Research Highlight

Stem Cell INVESTIGATION

Changes in CpG methylation marks differentiation of human myeloid progenitors to neutrophils

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The role of CpG methylation in the silencing of cancer-causing genes was established more than a decade ago (1). Yet, the role of CpG methylation in normal cellular differentiation is only beginning to be understood. Studies performed over the last 5 years have detailed essential changes of CpG methylation during mouse granulopoiesis (2,3). A recent article in Blood by Rönnerblad *et al.* (4), has added substantially to these data, showing temporal DNA methylation during human granulopoiesis regulates the expression of key genes including transcription factors.

Using DNA methylation arrays, mRNA expression arrays and cap analysis of gene expression (CAGE), this work by the FANTOM consortium curated the global CpG methylation profile in association with gene expression across four stages of human neutrophil differentiation: common myeloid progenitors (CMPs), granulocyte-macrophage progenitors (GMPs), promyelocytes/myelocytes (PMCs) and terminally differentiated human bone marrow neutrophils (PMNs). There were two key findings in this study. Firstly, Rönnerblad *et al.* discovered a progressive global hypomethylation of CpGs from CMPs to PMNs (4). These changes were predominantly observed between GMPs and PMCs, indicating that DNA methylation may be crucial for making the choice between monocyte and granulocyte lineages. Unlike changes of DNA methylation in cancer cells that occur typically in CpG-rich regions of gene promoters called CpG islands, methylation changes were mainly detected in low CpG areas during neutrophil differentiation. These low CpG areas were also far from promoter regions and transcription start sites of genes. This observation suggests in these cells that the majority of hypomethylation changes have a function other than allowing access of transcription factors or activators to promoter regions in order to regulate gene expression.

Analysis of expression and CAGE data, however, showed there were coordinated changes in transcription factor expression and motif activity (i.e., downstream target gene expression) for the myeloid-specific transcription factors *PU.1*, *GATA2*, *ETS1*, *GFI1* and *MEF2D*. These changes corresponded with reciprocal levels of methylation at the promoter regions, supporting the role of methylation in regulating transcription factor expression and function. Thus, despite the rarity of methylation changes in the gene promoter regions, these changes may be essential to regulate the expression of key myeloid differentiation genes.

The second and most novel finding from this work was that dynamic changes of DNA methylation at enhancers regulate the expression of key neutrophil genes. Rönnerblad *et al.* found a significant enrichment of CpG hypomethylation in enhancers that were preferentially activated in PMNs compared to progenitor cells, in conjunction with the increased expression of genes associated with these enhancers (4). Importantly, these methylation-regulated enhancers are associated with genes that encoded proteins for neutrophil development and function including CSF2Rb, IL1b, IL8Ra/b, TLR1 and TLR6. Thus, decreased enhancer methylation levels were associated with reciprocal expression of associated neutrophil genes, indicating their role in gene regulation during granulopoiesis.

Overall, Rönnerblad *et al.* (4) have presented the most rigorous study to date that identified changes in DNA methylation in association with gene expression during human neutrophil differentiation. Several previous studies in mouse cells have included few or no intermediate cell types between

hemopoietic stem cells and PMNs, and therefore the timing of DNA methylation changes in any species has not been established (2,3). It is however important to highlight that the PMCs used in Rönnerblad *et al*. consisted of a mixture of promyelocytes and myelocytes. Our recent work showed that the gene expression profile for mature granulocytes was more similar to myelocytes than promyelocytes in mice (5). This high similarity may explain the lack of changes in methylation and gene expression between PMCs (containing myelocytes) and PMNs (granulocytes). To better dissect any changes occurring in these cells, future studies should isolate pure populations of promyelocytes, myelocytes and granulocytes for comparison of DNA methylation. Another important question that was not covered by Rönnerblad *et al*. is the role of DNA methylation in the gene body. Changes of DNA methylation in exons and introns have been associated with mRNA splicing in many cell types (1). Whether or not differential DNA methylation in these regions regulates splicing of key genes during neutrophil differentiation remains to be determined.

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Footnote

Conflicts of Interest: JJ Wong is a fellow of the NSW Cancer Institute. J Holst is a National Breast Cancer Foundation Fellow.

References

1. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012;13:484-92.
2. Ji H, Ehrlich LI, Seita J, et al. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. Nature 2010;467:338-42.
3. Bock C, Beerman I, Lien WH, et al. DNA methylation dynamics during in vivo differentiation of blood and skin stem cells. Mol Cell 2012;47:633-47.
4. Rönnerblad M, Andersson R, Olofsson T, et al. Analysis of the DNA methylome and transcriptome in granulopoiesis reveals timed changes and dynamic enhancer methylation. Blood 2014;123:e79-89.
5. Wong JJ, Ritchie W, Ebner OA, et al. Orchestrated intron retention regulates normal granulocyte differentiation. Cell 2013;154:583-95.

