Deconstruction of oncogenic networks in APL sets the stage for the development of novel targeted cancer therapies

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Abstract

Acute promyelocytic leukemia (APL) is associated with reciprocal and balanced chromosomal translocations always involving the Retinoic Acid Receptor α (RARα) gene on chromosome 17 and variable partner genes (X genes) on distinct chromosomes. RARα fuses to the PML gene in the majority of APL cases, and in a few cases to the PLZF, NPM, NuMA and STAT5b genes. As a consequence, X-RARα and RARα-X fusion genes are generated encoding aberrant chimeric proteins that exert critical oncogenic functions. Here we will integrate some of the most recent findings in APL research in a unified model and discuss some of the outstanding questions that will need to be addressed in the future years.

Introduction

Cancer is characterized by a tremendous genomic heterogeneity. In this Grand Round I will discuss the hypothesis that the genotype of cancer cells determines not only their biological characteristics but also their response to treatment.

Acute promyelocytic leukemia is a form of leukemia characterized by the presence in the blood and bone marrow of leukemic blasts blocked at the promyelocytic stage of myeloid differentiation. Over the last 20 years there has been a tremendous advancement in the understanding of the molecular lesions associated with this disease and how these impact therapy. As a result, APL has become the first malignancy that is cured entirely by drugs that target the mechanisms underlying the pathogenesis of this disease. In addition, a detailed understanding of the mechanisms that lead to APL, has revealed novel oncogenic pathways that play a role in a broad spectrum of malignancies. Therefore, APL has become a paradigm for the study of molecular mechanisms that underlie tumorigenesis and determine the response to cancer therapy [1, 2].

Acute promyelocytic leukemia (APL) is associated with reciprocal and balanced translocation always involving the retinoic acid receptor α (RARα) gene on chromosome 17, which translocates to the promyelocytic leukemia gene (PML) on chromosome 15 in the vast majority of APL cases [3-6]. In a few variant cases, RARα fuses to the promyelocytic leukemia zinc finger (PLZF) gene, to the nucleophosmin (NPM) gene, to the nuclear mitotic apparatus (NuMA) gene and to the signal transducer and activator of transcription 5b (STAT5b) gene located on chromosome 11, 5, 11, or 17 respectively [7-10]. In view of the reciprocity of these translocations, X-RARα and RARα-X fusion genes are generated and co-expressed in the APL

blasts. Although rare, these variant translocations have been tremendously informative, allowing a comparative analysis of molecular and biological similarities and differences among the various fusion proteins. Since the RARα gene always breaks within the same intron, the various fusion proteins share and identical RARα moiety but do not bear structural similarities in the X moiety, and yet the disease associated with these molecular lesions is APL (Fig.1).

The RARα portion of the X-RARα fusion protein can mediate heterodimerization with RXR and bind to DNA through the retinoic acid receptor DNA binding domains [11]. Therefore, the X-RARα fusion products invariably retain the ability to potentially interfere with the RAR/RXR pathways.

The various X-RARα proteins also display the capacity of heterodimerizing with the respective X proteins (e.g. PML-RARα with PML etc.). This is due to the fact that X proteins are normally capable of homodimerizing and that the region that mediates X proteins homodimerization is invariably retained in the X moieties fused to RARα. Thus, X-RARα can potentially interfere with both X and RARα pathways.

Indeed, since its discovery it has been hypothesized that PML-RARα acts as a double dominant negative mutant on PML and RARα pathways. For example PML-RARα, unlike RARα, acts as a transcriptional repressor at physiologic concentration of the ligand (Retinoic Acid: RA), and that PML-RARα can also cause the disruption of the PML nuclear bodies (NBs) where PML normally accumulates [1, 2, 12] (Fig.2). As we will discuss, this notion has now been integrated on the basis of more recent findings that defined a "gain of function" aberrant transcriptional activity for PML-RARα.

Cell of origin of APL

The cell of origin of APL has been sought, but not unambiguously identified. In human APL, the cell of origin might be a pre-T cell or a myeloid progenitor. Therefore, expression of PML–RARα might both induce lineage switching and confer self-renewal to committed cells [13]

Modeling APL in the mouse

The generation of mouse models that recapitulate faithfully APL pathogenesis led to important conclusions regarding the role of various X-RARα and RARα-X fusion proteins in APL pathogenesis. Expression of the PML-RARα fusion protein in the myeloid promyelocytic compartment leads to APL like disease in transgenic mice [14-16]. Moreover, retroviral transduction of PML-RARα in murine hematopoietic progenitors results in APL development as well [17].

The generation PML and PLZF knock-out (KO) mice genes has also been extremely informative. in elucidating the function of these genes.

X-RARα proteins are biologically distinct RARα mutants that mediate differential response to RA.

Characterization of PML-RARα, PLZF-RARα, NPM-RARα and NUMA-RARα transgenic mice (TM) in which the expression of the fusion gene is restricted to the promyelocytic compartment has revealed that the X-RARα fusion proteins play a critical role in

leukemogenesis. However, X-proteins cause distict diseases in the mouse: PML-RARα and NuMA- RARα TM develop APL leukemia; NPM-RARα a myelo-monocytic acute leukemia, while PLZF-RARα TM develop a myeloprolipherative disorder reminiscent of human chronic myelogenous leukemia (CML) rather than of human APL [16, 18, 19]. In addition, X-RARα molecules dictate sensitivity to RA treatment. For example PLZF-RARα mice develop RA-resistant, while PML-RARα mice develop RA-responsive APL leukemia [14, 15, 20] (Fig.1). These observations indicate that X-RARα molecules do not represent identical RARα mutants and strongly suggest that the X-moiety lends the X-RARα fusion proteins distinct biological properties.

RARα-X proteins play a critical role in APL leukemogenesis, but are not sufficient for full-blown transformation.

Comparative characterization of RARα-X TM has revealed an important role for these molecules in APL leukemogenesis. RARα-PML and RARα-PLZF TM develop myeloproliferative disorder without an apparent block in myeloid differentiation, but never full-blown leukemia [21, 22].

In PML-RARa/RARα-PML double TM, the RARα-PML transgene increases the penetrance and the onset of leukemia, thus acting as a classic tumor modifier [22]. Similarly, in PLZF-RARa/RARα-PLZF double TM, RARα-PLZF metamorphoses the CML phenotype into an APL-like acute leukemia. Surprisingly, leukemia onset in PLZF-RARa/RARα-PLZF double TM is still preceded by a long preleukemic phase [21].

These findings underscore the "qualitative nature" of the multi-step process towards leukemogenesis. In fact, the phenotype observed in the PLZF-RARα/RARα-PLZF double TM is not due the mere addition of the phenotypes observed in the single TM, since neither of these mutants display the characteristic block of differentiation at the promyelocytic stage. APL in double TM is instead the qualitatively novel biological outcome of two concomitant aberrant activities affecting distinct molecular pathways [21].

Multiple hits in APL pathogenesis

The long latency observed in leukemia mouse models of APL strongly suggests that additional genetic events may cooperate with the APL fusion proteins towards leukemogenesis. The same events could contribute to the pathogenesis of human APL. For example, spectral karyotyping (SKY) has allowed the demonstration that leukemic cells from PML-RARα and PLZF- RARα TM do harbor multiple recurrent chromosomal abnormalities [23, 24]. These studies indicated that an interstitial deletion of chromosome 2 is one of the most one of the most common recurrent chromosomal abnormalities in murine APL models. Genetic characterization of this region led to the discovery that loss of one copy of PU.1, a master regulator of hemopoietic development, leads to increased APL penetrance in mice expressing PML-RARα [25]. This finding suggests that a similar mechanism may contribute to human APL leukemogenesis.

Other co-operating mutations have been implicated in the progression of APL: N-RAS mutation, fms-related tyrosine kinase 3 (FLT3) activation [26, 27]

X-RARα molecules are involved in the control of mitogenic and survival signals.

Analysis of $PML^{-/-}$ and $PLZF^{-/-}$ mice and cells has corroborated the notion that the blockade or the interference with pathways normally regulated by these molecules can indeed play a critical role in APL pathogenesis. Primary PML KO cells such as mouse embryonic fibroblasts (MEFs) or primary thymocytes display a marked proliferative advantage [28]. Furthermore, $PML^{-/-}$ cells of various histological origins including hemopoietic cells and $PML^{-/-}$ mice are protected from multiple apoptotic stimuli such as for instance ionizing radiation [28, 29]. In this respect, PML has been found to modulate both p53 dependent [30, 31] and independent [32, 33] apoptotic pathways. PML inactivation markedly impairs cellular senescence induced by oncogenic *Ras* [34, 35], and renders the cells genetically unstable [36]. Finally, $PML^{-/-}$ mice are more susceptible to tumorigenesis when challenged with carcinogens [29].

Analysis of $PLZF^{-/-}$ mice and cells revealed that the inactivation of this gene can also result in a proliferative and survival advantage throughout embryonic development [37].

Thus, inactivation of X gene in the mouse is uncovering functional commonality between the partners of RARα involved the various APL associated chromosomal translocation. In addition, these findings suggest that X functions may be deregulated and play a causative role in cancers other than APL.

The functional interference of X-RARα and RARα-X with X and RAR/RXR pathways is critical for APL leukemogenesis.

X-RARα proteins are expected to interfere with X and RARα pathways. This hypothesis implies that in human APL blasts the oncogenic function of the various X-RARα fusion proteins can be greatly facilitated by the reduction to heterozygosity of X and RARα because one allele of these genes is involved in the chromosomal translocation.

This hypothesis was tested in germline mutant mice. In this setting, the reduction to heterozygosity or the inactivation of PML and other X proteins or RARα should accelerate/exacerbate leukemogenesis by the various fusion proteins in TM.

Indeed, crosses of PML-RARα TM with $PML^{-/-}$ mice or PLZF-RARα TM with $PLZF^{-/-}$ mice have been extremely informative and totally supported this notion. The progressive reduction of the dose of PML resulted in a dramatic increase in the incidence of leukemia, and in an acceleration of leukemia onset in PML-RARα TM. Furthermore, in hemopoietic cells from PML-RARα TM, PML inactivation resulted in impaired response to differentiating agents such as RA and vitamin D_3 as well as in a marked survival advantage upon pro-apoptotic stimuli. These results demonstrated that PML acts *in vivo* as a tumor suppressor by rendering the cells resistant to pro-apoptotic and differentiating stimuli and that the functional impairment of PML by PML-RARα is a critical event in APL pathogenesis [38].

Moreover, the data obtained by crossing PLZF-RARα TM with $PLZF^{-/-}$ mice totally supported the notion that RARα-PLZF acts as a dominant negative RARα mutant and suggested once again that PLZF haploinsufficiency is critical for APL pathogenesis. In fact, $PLZF^{-/-}$/PLZF-

RARα mutants develop APL-like leukemia indistinguishable from the one observed in PLZF-RARα/RARα-PLZF double TM [21].

Classic model to explain the pathogenesis of APL

Most of the mechanistic understanding of the pathogenesis and response to therapy pertains to the PML-RARα and to a lesser degree to the PLZF-RARA fusion proteins.

RARα is a RA-responsive transcription factor, and PML is the organizer of nuclear subdomains that are linked to post-translational modifications and the control of stem cell self-renewal [12]. The PML–RARα fusion protein retains all the functional domains of RARα (notably the DNA-binding, hormone-binding and retinoid X receptor (RXR)-binding domains) and PML (in particular the RING finger and coiled-coil domains).

The classic mechanistic explanation of APL pathogenesis proposes that PML–RARα transforms haematopoietic progenitors through the transcriptional repression of RARα target genes in a dominant-negative manner [39]. This reflects PML–RARα homodimer formation through the PML coiled-coil domain, resulting in enhanced recruitment of co-repressors and histone deacetylases (HDACs) onto RARα target genes, enforcing DNA methylation [40-42]. As RARα signalling regulates myeloid differentiation, its inhibition could explain the block in differentiation that is observed in APL cells (Fig.3). Indeed, pharmacological doses of RA convert PML–RARα into a transcriptional activator, thus enhancing expression of crucial RARα targets and restoring the normal differentiation [39, 43, 44] (Fig. 4). However, this classic model does not explain why RA must be combined with chemotherapy to cure patients [45, 46]. It also

fails to explain why single-agent arsenic trioxide therapy cures most patients in the absence of clear-cut transcriptional activation and differentiation, at least *ex vivo*.

A revised model for APL pathogenesis

Several studies have demonstrated that transcriptional repression at RA responsive sites is not sufficient to induce APL [47, 48]. Furthermore, direct analyses of PML–RARα binding sites by chromatin immunoprecipitation followed by deep sequencing have demonstrated the presence of RXRα in the DNA-bound complex, and the binding to non-canonical sites (see below), but have only marginally supported the idea that PML–RARα induces massive histone modifications or DNA methylation [49]. Taken together, these results establish that deregulated RARα transcriptional regulation alone does not sufficiently explain APL pathogenesis.

Recent studies have identified several novel features of PML-RARA that contribute to a better understanding of APL pathogenesis.

First, it has been shown that PML–RARα bound to RXRα allow specific binding to a surprisingly large variety of *de novo* DNA sites, contributing to widespread transcriptional deregulation [49, 50]. This expanded repertoire of binding sites includes sites that are recognized by other nuclear receptors controlling myeloid differentiation or stem cell self-renewal, such as retinoic acid receptor-γ (*RARG*), vitamin D receptor (*VDR*) and thyroid hormone receptors. This finding illustrate that fusion of RARA to PML leads to a dramatic PML-RARA has a dramatic gain-of-function.

Second, PML–RARα undergoes post-translational modifications, most notably PML SOMOylation, which is important for APL initiation. This event induces transcriptional repression [51].

Last, RARα fusion proteins interact with key chromatin remodelling complexes, notably polycomb group repressive complex 1 (PRC1) and PRC2, which have a crucial role in stem cell maintenance. Thus PML–RARα could recruit PRC2 onto differentiation genes, resulting in their polycomb-dependent repression through histone modifications [52].

PML, PML-RARα and stemness.

PML–RARα may also function by interfering with PML-controlled pathways, which have been repeatedly implicated in apoptosis resistance and have been recently linked to stem cell self-renewal [53-56]. At least some of the functions of PML are mediated through PML nuclear bodies, which recruit a large number of partner proteins. These domains have been implicated in partner sequestration and/or post-translational modifications, notably phosphorylation, SUMOylation and ubiquitylation [56, 57]. PML, probably through nuclear body formation, is required for optimal transcriptional activity of the p53 tumor suppressor, which could explain both apoptosis resistance and alteration of progenitor self-renewal [35, 56].

PML–RARα disrupts PML nuclear bodies through its ability to heterodimerize with PML [58, 59] and could abrogate PML functions that are dependent on nuclear body formation [12]. Accordingly, PML–RARα expression was conclusively linked to defective p53 activation [35, 60]. However, other pathways that play crucial roles in tumorigenesis are under the influence of

PML nuclear bodies, such as protein phosphatase 2A (PP2A), which regulates the AKT–PTEN pathway, a crucial regulator of stemness [61].

Therapy for APL

Treatment with RA triggers rapid APL cell differentiation into granulocytes ex vivo and in vivo. In patients RA induces remission in requires treatment for 3 to 5 weeks, however, RA treatment generally results in transient disease clearance, as only a few patients with APL have been cured by RA alone. By contrast, the combination of RA with anthracyclines cures 70% of patients [45].

Arsenic trioxide is another agent with striking activity in APL. Arsenic trioxide therapy yields long-term survival and cures many patients [62, 63]. As a result, RA or arsenic trioxide has been incorporated with chemotherapy in every modern treatment regimen for APL leading to a cure rate of more than 90%. Moreover, combination therapy with these two agents in the absence of chemotherapy drugs has resulted in complete remission and cure rates exceeding 90%. Therefore, APL is the first malignancy that can be cured with treatment regimens that do not contain chemotherapy [64, 65].

Mechanism of action of RA and Arsenic trioxide

RA and arsenic trioxide, which are chemically unrelated, both degrade PML–RARα [66, 67]. This is sole property shared by these two agents was discovered through the observation of therapy-induced restoration of nuclear bodies [58, 59, 66] (Fig. 5 and 6).

Several mechanisms have been reported to be responsible for RA induced degradation of PML-RARA. The first PML–RARα degradation pathway identified involves the neutrophil elastase protease that cleaves the PML moiety of PML–RARα. These proteases are activated in myeloid cells by RA-induced differentiation [68-70]. Indeed, protease-resistant mutants of PML–RARα are more potent inducers of the disease in transgenic mice [71].

The second degradation pathway directly couples RA-induced transcriptional activation to proteasome-mediated RARα degradation [67]. A third pathway involves formation of autophagic vesicles [72].

By contrast, arsenic trioxide degrades PML–RARα by targeting its PML moiety and accordingly also degrades the normal PML protein. As_2O_3 physically interacts with the PML RING domain of PML and of the PML-RARA. This event causes a conformational change that triggers the recruitment of the SUMO E2 ligase UBC9 to PML-RARA, promoting PML-RARA SUMOylation, which promotes the partitioning of PML to PML-nuclear bodies [2, 73, 74]. PML SUMOylation in turn, recruits PML to PML nuclear bodies. In addition, PML SUMOylation recruits RING finger protein 4 (RNF4) onto SUMOylated PML. RNF4 is a SUMO-dependent ubiquitin ligase that polyubiquitylates PML and targets it to the proteasome [75, 76].

Thus, PML multimerization and arsenic trioxide binding, followed by PML hyperSUMOylation, constitutes the primary mechanism of arsenic trioxide-induced APL cure.

However, the PML SUMO E3 ligase responsible for mediating PML and PML-RARA SUMOylation in the presence of arsenic trioxide has remained unknown. We will present alter in this proposal, data demonstrating the PIAS1 SUMO E3 ligase is responsible for this effect.

Modelling APL therapy response in mice.

Mouse models of APL have been instrumental in developing successful drug therapies to treat patients with APL [77]. Preclincial trials have demonstrated that achieving maximal degradation of PML-RARA though RA and arsenic trioxide combination therapy clears leukemia initiating cells promoting rapid APL clearance [78-82].

RA–arsenic trioxide synergy was confirmed in clinical trials in APL patients [64, 83]. The fact that RA does not induce complete degradation in APL leukemia initiating cells explains why RA rarely cures APL when used as a single agent [78, 84]. Indeed, liposomal RA (which yields high intracellular concentrations) is much more potent than standard RA and cures half of the patients treated, even in the absence of chemotherapy [85].

Response to RA and arsenic treatment in PLZF-RARA APL

X-RARA fusion proteins account for 1–2% of patients with APL [39]. The most frequent of these is the PLZF–RARα fusion protein, which is associated with RA-resistant APL [7].

Resistance was proposed to result from an additional co-repressor binding site in PLZF, which precludes RA-dependent target gene activation and thus differentiation [86, 87]. Also in APL that arises in *PLZF–RARA;RARA–PLZF* transgenic mice is clinically resistant to treatment with RA, like their human counterparts. This is due to the fact that PLZF–RARα-expressing LICs are much less sensitive to RA than those from PML–RARα APL [78]. Indeed, high-dose RA treatment results in PLZF–RARα degradation [88], by the same mechanism as RARα and PML–RARα, triggering some progressive loss of LIC activity. Accordingly, in patients with PLZF–RARα APL, there is evidence for RA-induced differentiation and partial response [78, 89], although this is insufficient to clear the disease. Such RA resistance of LICs are most likely due to irreversible chromatin changes enforced by PRC1 [90]. Importantly, arsenic trioxide or ROS have no effect on PLZF–RARα-driven human or murine APL cells [73, 81, 88].

Role of PML deficiency in the promotion of malignancies other than APL

There is ample evidence that PML is a tumor suppressor. *Pml* deficiency promotes APL induced by PML-RARA. Moreover, *Pml* deficient mice are tumor prone. These observations complement data obtained in tissue culture systems indicating that PML is a critical regulator of apoptosis, OIS induction, and of the response to DNA damaging agents [12]. Therefore, PML has been the subject of intense investigation.

PML is the essential component of subnuclear structures identified as PML nuclear bodies (PML-NBs) [12, 91, 92]. Many proteins implicated in the regulation of apoptosis, cellular senescence, inhibition of cellular proliferation and maintenance of genomic stability reside, either

constitutively or transiently, in PML-NBs [12]. Therefore, it seems reasonable to speculate that PML deficiency would affect the function of multiple tumor suppressive pathways.

PML is a multidomain protein containing a RING-B-Box-Coiled-Coil region (RBCC), a nuclear localization signal, a C-terminal degron and a SUMO binding domain. The RBCC region mediates protein-protein interactions and is essential for PML homodimerization and localization to the PML-NBs [12, 91, 93]. PML is ubiquitously expressed and exists in multiple isoforms [94]. PML IV has been the most studied isoform because of its ability to induce OIS and apoptosis. It is believed that this property is due to PML's ability to faciltiate the transctiptional activity of the p53 tumor suppressor [30, 31].

PML undergoes several post-transcriptional modifications. For example, phosphorylation events determine its localization to the PML-NBs during arsenic induced apoptosis. Alternatively, direct phosphorylation of PML by casein kinase 2 (CK2) at serine 517, mediates its ubiquitination and degradation facilitating tumorigenesis [93, 95]. Moreover, PML SUMOylation is essential for proper assembly of the PML-NBs and PML tumor suppressive activity [91, 92].

SUMOylation is a process analogous to ubiquitination, where the SUMO protein is covalently conjugated to substrates through an enzymatic cascade that involves E1, E2 and E3 enzymes. To date, four types of SUMO E3 ligases have been identified in mammalian cells. One of these is the PIAS (protein inhibitor of activated STAT) family of proteins which comprises four members: PIAS1, PIAS3, PIASy, PIASxa and PIASxb [96]. It is well recognized that SUMOylation plays an important role in the regulation of a wide range of cellular processes including transcriptional regulation, protein-protein interactions and subcellular localization [97].

However, only recently it has been suggested that PML deficiency plays a role in the pathogenesis of malignancies other than APL.

Notably, PML deficiency is a common event in a wide spectrum of human cancers. This hypothesis is further supported by our observation that *Pml* loss accelerates tumor initiation in mouse models of NSCLC and prostate cancer [29, 61, 93, 98].

Regulation of PML protein ubiquitination and SUMOylation

We reported that PML is aberrantly poly-ubiquitinated and degraded upon phosphorylation by the serine-threonine kinase CK2, an oncogenic protein kinase frequently overexpressed or aberrantly activated in human tumors. Moreover, we have described that PML is often partially or completely lost in NSCLC [98, 99], while CK2 is overexpressed and amplified in NSCLC, predicting poor patient survival [100]. Indeed, we found a statistically significant inverse correlation between PML protein levels and CK2 kinase activity in NSCLC derived cell lines and in primary NSCLC) [93].

These experiments strongly suggest that elevated CK2 kinase activity leads to PML degradation in both NSCLC derived tumor cells and primary human specimens. As a result, pharmacologic CK2 inhibition may lead to significant anti-tumor effects.

PML and PML-RARA are SUMOylated on three major SUMOylation residues (lysine 60, 160 and 442). PML also contains a functional SUMO interacting motif (SIM) [91, 101]. Interactions involving SUMOylated proteins and SIM modules promote the recruitment of PML partner proteins and PML-NB biogenesis [2, 12, 91].

As we have discussed arsenic trioxide treatment leads to degradation of PML and PML-RARA. This protein involves PML-RARA SUMOylation and consequent ubiquitination. Moreover, PML is also degraded in tumor cells due phosphorylation by CK2.

To gain insight into the biological significance of PML SUMOylation, we aimed at the identification of the PML SUMO-E3 ligase. We discovered that PIAS1, a member of the Protein Inhibitor of Activated STAT family, physically interacts and co-localizes with PML in PML nuclear bodies. PIAS1 promotes SUMOylation of PML and PML-RARA in cell free systems. Furthermore, PIAS1 dependent PML SUMOylation leads to ubiquitin mediated PML degradation in non-small cell lung cancer (NSCLC) and prostate cancer cells. Moreover, an inverse correlation exists between the levels of PIAS1 and PML proteins in NSCLC and prostate cancer cells.

Finally, we have determined that PIAS1 is essential to mediate SUMOylation of PML-RARA and consequent degradation in APL cells treated with arsenic trioxide.

These data reveal a novel and unexpected function of PIAS1 and the SUMOylation machinery in the regulation of oncogenic networks and in regulating the response to targeted APL therapy (Fig. 7). Remarkably, the biological output of PIAS1 depends on PML status. PIAS1 promotes tumorigenesis in cells expressing PML, but exerts a tumor suppressive function in APL by degrading PML-RARA. We conclude that PIAS1 is a key regulator of both PML and of PML-RARA. These data provide the rationale for the development of pharmacologic inhibitors of PIAS1 to prevent aberrant degradation of PML in cancer cells. Conversely, strategies that stimulate PIAS1 activity may have a beneficial role in the therapy of APL.

Conclusions

From a clinical point of view, 30 years of clinical and basic research have allowed most patients with APL to be definitively cured, even with some treated without DNA-damaging agents [102].

From a basic science and translational science point of view, APL has been a source of many discoveries that have elucidated critical oncogenic networks and provided novel tools to study cancer biology (Fig. 8). A partial list of the accomplishments of this field are: 1. APL has been the first disease modeled with genetically modified mouse models; 2. The study of APL has illustrated that cancer is an heterogenic disease and that the genotype of cancer cells impacts treatment response to specific agents; 3. APL has elucidate novel epigenetic mechanisms governing transcription; 4. PML and PML-RARA illustrate the importance of SUMOylation in regulating oncogenic networks and the response to targeted therapy.

Thus, research on the mechanisms, genetics, and therapy of this paradigmatic form of leukemia will undoubtedly continue to greatly contribute to the fight against cancer in the years to come.

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Figure legends

Figure 1. Schematic representation of APL translocations. Translocation nomenclature, phenotype in transgenic mcie and response to ATRA/Arsenic are indicated.

Fig. 2. Model proposing that PML-RARa exerts a double dominant negative effect on both PML and RAR/RXR signaling. According to this view, PML-RARa by inhibiting PML dependent tumor suppressive functions, leads to resistance to apoptosis in the leukemic blasts. At the same time, PML-RARa would interfere the RAR/RXR, which leading to a block of differentiation of myeloid progenitors.

Fig. 3. PML-RARα recruits co-receptors at RAR/RXR responsive elements inducing transcriptional repression.

Fig. 4. Pharmacologic doses of ATRA (all transretinoic acid) relieve the transcriptional repression mediated by PML-RARα.

Figure. 5. RA induced degradation of PML-RARα both through neutrophil elastase protease or proteasomal degradation. This processes leads to degradation of the PML-RARa oncoprotein and APL remission.

Figure. 6. Arsenic trioxide leads to PML-RARα degradation through the proteasome. This processes leads to degradation of the PML-RARα oncoprotein and APL remission.

Fig. 7. PIAS1 promotes SUMOylation of PML and PML-RARα and consequent degradation. (A) Model of the mechanisms controlling PML and PML-RARα SUMOylation and ubiquitination. In cancer cells (left panel), PIAS1 leads to PML SUMOylation that, in turn, recruits CK2 to PML promoting the phosphorylation of the PML degron. This event triggers PML ubiquitin-mediated degradation with consequent loss of PML-tumor suppressive functions and promotion of tumorigenesis. CK2 and PIAS1 interact (dashed arrow), thus it is possible that a tertiary PML-CK2-PIAS1 protein complex exists. It is presently unknown whether RNF4 or other ubiquitin E3-ligase mediates the degradation of PML in cancer cells in the absence of arsenic. In APL (right panel), PIAS1 promotes SUMOylation of PML-RARα, which in the absence of arsenic is not sufficient to trigger ubiquitination of PML-RARα. The presence of arsenic allows the recruitment of the machinery involved in PML-RARα degradation. ATO = arsenic.

Fig. 8. Unified model for the molecular pathogenesis of APL. In vivo analysis of TM and KO mice supports a model by which the concomitant activity of X-RARα and RARα-X fusion

proteins is essential in APL pathogenesis. The APL fusion proteins can deregulate X and RARα functions in a dominant-negative fashion. At the same time, the APL fusion proteins may exert novel gain-of-function properties (*?*). These activities may be necessary but not sufficient in APL leukemogenesis as suggested by the fact that cooperative genetic events occur toward overt full-blown transformation in mouse models of APL. X_1-*n*, the various RARα fusion partners; *arrow:* transcriptional activation; *arrow:* transcriptional repression; *?*, unknown target genes/molecular events

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Figure 1

t(15;17)

APL

Yes/Yes

t(11;17)

MPD/APL

No/No

5(5;17)

Monocytic Leukemia

Yes/No

t(11;17)

APL

Yes/No

der(17)

?

No/No

Figure 2

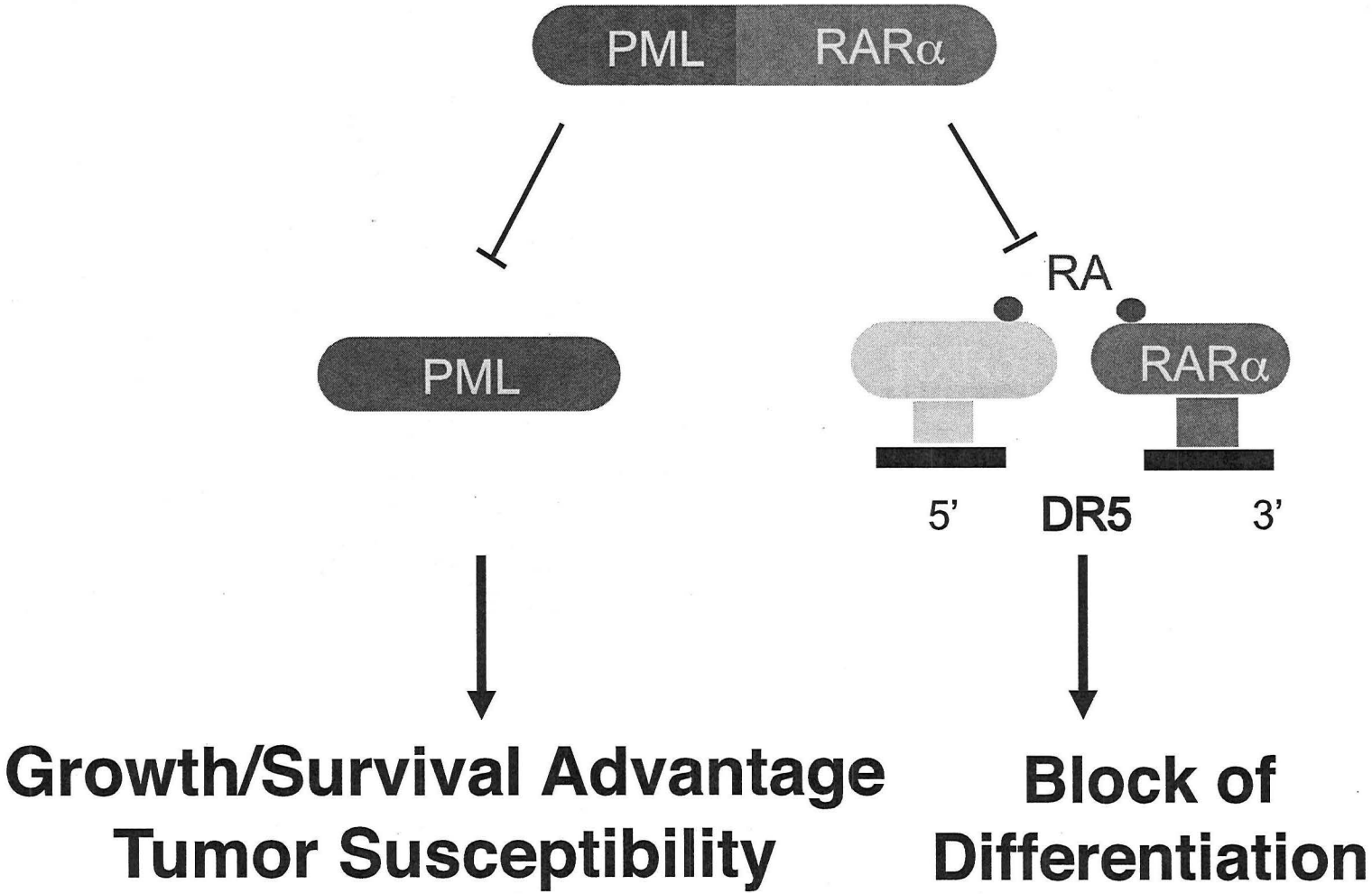

PML
RARα
PML
RA
RXR
RARα
5'
DR5
3'
Growth/Survival Advantage
Tumor Susceptibility
Block of
Differentiation

Figure 3

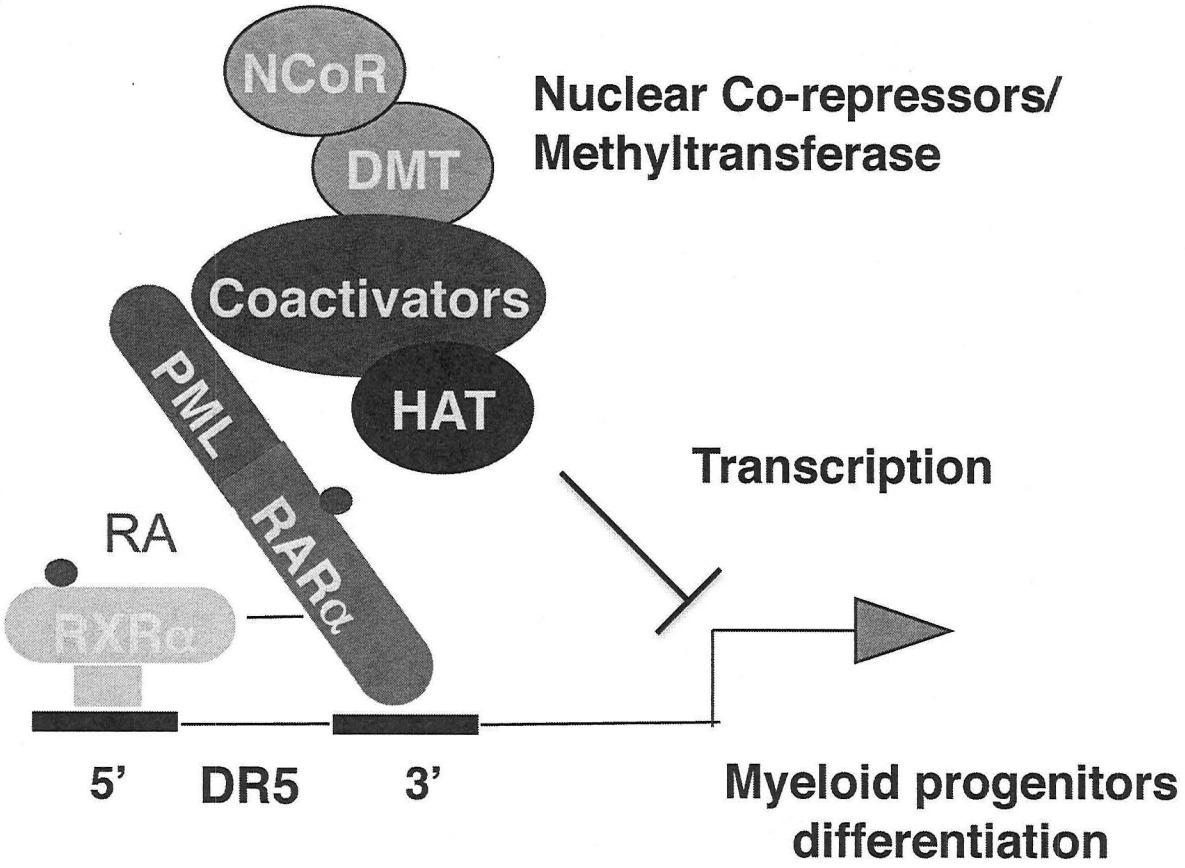

NCoR
DMT
Nuclear Co-repressors/
Methyltransferase
Coactivators
HAT
PML
RARα
Transcription
RA
RXRα
5'
DR5
3'
Myeloid progenitors
differentiation

Figure 4

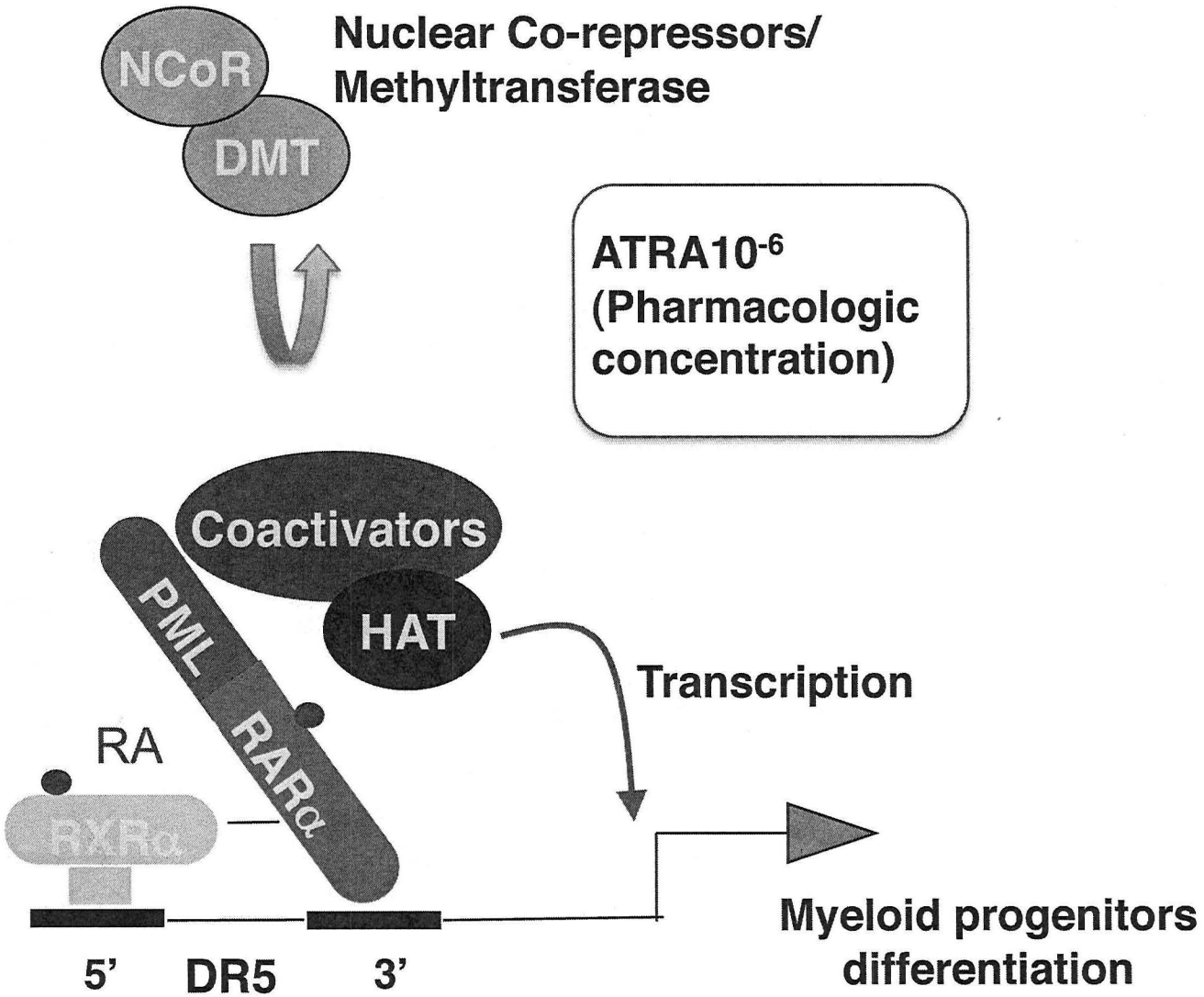

NCoR
DMT
Nuclear Co-repressors/
Methyltransferase
ATRA10-6
(Pharmacologic
concentration)
Coactivators
HAT
PML
RARα
RA
RXRα
Transcription
5'
DR5
3'
Myeloid progenitors
differentiation

Figure 5

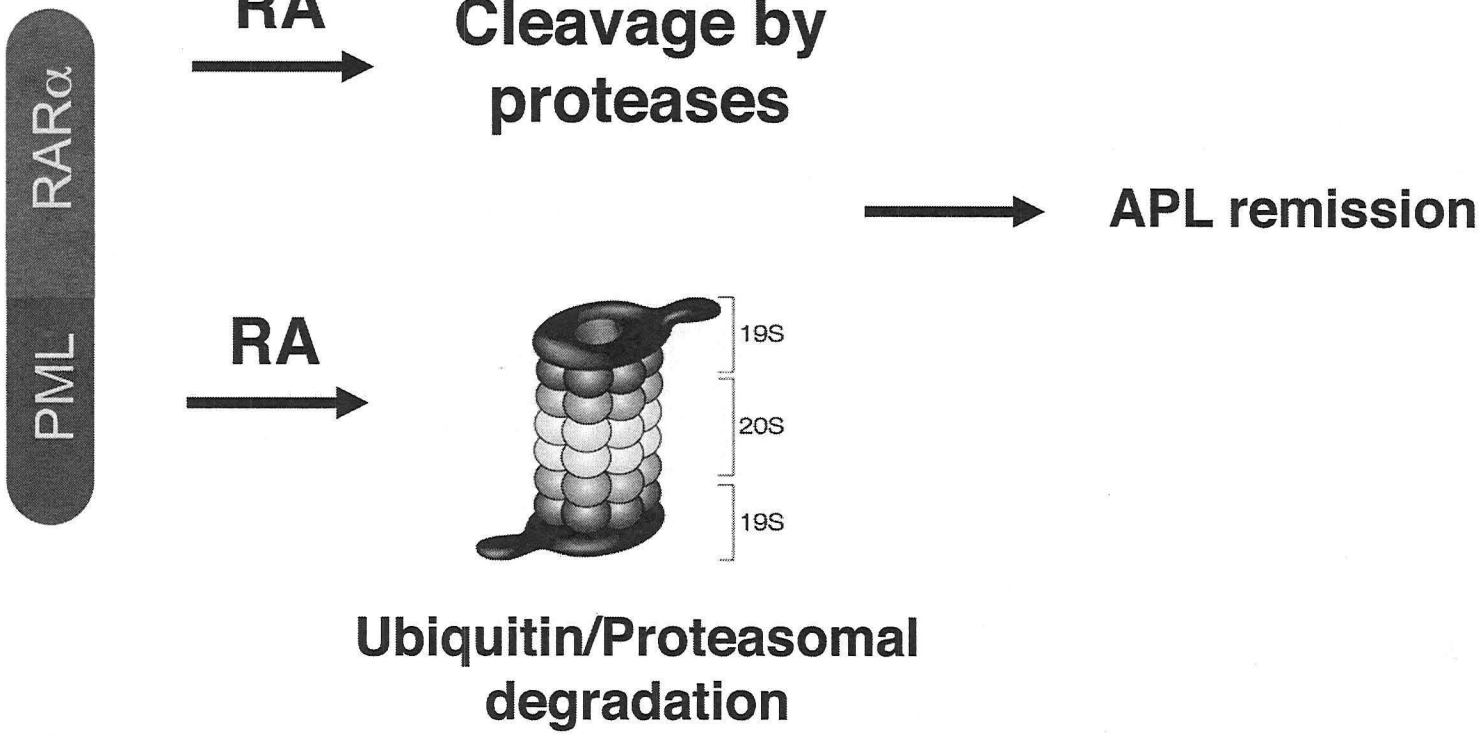

PML
RARα
RA
Cleavage by proteases
APL remission
RA
19S
20S
19S
Ubiquitin/Proteasomal degradation

Figure 6

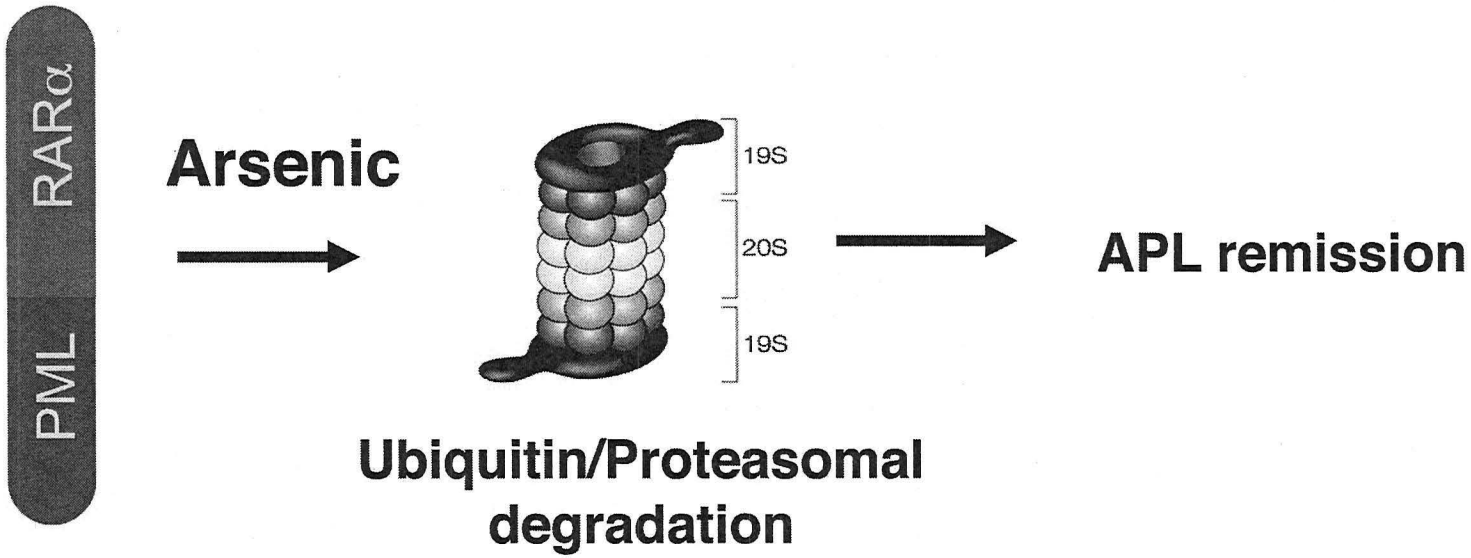
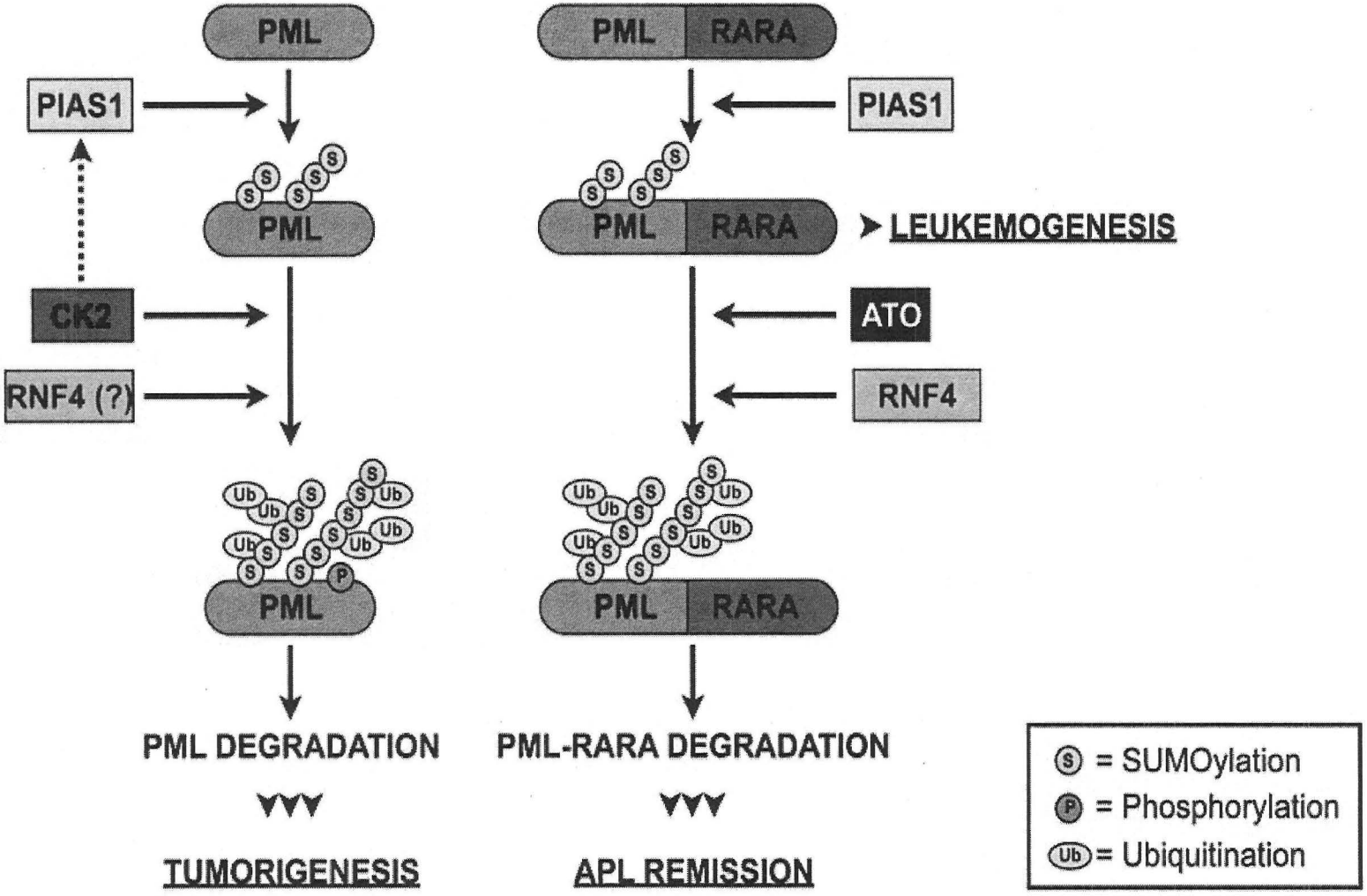
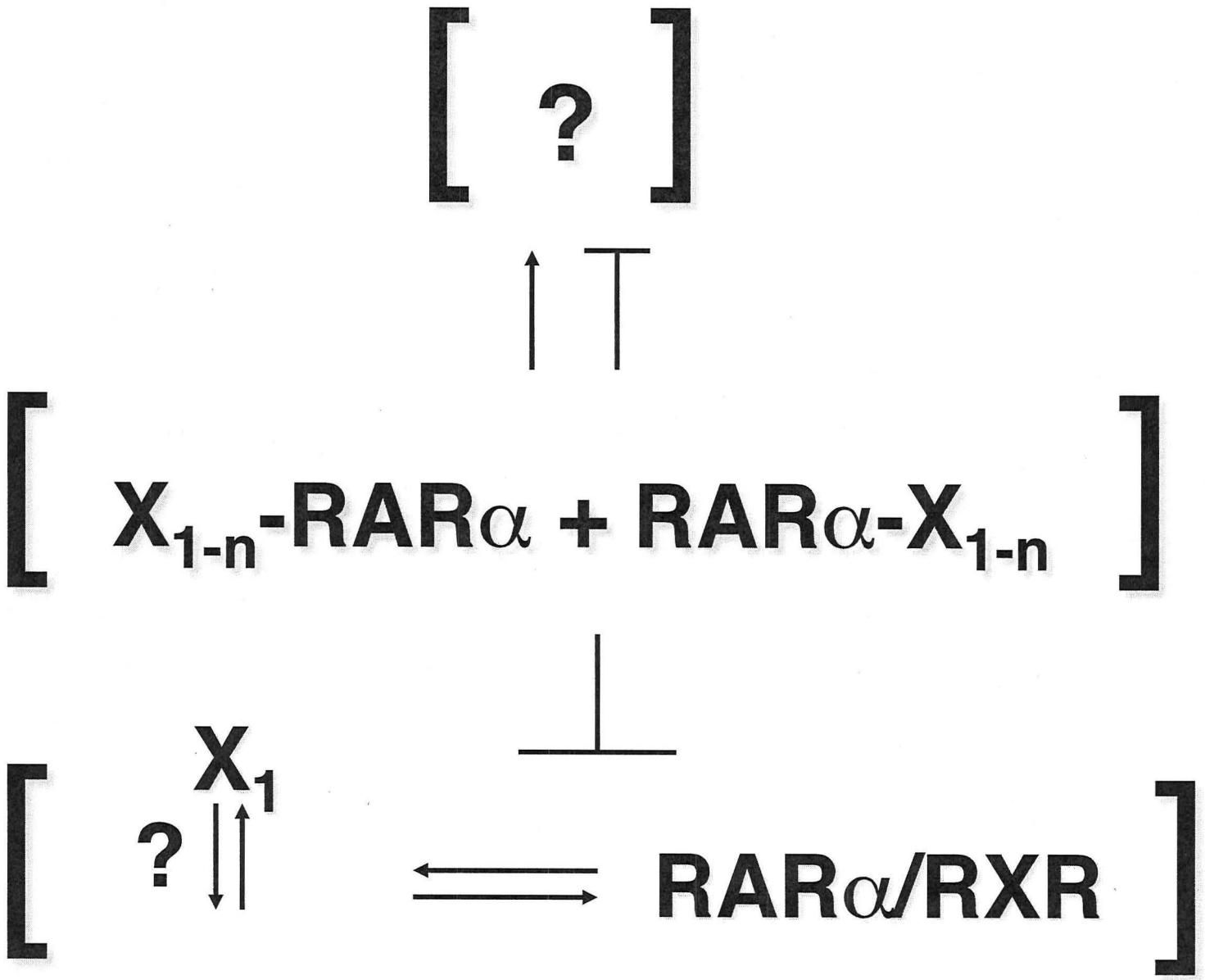

PML
RARα
Arsenic
19S
20S
19S
APL remission
Ubiquitin/Proteasomal
degradation

Figure 7


PML
PIAS1
PML
CK2
RNF4 (?)
PML
PML DEGRADATION
TUMORIGENESIS
PML RARA
PIAS1
PML RARA
LEUKEMOGENESIS
ATO
RNF4
PML RARA
PML-RARA DEGRADATION
APL REMISSION
= SUMOylation
= Phosphorylation
= Ubiquitination


[?]
[X1-n-RARα + RARα-X1-n]
X1
?
RARα/RXR