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PERSPECTIVE

The role of Pbx1 in T cells

Mayami Sengupta, Laurence Morel ✉

Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL 32610, USA
✉ Correspondence: morel@pathology.ufl.edu

Tissue and organ differentiation is tightly controlled to ensure proper development and function of the growing embryo as well as cells such as lymphocytes that differentiate throughout the adult stage. Therefore it is vital that the genes and the protein they encode that are involved in these processes function accurately. Hence, any mutation or error that occurs along the way can result in extensive damage, which is expressed in various ways in the embryo and can result in immune pathogenesis, including immunodeficiency and autoimmune diseases, when lymphocyte development is altered. A number of studies have been carried out to look at the genes regulating transcription in tissue differentiation, including the transcription factors Pbx1. This gene is of particular interest to us as we have identified that it is associated with systemic lupus erythematosus susceptibility (Cuda et al., in press). This perspective summarizes the known roles of Pbx1 in tissue differentiation as well as our recent findings associating genetic variations in Pbx1 to lupus susceptibility, and we will speculate on how this gene controls the maintenance of immune tolerance in T cells.

The TALE (three-amino-acid-loop-extension) class homeodomain proteins, Pbx (pre-B-cell-leukemia homeobox) and Meis (myeloid ecotropic viral integration site 1 homologue), allow embryo development by forming multimeric complexes with Hox factors (Gordon et al., 2010), with Meis proteins first binding directly to Pbx proteins (Chang et al., 1997; Knoepfler et al., 1997). Theses Pbx proteins are regulated actively by other TALE proteins that form transcriptionally active complexes, which are pivotal during this whole process (Chang et al., 1997; Knoepfler et al., 1997; Fognani et al., 2002). It is the interaction between Pbx-Meis partners that facilitates cooperative binding to a composite DNA element, and displays unique DNA-binding preferences for bipartite DNA sequences that adhere to a consensus, consisting of 5′ Pbx and 3′ Meis half sites (Chang et al., 1997). The interactions of Pbx-Meis complexes with both Hox and non-Hox factors ultimately contribute to gene activation (Zhang et al., 2002) and repression (Eklund et al., 2000); however, it is important to note that not all Pbx targets require the DNA-binding activity of Meis partners (Ferretti et al., 2000). Meis proteins can also be recruited to DNA by interaction with a Pbx partner and other homeodomain proteins, bypassing the need for Meis to bind DNA (Shanmugam et al., 1999; Liu et al., 2001). Pbx proteins are thought to act as the central developmental factors with a role in incorporating transduction signals by acting together with numerous proteins, thereby controlling gene expression programs throughout development. Transcription of Pbx target genes can be controlled by dynamic combination of transcription factors that physically interact with Pbx proteins in response to numerous cell signals (Laurent et al., 2008). A number of authors have also implied that heterodimers or multimers made up of Pbx/Meis and Pips (Pbx-interacting proteins) are more efficient transcriptional regulators than Pbx/Meis heterodimers alone, suggesting that there is need for the latter to co-operate with other transcription factors (Mojsin and Stevanovic, 2010).

Pbx1, 2 and 3 proteins are usually regarded as Hox ancillary factors that enhance Hox DNA-binding specificity (Tümpel et al., 2009) and are required for organogenesis (Selleri et al., 2001; Vitobello et al., 2011) and skeletal development (Capellini et al., 2006). We already know from work done by various investigators that it is, in fact, Pbx1 and not Pbx 2 or 3 that affects organogenesis. Pbx1 is a particularly important transcription factor known to prevent lineage-specific functional differentiation. Specifically, Pbx1 maintain self-renewal of hematopoietic stem cells (Ficara et al., 2008) and represses osteoblastogenesis, by maintaining cell renewal and preventing differentiation (Gordon et al., 2010). Lineage analysis has shown that Pbx1 expression is higher during the proliferative stage than during maturation. This was not the case for Pbx2, Pbx3 or Meis 1 which was measurable at low levels throughout, thus, suggesting that Pbx1 is specifically down regulated upon differentiation through the mature osteoblast formation (Gordon et al.,

2010). The shRNA used for target depletion of Pbx1 increased the expression of the bone marker genes, i.e. alkaline phosphatase, Bsp and Ocn. Chromatin-associated Pbx1 and Hoxa10 were present at osteoblast-related gene promoters preceding gene expression, but Pbx1 was absent from promoters during the transcription of bone-related genes. Additionally, Pbx1 complexes were associated with histone deacetylases which maintain chromatin inactivation. Loss of Pbx1 but not of Hoxa10 from the Osx promoter was associated with increased recruitment of histone acetylases (p300), as well as decreased H3K9 methylation, reflecting transcriptional activation. It was proposed that Pbx1 plays a central role in attenuating the activity of HOXA10 as an activator of osteoblast-related genes and functions to establish the proper timing of gene expression during osteogenesis, resulting in proper matrix maturation and mineral deposition in differentiated osteoblasts (Gordon et al., 2011).

Pbx proteins are not able to activate transcription alone, but contribute to the binding of Meis proteins to DNA and may also facilitate transcriptional activation by Meis partner proteins (Lu and Kamps, 1996). The Meis relative Prep1 plays a role in hematopoietic stem cell function and in early T cell development (Penkov et al., 2005; Di Rosa et al., 2007; Penkov et al., 2008). The Pbx1 gene is linked to the transcription factor E2A as a result of the t(1;19) translocation in pre-B cell leukemia (Kamps and Baltimore, 1993). This union prevents interaction with Meis proteins and converts Pbx1 to a transcriptional activator. Therefore, there is significant evidence for deregulation of Meis and Pbx family proteins promoting tumorigenesis in lymphoid and hematopoietic cells.

We have found that Pbx1 is the gene corresponding to the lupus susceptibility locus *Sle1a.1* in the NZM2410 mouse model (Cuda et al., in press). The NZM2410 allele of *Sle1a.1* corresponds to the expression of a novel splice isoform of *Pbx1*, *Pbx1-d*. Pbx1-d overexpression is sufficient to induce an activated/inflammatory phenotype in Jurkat T cells, and to decrease their apoptotic response to retinoic acid (RA). Vitamin A is an important nutrient known to play a significant role in cell differentiation, immune function, and embryonic development. *In vivo*, it functions in its most biologically active form RA (Qin et al., 2004).

Pbx1-d lacks both exons 6 and 7, corresponding to the DNA binding domain and the Hox binding domain, respectively. *Sle1a.1*-expressing CD4+ T cells that overexpress the Pbx1-d isoform are more activated and autoreactive than the congenic C57BL/6 (B6) T cells that express low levels of Pbx1-d. In addition, *Sle1a.1* CD4+ T cells present an impaired induction of Foxp3, the master regulator of regulatory T cells (Tregs) in response to RA, leading to a decrease in peripheral Tregs. These findings indicate that *Pbx1* is a novel lupus susceptibility gene that regulates T cell activation and tolerance (Cuda et al., in press). The amino acid sequence of Pbx1 is identical between mice and humans, and Pbx1-d was expressed more frequently in the T cells of lupus patients (Cuda et al., in press). In addition, Pbx1-d expression is associated with T cell abnormal responses to TGF and RA (Sobel et al., 2011). RA is essential for the normal growth and organogenesis of the vertebrate (Niederreither et al., 1999; Duester, 2008). This highlights a feed-forward mechanism linking Hox-Pbx-dependent RA synthesis during early axial patterning with the establishment of spatially restricted Hox-Pbx activity in the development. Sustaining normal levels of RA is vital as too much or too little can affect division and patterning of the embryo (Vitobello et al., 2011). Vitobello et al. also showed that the ultimate expression of RA is under the transcriptional control of Hox, Pbx and Meis factors *in vivo*. On the contrary, absence of Pbx1 resulted in a progressive reduction of endogenous RA activity. The function of *Pbx1* in T cells is still not known and needs to be studied in detail to uncover the underlying mechanisms linking *Pbx1* expression and T cell phenotypes (Perry et al., 2011). Our results however suggest that the link between RA and Pbx1 function is conserved in T cells.

Worldwide studies of sequence-specific binding of transcription factors and chromatin structure in a variety of cell types and species have been able to generate comprehensive information with which to recognize dynamic processes such as cell differentiation, the development of immune memory, disease progression and responses to external environmental stimuli. Chromatin immunoprecipitation (ChIP) is a technique whereby a protein of interest is selectively immunoprecipitated from a chromatin preparation to determine the DNA sequences bound to it (Collas, 2010). Genome-wide ChIP combined with next generation sequencing (ChIP-Seq) is a fairly new tool with which we can gauge protein-DNA interactions in a comprehensive and unbiased manner (Jothi et al., 2008). ChIP-Seq has certainly bettered the knowledge of the epigenetics that regulate chromatin structure and the transcriptional profiles. This novel combination allows us to investigate the genome wide distribution of chromatin binding proteins and histone modifications in any genome with a known sequence; done accurately very high quality data can be generated (Kidder et al., 2011). It would be of even greater benefit if data sets generated from various laboratories across the world could be directly compared (Kidder et al., 2011).

Previously, ChIP assays have been used to validate that Pbx proteins directly bind redundantly to the promoter regions (Qin et al., 2004); more specifically to the enhancer regions (Hollenhorst et al., 2009). To reveal how genomic sequences build transcriptional control networks, we need to understand the connection between DNA sequences and transcription factor binding and function. Genomes contain sequences encoding both gene products and the instructions for where and when each gene is expressed and this is mandatory for normal development. The gene expression and code is interpreted by transcription factors that bind to particular DNA

sequences which harbor instructions for gene activation and repression. Studies in living cells point toward individual transcription factors having significant flexibility in sequence recognition (Hollenhorst et al., 2009). To uncover the sequence motifs that mediate specific function, genomic approaches to transcriptional networks must go further than a description of factor occupancy to include correlates of functionality. In 2009, Hollenhorst applied this technique to govern the specificity of ETS1 in the promoter and enhancer regions of Jurkat T cells. Earlier in 2008 Lampe and colleagues identified a putative Hox-Pbx responsive cis-regulatory sequence, which resides in the coding sequence of Hoxa2 and is an important component of Hoxa2 regulation in rhombomere 4. By using cell transfection and ChIP assays, they showed that this regulatory sequence is responsive to paralogue group 1 and 2 Hox proteins and to their Pbx co-factors. Importantly, they also showed that the Hox-Pbx element cooperates with a previously reported Hoxa2 r4 intronic enhancer and that its integrity is required to drive specific reporter gene expression in r4 upon electroporation in the chick embryo hindbrain (Lampe et al., 2008).

To understand the specific role of Pbx1 in T cells, we want to identify the genes it regulates in T cells. Hence, we hypothesize that Pbx1 will have both T-cell specific and non-specific binding sites, associating it with a wide array of functions. Through ChIP and high throughput sequencing we hope to get an insight into the specific role of Pbx1 in T cells. ChIP-seq allows us to approach Pbx1 function in T cells with an unbiased genome-wide approach, as well as to overcome the tedious method of cloning each individual fragment of interest. Using various bioinformatic tools we hope to identify the Pbx1 binding sequences, which will be filtered and prioritized by comparing the data to existing databases. Genes of interest will then be studied in detail with a combination of molecular and functional assays. Hopefully this will bring us closer to deciphering the specific role that Pbx1 has in T cells.

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