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PML-RARα/RXR Alters the Epigenetic Landscape in Acute Promyelocytic Leukemia

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SUMMARY

Many different molecular mechanisms have been associated with PML-RARα-dependent transformation of hematopoietic progenitors. Here, we identified high confidence PML-RARα binding sites in an acute promyelocytic leukemia (APL) cell line and in two APL primary blasts. We found colocalization of PML-RARα with RXR to the vast majority of these binding regions. Genome-wide epigenetic studies revealed that treatment with pharmacological doses of all-*trans* retinoic acid induces changes in H3 acetylation, but not H3K27me3, H3K9me3, or DNA methylation at the PML-RARα/RXR binding sites or at nearby target genes. Our results suggest that PML-RARα/RXR functions as a local chromatin modulator and that specific recruitment of histone deacetylase activities to genes important for hematopoietic differentiation, RAR signaling, and epigenetic control is crucial to its transforming potential.

INTRODUCTION

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia characterized by severe bleeding tendency and a fatal course of only few weeks' duration (Wang and Chen, 2008). It is mostly triggered by the chromosomal translocation of the PML gene on chromosome 15 and the retinoic acid receptor α (RARα) on chromosome 17 that results in expression of the PML-RARα oncofusion gene in hematopoietic myeloid cells (de The et al., 1990; Kakizuka et al., 1991). The PML-RARα oncofusion protein acts as a transcriptional repressor that interferes with gene expression programs involved in differentiation, apoptosis, and self-renewal (Sell, 2005).

At the molecular level, PML-RARα behaves as an altered RAR. In absence of all-*trans* retinoic acid (ATRA), RARα interacts with RXR, itself a nuclear receptor, and binds to DNA. In contrast, PML-RARα has been reported to function by oligomerization without RXR (Minucci et al., 2000) or to require RXR in conjunction with oligomerization (Kamashev et al., 2004; Perez et al., 1993). The normal RARα/RXR heterodimer recruits corepressor complexes and represses transcription of its target genes. A conformational change caused by binding of ATRA triggers the dissociation of the corepressors and promotes the recruitment of coactivators. In contrast, PML-RARα acts as a constitutive repressor that is insensitive to physiological concentrations of ATRA (Licht, 2006; Kwok et al., 2006; Sternsdorf et al., 2006). Under physiological concentrations of ATRA, PML-RARα complexes have been reported to bind RXR (Perez et al., 1993), which may be essential for its oncogenic potential as it facilitates binding to widely spaced direct repeats (DRs) (Perez et al., 1993; Kamashev et al., 2004) and has recently been shown to be a critical determinant for the transforming potential of PML-RARα complexes (Zeisig et al., 2007; Zhu et al., 2007).

Significance

Since its discovery in 1990, PML-RARα has been one of the most intensely investigated translocation fusion proteins for understanding epigenetic repression during oncogenesis. Throughout the years many mechanisms have been proposed for PML-RARα function, including homodimerization, oligomerization, interaction with RXR, expanded DNA binding affinity, and recruitment of a wide spectrum of epigenetic enzymes based on studying a few genomic regions, mostly the promoter of the *RARβ* gene. In this study we provide genome-wide analyses of PML-RARα binding, colocalization with the nuclear receptor RXR, and epigenetic changes associated with ATRA-mediated PML-RARα degradation both in cell lines and ex vivo patient blasts. Our results show that alterations of H3 acetylation are intimately intertwined with the PML-RARα/RXR complex.

To overcome the transforming potential of PML-RARα, human APL patients are treated during the early phase of the disease with pharmacological doses of ATRA (Di Croce, 2005; Licht, 2006). This treatment has been shown to degrade PML-RARα (Raelson et al., 1996) and suggested to dissociate various epigenetic enzymes from chromatin, such as histone deacetylases (HDACs) (Lin et al., 1998; Grignani et al., 1998), DNMTs (Di Croce et al., 2002), MBDs (Villa et al., 2006; Morey et al., 2008), and histone methyltransferases (Carbone et al., 2006; Villa et al., 2007). Loss of these proteins has been suggested to severely remodel the repressive chromatin environment at PML-RARα binding sites, creating a more accessible chromatin structure. Unfortunately, until now the majority of these epigenetic changes have only been reported for a small region surrounding the promoter of the *RARβ* gene due to the lack of other high confidence genomic binding sites. Thus far, the identification of additional PML-RARα binding sites has been restricted to one chromatin immunoprecipitation (ChIP)-chip study in which PML-RARα was overexpressed in 6 days 5-aza-dC-treated U937 cells (Hoemme et al., 2008), thus unlikely reflecting the canonical disease. Similarly, only one study using CpG island arrays addressed large-scale epigenetic effects associated with ATRA treatment (Nouzova et al., 2004).

In the present study we used ChIP-seq to identify PML-RARα binding sites in the leukemic cell line NB4 and in blasts from two patients with newly diagnosed APL. In addition, we examined ATRA-induced genome-wide epigenetic alterations. Our results support a model in which recruitment of PML-RARα in conjunction with RXR and HDACs is crucial for oncogenic transformation.

RESULTS

Identification of PML-RARα Binding Sites in NB4 Leukemic Cells

To identify targets of the PML-RARα oncofusion protein, ChIP followed by deep sequencing (ChIP-seq) was performed using specific antibodies against PML and RARα in the PML-RARα-expressing leukemic cell line NB4 (Lanotte et al., 1991); in addition, total genomic DNA was sequenced to obtain a reference input profile. Overlapping tags were joined into peaks and displayed as tag-density files in the University of California Santa Cruz browser (http://genome.ucsc.edu/). The classical target *RARβ* shows enrichments of both PML and RARα tags over a narrow range at the promoter region (Figure 1A, top left). PML and RARα peaks were also detected at regions that have been previously described as potential PML-RARα targets, such as *TGM2*, *ID1*, *SPI1*, and microRNA-223 (Table S1 and Figure S1A, available online) and numerous targets for which no PML-RARα binding has been described before, such as for the hematopoietic regulators *GFI1* and *RUNX1* and the *RARα* gene (Figure 1A). These results suggest that, apart from regulating important factors involved in hematopoietic differentiation, PML-RARα influences retinoic acid signaling through regulation of *RARα* and *RARβ* expression. We used MACS (Zhang et al., 2008) at a false discovery rate of 10^{-6} to identify all PML and RARα binding regions and counted the number of PML and RARα tags within these regions. For each binding region we calculated the relative tag density, i.e., density at one region divided by average density at all regions. Regression curve analysis (Figure 1B) revealed a set of 2722 genomic regions at a cut off of 0.00012 (>14 tags/kb) to which both PML and RARα bind with high confidence (Table S2), while no high confidence PML- or RARα-only binding sites were found. As wild-type PML is expected to colocalize with RARα in APL cells (Dyck et al., 1994; Koken et al., 1994; Weis et al., 1994), these findings suggest that the 2722 high-confidence PML and RARα binding sites represent bona fide PML-RARα targets.

PML-RARα Colocalizes with the Nuclear Receptor RXR

Although PML-RARα has been reported to bind DNA as a homodimer or oligomer (Kamashev et al., 2004; Minucci et al., 2000; Perez et al., 1993), more recent findings indicate that RXR is present within a functional PML-RARα complex (Zeisig et al., 2007; Zhu et al., 2007). To investigate whether PML-RARα and RXR colocalize at a genome-wide level we performed ChIP-seq using a specific RXR antibody. As for PML and RARα, we detect enrichments of RXR tags at the *RARβ*, *RARα*, *RUNX1*, and *GFI1* and at the previously described genomic binding regions (Figure 1A and Figure S1A). Including the RXR data in our regression analysis revealed that the vast majority of PML-RARα peaks contain high densities of RXR tags, indicating a highly significant overlap between PML-RARα and RXR binding (Figure 1B). Partitioning our 2722 binding sites according to RXR tag density revealed that tag densities of PML, RARα, and RXR closely correlate, indicative of stoichiometric binding (Figure 1C). To further validate the colocalization of PML, RARα, and RXR we performed re-ChIP experiments to confirm binding of PML and RARα to the same locus (Figures S1B and S1C). In addition, we validated occupancy of PML, RARα, and RXR at 13 randomly selected regions identified in our ChIP-sequencing approach in comparison to a control region within the *MYOGLOBIN* gene (Figure S1D).

The distribution of all 2722 binding sites (Table S2) shows that PML-RARα/RXR peaks have statistical significant enrichment toward promoter regions as compared to the distribution of genomic DNA (19% for PML-RARα and 5% for genomic DNA) and a decrease (13% for PML-RARα and 31% for genomic DNA) toward distant regions (Figure 1D).

PML-RARα/RXR Binding Regions Comprise a Wide Variety of DR Response Elements

In vitro evidence suggests that the PML-RARα-containing complexes have gained an expanded DNA binding capacity away from DR5, DR2, and DR1 motifs toward more widely spaced DRs (Kamashev et al., 2004). To examine whether this gain of function is represented in our in vivo data, we interrogated the binding site sequences for overrepresentation of DR motifs with different spacing (between 0 and 13) and architecture (direct, inverted, or everted repeat) (Figure 2A). Indeed, we find all possible combinations of two AGGTCA half sites (Figure 2B). While a DR1–5 motif is detected within the majority of the binding sites (58.1%), a large number only contains DR motifs with altered spacing and/or architecture: 21.9% contain only a DR0 or DR6–13 motif and 10.0% contain only an ER or IR motif. Comparison with a similar analysis for RARα binding sites in ATRA-treated mouse embryonic stem cells revealed that nearly 80% of binding sites have a DR5 or DR2 motif, while 90% have