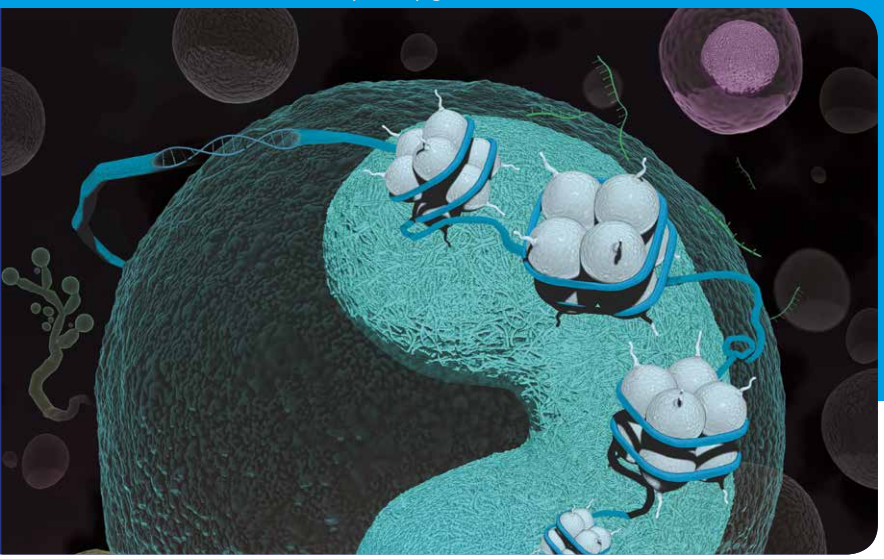


The life of a B cell

BLUEPRINT researchers track the entire epigenetic history of crucial immune system cells

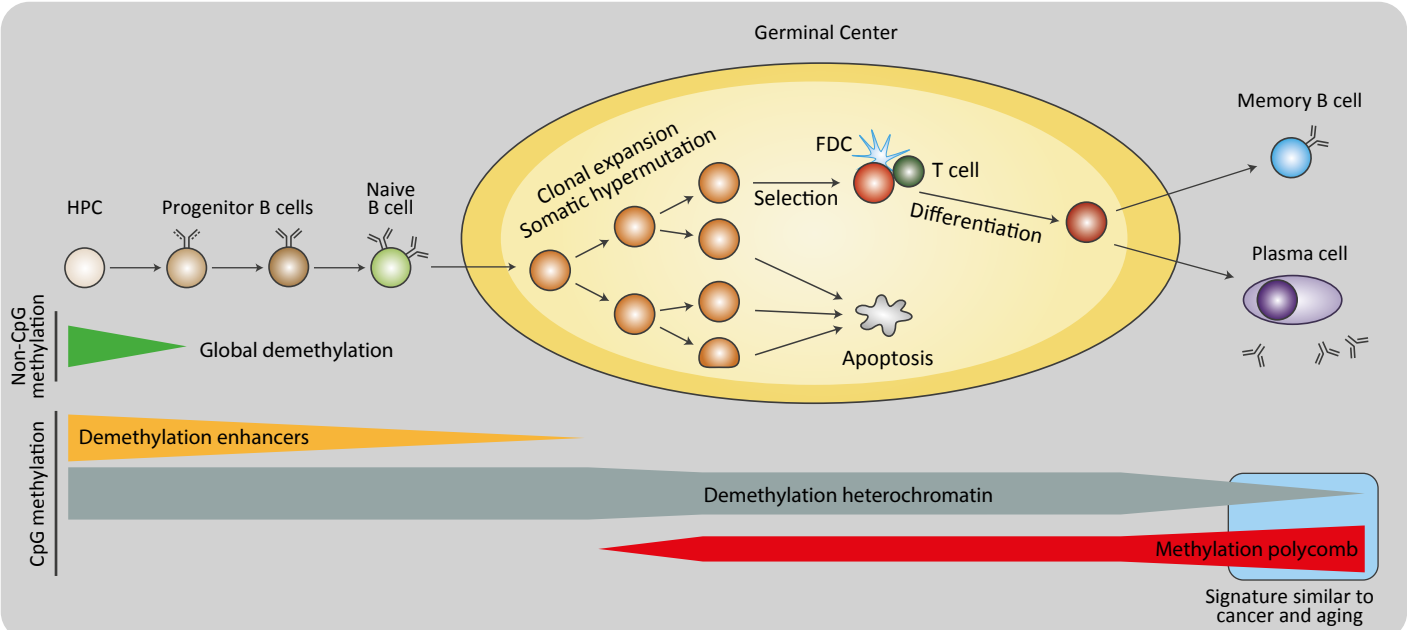
It has never been done before: to follow the entire epigenetic history of an immune stem cell through to its final differentiation and functional destination. B cells play crucial roles in the adaptive immune system of humans and other animals. They develop and retain a long-term memory to defeat infections by producing high quantities of specialized antibodies. During their life, B cells express new programs for their "immune police job".

Iñaki Martín-Subero and his team at the Institute of Biomedical Research August Pi i Sunyer (IDIBAPS) and University of Barcelona, working with other BLUEPRINT members, isolated B cells at each stage of their development to follow the epigenetic changes that mark each phase of their lives. The results have been published online in the June 8 edition of Nature Genetics. The researchers not only define the precise molecular changes during B cell development but provide general new insights into mechanisms that define cell fate in the human body.

The project focused on an epigenetic mechanism called DNA methylation. Enzymes in cells attach small chemical tags called methyl groups to specific DNA sequences. These tags are important for the regulation of genes and they accordingly change during development. In vitro data showed that stem cells start with a very general set of tagged regions/genes which become more and more specialized as the cells mature and develop their fates. So far, however, no one had followed this process in detail in cells isolated from the human body.

Marta Kulis and other members of Iñaki's group collaborated with other BLUEPRINT partners in Spain, Germany, Holland, and the UK and labs in the US to track DNA methylation across the entire genome of cells as they became fully differentiated B cells. The scientists performed a very deep and careful analysis of the changes that occur in DNA methylation during B cell differentiation. Most previous analyses paid attention to the methylation of bases called cytosines in their "canonical" context, neighboring a guanine base to form what is called a CpG. But Marta and her colleagues also examined DNA methylation outside of such CpGs – at non-CpG sites – a decision that turned out to be important.

In our immune system, B cells undergo a complex process of development and selection. Early progenitor B cells originate from hematopoeitic stem cells (HSCs) in the bone marrow that spawn the many types of the blood and lymph system cells. Through a genetic engineering trick, precursor B cells rearrange part of their genome to be able to recognize millions of damaging molecules from pathogens, the so called antigens. Then, they are released to circulate through the bloodstream and lymphatic system as naïve B-cells. Unless they encounter such an antigen, they remain naïve and die after a few days. But if an antigen is bound, the cell becomes stimulated, begins to proliferate and modify the sequence of its antibody genes so that it can better recognize the invading antigen. Some of these go on to become



plasma B cells – factories that churn out vast copies of free-floating antibodies that can bind to the foreign molecule. Others become memory B cells, which preserve the memory to quickly destroy a pathogen that infects the body a second time.

The researchers tracked methylation in the cells' DNA through all of these maturation stages. They made a number of important discoveries. First, they discovered a strong association between differences in DNA methylation and cell stages, revealing that the cell's degree is epigenetically determined. Secondly, a huge number of DNA methylation tags change – nearly five million of the sequences had tags applied or stripped from them over the process of development. The most dramatic changes occurred when the cells were stimulated by encounters with antigens.

"We observed these changes in rapidly reproducing B cells in the lymph nodes, in memory B cells, and in plasma cells," Iñaki says. "Many of them were clustered in genes. The most common event was that methyl tags that had been applied earlier in development were stripped off these sites. This distinguished activated cells from their naïve sisters, which retained most of the methyl tags."

Similarly to changes at CpG sites, an analysis of non-CpG methylation revealed that many of these tags are also changed. But the two events didn't happen at the same time. Tags were usually removed from non-CpG sites first, leaving tags on nearby CpGs that would be stripped off only later. The finding was important, Iñaki says, because it reveals that different mechanisms are responsible for the two events.

"Most non-CpG methylation is removed when HSCs take their first steps toward becoming B cells, meaning that these sites may play a crucial role in preserving stemness," Iñaki says. Another important finding concerned proteins called transcription factors that bind to specific sites in DNA and stimulate the activation of nearby genes. "Different types of cells use different transcription factors," Iñaki says. "We found that the transcription factors in B cells seem to be involved in removing methyl tags from the sites they bind to. Once that happens, the sequence remains free of tags in subsequent stages of development. This means that the sequence preserves an epigenetic memory that a transcription factor has been bound to it in the past."

One of the most significant findings of the study had to do with the functions of the changes in methyl tags. Some changes were linked to B-cell development, others to cell reproduction while a third "class" seemed to be associated with cell longevity. Remarkably, these changes were distinct in cells with extended lifespans, such as memory and plasma B cells.

This may represent an intriguing link to the many changes observed in blood cell cancers; cancer cells have a much-extended life span (or are even immortal) allowing them to outcompete healthy cells in the bloodstream and lymphatic system and hence form life-threatening situations. "Long-lived B cells and B-cell tumors share key DNA methylation patterns that all seem to be related to their long life span," Iñaki says.

Overall the study by Marta, Iñaki and their colleagues provides us with many novel insights into the life and functionality of normal and abnormal epigenetic tags in B cells. On the one hand, their study shows how well the biological selection and proliferation of cells is regulated by setting and removing of epigenetic tags. On the other hand, the study shows that cancer cells emerging from B cells acquire parts of the "normal" B-cell developmental program to become long lived or immortal. Both findings are a big step forward in the understanding of the molecular mechanisms underlying normal cell maturation and cancer development, and may provide the basis to develop new drugs for combating blood malignancies such as leukemia.

Reference:

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