promotion of angiogenesis and myelomonocytic cell recruitment. Macrophages directly enhance tumorigenicity in an FcγR-dependent fashion. Interestingly, here the culprit of tumor promotion was on mature TAMs rather than on immature elements in the myelomonocytic pathway such as MDSC. Tumor-promoting TAMs have a M2-like transcriptional profile. B cell-instructed innate cells are a source of IL-1, which activates the proinflammatory properties of CAF (Erez et al., 2010).

The TAM profile reported by Andreu et al. and previous profiles (Mantovani et al., 2008) include T cell-attracting anti-angiogenic chemokines (CXCL10 and CXCL11). This finding emphasizes the yin-yang dual potential of the macrophage-tumor cell interplay (Allavena et al., 2008; Mantovani et al., 2008; Ostrand-Rosenberg, 2008).

These observations raise important general issues. B cells are a valuable target in the therapy of autoimmune disorders. Therefore, the definition of a B cell/antibody/FcγR/macrophage pathway in cancer-promoting inflammation identifies potential targets for therapeutic intervention. Similarly, IL-1-blocking strategies are available or being developed for inflammatory disorders, and in both here and elsewhere (Dinarello, 2009), IL-1 is emerging as a key player in CRI.

Myelomonocytic cells are part of a common pathway of inflammation-mediated cancer promotion (Allavena et al., 2008; Mantovani et al., 2008; Pollard, 2004). However, the subsets involved (from classic mature macrophages or neutrophils to immature myelomonocytic cells) differ considerably in different settings (e.g., Andreu et al., 2010; DeNardo et al., 2009; and Fridlender et al., 2009). In a mouse model of metastatic breast cancer, DeNardo and colleagues (DeNardo et al., 2009) reported that macrophage M2 polarization and tumor promotion is driven by T cell-produced IL-4. Thus, not only can the subsets be different but so can the orchestrating signals in different tumors (Figure 1). Therefore, careful dissection of the players, conductors and themes in different human cancers will be required for the clinical exploitation of our understanding of CRI at the bedside.

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Discovery of Novel Transcriptional and Epigenetic Targets in APL by Global ChIP Analyses: Emerging Opportunity and Challenge

Jan-Henrik Mikesch,[1] Hinrich Gronemeyer,[2] and Chi Wai Eric So[1,*]
[1]Leukemia and Stem Cell Biology Lab, Department of Haematological Medicine, King's College London, London SE5 9NU, UK
[2]Department of Cancer Biology, Institut de Genetique et de Biologie Moleculaire et Cellulaire, IGBMC/CNRS/INSERM/ULP, 67404 Illkirch Cedex, Communauté Urbaine de Strasbourg, France
*Correspondence: eric.so@kcl.ac.uk
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Identifying transcriptional program(s) deregulated by oncoproteins is key to understanding the molecular basis of the disease. In this issue of *Cancer Cell*, two studies by Martens et al. and Wang et al. provide global blueprints for transcriptional targets and epigenetic modifications mediated by PML-RARα in acute promyelocytic leukemia.

Acute promyelocytic leukemia (APL) is characterized by the expression of RARα fusions and unique sensitivity to all-*trans* retinoic acid (ATRA) treatment. As a result, it has been the paradigm for studying differentiation therapies and more recently for epigenetic therapies. In the past decades, a tremendous amount of effort has been made to identify the aberrant

transcriptional targets and activities that account for the leukemogenic functions of RARα fusions. Although some important targets and principles have been established from various in vitro studies or targeted analyses of a small number of genomic loci, the majority of the in vivo direct downstream targets of, and the global epigenetic modulation by, RARα fusions remain largely unknown. Using state-of-the-art ChIP-sencing and ChIP-on-chip technologies, two recent studies by Martens et al. (2010) and Wang et al. (2010) have shed light on this issue (Figure 1).

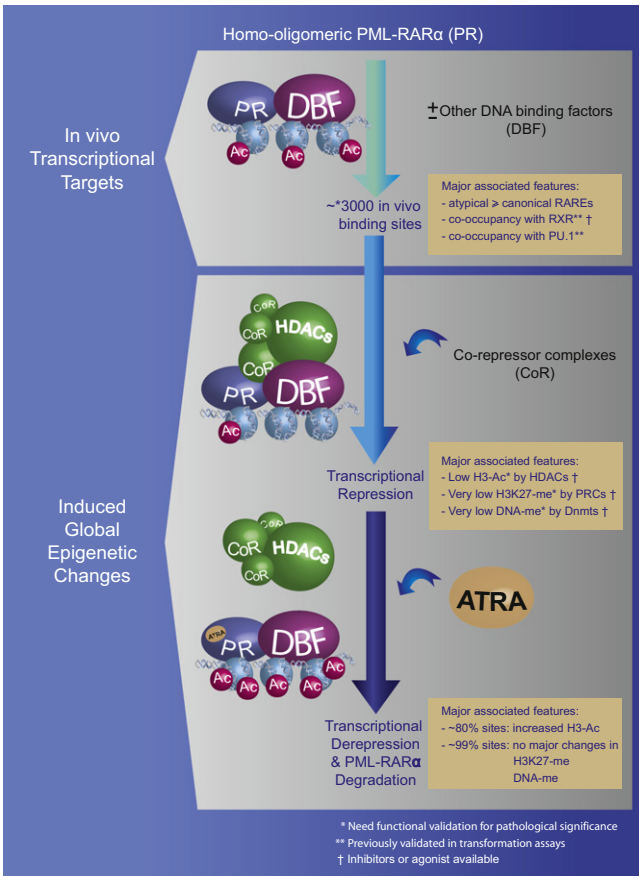

Figure 1. Transcriptional and Epigenetic Paradigms of PML-RARα Function

As shown in the upper panel, in the absence of ligand, PML-RARα binds to over 3000 binding sites in vivo as complexes with interacting DNA binding factors (DBF), such as RXR and/or PU.1, probably depending on the configuration of individual sites. The majority of these sites do not correspond to canonical RAREs. As shown in the lower panel, PML-RARα/DBF complexes associate with multicomponent corepressor complexes including HDACs, resulting in a low level of histone acetylation. In the presence of ATRA, corepressor complexes dissociate from PML-RARα/DBF, resulting in increased histone acetylation. Although a few PR target sites show decreased H3K27 trimethylation and DNA methylation, probably as a result of PRC and Dnmt recruitments, Martens et al. report that the majority of the sites do not undergo such changes. The role of the few modulated sites in the pathological and pharmacological response of the disease remains to be established. Dissociation of corepressor complexes allows access of coactivators and degradation of the PML-RARα protein. All together, this results in transcription derepression. Illustration by Pui Yi Tse.

Co-occupancy of Atypical RARE Binding Sites by PML-RARα/RXR Complexes

Martens et al. have discovered over 2700 high confidence PML-RARα in vivo binding sites in NB4 cells, of which 600 overlapped among three different cellular APL systems including primary APL blasts. In contrast to wild-type RARα binding targets, of which 90% contained canonical DR1-5 (direct repeats of the hexameric recognition motif spaced by 1 to 5 bp) retinoic acid response elements (RAREs), only 50% of PML-RARα binding sites contained such canonical RAREs, whereas over 30% contained atypical motifs. An independent study by Wang et al. also investigated this issue using zinc-inducible PML-RARα expression in U937 cells. Among 3000 identified putative PML-RARα binding sites, they found only a very low frequency (13%) of canonical RAREs, most of these sites instead contained atypical motifs with one or more RARE half-sites (RAREh) variably spaced in different orientations. Both studies revealed that RXR coexists with PML-RARα in most of the ChIPed regions. RXR also exhibited very similar binding characteristics as PML-RARα both pre- and post-ATRA treatment (Martens et al., 2010). Together, these results provide the first global in vivo evidence both consolidating and extending the hypothesis that DNA binding targets by homo-oligomeric PML-RARα fusion protein are significantly different from those of wild-type RARα (Perez et al., 1993). They also reinforce the idea of RXR as an essential component for APL pathogenesis (Zeisig et al., 2007; Zhu et al., 2007), although the actual functions of RXR in the complexes still need to be determined.

PU.1-Guided PML-RARα Binding Suppresses PU.1 Functions

Bioinformatics analysis by Wang et al. revealed that the majority of the PML-RARα binding sites contained at least one RAREh together with consensus binding sites for specific transcription factors including PU.1, ETS, and AP-1, suggesting that PML-RARα collaborates with other factors for DNA binding. Among them, the PU.1 motif is by far the most common neighbor of RAREh. Although it was previously observed that PU.1 is upregulated in APL during ATRA treatment and that forced expression of PU.1 released the differentiation block (Mueller et al., 2006), the underlying mechanisms are not completely understood. In this study, Wang and colleagues show that PU.1 interacts with PML-RARα and that its DNA binding is required for PML-RARα binding and suppression of PU.1/RAREh promoters. The study provides a potential mechanism for the reported function of PU.1 as a myeloid differentiation factor in APL and unravels a previously unrecognized role of cooperating transcription factors in DNA targeting of RARα fusions.

Histone Acetylation Rather Than H3K27 Methylation Associates with ATRA Response in APL

A key question of APL pathogenesis is how oncogenic RARα fusions (de)regulate gene programs. Although they can recruit different epigenetic modifiers including histone deacetylases (HDACs) (Grignani et al., 1998; Lin et al., 1998), polycomb repressive complexes (PRCs) (Villa et al., 2007), and DNA methyltransferases (Di Croce et al., 2002), it is not clear which

(combination) mediates the oncogenic function(s) of RARα fusions. To gain insights into this issue, Martens et al. performed ChIP sequencing against a number of histone modifications and DNA methylation. They discovered that histone acetylation was the only investigated mark associated with 80% of PML-RARα in vivo binding sites and changed dynamically upon ATRA treatment. Remarkably, H3K27 trimethylation and DNA methylation were very low and ATRA did not induce any significant changes in the vast majority (99%) of the PML-RARα binding sites. Although histone acetylation concurred with >500 PML-RARα/RXR binding sites, only 15 PML-RARα/RXR binding sites showed differential H3K27 trimethylation or DNA methylation. Moreover, H3 acetylation but not H3K27 trimethylation or DNA methylation showed strong correlation with RNA polymerase II occupancy near PML-RARα/RXR binding sites. Together, these analyses provide genome-wide evidence suggesting that histone deacetylation rather than H3K27 or DNA methylation is responsible for the major transcription repressive activity of the onco-fusion protein and endorses the use of HDAC inhibitors and RXR agonist for APL.

What Have We Learnt about the Oncogenic Function of PML-RARα?

Although the formation of PML-RARα on its own is not sufficient to induce APL, which requires additional (epi)genetic event(s) to progress from a myeloproliferative disease to full leukemia, recent work shows that eradicating PML-RARα is apparently sufficient to cure the disease by targeting the fusion protein in the leukemia-initiating cells (LICs) (Nasr et al., 2008). The fact that bulk leukemia cells and LICs express PML-RARα raises the question of whether the PML-RARα program shown in the study by Martens et al. is the same as that in LICs. Another key question that remains open even after the elegant global studies is whether it is indeed the aberrant gene interaction pattern of PML-RARα or nongenomic function(s) of PML-RARα, such as interaction with p53, that is crucial for leukemogenesis.

So Much Data, So Little Time. What To Do Now and Where To Go Next?

The two studies exemplify the unmatched opportunity originating from the recent explosion of array and sequencing technologies for discovery of novel chromatin binding/regulating factors and epigenetic marks. However, this new (epi)genomic era also poses novel challenges and risks. There is the technical aspect of how to analyze millions of DNA sequences/binding signals to distinguish "true" ones from noise. Too little noise indicates lack of sequencing depth, whereas too much noise can mask proper peak detection. Albeit continuously optimized, virtually all peak calling programs have limitations. This is particularly important for weak binding sites, which may well be the functionally important ones we are looking for. Given that antibodies are key tools, quality criteria for ChIP-seq compatible antibodies are required. Also, the issue of epitope masking deserves attention at global scale. The research community will soon need to come up with a set of standards to guarantee that high-quality data are generated and accessible to wide scientific communities. While these genomic technologies when applied appropriately will swiftly provide us with a huge database of potentially novel targets and molecular changes in cancer cells, it is absolutely essential to critically investigate at the onset what new functional insights these data will provide and then to identify and validate key candidate pathways in biologically relevant models related to the (patho)physiological phenomenon studied. Unfortunately, this ultimate functional validation remains the time-limiting step and the bottleneck for most of the current efforts in identifying critical cancer genes and their associated pathways/targets. Nevertheless, these new technologies enabling global assessment of molecular changes have given an unprecedented opportunity to interrogate entire cancer genomes and epigenomes. Not only will this provide unique insights into the underlying transformation mechanisms but also facilitate future drug development, particularly for targeting epigenetic enzymes that are key for transformation functions of oncogenic transcription factors, which themselves are otherwise intractable targets of small molecule inhibitors.

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