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Environmentally-defined enhancer populations regulate diversity of tissue-resident macrophages

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Abstract

Macrophages represent a class of cells specialized for phagocytosis that occurs in multiple, phenotypically distinct populations throughout the body. Two studies published in *Cell* demonstrate that these phenotypic differences reflect drastic differences in the populations of enhancers that regulate transcription, and that this epigenomic diversity is, in fact, highly plastic and sensitive to environmental cues.

Keywords

Macrophages; enhancers; histone modification; transcription factors; cellular plasticity

Two recent studies [1,2] utilize macrophages as a unique system for investigating the contributions of ontogeny and environment in shaping the phenotype of a specific cell type via epigenomic mechanisms. Macrophages constitute a diverse set of phagocytic cells that are widely distributed in the body and play important roles in tissue homeostasis and in innate immune responses. Specialized populations of macrophage cells residing within microenvironmental niches additionally perform tissue-specific functions, such as microglia in the brain that promote neuronal survival and red pulp macrophage cells in the spleen that clear senescent red blood cells [3]. Consistent with their diverse locations and functions, tissue-resident macrophages in different organs display distinct gene expression profiles [4]. Adding to the complexity of macrophage biology are recent findings clarifying their developmental origins and tissue maintenance. Thought for the past several decades to be derived from and maintained by hematopoietic stem cells through monocytic intermediates, it is now evident from lineage-tracing studies that most tissue-resident macrophage populations, entirely or in part, are derived from early embryonic hematopoietic progenitors that emerge in the yolk sac prior to hematopoietic stem cells [5,6]. Amazingly, these embryonic macrophages persist into adulthood, maintaining themselves as long-term residents of specific organs.

The diversity of these different tissue-resident macrophage populations raises several questions. How is this diversity achieved? What roles do specific tissue microenvironments

play in the establishment of macrophage diversity? How plastic is this diverse macrophage identity?

The production of multiple, phenotypically distinct cell types from a common genome is one of the central features of metazoan biology. While it has long been recognized that cellular diversity originates from differential gene regulation, the general basis for this has only emerged with the cataloguing of genetic regulatory elements and epigenomic chromatin states. As a result, we now appreciate that differential gene regulation arises largely from distinct populations of enhancers, bound by transcription factors, which are often located far from the sites of gene transcription. Enhancers occur in large numbers – 10^4 or more for each cell type – and genome-wide enhancer maps, in which the topography is determined by specific epigenomic modifications (usually H3K4 monomethylation and H3K27 acetylation), can serve to define specific cell types.

It is therefore interesting that both of these recent studies find drastic differences in enhancer populations among macrophage subtypes. In comparisons of brain microglia and large peritoneal macrophages (LPM), Gosselin *et al.* find that only 4% of promoters differ with respect to histone modification signatures, while 40% of active enhancers are specific to one type or the other [2]. Similarly, Lavin *et al.* find that the majority of active promoters are shared, not only among macrophage subtypes, but also between macrophages, monocytes and neutrophils. The majority of enhancers, however, are unique to each cell type, and very few (<2%) of macrophage-specific enhancers are shared across the seven subtypes canvassed by the authors [1]. Thus, differential enhancer usage marks not only distinct cell types, but also phenotypic variants within a particular cell type.

Such results imply that enhancer populations, and the epigenomic landscape of which they are a part, are influenced by the tissue-specific environments within which they reside. Lavin *et al.* test this directly by transplanting adult mouse bone marrow into irradiated recipients and subsequently isolating macrophages that repopulate the lung, spleen, liver and peritoneum [1]. They find that enhancer populations within the newly derived macrophages are similar to those of macrophages that normally reside in these environments. In a separate experiment, they transfer peritoneal macrophages to mouse lung and observe a general upregulation of lung macrophage-specific genes, with 70% of differential gene expression switching to the lung macrophage pattern.

Gosselin *et al.* examined the epigenomic plasticity of macrophages in cell culture [2]. Brain microglia and LPM cultured in the presence of appropriate cytokines (IL-34 and M-CSF, respectively) exhibit drastically altered gene expression, with microglia- and LPM-specific patterns preferentially lost. Noting that peritoneal macrophages are associated with retinoic acid (RA) signaling, while microglia are associated with signaling by TGF β , the authors then culture LPM in RA or TGF β . In the presence of either factor, they observe that more than half of LPM-specific genes are induced by RA, while half of the genes induced by TGF β are specific to microglia *in vivo*. These gene expression results are paralleled by histone modification signatures at enhancers: around half of enhancer regions normally observed in LPM show significantly reduced levels of H3K4 methylation and H3K27

acetylation upon transfer to cell culture, but one third of these regions are maintained in the presence of RA.

Taken together, the results indicate that although macrophage subtypes display specific enhancer signatures, associated with differential gene expression patterns, these features are highly responsive to local microenvironments. Enhancers can be gained or lost, and gene expression concomitantly modulated, on the basis of local signals. In particular, the transplantation experiments in Lavin *et al.* [1] show that a substantial proportion of the epigenomic signatures and gene expression patterns exhibited by normal, embryo-derived macrophages in a specific locale can be reproduced with adult hematopoietic stem cell-derived or even fully differentiated macrophages simply by placing them in that locale.

At the level of gene regulation, such distinct patterns of gene expression suggest that macrophage subpopulations express different transcription factors, and both studies employ bioinformatics-based approaches to identify some of these factors. PU.1 has previously been shown to occupy enhancers in macrophages [7,8], and both groups identify PU.1 binding motifs at the macrophage enhancers they catalogue. Lavin *et al.* also find that macrophage enhancers are often associated with Maf binding motifs, consistent with high expression of MafB and Maf in differentiating macrophages. In addition, they find specific motifs enriched in enhancers active within each macrophage subtype, including Gata and MEF2 motifs in peritoneal macrophage and microglial enhancers, respectively.

Gosselin *et al.* take this analysis one step further by performing ChIP-seq for PU.1 and enhancer-associated histone marks in microglia and LPM isolated from different mouse strains [2]. Genetic variation between the strains provides a sort of natural mutagenesis strategy for examining the effects of enhancer mutations [9]. To take advantage of this, the authors look for strain-specific PU.1 binding not associated with mutations in PU.1 motifs; such strain specificity should arise from mutations in associated binding motifs, which will correspond with macrophage subtype-specific transcription factors. Notably, the motifs identified in this way in LPM include KLF4, AP-1 and Gata, the last of these paralleling the results of Lavin *et al.* [1], and consistent with a recent study revealing a crucial and specific role for Gata6 in peritoneal macrophages [10].

Taken together, these studies show that tissue-resident macrophages are defined by distinct enhancer populations that in turn originate from differential expression of specific transcription factors working in conjunction with PU.1. Transcription factor expression in each macrophage population represents, in part, a response to the local environment in which each macrophage population resides, and thus these results argue for a high degree of plasticity among macrophage subtypes. These findings also lead to further questions. Conclusions drawn from bioinformatics-based analyses need to be validated – by ChIP-seq for the predicted subtype-specific factors, by *in vivo* mutagenesis of macrophage enhancers, etc. Furthermore, it is clear from both studies, but in particular the cell culture experiments, that the full repertoire of signals within each macrophage environment is not yet defined, and so the relative contribution of ontogeny to epigenomic and gene expression patterns cannot yet be measured. Future work will be required to address these and other questions, leveraging the unique and useful system provided by tissue-resident macrophages for the

study of the origins of epigenomic landscapes and the degree to which they can respond to external signals.

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