiling

242 clinically annotated CLL patients

Fang et al, unpublished
A common epigenetic signature may be required to reprogram normal HSCs to a leukemic phenotype.
45 genes almost universally hypermethylated in AML regardless of genetic background

- ZNF proteins
- Nuclear import proteins
- Regulators of myeloid cytokines
- Members of the mediator complex
- Retinoic acid signaling
- Membrane anchor proteins
- Tumor suppressors
- Regulators of STAT signaling

Figueroa et al, Cancer Cell, Jan 2010
Hypermethylation signature genes have preleukemic phenotype

90% are also silenced

shRNA induces
Serial replating,
Differentiation block
LSK expansion
increased colony formation

Overexpression induces
Growth arrest
Impairs transformation by MLL fusions

Maria Figueroa

Figueroa, et al, unpublished
A common signature of hypermethylated genes in B-ALL

- Inversely correlated with GE
- Not inversely correlated

The common DNA methylation signature of B-ALL patients

Figueroa, et al, in preparation
Do Hematopoietic Cells Have to Jump Over an Epigenetic Barrier to Become Transformed?
The common signature of CLL involves activation of the B-cell receptor signaling pathway.

Fang, et al, unpublished
Epigenetic signatures and classifiers are powerful independent clinical biomarkers
Cytosine methylation classifiers and somatic mutation of epigenetic modifiers allows superior risk stratification in AML

Epigenetic signatures define risk independent of genetic and clinical

Log rank p<0.003

5mC classifier most potent independent biomarker of outcome in AML

A Effect of Mutational Profiling

<table>
<thead>
<tr>
<th>Cytogenetic Classification</th>
<th>Mutational Analysis</th>
<th>Integrated Classification</th>
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<tbody>
<tr>
<td>Favorable: 19% of cohort (3-yr OS: 58%)</td>
<td>Favorable: 26% of cohort (3-yr OS: 64%)</td>
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<tr>
<td>Intermediate: 63% of cohort (3-yr OS: 36%)</td>
<td>Intermediate: 35% of cohort (3-yr OS: 42%)</td>
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<tr>
<td>Unfavorable: 18% of cohort (3-yr OS: 11%)</td>
<td>Unfavorable: 39% of cohort (3-yr OS: 12%)</td>
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Somatic mutations in epigenetic modifiers - New risk stratification classification for AML

Specific DNA methylation signature in BCR-ABL positive B-ALL features hypomethylation of an IL2RA network

- **BCR-ABL DNA methylation signature**
  - 192 probesets, 170 genes, adjusted p<0.01, dx>1
  - BCR/ABL(+) 74+9=83
  - BCR/ABL(-) 18+114=132
  - Accuracy: 87%

- **MassArray IL2RA**
  - p<1e-6
  - 2kb

- **IL2RA network**
  - IL2RA/CD25
  - p=0.0064

- **IL2RA expression**
  - Relative expression
  - Me  Exp

- **K-M curve OS in BCR-ABL+ B-ALL**
  - BCR/ABL(+) CD25(-), n=44
  - BCR/ABL(+) CD25(+), n=69

- **K-M curve OS in AML**
  - CD25 - (n=321)
  - CD25 + (n=75)
Epigenetically defined subtypes in CLL have distinct clinical outcomes and are linked with specific biological pathways.

Fang et al Unpublished

Aberrantly methylated network in poor risk cluster

Aberrantly methylated network in low risk cluster

K-M OS of CLL patients

p-value:2.98e-07

NFκB

ZAP70

BCL6

HATs
Epigenetic heterogeneity and evolutionary fitness
Germinal Center B-cell Differentiation

Naive B-cell

MZ B-cells

Notch2

BCL6

Centroblasts

AID

SHM

DARK ZONE

GERMINAL CENTER
DNMT1 is required for germinal center formation

Shaknovich et al Blood 2011
AICDA and DNMT3A associated hypomethylated gene signature in germinal center B-cells

DNA methylation heterogeneity in GC B-cells, a precursor to lymphomagenesis?

Shaknovich et al Blood 2011
Progression of epigenetic heterogeneity from normal B-cells to malignant lymphomas

De et al PLoS Genetics 2013
DNA methylation heterogeneity is an independent outcome predictor in GC derived B-cell lymphomas

De et al PLoS Genetics 2013
Large variable methylation regions linked to chromatin landscapes in malignant transformation

VMRs in Cancer Are LOCKs in Developmental Reprogramming

(A–D) Large variably methylated regions (VMRs) in cancer (C) were identified by whole-genome bisulfite sequencing. These VMRs are hypomethylated in cancer and largely correspond to nuclear lamina-associated large organized chromatin lysine (K)-modifications (LOCKs), which can be visualized by electron microscopy (A, bottom) and native chromatin immunoprecipitation (D). The same well-defined chromatin compartments that are reprogrammed during developmental cell fate transitions display altered variance across many types of cancer (B and C). Images reprinted with permission from Hansen et al. (2011) and McDonald et al. (2011).

Leukemia oncoproteins directly drive aberrant cytosine methylation patterning
EVI1 drives a malignant hypermethylation signature through recruitment of DNMT3A and DNMT3B

EVI1 is bound to methylated genes

EVI1 interacts with DNMTs

Hypermethylated genes feature Evi1 binding sites

EVI1 signature is hypermethylated

Methylation profile featuring Evi1 expression

Lugthart et al, Blood 2011
EVI1 is a mediator of aberrant epigenetic programming in AML
**MLLr B-ALLs feature aberrant DNA methylation signature**

430 probesets, 379 genes, adjusted p<0.01, dx>1

- **MLLr(+)**: 25+3=28
- **MLLr(-)**: 1+96=97

**Accuracy**: =97%

- **Probability MLLr-positive**
- **Probability MLLr-negative**

**MLLr DNA methylation and gene expression signatures**

**FLT3**

**Expression**

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<th>MLL fusion targets</th>
<th>Non-targets</th>
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<td>p &lt; 1e-6</td>
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**Methylation**

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Log2 (HpaII/MspI)
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**Geng et al, Cancer Discovery 2012**
MLLr binding is linked to hypomethylation, H3K79me, and transcriptional activation

competition? DOT1L MLL AF4 DNMTs

RNA Pol II H3K79me2 Nucleosome

Geng et al, Cancer Discovery 2012

Jay Hess, Scott Armstrong, Nancy Zeleznik-Le
MLLr drive BCL6 expression in B-ALL, which in turn plays a central role in maintaining the survival and proliferation of these tumors.

Geng et al, Cancer Discovery 2012

MLL-AF4 siRNA causes downregulation of HOXA9 and BCL6

BCL6 is hypomethylated in MLLr vs non-MLLr and vs. normal pre B-cells

Development of specific BCL6 inhibitors

Polo et al Nature Medicine 2004
Cerchietti et al Cancer Cell 2010

RI-BPI suppresses colony formation by primary E2993 MLLr cells

RI-BPI kills primary MLLr cells
Novel epigenetic cluster enriched in AMLs with IDH1/2 mutations

Figueroa et al, Cancer Cell, Dec 2010
Mice with IDH<sup>R132H</sup> knockin develop MPN and precisely recapitulate the DNA methylation defect of the human disease.

ERRBS

Shore | CpGi | Shore

Akalin et al, PLoS Genetics 2012,
Alkalin et al, Genome Biology 2012
Li et al BMC Bioinformatics, 2013
Sasaki et al Nature 2012

MPN phenotype

Profound hypermethylation

TSS-centered hypermethylation

WNT/Notch/TGFb
Mutant IDH produces aberrant oncometabolite 2HG, which suppresses TET mediated hydroxymethylation.

Figueroa et al, Cancer Cell, Dec 2010

**TET2 hydroxylates 5 mC**

**Global 5-hmC levels**

IDH and TET2 mutations are mutually exclusive.
Loss of TET2 leads to increased stem cells, self renewal, MPN phenotype and massive DNA hypermethylation.

**Hypermethylated signature in TET2 AMLs**

**Increased self-renewal in TET2 -/- HSCs**

**MPN phenotype in TET2 -/- mice**

Figueroa et al, Cancer Cell, Dec 2010
Moran-Cusio et al, Cancer Cell 2011, and unpublished data
Discovery of a class of AMLs driven by aberrant DNA 5’hydroxymethylation
TET2 and IDH AMLs display overlapping 5hmC and 5mC profiles

5hmC peaks form overlapping subsets in IDH1,2 and TET2 AMLs

Distinct 5hmC profiles in TET2 and IDH AMLs

Altuna Akalin, unpublished
Positive and negative correlation with gene expression of 5hmC and 5mC respectively

Altuna Akalin, unpublished
IDH and TET2 are not the only leukemias with perturbed 5hmC
Forces driving epigenetic gene regulation in leukemia

Genetic lesions (IDH, TET2, etc)

Epigenetic instability

Stochastic variation

Oncoprotein specific signature (MLL, IDH, EVI1 etc)

Natural Selection (45 gene AML sig, etc)

Random noise (MDS, secondary AML)
Epigenetic targeted therapy
Rationale for DNMT-i therapy in DLBCL

DLBCLs are highly proliferative

Relatively short exposure should allow excellent penetration of tumor mass

DNMT1 is required for normal GC B-cells

Epigenetic clonal complexity may contribute to pathogenesis

Aberrant hypermethylation of tumor suppressors documented as being clinically significant

Low demethylating dose drug should not have cross-toxicity with chemotherapy
DNMTi overcome chemotherapy resistance in DLBCL by inducing a SWING phenotype

Cell line characterization

Low dose, long exposure induces SWING phenotype

Low dose, long exposure induces chemosensitization

Chemosensitization of primary DLBCLs

In vivo enhancement of chemotherapy
SMAD1 methylation is a biomarker for chemo-resistant disease and biologically contributes to resistance.

- SMAD1 methylation silencing linked to resistance
- SMAD1 hypermethylation in resistant cell lines
- SMAD1 induced by DNMT-i

**Graphs and Figures:**

- SMAD1 methylation and expression linked to ABCs
- SMAD1 methylation linked to primary refractory DLBCL
- SMAD1 complements chemotherapy resistance

*Clozel et al, Submitted*
Phase I trials in newly diagnosed and relapsed DLBCL

**Newly Diagnosed DLBCL**
- **5 Aza + RCHOP**
  - Day 1: 5 Azacytidine
  - Day 5: Biopsy
  - Day 7: Biopsy

**Relapsed DLBCL**
- **Oral 5 Aza + RDICE**
  - Day 1: 5 Azacytidine
  - Day 5: Biopsy
  - Day 7: Biopsy
AZA-RCHOP well tolerated and apparently effective in high risk newly diagnosed DLBCL

Clozel et al, Submitted but still being blocked by reviewer #2
Aberrant epigenetic programming is hallmark of leukemia, and can be used to classify the disease into biologically relevant subtypes. A common epigenetic signature may be required to reprogram normal HSCs to a leukemic phenotype. In CLL, a common epigenetic signature is massive and involves BCR signaling pathway. Epigenetic signatures and classifiers are powerful independent clinical biomarkers. Epigenetic programming points towards novel disease mechanisms.

EVI1: the first example of a leukemogenic factor that induces a specific epigenetic program. MLLr B-ALL feature coordinated hypomethylation, fusion protein binding and H3K79me3 driving expression of oncoproteins. IDH1/2 and TET2 mutations define a new subtype of AML featuring deregulated 5hmC patterning.
DNMT1 required for GC formation - perhaps because of replication function?

Hypomethylation signature typical in GC - AICDA and DNMT3A

Unique epigenetic instability phenotype in GC B-cells, evidence of epigenetic clonality in GC B-cells

Progressive heterogeneity associated with disease phenotype

Epigenetic heterogeneity is an independent clinical risk factor

GCB and ABC DLBCLs have unique epigenetic signatures

Demethylating dose MTI induce SWING phenotype to chemosensitize resistant DLBCLs

Hypermethylation and repression of SMAD1 contributes to and is a biomarker for epigenetically mediated chemotherapy resistance

AZA-RCHOP is well tolerated - CALGB multicenter phase II starting