DNA Methylation in MDS/MPD/AML: Implications for application

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Disclosures

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Scientific Advisory Board: OncoMethylome Sciences

I will not be discussing off-label use.
Methylation changes in Neoplasia

When do changes in DNA methylation develop?
Is the detection of DNA methylation of prognostic or predictive use?
Are epigenetic changes targets for therapy?
Gene Specific Methylation in MDS: p15/CDKN2B

Table 1  
Hypermethylation of p15 in MDS with and without blast expansion

<table>
<thead>
<tr>
<th>Authors</th>
<th>All MDS</th>
<th>RA, RARS</th>
<th>RAEB, RAEB-t, CMMoL, OL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uchida et al (1997)</td>
<td>16/32 (50)</td>
<td>1/12 (8)</td>
<td>15/20 (75)</td>
<td>15</td>
</tr>
<tr>
<td>Quesnel et al (1998)</td>
<td>20/53 (38)</td>
<td>0/12 (0)</td>
<td>20/41 (49)</td>
<td>16</td>
</tr>
<tr>
<td>Tien et al (2001)</td>
<td>9/45⁹ (20)</td>
<td>0/13 (0)</td>
<td>9/32 (28)</td>
<td>19</td>
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<tr>
<td>Preisler et al (2001)</td>
<td>11/14 (79)</td>
<td>3/6 (50)</td>
<td>8/8 (100)</td>
<td>20</td>
</tr>
<tr>
<td>Daskalakis et al (2002)</td>
<td>15/23 (65)</td>
<td>3/5 (60)</td>
<td>12/18 (67)</td>
<td>21</td>
</tr>
<tr>
<td>Chen and Wu (2002)</td>
<td>14/22 (64)</td>
<td>5/13 (38)</td>
<td>9/9 (100)</td>
<td>22</td>
</tr>
<tr>
<td>Hofmann and Koeffler (2002)</td>
<td>34/57 (60)</td>
<td>19/34 (56)</td>
<td>15/23 (65)</td>
<td>17</td>
</tr>
<tr>
<td>Au et al (2003)</td>
<td>6/10 (60)</td>
<td>3/3 (100)</td>
<td>3/7 (42)</td>
<td>23</td>
</tr>
<tr>
<td>Aoki et al (2003)</td>
<td>9/24 (38)</td>
<td>0/9 (0)</td>
<td>9/15 (60)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>134/280 (48)</td>
<td>34/107 (32)</td>
<td>100/173 (58)</td>
<td></td>
</tr>
</tbody>
</table>

RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; RAEB-t, refractory anemia with excess blasts in transformation; CMMoL, chronic myelomonocytic leukemia; OL, overt leukemia after previous MDS; mC, hypermethylation. For Hofmann and Koeffler⁷ see also: Hofmann WK et al. Blood 2000; 96: (Suppl 1). 834 (abstract).

⁹Methylation determined at initial study.
p15/CDKN2B in MDS/AML:
Increase with progression and use for predicting survival

Christiansen,
Leukemia, 2003, 1813
p15/CDKN2B in MDS/AML:
Interaction with Important Genetic Abnormalities

Table 4  Methylation of p15 related to clinical, cytogenetic and genetic characteristics of 81 patients with t-MDS or t-AML

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Methylation, Unmethylation, N=55</th>
<th>N=26</th>
<th>P^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) (median)</td>
<td>60</td>
<td>52.5</td>
<td>0.07^d</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>30/25</td>
<td>13/13</td>
<td>0.81</td>
</tr>
<tr>
<td>Previous treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkylating agents+cisplatin</td>
<td>42</td>
<td>21</td>
<td>0.78</td>
</tr>
<tr>
<td>Topoisomerase II inhibitors</td>
<td>23</td>
<td>12</td>
<td>0.81</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>25</td>
<td>14</td>
<td>0.63</td>
</tr>
<tr>
<td>Latent period (median months)</td>
<td>51</td>
<td>51</td>
<td>0.54^d</td>
</tr>
<tr>
<td>Platelet counts (median)^b</td>
<td>50</td>
<td>46</td>
<td>0.47^d</td>
</tr>
<tr>
<td>Cytogenetic and genetic abnormalities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deletion or loss of 7q</td>
<td>31</td>
<td>4</td>
<td>0.0006</td>
</tr>
<tr>
<td>Deletion or loss of 5q</td>
<td>17</td>
<td>3</td>
<td>0.096</td>
</tr>
<tr>
<td>Deletion or loss of 17p</td>
<td>7</td>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>Recurrent balanced aberrations</td>
<td>7</td>
<td>5</td>
<td>0.51</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>6</td>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>One or two aberrations</td>
<td>19</td>
<td>13</td>
<td>0.22</td>
</tr>
<tr>
<td>(\geq 3) Aberrations</td>
<td>30</td>
<td>7</td>
<td>0.03</td>
</tr>
<tr>
<td>FLT3/ITD and MLL/ITD</td>
<td>5</td>
<td>0</td>
<td>0.17</td>
</tr>
<tr>
<td>p53 mutations^c</td>
<td>16</td>
<td>5</td>
<td>0.42</td>
</tr>
</tbody>
</table>

^aIf not otherwise indicated, Fisher’s exact test (two sided).
^bPlatelet counts were available in 65 of 81 cases.
^cP53 mutational status was available in 75 of 81 cases.\(^24\)
^dWilcoxon’s two-sample test (two sided).
Why Has Methylation Been Inconsistent as a Prognostic Marker?

• No systematic analysis of methylation and prognostic, cytogenetic and molecular markers

• Gene selection and methods
  – How do we choose the right genes?
  – Does method effect use as marker? Is it presence of methylation or density (level) that affects prognosis?

• Confounders
  – good and poor outcome groups as the basis for comparison, differences in terms of age, WBC, and cytogenetics.
Study Design- Primary AML Samples
(Elizabeth Griffiths, Hetty Carraway)

• 207 1° AML samples from the JHU tumor bank, 1998-2008
  – 114 with normal karyotype: 73 diagnostic, 40 relapse specimens
• Methylation analysis by MSP was performed for:
  – 11 known genes, 12 Wnt pathway inhibitor genes
• Samples annotated for:
  – age, specimen type, karyotype, WBC at diagnosis, induction therapy, antecedent MDS/MPD, primary refractoriness, FLT3-ITD status and survival.
• $NPM1^{\text{mut}}$ and FLT3-ITD status assessed for samples without information
Methylation in Cytogenetically Normal and Abnormal AML

* Statistically different between two groups, p<0.04
Univariate Hazard Ratio for Death: Known genes (n=73)
Multivariate Hazard Ratio for Death (n=73)

Corrected for age, *NPM1/FLT3-ITD* status, antecedent MDS, and WBC at diagnosis.
n= 73 uniformly treated patients with normal karyotype AML, multivariate hazard ratio corrected for age, antecedent hematologic diagnosis, WBC, FLT3-ITD, NPM1^mut Status
Kaplan-Meier Survival Estimates
In patients with Normal Karyotype

Log Rank $p=0.04$

$n=73$, $p<0.05$
Why Has Methylation Been Inconsistent as a Prognostic Marker?

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  - How do we choose the right genes?
  - Does method effect use as marker? Is it presence of methylation or density (level) that affects prognosis?

- Confounders
  - good and poor outcome groups as the basis for comparison, differences in terms of age, WBC, and cytogenetics.
DNA Methylation Predicts Survival and Response to Therapy in Patients With Myelodysplastic Syndromes

Shen, JCO, 2009, 605
Quantitative DNA methylation predicts survival in adult acute myeloid leukemia
Quantitative DNA methylation predicts survival in adult acute myeloid leukemia

Bullinger,

Blood, 2010, 636
Epigenetic progression of MDS/MPD and AML

Normal Marrow

Low Risk MDS

High Risk MDS

AML

PV

Myelofibrosis

ET

Gene Methylation

Gene A

p15

CTNNA1

SOCS1

Gene B

Methylation Density

Gene A

p15

CTNNA1

SOCS1

Gene B
DNA methylation of CTNNA1 in AML cell lines
(Ying Ye, Mike McDevitt)

MSP

U

U

M

U

U/M

HL60

HNT34

KG1a

ML-1

U937

Bisulphite Sequence
0: Transcriptional start site
-303

+40

MSP 1

+43

+149

+273

+352

0

+43

+149

+273

+352

+352

0

+43

+149

+273

+352
Chromatin immunoprecipitation (ChIP) at CTNNA1 promoter in AML cell lines

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>HNT34</th>
<th>HL60</th>
<th>KG1</th>
<th>KG1a</th>
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<tbody>
<tr>
<td>H3K9Ac</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H3K4me2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H3K9me2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>H3K27me3</td>
<td></td>
<td></td>
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<tr>
<td>IgG</td>
<td></td>
<td></td>
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</tbody>
</table>

CTNNA1 expression:
- HNT34
- HL60
- KG1
- KG1a

CTNNA1 MSP:
- HNT34
- HL60
- KG1
+ KG1a
DNA methylation and repression of CTNNA1 in primary AML

CTNNA1/GAPDH

Patient group B (n=9)

P=0.01

Patient group A (n=17)

Normal (n=7)

CTNNA1

MSP --+

0 0.5 1 1.5 2 2.5 3

-250 -200 -150 -100 -50 0 50

DNA methylation and repression of CTNNA1 in primary AML
Chromatin immunoprecipitation (ChIP) of the CTNNNA1 promoter: Repression is associated with inactive histone modification (Primary AML)

A. CTNNNA1 expression by Real Time PCR

B. H3K9Ac

C. H3K4me2

D. H3K27me3
Model of progressive epigenetic inactivation

- **Active chromatin**: High gene expression
- **Compacted chromatin**: Depressed expression/Silencing
- **Invariant chromatin**: Gene silencing

H3K9Ac  H3K9Me2  Methylated DNA
H3K4Me2  H3K27Me3
## Acknowledgements

<table>
<thead>
<tr>
<th>AML prognosis</th>
<th>CTNNA1 studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elizabeth Griffiths</td>
<td>Ying Ye</td>
</tr>
<tr>
<td>Hetty Carraway</td>
<td>Michael McDevitt</td>
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<td>Steve Gore</td>
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<td>Hetty Carraway</td>
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<td>Oliver Galm</td>
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