Trends in Immunology

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Spotlight

Transcription factors combine to paint the methylation landscape

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There is paucity of information about DNA methylation dynamics in immune cells. Roy *et al*. mapped the DNA methylation status of several thousand differentially methylated CpGs in human immune cells. They reported that the extent of cell type-specific hypermethylation is intriguingly most prevalent in adaptive immune cells rather than innate cells.

Hematopoietic stem cells give rise to mature cells of the immune system through the combinatorial effects of transcription factors, which control cell fate through reorganization of epigenetic landscapes. Various studies have furthered our understanding of which lineage-determining transcription factors play fundamental roles in shaping chromatin accessibility [1,2]. In addition, there are reports of histone modification combinations that contribute to defining cellular identity in T cells [3] and B cells [4]. Nevertheless, despite these advances, there is paucity of information concerning DNA methylation dynamics in many immune cell types and, more importantly, on how such dynamic landscapes are controlled. Indeed, the interdependence and combinatorial relationship among factors controlling chromatin accessibility, histone modifications, and DNA methylation remain to be thoroughly characterized for such cell types and particularly in humans. The present study by Roy *et al*. [5] contributes to addressing this concern by generating DNA methylation maps for six immune cell types in humans, illustrating the association of these maps with other epigenetic and transcriptomic signatures.

Roy *et al*. utilized Illumina Infinium methylation EPIC arrays on bisulfite-treated genomic DNA of six human immune cell populations isolated from peripheral blood mononuclear cells taken from 24 healthy individuals (aged 22–83 years), including B cells, $CD4^+$ and $CD8^+$ T cells, NK cells, monocytes, and granulocytes [5]. This array-based technique was used to query the methylation status of 853 307 CpG (850 K) sites across the genome [6]. The authors identified several thousand differentially methylated CpG sites in pairwise comparisons between immune cells [5]. Every immune cell population examined had cell type-specific hypomethylated regions, which generally correspond to transcriptionally permissive regulatory elements, in addition to hypermethylation regions, which generally correspond to transcriptionally repressed regulatory elements. An unexpected finding was the variable extent of cell type-specific hypermethylation in adaptive immune cells, in particular in T cells, compared with innate immune cells such as NK cells and monocytes (Figure 1).

The authors examined the relationship between selective gain and loss of DNA methylation with other epigenetic features, including chromatin accessibility measured by the DNase I hypersensitivity assay and histone modifications H3K4me1 and H3K27ac marking poised and active enhancers, respectively. As expected, genomic regions that were only demethylated in B cells were accompanied by selective gain of chromatin accessibility and deposition of H3K4me1 and H3K27ac in B cells. By contrast, genomic regions that were selectively methylated in B cells were accessible in other immune cells, such as $CD4^+$ T cells and NK cells, suggesting their active regulatory status in opposite lineages to B cells. Although the dynamics of DNA methylation in B cells was in line with the gain and loss of active chromatin signature of enhancers in this cell type, DNA methylation and the active chromatin features did not follow this simplistic trend in T cells. Roy *et al*. found that T cell-specific demethylated regions coincided with accessible chromatin sites and H3K4me1 and H3K27ac deposition in T cells. Unexpectedly though, they seemed to be poised in NK cells due to the detection of accessible chromatin and deposition of H3K4me1, but not H3K27ac, in NK cells. By contrast, T cell-specific demethylated sites were accessible and marked with H3K4me1 and H3K27ac modifications prominently in innate cell lineages, especially monocytes. These data suggest that intriguing mechanisms may be in place whereby T cell methylation signatures at demethylated sites are also accessible in NK cells, while hypermethylation is detected in other lineages.

Roy and colleagues also mapped transcription factor binding motifs in regions that were specifically hypo- and hypermethylated regions in these immune cell types. Distinct key developmental factors were identified in differentially methylated regions, highlighting the combinatorial nature of cell type-specific transcriptional regulation. For example, PU.1 and EBF binding motifs were identified in B cell-specific hypomethylated regions, while PU.1 and ATF motifs were enriched in monocyte hypomethylated sites. Of note, RUNX and PU.1 motifs were associated with both hypo- and hypermethylation, depending on cell type, which was intriguing considering the cooperative role of these transcription factors across many hematopoietic cell lineages. The authors further identified a proportion of EBF1-bound sites that were specifically hypomethylated in B cells, while other EBF1-bound sites were hypomethylated and deemed chromatin-accessible in all immune cell types tested, as evidenced from ChIP-seq. Finally, the authors demonstrated a

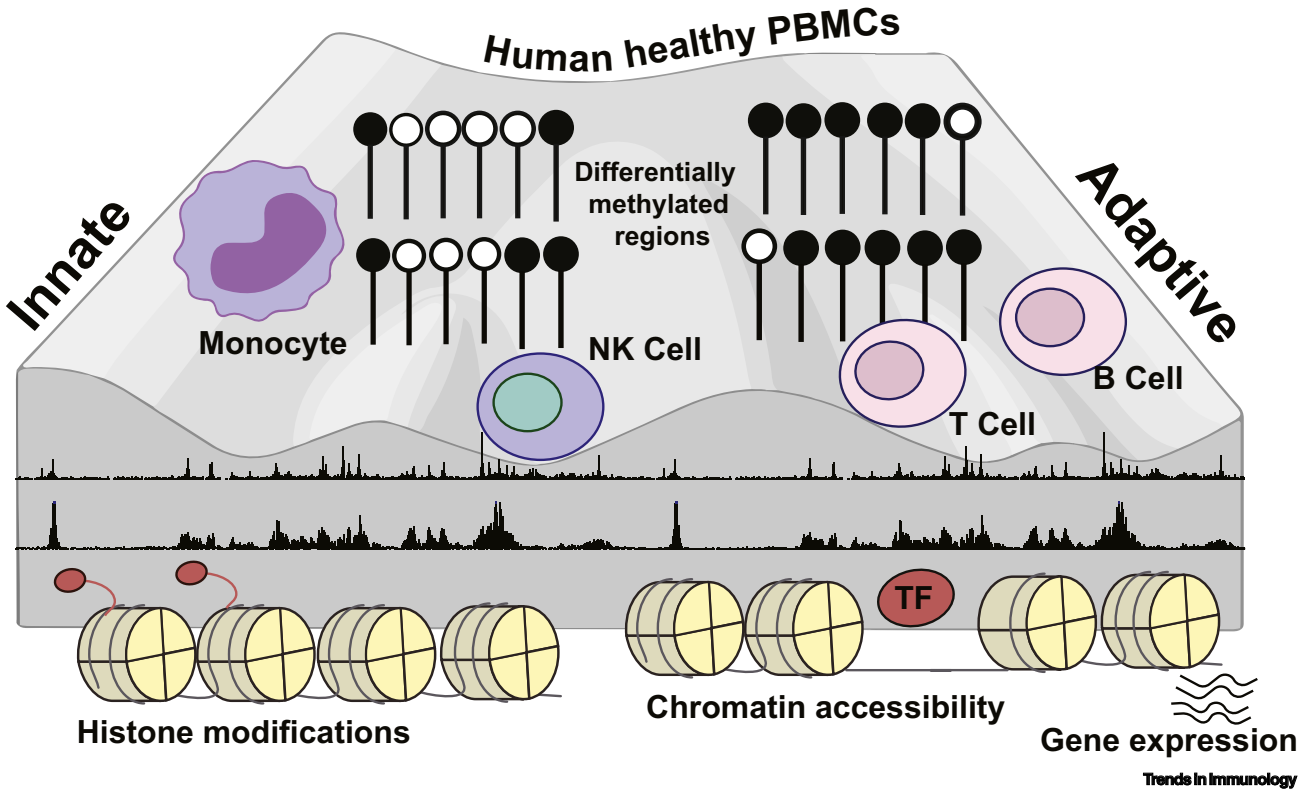

Figure 1. The extent of cell type-specific hypermethylation is most prevalent in human adaptive immune cells rather than in innate cells. Abbreviations: PBMC, peripheral blood mononuclear cell; TF, transcription factor.

relationship between B cell-specific hypomethylation and lineage-specific transcription factor binding corresponding to changes in gene expression. Overall, this work provides a comprehensive assessment of the methylation landscape of human immune cells and offers a host of candidate factors that might control DNA methylation in human hematopoiesis, yielding a valuable resource for further interrogation.

Few lineage-specific transcription factors have been directly implicated in interactions with DNA methylating or demethylating factors. This is relevant, as in this study, PU.1 motifs were found near sites of T cell- and NK cell-specific hypermethylation, suggesting a possible role for this factor in directing *de novo* methylation in these cells. Studies are actively delineating the factors that modulate methylation states in diverse cell types, most prevalently in B cell development [7,8]. Indeed, recent work showed that ten-eleven translocation (TET) demethylases were required for FoxA-mediated demethylation of regulatory elements required for liver cells [9]. But, from the immune cell perspective, the lineage-specific factors that have been identified by Roy *et al.* [5] certainly add a new perspective regarding the regulatory complexity of cell type-specific methylation patterns.

Much of the mechanistic insight in this research area has been carried out in murine models in which DNA methylating and demethylating enzymes can be genetically ablated. However, the extent to which these murine studies parallel human biology remains to be characterized. Nevertheless, the study discussed here provides an in-depth resource to establish a robust baseline for assessing methylation signatures in healthy donor human immune cell populations and for which diseased states might be eventually compared [5]. Further research can clarify the extent to which murine methylation mechanisms are conserved across human populations and how these states change with aging and/or with diseases such as cancers, or imprinting and genomic stability disorders, for which specific methylation patterns have been identified.

The work by Ranjan Sen's group and colleagues [5] illustrates the complex multilayered regulatory mechanisms underpinning hematopoiesis. The concordance between cell type-specific open chromatin and hypomethylation was evident in B cells

and monocytes. However, the authors describe T cell-specific hypomethylation as coinciding with open chromatin sites in both T cells and NK cells. These findings raise intriguing questions regarding the epigenetic control of transcriptional outputs, postulating that chromatin accessibility alone does not necessarily faithfully dictate gene expression. Further work should aim to uncover how multiple epigenetic mechanisms, including those presumably triggered by changes in the cellular environment, potentially synergize to finely tune transcriptional outputs.

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Declaration of interests

No interests are declared.

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