Epigenetics: A pioneer area for drug discovery across multiple therapeutic areas

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Ontario Cancer Institute
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Public-private consortium funding pre-competitive protein-based science
- GSK, Merck, Novartis
- Canadian, Swedish granting agencies
- Wellcome Trust, UK

**Goals:** 3D protein structures for biological and drug discovery (~160/year)

**Three sites:** Toronto, Oxford, Stockholm

All data and reagents are made publicly available, without restriction on use
- 3D structures
- Protein expression clones
- Protocols for expression and crystallization
- Other protein-based protocols and methodologies
- [www.TheSGC.org](http://www.TheSGC.org)

**New initiatives in developing chemical probes and protein capture reagents for biological discovery and potential new drug targets**
Epigenetics

Heritable changes in phenotype caused by mechanisms other than changes in the underlying DNA sequence
**Biologically attractive, “pioneer target area”**

- Play a key role in development, differentiation and stem cell biology
- Underlie many chronic diseases: cancer, inflammation, psychiatric disorders
- Directly impact transcriptional programs, DNA repair & metabolism
- Intense area of research for which there is a receptive community to test chemical probes and protein capture reagents

**Epigenetic targets appear to be Druggable**

- SAHA (HDAC inhibitor) approved for cutaneous T-cell lymphoma
- Inhibitors of DNA MTases shown to reactivate silenced genes
- nM inhibitors of Bromo domains have been developed and can affect transcriptional programs.

**Opportunity for discovery of new biology and new drug targets using chemical biology approaches**
Three basic mechanisms of epigenetic regulation

- Tranx regulation
- Chromatin & struct
- DNA repair & rep

Histone modifications

Gene regulation

RNA regulation

DNA methylation

Gene silencing
Gene expression is regulated by chromatin structure and its covalent modifications

The “Histone Code” hypothesis: Covalent modifications of histone tails encode heritable regulatory information

Strahl & Allis, 2000

Mohd-Sarip et al, Science, 2004

Luger et al., Nature, 1997

Histones
- H2A
- H2B
- H3
- H4
Acetyl- and methyl- lysines are an important component of the histone code.
Readers, Writers and Erasers of Histone Marks: Key Focus of SGC Structural Effort

**Histone Acetyltransferases**: not so specific

**HDACs**: Sirt5 and HDAC7 - substrates unknown

**Histone Methyltransferases**: site specific

**Readers**: mixed specificity

**Demethylase**: specific


HAT1 with H4K12Ac and CoA

**L3MBTL1 & 2**

**BRDT, BRD3, BRD4**

**SCMH1**

**SAHA**

**CBX2, CBX3, CBX4, CBX7**

**PRDM2, SUV39H2, EHMT1, EHMT2**

**WDR5, SETMAR**

**JMJD2**

**MYST3**

**SHUETZ**

**HAT1, TIP60**

**TIP60, MYST1**

**TIP60 HAT1**
Can we exploit the variability in Lysine binding sites?

MBT Domain (mono & di-methyl)

Tudor Domain (di- & tri-methyl)

Bromo Domain (acetyl)

Chromo Domain (tri-methyl)
Histone Methyltransferase Family Approach: Opportunities for selectivity

Conserved SET domain has unique substrate pockets

H3K4
H3K9
H3K79

I-SET
SAM

Post-SET

T-3
R-1
T+2
A-2

Assays

Histone Substrate

Structures

SGC
Others

B4
Arg

B3

B2

B1

B6

B5

B7
Case Study: G9a methyltransferase

Reversal of H3K9me2 by a Small-Molecule Inhibitor for the G9a Histone Methyltransferase


A Combined Chemical and Genetic Approach for the Generation of Induced Pluripotent Stem Cells

Yan Shi, Jeong Tae Do, Caroline Desponts, Heung Sik Hahm, Hans R. Schöler, and Sheng Ding.
But, also implicated in Addiction, Cognition/Behavior, Viral Response and Cancer

Essential Role of the Histone Methyltransferase G9a in Cocaine-Induced Plasticity
Ian Maze, 1 Herbert E. Covington III, 1 David M. Dietz, 2 Quinsey LePlant, 1, 2 William Renthal, 2 Scott J. Russo, 1 Max Mechanic, 2 Ezekiel Mouzon, 3 Rachael L. Neve, 3 Stephen J. Haggarty, 4, 5 Yanhua Ren, 6 Srihari C. Sampath, 9 Yasmin L. Hurd, 1 Paul Greengard, 1 Alexander Tarakhovsky, 6 Anne Schaefer, 7, 8 Eric J. Nestler 9

G9a and Glp Methylate Lysine 373 in the Tumor Suppressor p53
Jing Huang, 10 Jean Dorsey, 11 Sergey Churlkov, 12 Xinyue Zhang, 13 Thomas Jenuwein 14, 15, Danny Reinbe and Shelley L. Berger 16

Distinct Roles for Histone Methyltransferases G9a and GLP in Cancer Germ-Line Antigen Gene Regulation in Human Cancer Cells and Murine Embryonic Stem Cells
Petra A. Link, 1 Omkaram Gangisetty, 1 Smitha R. James, 1 Anna Woloszynska-Read, 1 Makoto Tachibana, 2 Yolchi Shinkai, 2 and Adam R. Karpf 1

EVI-1 interacts with histone methyltransferases SUV39H1 and G9a for transcriptional repression and bone marrow immortalization
S Goyama 1, 2, E Nitta 1, T Yoshino 1, S Kako 1, N Watanabe-Okochi 1, M Shimabe 1, Y Imai 1, K Takahashi 2 and M Kurokawa 1
Goal: Develop well characterized small molecules to be used to link pharmacological inhibition of an individual target (or small group of targets) with cellular biology/pathways/phenotype

- **Potent:** IC50 < 100 nM *in vitro*
- **Selective:** 30x over related proteins
- **Cell permeable:** IC50 < 1 uM in cell
- **Low/No cellular toxicity**

• Make available to research community
Objective: identify 40 probes and make compounds & data publicly available (no restriction on use) over 4 years

Participants:

• SGC – Toronto (HMTs, Royal Family, HATs)  
  Funder: Ontario  $4.6M
• SGC – Oxford (KDMs, Bromo domains)  
  Funder: Wellcome Trust  $8M
• SGC – Stockholm (PARPs)  
  Funder: Swedish Sci. F.  $3M
• GSK, Pfizer, Novartis Chemistry (8 med chemist FTEs each)  
  • commit to release “public probe” other compounds not disclosed
• NIH Chemical Genomics Center (20 HTS slots – data public)
• Ontario Inst. Cancer Research (2 FTE med chemists)
• Frye Lab, University N. Carolina (4 FTE med chem/assay dev)
Structure based design of potent and selective G9a antagonist

• UNC0638 occupies histone binding groove and does not interact with SAM binding pocket. Same binding mode as BIX01294 & UNC0224
UNC0638: Selective chemical probe for G9a/GLP methyltransferases

**UNC0638 Data Sheet**

**Biology of the G9a/GLP methyltransferases**

G9a (EHMT2) and GLP (EHMT1) catalyze the mono and dimethylation of lysine 9 of histone 3 (H3K9) and other non-histone substrates such as p53 and MIZ.

**Cellular Activity**

Significant reduction in H3K9 dimethylation at 100nM in MDA-MB231 cells as measured by fluorescence immunostaining without significant cellular toxicity.

**Selectivity Within Target Family**

<table>
<thead>
<tr>
<th>Protein</th>
<th>IC_{50} nM (Activity)</th>
<th>Tm shift °C</th>
<th>Tm shift °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>G9a (EHMT2)</td>
<td>&lt;15</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>GLP (EHMT1)</td>
<td>19 ± 1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>SETD7</td>
<td>&gt;10,000</td>
<td>nt</td>
<td></td>
</tr>
<tr>
<td>SETD8</td>
<td>&gt;10,000</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>PRMT3</td>
<td>&gt;10,000</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>SUV39H2</td>
<td>&gt;10,000</td>
<td>nt</td>
<td></td>
</tr>
<tr>
<td>DCT1L</td>
<td>nt</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>PRDM1</td>
<td>nt</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>PRDM10</td>
<td>nt</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>PRDM12</td>
<td>nt</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>SMYD3</td>
<td>nt</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>JMJD2E</td>
<td>4660 (AlphaScreen)</td>
<td>nt</td>
<td></td>
</tr>
<tr>
<td>HTATIP</td>
<td>nt</td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

(n=not tested, nd=not detected, 1 singlicate determination @ 100 μM)

**Selectivity Beyond Target Family**

>30% Inhib @ 1 μM


Well tolerated in variety of cancer cell lines

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>IC\textsubscript{50} (nM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>H\textsubscript{3}K9me2</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>MDA231</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>MCF7</td>
<td>70 ± 12</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td></td>
</tr>
<tr>
<td>PC3</td>
<td>59</td>
</tr>
<tr>
<td>22RV1</td>
<td>48</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td></td>
</tr>
<tr>
<td>HCT116 wt</td>
<td>210</td>
</tr>
<tr>
<td>HCT116 p53-/-</td>
<td>240</td>
</tr>
<tr>
<td>Human fibroblast</td>
<td></td>
</tr>
<tr>
<td>IMR90</td>
<td>120</td>
</tr>
</tbody>
</table>

Re-activates expression of retroviral GFP reporter protein

Δ GFP% between treated and non treated cells

**James Ellis, HSC**
Selective inhibition of BET bromodomains

Panagis Filippakopoulos\*, Jun Qi\*, Sarah Picaud\*, Yao Shen, William B. Smith, Oleg Fedorov, Elizabeth M. Morse, Tracey Keates, Tyler T. Hickman, Ildiko Felletar, Martin Philpott, Shonagh Munro, Michael R. McKeown, Yuchuan Wang, Amanda L. Christie, Nathan West, Michael J. Cameron, Brian Schwartz, Tom D. Heightman, Nicholas La Thangue, Christopher A. French, Olaf Wiest, Andrew L. Kung, Stefan Knapp, & James E. Bradner

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Inhibition (%) vs. log[conc. (μM)]

JQ1R betson1
JQ1S betsoff1

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\*These authors contributed equally to this work.
Regulation of P-Tefb(cdk9) mediated transcription
• KO of BRD4/2 results in G1 arrest and apoptosis and suppresses many genes required for growth
• P-TEFb and BRD functions with the oncogene c-Myc
• c-Myc interacts with the H3Kme3 specific demethylase JARID1A

BET in Disease: NMC
• *NUT* midline carcinoma (*NMC*) is a rare, highly lethal cancer that occurs in children and adults.
  • NMCs uniformly present in the midline, most commonly in the head, neck, or mediastinum, as poorly differentiated carcinomas
  • Rearrangement of the Nuclear protein in testis (*NUT*) that creates a *BRD4-NUT* fusion gene
  • Variant rearrangements, some involving the *BRD3* gene
  • NMC is diagnosed by fluorescence *in situ* hybridization. However, most cases of NMC currently go unrecognized.
BET Probe: effective against NMC xenograft

Cellular and in vivo studies

- Induces terminal differentiation of BRD4-NUT cell lines derived from MLC patients
- Anti-proliferative effects on cell lines that carry BRD-NUT fusion at 100nM
- Induces apoptosis in BRD-NUT cell lines
- Dissolves nuclear foci typically observed in BRD-NUT cell lines and biopsies
- Reduces tumour growth in xenograft models (50mg/kg IP, enantiomer)
- Displaces BRD4 from E-selectin and TNFα promoter in ChIP assays

Collaboration with J. Bradner Lab, Harvard Medical School
Summary

• Open access research tools
  • Proteins, structural info, production protocols
  • Chemical Probes
  • Protein capture reagents

• Proactive engagement of community for
  • biological discovery
  • target validation
  • ultimately new therapeutics
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Udo Opperman – PI Histone Lysine demethylases
Tom Heightman – PI Chemical Biology & Project Manager–Chem Probes
Peter Brown – Project Manager–Chemical Probes
Masoud Vedadi – PI Molecular Biophysics
Matthieu Schapira – PI Research Informatics/Comp Chem
Jinrong Min – PI Structural Chromatin Biology

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