The DNA methylation-demethylation cycle

DNA demethylation occurs in a replication-dependent manner if DNMT1 is prevented from remethylating cytosine in the newly replicated strand.
5hmC is highest in gene bodies of the most highly transcribed genes, and at active enhancers (H3K4me1+, H3K27Ac+).
TET2 deletions and mutations are frequently associated with haematologic malignancies.

Delhommeau et al., NEJM 2009

Ko*, Pape*, Huang* et al., Nature 2010

TET2 mutations are loss-of-function
Loss of 5-hydroxymethylcytosine is accompanied with malignant cellular transformation

Yotaro Kudo,1 Keisuke Tateishi,1,3 Keisuke Yamamoto,1 Shinzo Yamamoto,1 Yoshinari Asaoka,1 Hideaki Iijichi,1 Genta Nagae,2 Haruhiko Yoshida,1 Hiroyuki Aburatani2 and Kazuhiro Koike1

5-Hydroxymethylcytosine Is Strongly Depleted in Human Cancers but Its Levels Do Not Correlate with IDH1 Mutations

Seung-Gi Jin, Yong Jiang, Runxiang Qu, et al.

Cancer Res 2011;71:7360-7365. Published OnlineFirst November 3, 2011

Tumor development is associated with decrease of TET gene expression and 5-methylcytosine hydroxylation

H Yang1,2,8, Y Liu1,4,8, F Bai2, J-Y Zhang1, S-H Ma1,2, J Liu1,4, Z-D Xu3,4, H-G Zhu3,4, Z-Q Ling6, D Ye1, K-L Guan1,4,7 and Y Xiong1,2,5

Loss of 5-Hydroxymethylcytosine Is an Epigenetic Hallmark of Melanoma

Christine Guo Lan,1,2,13 Yueyi Xu,1,13 Craig Geol,1,3 Feizhen Wu,8 Allison Larson,6 Karen Dresser,7 Wengj Xu,9 Li Tarn,9 Yeguang Hu,1 Qian Zhan,2 Chung-wei Lee,7 Di Hu,1 Bill Q. Lian,1,8 Sonja Klef,7,5 Yijun Yang,10 James Neiswender,6 Abraham J. Khorasani,1 Rui Fang,1 Cecilia Lezcano,2 Lyn M. Duncan,4 Richard A. Scolyer,11 John F. Thompson,11 Hojjat Kakavandi,11 Yariy Hovanes,3,12 Leonard I. Zon,9 Martin C. Mihm Jr.,8 Ursula B. Kaiser,1 Tobias Schatten,9 Bruce A. Woda,7 George F. Murphy,2,10 and Yuliang G. Shif6,8

TET1 Suppresses Cancer Invasion by Activating the Tissue Inhibitors of Metalloproteinases

Chih-Hung Hsu,1,11,13 Kai-Lin Peng,1,12 Ming-Lun Kang,1,12 Yi-Ren Chen,3,12 Yu-Chih Yang,1,3,4 Chin-Hsien Tsai,3,4 Chi-Shuen Chu,1,6 Yung-Ming Jeng,6 Yen-Ting Chen,1,6 Feng-Miao Lin,7 Hsin-Da Huang,7 Yun-Yuh Lu,1 Yu-Ching Teng,1,2 Shinn-Tsuen Lin,8 Ruo-Kai Lin,1,14 Fan-Mei Tang,9 Sung-Bau Lee,1,15 Huan Ming Hsu,16 Jhyl-Chemg Yu,16,1 Pei-Wen Hsiao,5 and Li-Jung Juan1,2,5

HMGA2/TET1/HOXA9 signaling pathway regulates breast cancer growth and metastasis

Miao Sunab, Chon-Xiao Song1, Hao Huang6,1, Casey A. Frankenbergerab, Devipriya Sankarasharma1, Suzana Gomes8, Ping Chen6, Jianjun Chen6, Kiran K. Chada6, Chuan He, and Marsha R. Rosnerab,2

MicroRNA-Antagonism Regulates Breast Cancer Stemness and Metastasis via TET-Family-Dependent Chromatin Remodeling

Su Jung Song,1 Laura Polase,1,6 Min Sup Song,1,6 Ugo Ala,1 Kailyn Webster,1 Christopher Ng,1 Gary Beringer,3,4 Nicola J. Breslau,1 Xin Yuan,1 Lewis C. Cantley,6 Andrea L. Richardson,6 and Pier Paolo Pandolfo1,7

Cancer Science 2011
Cancer Research 2011
Oncogene 2011
Cell 2012
Cell Reports 2012
PNAS 2013
Cell 2013
Is acute loss of TET function associated with cancer? Yes

Two model systems in mice:

- deletion of Tet2 and Tet3 with Mx1Cre and polyI:polyC injection, or with Cre-ERT2 and tamoxifen injection
  effects of deletion first seen in haematopoietic stem/precursor cells → aggressive myeloid leukemia

- deletion of Tet2 and Tet3 with CD4Cre in T cells
  → aggressive antigen-driven T cell leukemia

Both: cell-intrinsic, polyclonal, transmissible indefinitely to recipient mice
Cancer develops rapidly (< 5 weeks)
**WGBS of WT and Tet2/3 DKO LSK haematopoietic stem/ precursor cells**

### Sequencing results

<table>
<thead>
<tr>
<th></th>
<th>Total Number of Reads</th>
<th>Mapped Reads</th>
<th>(%)</th>
<th>Covered Basepairs</th>
<th>Genome Coverage</th>
<th>Genome Coverage per Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONTROLS</strong></td>
<td></td>
<td></td>
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<tr>
<td>Ctr1</td>
<td>215,810,374</td>
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<td>Ctr2</td>
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<td>177,428,469</td>
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<tr>
<td>Ctr3</td>
<td>208,921,452</td>
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<td>19,479,366,414</td>
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<tr>
<td><strong>KNOCKOUTS</strong></td>
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<tr>
<td>KO1</td>
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<td>KO2</td>
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<td>KO3</td>
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<td>0.93</td>
<td>18,171,926,060</td>
<td>6.67</td>
<td></td>
</tr>
</tbody>
</table>

Covered basepairs = mapped reads x length of reads
mm9 Genome size = 2,725,765,481

### Graphs

- **a** TPR = True positive regions (sensitivity) → refDMRs
- **b** FDR = False Discovery Rate (specificity) → refPositives

Ziller et al. (2014)
Narrowing of “canyons” of DNA methylation in Tet2/3 DKO cells

Both genes downregulated in Tet2/3 DKO cells relative to WT
TET loss-of-function results in increased DNA methylation across the genome

TSS and gene body methylation (both strands, replicates separated)

Active enhancers (H3K4me1⁺, H3K27Ac⁺)
Increased DNA methylation in both classes of differentially-expressed genes (plotted as averages across all genes)

- Upregulated genes, n=721
- Downregulated genes, n=290

Position relative to gene body

Methylation (%)
Increased DNA methylation in both up- and down-regulated genes (plotted at the single-gene level)

Methylation change in differentially expressed genes (721 up, 290 down)
Bisulfite sequencing conflates five bases into just two

Approximate numbers of modified cytosines in the mouse ES cell genome:

- \( \sim 30 \times 10^6 \) 5mC
- \( \sim 5 \times 10^6 \) 5hmC
- \( \sim 6 \times 10^6 \) 5fC
- \( \sim 600 \times 10^6 \) C
- 5caC (1000-10,000)

Read as 5mC
Read as C
1. Include oxi-mC (or at least 5hmC) mapping in DNA methylation analysis

   DNA methylation is not binary as previously thought:
   5mC can be 5hmC; C can be 5fC or 5caC

   Also, a 20% change in methylation level using bisulphite sequencing means that
   20% of alleles have likely undergone a change in modification status

   and some undefined proportion have changed state, from 5mC to 5hmC or vice versa,
   but have not been counted

2. Include perturbations and kinetic measurements

   DNMTs and TETs are clearly sensitive to environment and metabolism

   Changes may happen on very rapid timescales, as seen in these cancers

3. Encourage the development of new sequencing methods to map all modified cytosines
   in unamplified genomic DNA

   8 bases altogether: A, G, T + 5 cytosine species: C, 5mC, 5hmC, 5fC, 5caC

   Ideally, long reads (10 kb) to allow unambiguous mapping of repetitive DNA
1. Shift some attention to purified primary cells, not just tissues or cell lines!

   Cells examined ex vivo or in situ can be very different from cultured cells, established cell lines, or even primary cells cultured for just a few days in vitro.

2. Corollary: enable technologies for looking at single cells or small numbers of cells preferably isolated ex vivo

   e.g. exhausted T cells in mouse models or from humans are available as thousands, not millions

   All the technologies: histone modifications, ChIP-seq, DNA modification mapping

3. Take-home message: model organisms are likely to be quite useful, even to a National “Human” Genome Research Institute
Collaborators

L Aravind, NCBI, NIH
Myunggon Ko
Jungeun An
Mamta Tahiliani
Ageliki Tsagaratou
Lukas Chavez  Ashu Chawla  Edahi Avalos

Peggy Goodell, Mira Jeong, Wei Li, Baylor College of Medicine

Tarmo Äijö  Härri Lähdesmäki

Aalto University, Finland