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硕 士 学 位 论 文

调控单核-巨噬细胞分化的组蛋白甲基化重建规律研究

Reprogramming of global histone methylation controls the
differentiation of monocytes into macrophages

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缩略语索引

缩略语	英文全名	中文全名
MPS	Mononuclear phagocyte system	单核巨噬细胞系统
PKC	Protein kinase C	蛋白激酶 C
PMA	Phorbol ester	佛波酯
M-CSF	Macrophage colony stimulating factor	巨噬细胞集落刺激因子
MLL	Mixed lineage leukemia	混合谱系白血病
EZH2	Enhancer of zeste homolog 2	zeste 基因增强子
HOXA	Homeobox A	同源框基因 A 簇
FOXO	Forkhead box O	叉头框基因 O 簇
BM	Bone marrow	骨髓
H3K4me3	Trimethylation of Histone H3 lysine 4	组蛋白 H3 第 4 位赖氨酸 三甲基化
H3K27me3	Trimethylation of Histone H3 lysine 27	组蛋白 H3 第 27 位赖氨酸 三甲基化
IL-6	Interleukin 6	白细胞介素 6
LPS	Lipopolysaccharide	脂多糖

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摘要

单核-巨噬细胞系统由一类具有吞噬功能的免疫细胞组成，包括单核细胞、巨噬细胞及树突状细胞，是机体免疫系统重要的组成之一。来源于造血干细胞单核细胞在趋化因子激活下，分化为巨噬细胞和树突状细胞，参与机体防卫。目前有关单核-巨噬细胞分化的细胞学过程已基本阐释，然而分化进程中关键分子及其调控网络仍需要深入阐明。此外，单核细胞如果过度增生，会导致正常造血功能衰竭。白血病的发生也与单核-巨噬细胞系统的异常发展有关。因此，深入探索和阐明单核/巨噬细胞形成过程中关键转录因子及其调控网络，为深入了解免疫系统发育及疾病发生提供重要的理论依据，并为白血病的临床诊断和治疗提供有效的分子靶点。

本研究采用了经典的佛波酯（PMA）诱导的髓系白血病细胞系分化模型，体外模拟单核向巨噬细胞分化过程。通过 ChIP-on-chip 结果发现，在 PMA 诱导的单核-巨噬细胞分化过程中，多数分化相关转录因子启动子区域富集丰富的正性组蛋白 H3K4me3 和负性组蛋白 H3K27me3 共价修饰，这些转录因子包括 *HOXAs*、*FOXOs*、*KLF4*、*IRF8* 等。转录水平检测发现，在 PMA 诱导的单核-巨噬细胞分化过程中，*HOXAs* 基因在分化早期就快速降低，且始终维持低表达。深入研究发现，其早期转录沉默主要与蛋白激酶 C（PKC）通路的激活有关；而分化过程中的低表达主要由正性组蛋白 H3K4me3 修饰降低调控，维持阶段的低表达则是由负性组蛋白 H3K27me3 修饰增高引起。同时，其它转录因子也受到不同程度的表观遗传调控。H3K27me3 组蛋白甲基化修饰调控的 *FOXOs* 作为负性调控因子，在分化中拮抗 *HOXAs* 沉默。利用 H3K4me3 和 H3K27me3 小分子抑制剂靶向干预染色质组蛋白修饰，明显促进单核-巨噬细胞分化效率。最后，我们从小鼠体内原代分离骨髓干细胞，在生理状态下验证了单核巨噬细胞分化进程中染色质组蛋白甲基化重建的关键而必要的生物学作用。

综上所述，本研究证明在单核-巨噬细胞分化过程中组蛋白重建调控关键转录因子的表达，从而使分化持续、不可逆地进行；阻断组蛋白修饰显著促进单核细胞分化进程；本课题从病/生理学水平研究了单核-巨噬细胞分化过程的表观遗传

传调控机制，为靶向调控单核-巨噬细胞分化过程提供理论依据，也为临床白血病的治疗提供新的思路。

关键词：单核细胞 巨噬细胞 表观遗传学

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Abstract

Monocyte-macrophage system is a part of the immune system in higher animals, which consists a class of phagocytosis immune cells, including monocytes, macrophages and dendritic cells. Mononuclear cells derive from hematopoietic stem cells, and can differentiate into macrophages and dendritic cells, which involved in the body defense. Currently the differentiation process has been mainly explained, but the key elements and regulatory networks participating in monocyte-macrophage differentiation is still unclear. In addition, excessively proliferated monocytes may do harm to normal hematopoietic function. The occurrence of leukemia is also related to monocyte-macrophage system abnormalities. Therefore, further identifying and clarifying the key transcription factors and regulatory networks during monocyte-macrophage formation will provide an effective molecular target for immune diseases and leukemia.

In this study, we use a classical differentiation model, phorbol ester (PMA) induced myeloid leukemia cell differentiation, to simulate monocyte to macrophage differentiation in vitro. And then we divide the differentiation artificially into start-up phase, differentiation phase and maintenance phase. By chromatin immunoprecipitation chip (ChIP-on-chip), we found the promoter regions of majority differentiation-related transcription factors is occupied by positive trimethylation of histone H3 lysine 4 (H3K4me3) and negative trimethylation of histone H3 lysine 27 (H3K27me3) in PMA-induced monocyte-macrophage differentiation. The transcription factors include *HOXAs*, *FOXOs*, *KLF4* and *IRF8*. The transcription levels of *HOXAs* is rapidly decreased in the early stage during PMA-induced monocyte-macrophage differentiation, and maintains in a low expression level. In-depth study, we find that the early transcriptional silencing of *HOXAs* is correlated with the activation of protein kinase C (PKC) pathway. And positive regulation of histone H3K4me3 modification participates in regulating *HOXAs* at differentiation

phage, then the H3K27me3 maintains low level expression of *HOXAs* at phygocytic periods. In addition, other transcription factors have also been found to be regulated by epigenetics. We also find that the transcription factor *FOXOs*, regulated by histone methylation, acts as an antagonistic factors. Small molecule inhibitors targeted histone modifications can significantly promote the differentiation of monocytes. Finally, bone marrow stem cells isolated from mice verified the important roles of the histone modifications in cell differentiation under physiological state.

In conclusion, our study shows that reprograms of global histone methylations regulates the expression of key transcription factors in the monocyte-macrophage differentiation, so that insuring differentiation in a sustained and irreversible manner. Blocking histone modifications will promote differentiation of monocytes. The research illuminates the epigenetic mechanism in cell differentiation from physiological and pathological perspectives, and provides a theoretical basis for targeted regulation of monocyte-macrophage differentiation and clinical treatment of leukemia.

Key Words: monocyte; macrophage; epigenetics

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