Epigenetic Deregulation in Haematologic Malignancies

Csaba Bödör

1. Centre for Haemato-Oncology, BCI
2. Semmelweis University, Department of Pathology and Experimental Cancer Research
INTRODUCTION: EPIGENETICS

“Same genotype, different phenotypes”
INTRODUCTION: EPIGENETICS

Haematopoietic cell development

Cedar et al, Nature Reviews Immunology, 11, 2011
GOD... THE HUMAN GENOME CODE'S BEEN UNRAVELLED

DAMN HACKERS!!! NOW, I HAVE TO CHANGE THE PASSWORD
THE EPIGENETIC CODE

- DNA methylation
- Histone modifications
  - Methylation
  - Acetylation
  - Phosphorylation ...

- Specific enzymes
- Deposition of chromatin marks

- Alterations in cancer epigenome

- Attractive therapeutic targets ...
- Proof of concept: DNA hypomethylating agents, HDACi

THE EPIGENETIC CODE

Druggable epigenetic regulators

STUDYING THE CANCER EPIGENOME

- From a single gene approach to global approaches

Single gene — Many genes

Interactions, hierarchy — Deregulation...
STUDYING THE CANCER EPIGENOME

- Advances in Next Generation Sequencing Technologies (Whole genome, exome, transcriptome sequencing)
  - Novel mutation targets in haematologic malignancies:
    - DNA methylation,
    - Histone methylation and acetylation machinery

- Array based and NGS technologies
  - Analysis of methylomes, histonomes ...
  - Correlation of methylation with gene expression

- Validation
  - False +ve, rare somatic variants
  - Passenger vs driver mutations
MUTATIONS IN THE EPIGENETIC MACHINERY

- **IN MYELOID MALIGNANCIES**
- *DNMT3A* mutations in 20% of AML
- *TET2* mutations in ~20% of AML and MDS

- Associated with poor prognosis
- Mechanism poorly understood ...

- Decreased *DNMT3A* activity, increased sensitivity to decitabine (*Metzeler et al, Leukemia, 2012*)

- *TET2* participates in the conversion of 5 mC to 5 hmC, required for DNA de-methylation (*Yang et al, Oncogene, 2012*)
MUTATIONS IN THE EPIGENETIC MACHINERY

- **IN LYMPHOID MALIGNANCIES**
  - **EZH2** Y646 mutations in FL and GC-DLBCL (*Morin et al, Nat Gen, 2010*)
  - Mutations in the catalytic domain (SET)

- **Gain of function vs loss of function**

  - **Rationale for EZH2i therapy**

---

*Bödör et al, Leukemia, 2011*

*Yap et al, Blood 2011*

*Sneeringer et al, PNAS 2010*

*Makishima et al, Leukemia, 2010*
The changing (epi)genetic landscape of B-cell lymphomas

<table>
<thead>
<tr>
<th>Gene</th>
<th>Feature of mutation</th>
<th>Follicular lymphoma</th>
<th>Diffuse large B-cell lymphoma</th>
<th>Multiple myeloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLL2</td>
<td>loss of function</td>
<td>89%</td>
<td>32%</td>
<td>5%</td>
</tr>
<tr>
<td>EZH2</td>
<td>gain of function</td>
<td>15%</td>
<td>21%</td>
<td>-</td>
</tr>
<tr>
<td>CREBBP</td>
<td>loss of function</td>
<td>33%</td>
<td>25%</td>
<td>-</td>
</tr>
<tr>
<td>P300</td>
<td>loss of function</td>
<td>15%</td>
<td>10%</td>
<td>-</td>
</tr>
<tr>
<td>UTX</td>
<td>loss of function</td>
<td>-</td>
<td>-</td>
<td>10%</td>
</tr>
</tbody>
</table>
Cancer epigenetics reaches mainstream oncology

Manuel Rodríguez-Paredes¹ & Manel Esteller¹−³

Epigenetic protein families: a new frontier for drug discovery

Cheryl H. Arrowsmith¹,²,³, Chas Bountra⁴, Paul V. Fish⁵, Kevin Lee⁶* and Matthieu Schapira¹,⁷
### Patient stratification

- Targeted epigenetic therapy ...

---

#### Table 2 | HDAC and sirtuin inhibitors in clinical development

<table>
<thead>
<tr>
<th>Compound</th>
<th>Targets</th>
<th>Indications</th>
<th>Highest clinical status</th>
<th>Further information</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>HDAC1, HDAC2, HDAC3 and HDAC6</td>
<td>Oncology</td>
<td>Approved</td>
<td></td>
<td>111,175</td>
</tr>
<tr>
<td>Romidepsin</td>
<td>HDAC1, HDAC2, HDAC3 and HDAC6</td>
<td>Oncology</td>
<td>Approved</td>
<td></td>
<td>111,177</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>HDAC1, HDAC2, HDAC3 and HDAC6</td>
<td>Oncology</td>
<td>Phase III</td>
<td>ClinicalTrials.gov identifiers: NCT01034183 and NCT01023308</td>
<td>111,175</td>
</tr>
<tr>
<td>Belinostat</td>
<td>HDAC1, HDAC2, HDAC3 and HDAC6</td>
<td>Oncology</td>
<td>Phase II</td>
<td>ClinicalTrials.gov identifiers: NCT0066333, NCT00826854, NCT00934050 and others</td>
<td>111,170</td>
</tr>
<tr>
<td>Entinostat</td>
<td>HDAC1 and HDAC2</td>
<td>Oncology</td>
<td>Phase II</td>
<td>ClinicalTrials.gov identifiers: NCT00866333, NCT00826854, NCT00934050 and others</td>
<td>111,180</td>
</tr>
<tr>
<td>Mocetinostat</td>
<td>HDAC1 and HDAC2</td>
<td>Oncology</td>
<td>Phase II</td>
<td></td>
<td>111,181,182</td>
</tr>
<tr>
<td>Retminostat</td>
<td>HDAC1, HDAC3 and HDAC6</td>
<td>Oncology</td>
<td>Phase II</td>
<td>ClinicalTrials.gov identifiers: NCT01034745</td>
<td>183</td>
</tr>
<tr>
<td>Givinostat</td>
<td>HDAC (class I and II)</td>
<td>Inflammation, oncology</td>
<td>Phase II</td>
<td>ClinicalTrials.gov identifiers: NCT00928707 and NCT01261624</td>
<td>184</td>
</tr>
<tr>
<td>SB939</td>
<td>Pan-HDAC</td>
<td>Myelofibrosis</td>
<td>Phase II</td>
<td>ClinicalTrials.gov identifiers: NCT0112384 and NCT01200496</td>
<td>185,186</td>
</tr>
<tr>
<td>CUDC-101</td>
<td>HDACs, EGFR and HER2</td>
<td>Oncology, solid tumours</td>
<td>Phase Ib</td>
<td>ClinicalTrials.gov identifiers: NCT01171924</td>
<td>187</td>
</tr>
<tr>
<td>PCI-24781</td>
<td>HDAC (class I and II)</td>
<td>Oncology</td>
<td>Phase I/II</td>
<td>ClinicalTrials.gov identifiers: NCT01027910</td>
<td>188</td>
</tr>
<tr>
<td>4SC-202</td>
<td>HDAC (class I)</td>
<td>Oncology</td>
<td>Phase I</td>
<td>ClinicalTrials.gov identifiers: NCT01344707</td>
<td>–</td>
</tr>
<tr>
<td>AR-42</td>
<td>HDAC (class I and II)</td>
<td>Oncology</td>
<td>Phase I</td>
<td>ClinicalTrials.gov identifiers: NCT01029033</td>
<td>180</td>
</tr>
<tr>
<td>CG200745</td>
<td>Pan-HDAC</td>
<td>Oncology</td>
<td>Phase I</td>
<td>ClinicalTrials.gov identifiers: NCT01226407</td>
<td>193</td>
</tr>
<tr>
<td>ACY-1215</td>
<td>HDAC6</td>
<td>Oncology</td>
<td>Phase I/II</td>
<td>ClinicalTrials.gov identifiers: NCT01323751</td>
<td>–</td>
</tr>
<tr>
<td>EVP-0334</td>
<td>HDAC (class I)</td>
<td>Alzheimer's disease</td>
<td>Phase I</td>
<td>See the EnViivo Pharmaceuticals website</td>
<td>191</td>
</tr>
<tr>
<td>RG2833</td>
<td>HDAC3</td>
<td>Friedreich's ataxia</td>
<td>Preclinical (investigational new drug)</td>
<td>See the RepliGen website</td>
<td>–</td>
</tr>
<tr>
<td>SEN196</td>
<td>SIRT1</td>
<td>Huntington's disease</td>
<td>Phase II</td>
<td>See the Siena Biotech website</td>
<td>127</td>
</tr>
</tbody>
</table>
“... will generate reference epigenomes of at least 50 specific blood cell types and their malignant counterparts and aim to provide high-quality reference epigenomes of primary cells from >60 individuals with detailed genetic and, where appropriate, medical records.”

“...generate reference epigenomes, including RNA-Seq for transcriptome analysis, bisulfite sequencing for methylome analysis, DNasel-Seq for analysis of hypersensitive sites and ChIPSeq for analysis of at least six histone marks.”

“...To foster the clinical relevance of epigenetic analysis by including a major effort in the biomarker area. The focus will be identifying biomarkers for more accurate prognosis and personalized therapy.”
THE HUMAN EPIGENOME PROJECT (HEP)

Current consortium members:

The Wellcome Trust Sanger Institute is a recognised leader in genome sequencing, high-throughput systems, informatics and analysis of gene function using genetic approaches in a variety of model organisms and humans.

Epigenomics AG is a transatlantic biotechnology company with headquarters in Berlin, Germany and its wholly owned subsidiary in Seattle, Washington, USA, pioneering tomorrow’s personalized medicine by exploiting the information of DNA methylation patterns.

The Centre National de Génotypage is a national research institute set up in 1998 by the French Government in anticipation of using the genome sequencing information for the identification of genes and gene function.