Laissez-FAIRE-Seq: Probing Chromatin Structure in Blood Progenitors

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Hematopoietic stem and progenitor cells (HSPC) are essential for sustained production of red and white blood cells. Although HSPC transplantation has a wide variety of clinical applications, HSPCs are difficult to expand *ex vivo*, highlighting the need to understand their basic biology. Protein methyltransferases are known to play key roles in regulating gene expression, but their part in regulating chromatin structure had not been clearly defined. G9a and GLP are two related protein methyltransferases that are required for de novo methylation of histone H3 on lysine 9, (H3K9me2), a histone mark associated with transcriptional repression. In a new study published in*Epigenetics and Chromatin*, Dr. Xiaoji Chen, a former graduate student in the lab of Dr. Patrick Paddison (Human Biology Division), along with their collaborators, addressed the role of G9a /GLP in controlling chromatin structure in HSPCs.

The authors had previously found that H3K9me2 marks are largely absent in HSPCs and are formed de novo in more committed HSPCs. In committed HSPCs, G9a/GLP activity nucleates H3K9me2 marks at CpG islands (cytosine nucleotide followed by a guanine; CGIs), as well as at other genomic sites, which then spread to form domains known as chromatin territories. They had further demonstrated that treatment of HSPCs with a small molecule inhibitor of G9a/GLP methyltransferase activity (UNC0638), resulted in genome-wide loss of H3K9me2 and improved retention of stem cell properties by HSPCs (Chen et al., 2012).

To address the role of G9a/GLP-dependent patterning of H3K9me2 on chromatin structure, the authors profiled chromatin accessibility of committed HSPCs treated with UNC0638 using a technique called FAIRE-seq (Formaldehyde-Assisted Isolation of Regulatory Elements), which enables detection of nucleosome-depleted regions of the genome. Integration of the FAIRE-seq data with ChIP-seq (Chromatin immunoprecipitation followed by high throughput sequencing) for H3K9me2 and DNA methylation data from the same cells revealed that loss of H3K9me2 caused by UNC0638 treatment led to an increase in chromatin accessibility. Importantly, increased chromatin accessibility was largely restricted to sites of H3K9me2 nucleation. "This paper helps cross validate our work on histone H3 methylation patterning during human blood lineage development, by showing that these marks have an impact on chromatin structure," said Dr. Paddison.

Previous results had demonstrated that about half of the H3K9me2 sites coincided with CGIs, but the sequence basis for the other half had remained unexplained. Further examination of non-CGI sites revealed that they had similar GC and CpG content to CGIs, yet did not meet the criteria for CGI classification. Next, the authors compared FAIRE-seq read counts at promoter-associated CGIs or at "orphan" CGIs, which have unknown functions. This analysis revealed that both sets of CGIs displayed an increase in chromatin accessibility in response to UNC0638 treatment. Finally, the authors investigated the role of DNA methylation in the response to UNC0638 treatment and found that the biggest changes in chromatin accessibility occurred at unmethylated CGIs, which have low rates of cytosine-to-thymine deamination.

In summary, this study showed that H3K9me2 patterning regulates chromatin structure at promoter and "orphan" CGIs, promoting "closed" chromatin states. "Although we still haven't deciphered their exact biological significance, the work sheds light on another feature of our genome: CpG islands. In this case, we find that CpG islands are sites where these histone methylation marks are initially laid down, which is really unexpected and enables us to assign a biological function to thousands of 'orphan' sites that previously did not have a function. Something interesting is afoot," explained Dr. Paddison.

Schones DE, Chen X, Trac C, Setten R, Paddison PJ. 2014. G9a/GLP-dependent H3K9me2 patterning alters chromatin structure at CpG islands in hematopoietic progenitors. *Epigenetics Chromatin*. 7(23).

See also: Chen X, Skutt-Kakaria K, Davison J, Ou YL, Choi E, Malik P, Loeb K, Wood B, Georges G, Torok-Storb B, Paddison PJ.2012 G9a/GLP-dependent histone H3K9me2 patterning during human hematopoietic stem cell lineage commitment. *Genes Dev*. 26(22):2499-2511.

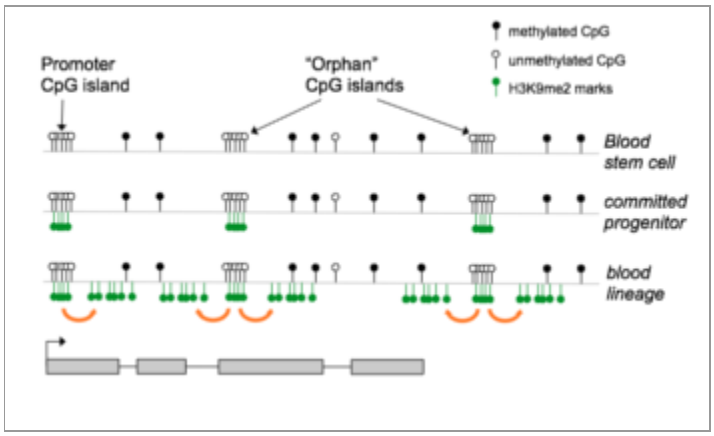

Image provided by Dr. Patrick Paddison

Previous work had shown that histone H3 lysine 9 dimethylation (H3K9me2) is absent or low in human adult hematopoietic stem cells and is progressively patterned during differentiation across most of the coding regions of the genome. Both previous and current work highlight the fact that these patterning events are nucleated at CpG islands by the G9a and GLP protein methyltransferases. Importantly, this work establishes a function for orphan CpG islands (previously unknown).