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Histone Methylation is Critical in Monocyte to Macrophage Differentiation

Jennifer Bermick*, Matthew Schaller and William Carson IV

Department of Pathology, University of Michigan Medical Center, Ann Arbor, MI, USA

*Corresponding author
jendalto@med.umich.edu

Abbreviations:
Mo, monocytes; Mφ, macrophages; KLF4, Krüppel-like factor 4; FOXO, Forkhead box O; HOXA, Homeobox A; HMTs, histone methyltransferases; MLL, mixed lineage leukemia; EZH2, enhancer of zeste homolog 2.

Cells of the mononuclear phagocyte system are part of the innate immune system and function as a first line of defense, sensing, ingesting and destroying foreign antigens and pathogens and directing subsequent inflammatory responses. Two of the main components of the mononuclear phagocyte system are monocytes (Mo) and macrophages (Mφ). Mo are derived from bone marrow precursors and provide immune surveillance in the bloodstream. Mφ primarily reside in the tissues, and are the main regulators of tissue homeostasis and repair. Tissue-resident Mφ have two distinct origins. Most tissue-resident Mφ are established prenatally and are maintained by self-renewal[1]. A smaller subset of tissue-resident Mφ develop from recruited Mo

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that differentiate into Mφ under the guidance of growth factors and other inflammatory stimuli[1]. Mo derived Mφ play a prominent role in tissue inflammation and pathology, including infection control and cancer development. It is therefore critical to better understand the pathways involved in the differentiation of Mo into Mφ, so targeted therapies can be developed to improve outcomes in diseases mediated by these cells. Mo derived Mφ subset heterogeneity is tightly controlled by defined transcriptional networks[2]. Although these networks have been relatively well mapped out, the regulation of specific transcription factors in stage- and lineage-specific Mo to Mφ differentiation is not well understood.

In this issue of *The FEBS Journal*, Jin and colleagues assessed how changes in histone modifications impacted the transcriptional regulation of Mo to Mφ differentiation[3]. Histone modifications stimulate or repress gene transcription by altering chromatin structure, and have been shown to regulate normal developmental changes in the immune system and contribute to cell lineage decisions and differentiation[4, 5]. This makes them an attractive area to explore in the regulation of Mo to Mφ differentiation. The present report provides important evidence that alterations in the Mo histone modification landscape underlie broad changes in transcription factor expression critical in the process of Mo to Mφ differentiation.

There are numerous transcription factors involved in the differentiation of Mo to Mφ, and these differ based on the specific Mφ subset. Regardless of the terminal Mφ differentiation state, the transcription factors involved need to be precisely regulated in order for appropriately functional Mφ to develop. Key transcription factors involved in general Mo to Mφ differentiation that were evaluated in the present study include the positive regulators Krüppel-like factor 4 (KLF4) and the Forkhead box O family (FOXO), as well as the Homeobox A gene cluster (HOXA) which acts to block this differentiation[6-8]. Aberrant expression or function of any of these or other transcription factors can lead to the development of acute myelogenous leukemia, highlighting the importance of transcriptional regulation in maintaining tissue homeostasis and appropriate Mφ differentiation and function[8].

Epigenetic modifications alter chromatin structure and change the ability of transcription factors to interact with the DNA, thus allowing for precise gene expression regulation without altering the underlying genetic code. The chemical modification of lysine residues in histone tails is one type of epigenetic modification, and the position of the modified lysine within the histone sequence, determines whether the added modification results in an active (open) or silenced (closed) gene configuration. Activation of gene transcription occurs when three methyl groups are added to lysine (K) 4 of histone (H) 3 (ie. H3K4me3). Conversely, H3K27me3 results in chromatin

compaction and gene repression[9]. There are numerous enzymes that add modifications to histone proteins, called histone methyltransferases (HMTs), controlling the gene expression profile of a cell and allowing for plasticity during cell activation and differentiation. The mixed lineage leukemia (MLL) protein family and the enhancer of zeste homolog 2 (EZH2) are HMTs that trimethylate histone 3 lysine 4 and histone 3 lysine 27, resulting in H3K4me3 and H3K27me3, respectively[10].

The roles of MLL and EZH2 have been most extensively studied in cancer states, but there has recently been increased interest in the contribution of these HMTs to immune cell activation and differentiation. In recent years, both MLL and EZH2 have been found to have crucial roles in T-cell proliferation, differentiation and maintenance of lineage phenotypes[11, 12]. Similarly, these HMTs have been shown to contribute to Mo to Mφ differentiation and the maintenance of specific Mφ phenotypes[8, 13]. The present study adds to this body of literature by demonstrating that both MLL and EZH2 cause site-specific changes in H3K4me3 and H3K27me3 at the promoters of the transcription factors KLF4, FOXO and HOXA, which are important in the regulation of Mo to Mφ differentiation (Figure 1). A strength of this study is the use of pharmacologic inhibition of these HMTs, which promotes Mo to Mφ differentiation and opens up the possibility of using an HMT inhibitor in disease states in which aberrant Mo to Mφ differentiation is involved. While further work is needed to dissect the exact upstream mechanisms underlying these results, this study supports the notion that site-specific histone methylation and subsequent chromatin remodeling at known immunologically important transcription factor promoters is a critical step in Mo to Mφ differentiation.

These findings set the stage for potential therapeutic targets to alter the process of Mo to Mφ differentiation, which is crucial to the development of many serious disease states, including leukemia, obesity and atherosclerosis[14]. HMT inhibitors are attractive treatment options for these diseases; some inhibitors have reached the early stages of clinical trials in cancer, including an EZH2 inhibitor used to treat B-cell lymphoma[15]. However, challenges remain when considering using HMT inhibitors to treat human disease states. One such challenge includes off-target effects and toxicities of HMT inhibitors due to the complicated biology of HMTs, which have numerous and diverse targets that differ between cell types. This makes systemic administration of an HMT inhibitor complicated and risky, highlighting the importance of studies that further detail the intricacies of HMT targets in varied cell types under different conditions. Only in further understanding how and why HMTs select their targets and why this differs

between cell types and upon different stimulating conditions can we move forward in developing safe and effective therapies to treat these diseases while minimizing off-target effects.

Mo derived Mφ play an important role in many disease states, and defined transcriptional networks tightly regulate the process of Mo to Mφ differentiation. Some of these networks are under the direct control of the HMTs MLL and EZH2, resulting in increased H3K4me3 and H3K27me3 and subsequent chromatin remodeling at the promoter sites of transcription factors crucial in Mo to Mφ differentiation. These findings identify potential therapeutic targets for diseases in which Mφ play a prominent role, although better understanding of the nuanced regulation of these HMTs and their specific targets is necessary before directed epigenetic treatments can be widely implemented.

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Figure 1. Schematic overview of histone methylation changes impacting transcription factor expression in monocyte to macrophage differentiation. The upper portion of the figure demonstrates that macrophage differentiation factors stimulate decreased monocyte H3K27me3 at the promoter sites of *KLF4*, *FOXO1* and *FOXO3*, causing the chromatin to open, allowing transcription factors to access the promoter sites, and resulting in increased gene transcription. The lower portion of the figure demonstrates that macrophage differentiation factors stimulate decreased monocyte H3K4me3 and increased H3K27me3 at the promoter sites of *HOXA6*, *HOXA7* and *HOXA13*, causing the chromatin to close, denying transcription factors access to the promoter sites, and resulting in decreased gene transcription.