Determinants of Oncogenic Transformation in Acute Promyelocytic Leukemia: The Hetero-Union Makes the Force

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Acute promyelocytic leukemia (APL) is caused by chromosomal translocations that involve the retinoic acid receptor α (RAR) and several other genes to yield X-RAR fusion proteins. Unlike wild-type RARs, which require heterodimerization with the retinoid X receptor (RXR) for their function as DNA-binding transcriptional regulators, X-RAR fusion proteins bind DNA and deregulate transcription as homo-oligomers. In this issue of *Cancer Cell*, however, Zeisig et al. and Zhu et al. show that RXR recruitment is a critical determinant for the transforming potential of oligomeric X-RAR fusion proteins and explore the possibility for targeted interventions in APL with either RAR or RXR ligands.

Pharmacological doses of the physiological RAR ligand retinoic acid (RA) induce differentiation of leukemic blasts and disease remission in APL patients (Licht, 2006). Since RA treatment of APLs represents the most successful example of differentiation therapy in cancer, exploration of molecular mechanisms of APL pathogenesis and RA response has become an area of intense investigation.

Mechanism of Action of APL Fusion Proteins

APLs are characterized by chromosome translocations, which generate oncogenic RAR fusion proteins. Most of the studies have been performed on PML-RAR, which is involved in >95% of cases. PML-RAR behaves as an altered RAR; it recruits several chromatin remodeling complexes endowed with enzymatic activities, such as histone deacetylases, DNA, and histone methyltransferases to RAR target genes and, due to a relaxed specificity of DNA binding, to novel targets (Licht, 2006). The net result is the establishment of a stably repressive chromatin structure at target genes, refractoriness to differentiating stimuli, and block of differentiation of PML-RAR-expressing cells. For maximal transcriptional repressive activity, PML-RAR must undergo specific posttranslational modifications: point mutations that abrogate sumoylation of PML-RAR render the fusion protein unable to induce leukemias in transgenic models (Zhu et al., 2005). Association of PML-RAR with histone deacetylases is also responsible for alterations in the acetylation of nonhistone substrates, such as p53, resulting in inhibition of their function (Insinga et al., 2004). One recognized mechanism of oncogenic activation of RAR involves its acquired capacity, contributed by the coiled-coil domain present in the PML moiety, to self-associate and to form oligomeric complexes (Licht, 2006; Kwok et al., 2006; Minucci et al., 2000; Sternsdorf et al., 2006). Through mechanisms that are only partially understood, oligomerization leads to an increased stoichiometry of association of PML-RAR with transcriptional corepressors and chromatin modifiers and is a critical (although not exclusive) determinant of oncogenic transformation. Artificial chimeric proteins where RAR is fused to heterologous oligomerization domains recapitulate several biological activities of PML-RAR, including leukemogenicity in vivo, although at a reduced efficiency, testifying additional functional requirements contributed by the fusion partner for full transforming activity (Sternsdorf et al., 2006). Using different synthetic RAR chimeras carrying one or four copies of the FKBP self-association domain, Zeisig et al. (2007) show that homodimerization of RAR is not sufficient to trigger RAR-mediated immortalization of murine hematopoietic progenitors, and that higher-order oligomerization is required (Zeisig et al., 2007). Since the isolated coiled-coil domain of PML forms trimeric structures in vitro, it appears that a minimum of three RAR moieties must self-interact to form the "docking platform" required for efficient recruitment of the relevant accessory factors (Minucci et al., 2000). Interestingly, the other X-RAR fusion proteins all contain an oligomeric (rather than dimeric) self-association domain (Licht, 2006; Kwok et al., 2006; Minucci et al., 2000). Other leukemia-associated chimeric transcription factors also share a similar mechanism of oncogenic activation, suggesting that oligomerization of transcription factors represents a common mechanism of oncogenic activation, as the well-known self-association of tyrosine kinases.

Table 1. Analysis of the Determinants for Oncogenic Transformation in APL

Name and Reference	Biochemical Properties	Transcription	Immortalization of Murine Progenitors	APL	RA Response	References
PML-RAR						
M883R;T886R	no RXR binding, reduced DNA binding[a]	+/–	+	–[b]	+	Zhu et al., 2007
K160R	defective sumoylation, defective DAXX recruitment	+/–[c]	–	–[b]	+	Zhu et al., 2005
ΔCCRAR	defective oligomerization, defective corepressor binding	–	NT[d]	NT	+	Minucci et al., 2000
CCRAR	forced oligomerization through PML coiled coil	+	NT[e]	NT	+	Minucci et al., 2000
PLZF-RAR						
CVII, *CVIII*	defective oligomerization, defective corepressor binding	NT	–	NT	NT[f]	Kwok et al., 2006
STAT5-RAR						
SR-ΔN	defective tetramerization	NT	–	NT	NT	Zeisig et al., 2007
Chimeric RAR						
1xFKBP-RAR	forced homodimerization	NT	–	NT	NT	Zeisig et al., 2007
4xFKBP-RAR	forced oligomerization (higher-order oligomers)	NT	+	NT	NT	Zeisig et al., 2007
F3-RAR	forced oligomerization	+	+	+[g]	NT	Sternsdorf et al., 2006

Key point mutants/deletion mutants for the various X-RAR fusion proteins referenced here have been listed for altered biochemical properties, capacity to repress transcription of exogenous reporters or of endogenous genes, capacity to immortalize murine hematopoietic progenitors, and ability to respond to retinoic acid in vivo/in vitro. NT, not tested.
[a]Only in vitro data.
[b]Myeloproliferative syndrome.
[c]Suggested from indirect data: in direct assays, no clear differences were observed compared to PML-RAR.
[d]Impaired ability to block differentiation.
[e]Able to block differentiation.
[f]PLZF-RAR does not respond to RA treatment.
[g]With severely reduced efficiency compared to PML-RAR.

Recruitment of RXR Is Essential for Transformation by X-RAR Fusion Proteins

X-RAR fusion proteins have been previously shown to associate with RXR in vitro, like wild-type RARs, but the actual contribution of RXR binding to the pathogenesis of APL remains unclear (Kamashev et al., 2004). The two studies in this issue of *Cancer Cell* address the relevance of RXR recruitment using different model systems. Making use of point mutants that eliminate the capacity of PML-RAR to associate with RXR (*M883R*;*T886R*), Zhu et al. (2007) show that many features of the original fusion protein are maintained in vitro, including association with DNA and corepressors as homo-oligomers; sumoylation; and, more important, ability to block differentiation and to immortalize murine primary hematopoietic progenitors (Zhu et al., 2007). A notable exception is the somewhat reduced transcriptional activity of the RXR-defective *M883R*;*T886R* mutant, which is presumably due to reduced binding to DNA response elements for the lack of PML-RAR/RXR hetero-oligomeric complexes, although conclusive evidence is still needed, such as chromatin immunoprecipitation of PML-RAR and RXR on endogenous target genes. Strikingly, transgenic mice harboring the *M883R*;*T886R* mutant under the control of the MRP8 promoter, a myeloid-specific promoter that has been extensively used to express PML-RAR in mice, fail to develop APL, although they show a mild myeloproliferative syndrome (Zhu et al., 2007). Zeisig et al. (2007) focus on the much rarer APL-associated STAT5-RAR fusion protein (Zeisig et al., 2007). First, by deletion mutant analysis, they show that only the STAT5-RAR constructs that conserve the capacity to bind DNA as hetero-oligomeric complexes with RXR retain the ability to transform primary murine hematopoietic progenitors in vitro. Second, by shRNA-mediated silencing of RXR in murine progenitors and NB4 cells (an APL-derived human cell line) they showed that STAT5-RAR fails to immortal-

ize murine hematopoietic progenitors in the absence of RXR, and that NB4 cells undergo apoptosis (Zeisig et al., 2007). It should be noted that the results from these two studies are—apparently—at odds: in one case, impairment of RXR recruitment abrogates leukemogenicity in vivo, yet the same RXR-defective *mutant* is still able to transform in vitro; in the second case, ablation of RXR expression prevents in vitro transformation. Although the use of different X-RAR fusion proteins does not allow a direct comparison, it raises the possibility that RXR-dependent, X-RAR-independent pathways play additional roles. In any case, these findings make a strong case for the relevance of RXR recruitment in the pathogenesis of APL and in the maintenance of the leukemic phenotype. Mechanistically, RXR appears to be required for full execution of the transcriptional program imposed by PML-RAR on its target cell, most likely due to an enhanced DNA binding and to the expansion of the repertoire of PML-RAR targets in the PML-RAR/RXR hetero-oligomeric context.

Modulation of the Activity of X-RAR Fusion Proteins by RAR and RXR Ligands

Pharmacological concentrations of RA induce release of transcriptional corepressors from PML-RAR, restarting the process of granulocytic differentiation in APL cells (Licht, 2006). Since RA also triggers degradation of PML-RAR, it is unclear whether RA-bound PML-RAR is actively involved in the process of differentiation or differentiation starts as the simple consequence of the disruption of the oncogenic brake. RA treatment of *M883R*;*T886R*-expressing murine hematopoietic progenitors leads to monocytic rather than granulocytic differentiation (Zhu et al., 2007). Since *M883R*;*T886R* is also degraded by RA treatment, these results suggest (1) that PML-RAR might contribute to the process of differentiation actively, otherwise the differentiation paths followed in the two cases should be identical, and/or (2) that titration of endogenous RXR might play a role in the determination of cell fate in PML-RAR-expressing cells before RA treatment, "pausing" the cells at a different stage of hematopoietic differentiation, thus triggering different differentiation pathways upon degradation of the fusion protein.

Similarly, RXR binding by specific ligands (rexinoids) might affect the activities of the PML-RAR/RXR complexes. Surprisingly, Zhu et al. (2007) did not observe major biological effects treating PML-RAR-expressing murine cells with rexinoids (even with cAMP, a combination which is known to lead to differentiation of human APL cells due to PKA-mediated facilitation of RXR signaling), while Zeisig et al. (2007), using a different rexinoid, found that RXR binding alone (without cAMP) is sufficient to trigger apoptosis of STAT5-RAR-expressing murine cells, and of human APL cells (Zeisig et al., 2007; Zhu et al., 2007; Altucci et al., 2005). Species specificity, X-RAR fusion specificity, and RXR ligand specificity provide multiple ways to interpret these data, which in any case point to a potential use of RXR ligands to modulate X-RAR functions.

Current Limitations in Our Understanding of APL

Though several studies have been carefully conducted to dissect the determinants of oncogenic transformation in APL, it is difficult to draw definitive conclusions so far. This is due, at least in part, to technical limitations of the biological assays used: as a typical example, in vitro assays of serial replating of murine hematopoietic progenitors in semisolid medium where X-RAR expressing cells show an indefinite ability to self-propagate and form colonies are—in our opinion—perhaps too emphatically considered as "transformation" assays (we have used the term transformation in our commentary according to this view) and should be better viewed as "immortalization" assays (and immortalization is necessary but not sufficient for transformation, as decades of studies in other cell systems have shown). Leukemias arise in vivo (e.g., in transgenic mice) after a long latency, suggesting that additional genetic or epigenetic lesions must accumulate for "full transformation" to occur. The in vitro phenotypes should therefore be considered as distinctive of a specific "preleukemic" phenotype, rather than indicative of complete oncogenic transformation. With this limitation in mind, the in vitro biological activities of fusion proteins cannot be easily reconciled with their in vivo leukemogenic potential, since they might not be sufficient to induce a full leukemic phenotype, as also revealed by the RXR-defective mutant described by Zhu et al. (2007). It is therefore critical to fill the holes in the available experimental data, especially for the part concerning biological data (see Table 1). Only at the end of this additional work might we be able to draw an "epistatic map" of events required for APL and apply these results to other forms of cancer.

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