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Epigenetic control of B cell differentiation

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Abstract

Gene expression, differentiation and the specialized function of various cell types are controlled epigenetically by post-translational histone modifications. These modifications establish a "histone code" that is recognized by various regulatory proteins, thereby creating a stable pattern of gene expression. The focus of this review is to discuss how the chromatin modifications regulate immunoglobulin gene rearrangement and B cell differentiation.

Keywords: Epigenetic regulation; Polycomb group protein; V(D)J recombination; Histone modification; Lymphocyte development

1. Introduction

The differentiation of B lymphocytes require controlled lineage- and locus-specific immunoglobulin gene recombination as well as developmental stage-specific transcription of genes contributing to lymphocyte development and function. Recombination of antigen receptor genes establishes the unique antigen specificity of B lymphocytes. In turn, transcription of the rearranged genes leads to expression of the antigen receptors that guide stepwise developmental progression of lymphoid cells and their terminal differentiation in peripheral lymphoid organs [1,2]. The transcriptional regulation of developmental stage-specific genes is responsible for lymphoid cell proliferation, survival and synthesis of protein mediators of humoral or cellular immunity [3].

In recent years a growing body of evidence has shown that immunoglobulin gene locus accessibility to V(D)J recombination and differentiation stage-specific transcriptional program are controlled epigenetically via chromatin modifications [4–6]. In multi-cellular organisms as evolutionarily divergent as worms and humans, a significantly shared epigenetic regulation is carried out by Polycomb-group (Pc-G) proteins [7,8]. Pc-G proteins were originally discovered in Drosophila as regulators of homeotic genes [9]. After gastrulation and during embryonic development, Pc-G proteins, which are transcriptional repressors, together with Trithorax group proteins (Trx-G), which have an activating role, maintain the expression pattern of homeotic genes for embryonic development [10]. While the role of Pc-G proteins in embryogenesis is well established, the function of these proteins in adult organisms is not well understood. In adult mice, the expression of Pc-G protein is particularly high in lymphoid cells [11,12]. Lymphopoiesis is similar to embryogenesis in the sense that stem cell differentiation generates a large cell mass that morphs into specialized cell types. In this review we will discuss how the Pc-G proteins, in cooperation with other epigenetic regulators, control differentiation of B lymphocytes.

2. Polycomb group genes as regulator of lymphopoiesis

At least two major Pc-G multiprotein complexes have been identified in mammals. The Polycomb Repressive Complex 1 (PRC-1), contains HPC2, HPH, Bmi-1 and RING1 proteins and negatively regulates chromatin accessibility promoted by the SWI/SNF complex [13]. The Polycomb Repressive Complex 2 (PRC-2), consists of Eed, Suz12, YY1, Ezh2 [14,15] and can be further divided into PRC-2-Eed1 and PRC-3-Eed3/4, depending on which of the four splice variants of Eed is present in the complex [16]. The importance of PcG protein in lymphocyte differentiation is demonstrated

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by defective development of lymphoid precursors deficient for PcG proteins such as Bmi-1, mel-18 or Ezh2, which stop development at the pro-B and pro-T cell stage [17–20]. Differentiation of lymphocytes in the early stage is critically dependent on antigen receptor expression and signaling as well as on signals induced by cytokines such as IL-7 [21]. Ezh2 in particular is likely to play a key role in antigen receptor expression by controlling IgH gene V(D)J recombination, since the deficiency of Ezh2 alters recombination of the largest V_H gene family within *Igh* gene locus [18].

While Ezh2 regulates IgH gene recombination in B cells, the lack of Ezh2 does not affect pro-B cell proliferation in vivo [18]. Irrelevance of Ezh2 for B cell proliferation is further supported by the normal antigen receptor-stimulated proliferation of Ezh2-deficient peripheral B cells [18]. In contrast to Ezh2, Bmi-1, mel-18 and rae28 are likely to control B cell development by regulating proliferative lymphocyte expansion in response to IL-7 [19,20,22]. These data suggest that although their absence produces similar developmental failure, individual PcG proteins contribute differently to various processes controlling lymphocyte development. The function of PcG proteins could also be influenced by cell context. For example, rae28 is required for B cell development, but is not essential for generation of T cells [22]; similarly, Ezh2 controls V(D)J rearrangement in B lineage cells, but is irrelevant for the same process in TCRβ locus of T lineage cells [18].

The described functions of the PcG proteins are deduced from the phenotypes of the mutant cells. The potentially wide range of genetic changes produced by PcG protein deficiency suggests that developmental anomalies in the absence of PcG proteins may simply reflect the mutant cell's failure to maintain the lineage-specific pattern of gene expression. This possibility is unlikely for Ezh2-deficient developing pro-B and pro-T cells since they show wild-type like patterns of gene expression [18]. However, these results should be taken with a caveat since they do not address the role of PcG protein in regulation of transcription of non-coding microRNAs (miRNAs). These RNAs control cell differentiation by regulating gene expression and protein translation [23,24]. It cannot be excluded that Pc-G proteins like Ezh2 control lymphocyte differentiation via regulation of miRNA expression.

3. Polycomb group proteins and immunoglobulin gene recombination

Epigenetic control of V(D)J recombination implies the existence of chromatin modifications that control the locus-, lineage- and differentiation stage-specific accessibility of recombination substrates to the RAG recombinase complexes [4,5,25,26]. In early developing bone marrow B lineage cells, D_H to J_H rearrangement precedes V_H to D_HJ_H joining. Prior to D_H to J_H recombination, histone H3 acetylation is abundant within a 120 Kb domain that encompasses the D_H gene segments and extends to the Cμ exons [27]. Afterwards the hyperacetylated domain spreads into distal V_H gene region in an IL-7 dependent manner accompanied by V_H to D_HJ_H recombination [27,28]. Analysis of the TCRα/δ locus in T lineage cells shows complete overlap between regions displaying histone H3 hyperacetylation and those accessible to recombinase [29]. Direct correlation between acetylation of the antigen receptor loci and V(D)J recombination suggests that histone H3 acetylation is likely to be a part of a "histone code" that either signals initiation of recombination or is needed to sustain it.

However, other studies indicate that acetylation alone is not sufficient to grant recombination factors access to the DNA template [30]. In B lineage cells, the wild-type like levels of acetylation of the V_H genes distal to the D_H segment do not correlate with their poor recombination in the absence of PcG protein Ezh2 [18]. This points to the involvement of additional chromatin modifications in V(D)J recombination. Indeed, methylation of histone H3 on different lysine residues has a distinct impact on V(D)J recombination. Di-methylation of lysine 4 in histone H3 (H3-K4) is directly correlated with V(D)J recombination in early B and T lineage cells thus suggesting the permissive nature of this modification [31]. H3-K9 methylation is correlated inversely with the efficiency of V(D)J recombination [31]. Recombinase access requires removal of this repressive methylation mark, a process which is regulated by Pax5 [32]. The repressive role of methyl H3-K9 in V(D)J recombination is further supported by inhibition of the *Tcrb* mini-locus recombination when tethered to histone H3-K9 methyltransferase G9a [33].

In addition to characterizing chromatin modifications that directly or inversely correlate with the V(D)J, two major problems remain to be resolved. First, what is the mechanism that targets chromatin-modifying enzymes to a particular locus? Second, how do histone modifications control locus accessibility to recombination machinery? Before chromatin modification became experimentally testable, transcription of the non-rearranged antigen receptor loci (germline transcription) was seen as a sign and/or cause of locus accessibility to recombination [34]. The significance of transcriptional control gained additional support from findings that demonstrated the regulatory role of enhancers and promoters, embedded within the antigen receptor loci, in recombination. Deletion of enhancers either reduces or abolishes the efficiency of V(D)J recombination, while incorporation of lineage-specific enhancers into transgenic recombination substrates allows their recombination in a lineage-specific fashion [29,35–39]. The assembly of the pre-initiation complex on the promoter may interfere with histone modification and promote accessibility to recombination enzymes. However, given the association of RNA Pol II with histone acetyltransferase [40,41], it is more probable that during germline transcription promoters help to install the histone modifications permissive for recombination.

It has been recently proposed that chromatin can be modified with the help of antisense transcripts originating from the *Igh* locus in early developing B lineage cells at the stage preceding V(D)J recombination [42]. While the sense tran-