Progressive G9a/GLP Methylation during Stem Cell Differentiation

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Epigenetic modifications, which are heritable changes in cell phenotype that are not encoded in DNA sequences, have profound effects on cell phenotype and may play a role in cellular development and cancer. These changes can occur by either modifying the DNA directly through methylation, typically the addition of a methyl group to the 5' position of cytosine, or by modifying proteins that interact with DNA. Histones are the basic protein subunits of nucleosomes, which help to condense DNA into chromosomes. Histone proteins are modified through several mechanisms, including methylation, ubiquitination, acetylation, and phosphorylation.

G9a and G9a-like protein (GLP) methylate the lysine at position 9 of the histone H3 subunit (H3K9), and have been described as "a gatekeeper of [stem cell] differentiation (Collins and Cheng, 2010)." Mono- and dimethylation of H3K9 (H3K9me1 and H3K9me2, respectively) by G9a/GLP are associated with transcriptional repression of genes normally expressed in stem cells, and G9a is necessary for mouse stem cells to differentiate into committed cell lineages. G9a/GPL methylation in fully differentiated cells occurs in large regions of chromatin spanning millions of nucleotides, and the pattern of these regions is cell-type specific. Interestingly, this methylation is lost in some cancer cell lines. Taken together, these results suggest that these methylated blocks of chromatin may be important for the development and/or maintenance of cellular differentiation.

This question is addressed in a recent study led by senior author Dr. Patrick Paddison and graduate student Xiaoji Chen (Human Biology Division) published in Genes and Development. The authors use hematopoietic stem and progenitor cells (HSPCs) as a model to investigate methylation patterns during the process of cellular differentiation. Five cell populations were investigated: 1) stem cells; 2) early but committed progenitor cells; 3) differentiated megakaryocytes, which produce platelets; 4) early differentiated T cells; and 5) bone marrow stromal cells, which are not hematopoietic (see figure).

Chen et al. immunoprecipitated DNA from each of these cell lines using an antibody specific to H3K9me2, and they sequenced the DNA that was associated with this type of methylated histone. The authors found that H3K9me2 chromatin territories are absent in primitive cells and are formed

de novo during lineage commitment. Interestingly, the H3K9me2 marks are nucleated preferentially at CpG islands in lineage committing progenitors and then further spread to regions outside of the nucleation site as cells becoming fully lineage committed to form H3K9me2 territories. It is possible that the progressive expansion of H3K9me2 during differentiation may influence higher-order chromatin structural changes that are important for lineage-specific differentiation.

The authors found that blocking G9a/GLP activity with the drug UNC0638 in committing HSPCs wiped out H3K9me2 patterning such that it remained like that of primitive cells. UNC0638 treatment also allowed for better retention of stem cell-like properties, slowing the differentiation of HSPCs in culture and producing more cells with stem cell markers during outgrowth.

One interesting outcome of the work is the notion that H3K9me2 marks are nucleated at CpG islands. Approximately half of CpG islands are located near transcription start sites (TSS), while the other half are either within or between transcription units. The function of non-TSS CpG islands is unknown. However, the work presented by Chen, *et al*. suggests that these “orphan” CpG islands may act as potential sites of H3K9me2 nucleation and spreading. It remains to be determined whether or not all adult stem cells become "reprogrammed" with respect to H3K9me2 patterning during differentiation, or if this is strictly a hematopoietic cell phenomenon. Chen and colleagues are currently examining other progenitor populations to determine if this is a general theme during development.

Finally, this work may lead to direct clinical applications. The effects of UNC0638 in this study may be used to improve hematopoietic stem cell transplantation by expanding the number of cells in vitro, and improving engraftment in the patient.

Chen X, Skutt-Kakaria K, Davison J, Ou YL, Choi E, Malik P, Loeb K, Wood B, Georges G, Torok-Storb B, Paddison PJ. G9a/GLP-dependent histone H3K9me2 patterning during human hematopoietic stem cell lineage commitment. Genes Dev. 2012 Nov 15;26(22):2499-511.

See also: Collins R, Cheng X. A case study in cross-talk: the histone lysine methyltransferases G9a and GLP. Nucleic Acids Res. 2010 Jun;38(11):3503-11.

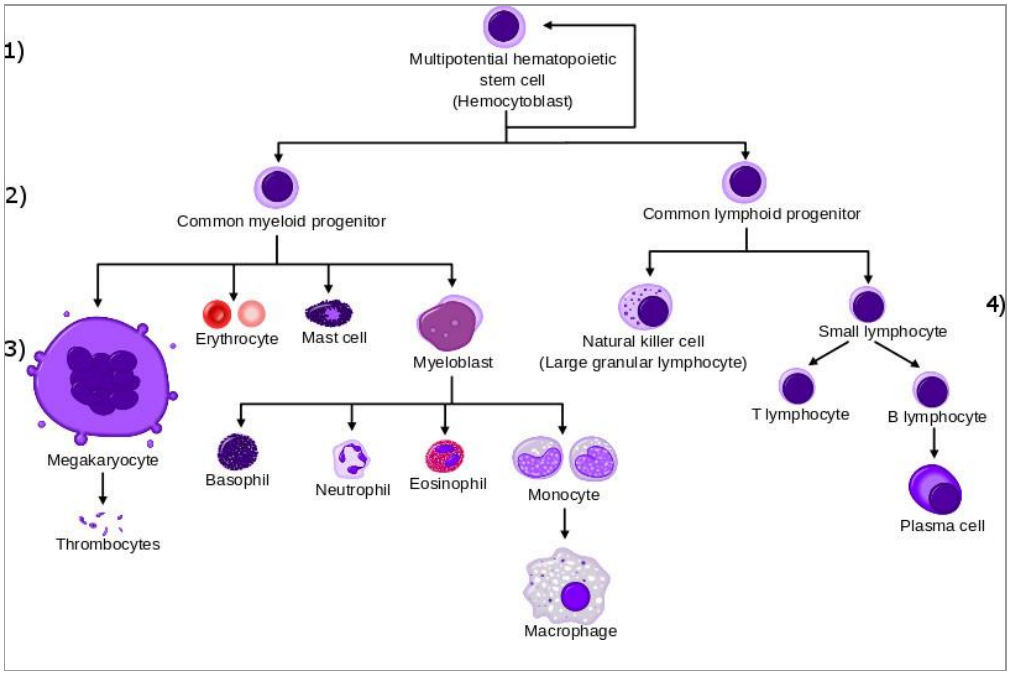

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Diagram of hematopoiesis. Numbers in the image correspond to the lineages examined in the paper.