Molecular Cell
Article

A Negative Feedback Loop of Transcription Factors Specifies Alternative Dendritic Cell Chromatin States

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http://dx.doi.org/10.1016/j.molcel.2014.10.014

SUMMARY

During hematopoiesis, cells originating from the same stem cell reservoir differentiate into distinct cell types. The mechanisms enabling common progenitors to differentiate into alternative cell fates are not fully understood. Here, we identify cell-fate-determining transcription factors (TFs) governing dendritic cell (DC) development by annotating the enhancer landscapes of the DC lineage. Combining these analyses with detailed overexpression, knockdown, and ChIP-Seq studies, we show that Irf8 functions as a plasmacytoid DC epigenetic and fate-determining TF, regulating massive, cell-specific chromatin changes in thousands of pDC enhancers. Importantly, Irf8 forms a negative feedback loop with Cebpb, a monocyte-derived DC epigenetic fate-determining TF. We show that using this circuit logic, a pulse of TF expression can stably define epigenetic and transcriptional states, regardless of the microenvironment. More broadly, our study proposes a general paradigm that allows closely related cells with a similar set of signal-dependent factors to generate differential and persistent enhancer landscapes.

INTRODUCTION

Eukaryotic DNA is organized into a higher-order chromatin structure in various levels of nucleosome compaction (Woodcock and Ghosh, 2010). During differentiation, chromatin is dynamically modified in a sequence of events leading to cell-type-specific chromatin landscape (Deaton and Bird, 2011; Xu and Zaret, 2012). However, the mechanisms regulating chromatin states across different immune cell types are not fully understood (Winter and Amit, 2014). Furthermore, there is a debate as to whether chromatin states mainly serve as epigenetic memory of earlier developmental decisions or whether they are representative of the ongoing activity of the microenvironment and sequence specific regulators (Cheedipudi et al., 2014). Studies have shown that specific TFs known as "pioneers" play a critical role in this process, by recruiting chromatin-modifying enzymes to cell-specific regulatory elements (Budry et al., 2012; Lupien et al., 2008; Wallberg et al., 2000; Zaret and Carroll, 2011). As the cell identity is established, pioneers are the earliest TFs to appear on regulatory regions prior to enhancer establishment and are sufficient to uncompact local chromatin structure and promote gene activation (Cirillo et al., 2002). In some cases, pioneer factors establish the chromatin state and are then dispensable for chromatin maintenance (Formosa, 2013). Alternatively, individual pioneer factors are essential for continuous maintenance of the chromatin state (Kadauke et al., 2012; Xu et al., 2007). Currently, the regulatory networks of pioneer TFs and, more specifically, the feedback circuits between pioneer TFs and cell fate decisions in the immune system have not been thoroughly examined (Lara-Astiaso et al., 2014).

Here, we examine these questions through the differentiation of one branch in the innate immune lineage. Dendritic cells (DCs) are antigen-presenting cells crucial for both the innate and adaptive immune response to infections (Banchereau and Steinman, 1998). DCs represent a functionally and genetically heterogeneous population of innate immune cells originating either from common DC precursors (CDPs) or monocytes (Reizis, 2012). While DCs share many common functional features, multiple DC subtypes with distinct immune functions have been identified in both mice and humans (Shortman and Liu, 2002). CDP-derived, Flt3-ligand (Flt3L)-dependent DCs include type 1 interferon-producing plasmacytoid DCs (pDC) and classical DCs (cDCs). The latter have been further subdivided into CD8a+ and CD11b+ subsets. Under conditions of inflammation, but potentially also under certain steady-state conditions, the cDC compartment is complemented by monocyte-derived DCs (moDC) (Mildner et al., 2013b; Segura and Amigorena, 2013). Although all DCs respond to infections, pDCs are specialized in antiviral immunity through their ability to produce large amounts of type I interferons (IFNs) (Gilliet et al., 2008). On the other hand, moDCs and cDCs are important mediators of antibacterial responses and antigen presentation. Phenotypically, pDCs differ from cDCs and moDCs in several key aspects: pDCs have a round morphology with a highly developed secretory compartment, while cDCs and moDCs exhibit typical dendrite morphology with prominent cytoplasmic protrusions. Molecularly, pDCs display many features of lymphocytes such as the B cell marker (B220), nucleic acid-sensing TLRs (TLR7,

TLR9), and transcription factors (Tcf4, SpiB, Bcl11a), whereas cDCs and moDC express high levels of receptors that sense bacterial components (TLR2, TLR4) and inflammatory response genes (Il1b, Tnf, Cxcl1) (Crozat et al., 2010; Gilliet et al., 2008). In contrast to the rich expression profiling and various TFs documented in the regulation of mRNA expression (Cisse et al., 2008; Ghosh et al., 2010; Spits et al., 2000; Tsujimura et al., 2002; Vander Lugt et al., 2014), the role of chromatin regulation in DC specification is mostly unknown.

Over the years, a variety of TFs were shown to play important roles in controlling the development of DC populations. In particular, mice lacking the interferon regulatory factor 8 (Irf8) are pDC deficient (Tamura et al., 2005; Tsujimura et al., 2002), and absence of Irf8 from DC progenitors causes DC-to-neutrophil reprogramming (Schonheit et al., 2013). The TF Pu.1, an ETS family member, is required for the development of the myeloid lineage (Carotta et al., 2010; Heinz et al., 2010): Pu.1-deficient mice have impaired development of macrophages, monocytes, and DCs in the embryo and neonate (Guerriero et al., 2000). Further studies demonstrate that Pu.1 and Cebpb function as chromatin-regulating factors of both macrophages and moDC (Garber et al., 2012; Heinz et al., 2010; Natoli et al., 2011). While these and other TFs have been shown to regulate DC function, we do not fully understand how these TF circuits are associated with regulation of alternative chromatin dynamics during DC specification.

To further understand the mechanism regulating cell fate in the DC lineage, we analyzed the transcriptional and epigenetic states of two DC subsets. By comparing the chromatin landscape in the DC lineage, we find thousands of differential regulatory regions with enrichment of Irf and Cebp motifs in pDC- and moDC-specific enhancers, respectively. Combining these results with genome-wide expression data and ChIP-Seq, we find that Irf8 exhibits many characteristics of an epigenetic fate determining TF in pDC, similar to those of Cebpb in moDC (Garber et al., 2012; Heinz et al., 2010; Heinz et al., 2013): Irf8 binds over 30,000 regulatory regions and is highly enriched in pDC-specific enhancers. Further, using knockdown and overexpression experiments, we show that Irf8 is both necessary to maintain pDC specific enhancers and sufficient to transform moDC into cells with pDC characteristics via de novo activation of pDC enhancers. Importantly, we find that Irf8 and Cebpb form a self-reinforcing double-negative feedback loop to establish and maintain a moDC- or pDC-specific enhancer state. We show that, similar to the predicted model, the properties of this circuit enable the generation of persistent chromatin states in the DC lineage regardless of the environmental cytokine milieu. Together, our findings establish the existence of a general mechanism that allows immune cells to generate differential enhancer landscapes and stable functional states by a combination of negative feedbacks and positive autoregulation of chromatin-regulating TFs (Graf and Enver, 2009).

RESULTS

pDC and moDC Are Distinguished by Thousands of Differential Enhancers

To define the regulatory events leading to specific chromatin states in the DC lineage, we took advantage of two commonly used in vitro models for DC differentiation. Addition of granulocyte-macrophage colony-stimulating factor (GM-CSF, CSF-2) to naive bone marrow cultures differentiates precursors into a monocyte-derived DC state (denoted from here on as moDC) (Mildner et al., 2013b). In contrast, addition of the FMS-like tyrosine kinase 3 ligand (Flt3L) directs precursor development toward a plasmacytoid DC state (denoted from here on as pDC) (Chen et al., 2013; Karthaus et al., 2013; Tamura et al., 2005). Following in vitro differentiation, DCs were activated with four types of pathogen components: LPS (a component of Gram-negative bacteria, TLR4 ligand), PAM3CSK (a component of Gram-positive bacteria, TLR2 ligand), and CpG oligodeoxynucleotides A and B (synthetic DNA components, TLR9 ligands).

We analyzed gene expression using RNA-Seq and quantitative real-time PCR (qPCR) to assess differentiation-associated markers and activity-dependent cytokines (Figures 1A–1C and see Figures S1A and S1B available online). In confirmation of the in vitro model, the GM-CSF-driven cells induced known moDC markers, including Itgam (CD11b), coagulation factor VII and X (F7, F10), Tlr2 and Tlr4, and robustly induced inflammatory cytokines (e.g., Cxcl2, Il1a, and Il12b) in response to LPS activation. In contrast, Flt3L-driven cells expressed markers of in vivo pDC, such as Flt3, Siglech, Bcl6, and Ly6d, and responded robustly to CpG A by activation of multiple type I interferon antiviral response genes including Isg15, Ifit1, Irf7, and Ifna1, as shown previously for both in vitro and in vivo models (Blasius and Colonna, 2006; Cisse et al., 2008; Okada et al., 2003; Tamura et al., 2005). Genome-wide analysis of gene expression (Figure 1C; Experimental Procedures) showed that the sets of genes highly expressed in moDC were enriched for inflammatory response genes (clusters I and III); in contrast, gene sets highly expressed in pDC were enriched in antiviral response genes (clusters IV and VII) (Experimental Procedures; Table S3). In addition, we compared the in vitro pDC model to in vivo mPDCA$^+$ pDC isolated from spleens using RNA-Seq and qPCR, corroborating the high similarity in these gene programs (Figures S1C and S1D). As previously shown, pDC and moDC activate different gene programs prior to and upon stimulation. Overall, we detected 855 differentially expressed genes between pDC (381 genes) and moDC (474 genes) in resting state and across the first 6 hr of stimulations (Figure 2A; Table S1; Experimental Procedures). From this point on, we refer to these differentially expressed groups of genes as pDC and moDC specific.

We next characterized the cell-type-specific epigenetic landscapes of moDC and pDC, using chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-Seq) (Blecher-Gonen et al., 2013). Specifically, we profiled genome-wide epigenetic modifications corresponding to candidate enhancers (monomethylation of histone 3 lysine 4; H3K4me1), active enhancers (H3K27ac), and promoter regions (H3K4me3) in both moDC and pDC (Figure 2). In accordance with other cell-type-specific epigenetic studies, analyses of H3K4me3 regions identified a relatively small number of differential sites between the two cell types (Heintzman et al., 2009; Experimental Procedures; Figure S2A), whereas H3K4me1 and H3K27ac analysis revealed a large number of differential sites (Figures S2B and S2C). Consistent with the regulation of cell-type-specific genes by differential enhancer usage, we found that the surrounding K4me1

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