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Acute Promyelocytic Leukemia: A Model Disease for Targeted Cancer Therapy

Emma Lång and Stig Ove Bøe
Oslo University Hospital, Oslo, Norway

1. Introduction

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) characterized by a severe bleeding tendency, accumulation of abnormal promyelocytes in the bone marrow and a reciprocal t(15;17) chromosomal translocation that fuses the gene encoding the promyelocytic leukemia protein (PML) to that encoding retinoic acid receptor alpha (RARA) (de Thé & Chen, 2010). During the past 30 years two therapeutic drugs have been introduced into the clinic that have dramatically improved the treatment outcome of this disease (Wang & Chen, 2008). The first of these components was all-trans retinoic acid (ATRA), a vitamin A derivative that significantly increased clinical remission and improved the 5-years disease-free survival rates from below 40% to more than 80% (Huang *et al.*, 1988). The second drug was arsenic trioxide (ATO), a component that was discovered to be remarkably effective in treating APL as a single agent (Sun *et al.*, 1992). Today, most hospitals employ ATRA in combination with chemotherapy as frontline therapy, while ATO is being used for refractory or relapsed patients. Recent clinical studies have also revealed a positive synergistic effect between the two drugs, suggesting that future therapy of newly diagnosed patients may involve a combination of the two reagents (Estey *et al.*, 2006; Hu *et al.*, 2009; Shen *et al.*, 2004; Wang *et al.*, 2004).

The success of using ATRA and ATO in APL therapy appears to be linked to the ability of these drugs to interact with the fusion oncoprotein PML/RARA, which is produced by the APL-associated t(15;17) translocation, and that causes the disease. ATRA contacts a ligand binding domain present within the RARA moiety of this chimeric protein and promotes differentiation of APL cells along the granulocyte linage (Huang *et al.*, 1988). ATO, on the other hand, has recently been shown to bind one or more cysteine rich motifs within the PML protein (Jeanne *et al.*, 2010; Zhang *et al.*, 2010) and contributes to the cure of APL through a mechanism that involves eradication of leukemic-initiating cells (LICs) (Nasr *et al.*, 2008; Ito *et al.*, 2008; Zheng *et al.*, 2007).

Due to the success of using ATRA and ATO in the clinic, and because of the ability of these drugs to promote clinical remission through a direct contact with PML/RARA, APL has become one of the most attractive model diseases for the development of targeted cancer therapy. The APL cure offers a proof of principle that a cancer can be cured through targeted inactivation of an oncoprotein, and it provides a rationale for the development of novel therapeutic strategies that target fusion oncoproteins produced by chromosomal translocations. In this chapter we will summarize the current knowledge of the biological properties of PML, RARA and PML/RARA with particular emphasis on tumorigenesis in APL patients and the molecular mechanisms that underlie the response to ATRA and ATO.

2. APL treatment – a historical perspective

2.1 The discovery of ATRA-based APL therapy

APL was first characterized as a distinct clinical entity in 1957 (Hillestad, 1957). Throughout the 1950s and 1960s, this disease had a 100% mortality rate and no effective treatment options. In 1973, chemotherapy by the topoisomerase inhibitor daunorubicin was shown to have some curative effect, yielding a complete remission (CR) rate of 55% (Bernard *et al.*, 1973), and in the early eighties induction therapy based on anthracyclins (daunorubicin, idarubicin among others) and the nucleocide analogue cytosine arabinoside (Ara-C) was found to yield CR rates of up to 80% in newly diagnosed patients (Cunningham *et al.*, 1989; Sanz *et al.*, 1988). However, the patients frequently suffered from one of the inherent drawbacks with induction therapy, namely the release of coagulation factors from dead leukemic cells, causing severe bleedings and increased risk of fatal outcome (Cordonnier *et al.*, 1985; Drapkin *et al.*, 1978; Ruggero *et al.*, 1977). Consequently, most APL patients required intensive platelet and fibrinogen support, and based on the criterion of 5-years disease-free survival (DFS), only 35-45% of the cases were cured (Fenaux *et al.*, 2007). The focus on APL therapy changed in 1978, as it became clear that leukemic cells undergo terminal differentiation upon treatment with differentiating-inducing agents, such as ATRA, Ara-C and 13-cis retinoic acid (Breitman *et al.*, 1981; Degos *et al.*, 1985; Gold *et al.*, 1983; Koeffler *et al.*, 1985; Sachs, 1978). Such differentiation therapy showed an advantage over induction therapy, with respect to incidences of severe bleedings, and led to reduced mortality rates. In 1985, the first attempt to treat APL patients with ATRA was made with promising results, but the percentage of patients with 5-years DFS was still relatively low (less than 50%) (Huang *et al.*, 1987; Huang *et al.*, 1988). Subsequently, optimization trials, combining ATRA with chemotherapy, raised the CR rates up to 90-95% and the 5-years DFS to 86% (Wang & Chen, 2008). In addition, the combination of ATRA and chemotherapy, which currently represents standard frontline APL therapy, helped reducing retinoic acid syndrome (RAS), a potentially fatal side effect caused by induction therapy and manifested in a burst of inflammatory cytokines released from malignant promyelocytes (de Botton *et al.*, 2003; Fenaux *et al.*, 1999; Sanz *et al.*, 1999; Tallman *et al.*, 1997).

2.2 The discovery of ATO-based APL therapy

Arsenic, in the form of arsenic trioxide (ATO), was first described as an agent that possesses antileukemic properties in the year 1878. In this study, Fowler's solution, a solution of ATO in potassium bicarbonate, was shown to dramatically reduce the number of white blood cells in a patient with chronic myelogenous leukemia (CML) (Cutler & Bradford, 1878). Subsequently, this remedy was used as a primary antileukemic agent until the discovery of radiation therapy in the early 20th century (Forkner & Scott, 1931; Kwong & Todd, 1997). In the 1970s, ATO reappeared as a therapeutic agent for APL as Chinese researchers showed that ailing-1, a mixture of ATO and crude herbal extracts, was effective in the treatment of both *de novo* as well as relapsed cases (Shen *et al.*, 1997; Sun *et al.*, 1992; Zhang *et al.*, 1996). Additional clinical studies showed that ATO, as a single agent, caused complete remission in up to 90% of patients and reduced the relapse rate for high risk patients (Niu *et al.*, 1999; Shen *et al.*, 1997). A research group in the United States confirmed these preliminary studies and further showed that ATO treatment induced partial differentiation of leukemic cells, caspase activation and subsequently apoptosis (Soignet *et al.*, 1998).

2.3 Present and future APL therapy

Currently, ATRA in combination with chemotherapy is being employed as frontline therapy for APL, whereas ATO primarily is being used for treatment of cases that are resistant to ATRA or patients suffering from frequent relapses. However, several clinical trials are now assessing the synergistic effect of combining ATRA and ATO with and without chemotherapy. These trials are conducted mainly on the basis of successful studies in animal models, showing a positive effect of ATRA/ATO combinations in APL mice (Jing *et al.*, 2001; Lallemand-Breitenbach *et al.*, 1999). The main conclusion so far from the ongoing clinical studies is that newly diagnosed patients are likely to benefit from ATRA/ATO combination treatment in addition to low-dose chemotherapy (Estey *et al.*, 2006; Hu *et al.*, 2009; Shen *et al.*, 2004; Wang *et al.*, 2004).

3. The mechanism of PML, RARA and PML/RARA

3.1 The role of PML/RARA in APL pathogenesis

The molecular hallmark of APL is the t(15;17) chromosomal translocation that expresses the fusion oncoprotein PML/RARA. While this genetic aberration is identified in more than 97% of all APL cases, the remaining patients diagnosed with this disease harbor variant translocations that all involve the *RARA* gene in fusion with alternative partners such as the genes encoding promyelocytic leukemia zinc finger (*PLZF*) (Chen *et al.*, 1993), nucleophosmin (*NPM*) (Redner *et al.*, 1996), nuclear matrix associated (*NUMA*) (Wells *et al.*, 1997), or signal transducer and activator of transcription 5b (*STAT5B*) (Arnould *et al.*, 1999). The most compelling evidence that PML/RARA alone can contribute directly to APL development comes from studies in mice showing that expression of this oncoprotein as a transgene leads to development of an APL–like disease. However, these experiments also show that a relatively long latency period is required prior to onset of disease, suggesting the involvement of acquired genetic aberrations in addition to the t(15;17) translocation (Brown *et al.*, 1997; Grisolano *et al.*, 1997).

3.2 The function of PML

The first component of the PML/RARA fusion, the PML protein, is a tumor suppressor (Bernardi *et al.*, 2006; Salomoni & Pandolfi, 2002; Trotman *et al.*, 2006) that functions in multiple cellular processes, including apoptosis (Wang *et al.*, 1998), differentiation (Ito *et al.*, 2008), DNA repair (Bøe *et al.*, 2006; Dellaire *et al.*, 2006a), senescence (Ferbeyre *et al.*, 2000; Pearson *et al.*, 2000), angiogenesis (Bernardi *et al.*, 2006) and virus defence (Everett & Maul, 1994). The human *PML* gene is located on chromosome 15, consists of nine exons and produces several alternatively spliced protein isoforms designated PML I through VII. All of these PML variants contain an identical tripartite (TRIM) motif in their N-terminal region, and a C-terminus that varies due to alternative splicing (Borden, 2002; Fagioli *et al.*, 1992; Jensen *et al.*, 2001; Jul-Larsen *et al.*, 2010; Reymond *et al.*, 2001). The TRIM motif, which comprises a RING finger, two B-boxes and a predicted coiled coil domain, has been shown to be important for PML multimerization, a feature responsible for one of the most striking properties of this protein, namely the ability to generate nuclear structures termed PML nuclear bodies (PML NBs) (Lallemand-Breitenbach & de The, 2010). These bodies are highly dynamic and change their morphology and biochemical composition in a cell cycle-dependent manner. For example, during entry into mitosis, several PML NB resident components, including Daxx, Sp100 and SUMO, are lost concomitant with formation of PML NB aggregates called mitotic assemblies of PML proteins (MAPPs), whereas transition from mitosis to G1-phase coincides with exclusion of PML NBs from the progeny nuclei and complex formation with nucleoporins and microtubule filaments to form cytoplasmic assemblies of PML and nucleoporins (CyPNs) (Chen *et al.*, 2008; Dellaire *et al.*, 2006b; Jul-Larsen *et al.*, 2009). Although, PML NBs have the capacity to recruit a large number of different protein components, PML is the only protein so far that has been shown to be required for their formation. For this reason, it is widely assumed that the ability to assemble these cellular compartments represents an integral part of PML biogenesis. It still remains, however, to clearly define the molecular mechanism involved in PML NB assembly and function.

3.3 The function of RARA

The second fusion partner, RARA, is a ligand binding transcription factor that contains a DNA binding motif in its central region and a retinoid binding domain at the C-terminus. To generate an active protein complex, this nuclear receptor forms a heterodimer with the RXR family of transcription factors. Upon direct binding to a RA responsive element (RARE) within the regulatory region of a target gene, RARA/RXR complexes promote transcriptional silencing by recruiting co-repressor proteins such as NCOR1, SMRT and histone deacetylase to the promoter-binding complexes. In the presence of physiological concentrations of ligand (i.e. retinoids), a conformational change occurs within the RARA/RXR heterodimer that leads to dissociation of co-repressors and concomitant recruitment of histone acetylases and components of the basic transcription machinery, thus transforming the protein complex from a gene silencer to a gene activator (Bastien & Rochette-Egly, 2004). RARA regulates several genes involved in myeloid progenitor cell differentiation, including c-myc (Bentley & Groudine, 1986; Gowda *et al.*, 1986), C/EBPβ (Duprez *et al.*, 2003), C/EBPε (Park *et al.*, 1999) and PU.1 (Mueller *et al.*, 2006), suggesting an important role of this protein in blood cell maturation.

3.4 The function of PML/RARA

Upon fusion between PML and RARA, the variable C-terminus of the PML protein is lost, whereas the constant N-terminal TRIM motif generally remains intact. In the case of RARA, fusion to PML leads to loss of the first 50 to 60 N-terminal amino acids, a deletion that does not appear to affect the DNA and ligand binding activities of this protein (de Thé *et al.*, 1991). Thus, PML/RARA retains the powerful protein-protein interaction domain of the PML protein, whereas the variable isoform-specific region is replaced by the trans-activating functions of RARA (Fig. 1.).

One of the gained PML/RARA functions that is thought to contribute largely to APL development is the ability of this chimeric protein to form stable transcription repression complexes that are irresponsive to physiological concentrations of retinoids. As a consequence, gene promoters that are targeted by PML/RARA become constitutively repressed, an observation that has led to the general assumption that this oncoprotein causes a block in blood cell differentiation through transcriptional inhibition of key genes

involved in hematopoietic maturation. Consistent with a role in gene repression, PML/RARA has also been shown to recruit the histone methyl transferase SUV39H1 (Carbone *et al.*, 2006), members of the polycomb repressive complex 2 (PRC2) (Villa *et al.*, 2007) and DNA methyltransferases (DNMTs) (Di Croce *et al.*, 2002), proteins that are known to induce a repressive chromatin structure. In addition to increased repressor activity, the PML/RARA fusion also appears to possess a considerable expanded repertoire of target genes compared to the normal RARA protein. This notion is supported by *in vitro* binding studies showing that PML/RARA has a broader and more relaxed DNA binding specificity compared to RARA (Hauksdottir & Privalsky, 2001; Kamashev *et al.*, 2004), and by a genome wide screen revealing a wide range of PML/RARA target genes (Hoemme *et al.*, 2008). The altered DNA binding and transcription repression properties of PML/RARA are partially due to the ability of this chimeric protein to form homodimeres through protein-protein interactions mediated by the TRIM motif of PML (Jansen *et al.*, 1995; Perez *et al.*, 1993). In addition, this chimeric protein has also been shown to form functional complexes with other transcription factors such as RXR and Daxx, a feature that may further contribute to the expanded promoter binding capacity (Zeisig *et al.*, 2007; Zhu *et al.*, 2005; Zhu *et al.*, 2007).
PML/RARA is also thought to contribute to malignant transformation and development of APL through inhibition of PML tumor suppressor functions. A dominant negative effect of PML/RARA on this protein is evident by studies demonstrating disruption of nuclear PML bodies into a dispersed microspeckled pattern in cells expressing this oncoprotein (Dyck *et al.*, 1994; Koken *et al.*, 1994; Weis *et al.*, 1994). Interestingly, while disruption of PML NBs by PML/RARA in the nucleus is evident, this oncoprotein readily assembles into MAPPS and CyPNs, the mitotic and cytoplasmic versions of PML NBs, respectively (Jul-Larsen *et al.*, 2009). The disruption of PML NBs in the nucleus may reflect the role of this oncoprotein in repression of gene activity.

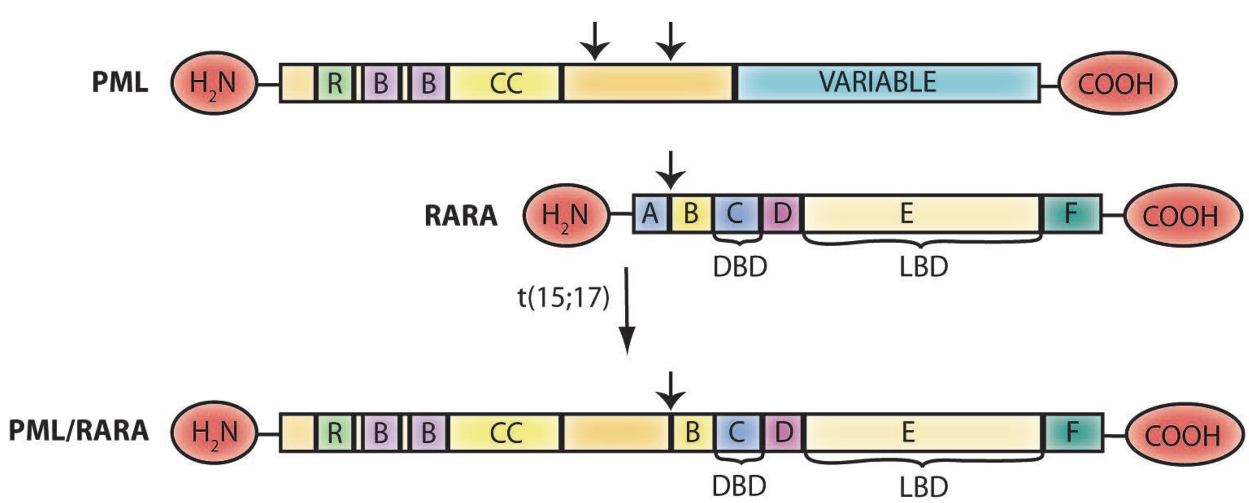

Fig. 1. Structural organization of PML, RARA and PML/RARA. PML contains a RING domain (R), two B boxes (B), a coiled coil (CC) and a variable C-terminus. RARA consists of six regulatory domains (A-F), of which domain C and E harbor the DNA binding domain (DBD) and the ligand binding domain (LBD), respectively. The t (15;17) translocation produces PML/RARA, which retains the N-terminal PML motifs as well as RARA DNA and ligand binding activity. Arrows indicate protein breakpoints.

While PML/RARA is constantly expressed in more than 97% of all APL patients, the reciprocal fusion protein RARA/PML, which contains the N-terminus of RARA and variable lengths of the PML C-terminus, is identified in only 70-80% of the cases (Alcalay *et al.*, 1992; Grimwade *et al.*, 1996). Not much is known about the role of this protein in the pathogenesis of APL. However, one study has described a possible link between RARA/PML fusion gene deletions and resistance to ATRA-based therapy (Subramaniyam *et al.*, 2006).

4. The mechanism of ATRA and ATO-mediated APL therapy

4.1 The mechanism of ATRA-based APL therapy

Phenotypically, pharmacological concentrations of ATRA lead to effective differentiation of immature APL cells to terminally differentiated granulocytes. From a therapeutic point of view this may be beneficial since the immature malignant cells progress from being highly proliferative and long-lived to arrested and short-lived. In addition, *in vitro* cell culture experiments have shown that ATRA-induced differentiation also coincides with activation of apoptosis (Altucci *et al.*, 2001; Grignani *et al.*, 1998; Martin *et al.*, 1990). The relative contribution of apoptotic cell death versus increased turnover of mature granulocytes to ATRA-induced clearance of tumorigenic cells is not clear. Although ATRA appears to be highly effective in clearing the bulk of proliferative tumor cells, a residual population of cells with detectable t(15;17) translocation almost invariably persist following treatment with this reagent alone, a feature that probably explains the additional need for chemotherapy in order to achieve complete remission (Chen *et al.*, 1991; Chomienne *et al.*, 1990; Huang *et al.*, 1988; Zhu *et al.*, 1995).

At the molecular level, therapeutic doses of ATRA reverse the differentiation block caused by PML/RARA through a direct interaction with the ligand binding site present on the RARA moiety. As for normal RARA, the ligand-receptor interaction induces a change in the PML/RARA protein structural conformation, which leads to release of transcription repressors and subsequent activation of the basal transcription machinery. Coincident with transcription activation, ATRA also induces recruitment of the proteasome to the ligand binding transcription activation domain AF2 of RARA, and subsequent proteasome-dependent degradation (Kopf *et al.*, 2000; Zhu *et al.*, 1999). A protein that has been proposed to participate in this pathway is the ubiquitin-activating enzyme E1-like (UBE1L) protein, which itself represents one of the ATRA-induced proteins (Kitareewan *et al.*, 2002). ATRA-mediated degradation appears to affect RARA and PML/RARA equally well and may be functionally linked to transcription activation, since mutations in RARA that impairs its DNA binding activity also inhibits ATRA-mediated catabolism (Zhu *et al.*, 1999). The relative contribution of transcriptional activation, differentiation and degradation on therapy remains to be fully elucidated.

4.2 The mechanism of ATO-based APL therapy

Compared to ATRA, ATO has a more limited ability to induce terminal differentiation of APL cells. *In vitro* studies using cultured cells have revealed a dose-dependent effect of this drug on differentiation and apoptosis (Chen *et al.*, 1997). At high concentrations (0.5-2.0 μM) ATO induced cell death by apoptosis, while at low concentrations (0.1-0.25 μM) this drug caused partial differentiation of APL cells along the granulocyte linage (Cai *et al.*, 2000; Chen *et al.*, 1997). The results from these experiments appear to be in good agreement with studies demonstrating ATO-induced partial differentiation and apoptosis in APL patients or animal models, where the effective serum concentrations of ATO generally ranges from 0.1 to 1.0 μM (Chen *et al.*, 1997; Lallemand-Breitenbach *et al.*, 1999). Interestingly, ATO-mediated differentiation has been shown to become dramatically enhanced in the presence of cyclic adenosine monophosphate (cAMP). The mechanism responsible for this synergistic effect was proposed to be the combined effect of ATO-induced PML/RARA degradation and cAMP-mediated inhibition of cell cycle progression (Guillemin *et al.*, 2002; Zhu *et al.*, 2002).

At the molecular level, ATO exerts its therapeutic effect on APL in part by initiating a cascade of biochemical alterations that primarily affect the PML moiety of PML/RARA. Firstly, the presence of arsenic in the cell culture medium has been shown to increase PML and PML/RARA multimerization, an effect that is manifested by decreased solubility of these proteins upon preparation of cell lysates and reduced mobility within PML NBs as determined by analysis of GFP-tagged PML in living cells (Jeanne *et al.*, 2010; Zhang *et al.*, 2010). Concomitant with increased aggregation, PML becomes extensively SUMOylated on at least three different lysine residues. All of the three different SUMO isoforms, including SUMO1, 2 and 3, appear to participate in this reaction, and both mono and poly-SUMOylation events have been reported (Lallemand-Breitenbach *et al.*, 2001; Lallemand-Breitenbach *et al.*, 2008; Muller *et al.*, 1998; Tatham *et al.*, 2008). Subsequent to SUMOylation, a protein called RNF4 binds SUMOylated residues on PML in order to catalyze poly-ubiquitination, a modification that directs PML and PML/RARA to the proteasome for degradation (Lallemand-Breitenbach *et al.*, 2008; Tatham *et al.*, 2008). Recently, a direct interaction between PML and ATO, that potentially triggers this SUMO-mediated degradation pathway, was mapped to cysteine residues located in the TRIM and B-box motifs of PML (Jeanne *et al.*, 2010; Zhang *et al.*, 2010).

In addition to affecting differentiation of leukemic cells, recent studies have also implicated ATO in clearance of leukemic-initiating cells (LICs), a small population of malignantly transformed cells with stem cell characteristics that frequently are refractory to cancer therapeutic drugs. Consistent with this, PML/RARA expression has been reported to support properties of self-renewal of LICs (Wojiski *et al.*, 2009), and certain characteristics of promyelocytic phenotypes provide the basic properties for the development of APL-initiating LICs (Guibal *et al.*, 2009). Furthermore, a recent study demonstrated LIC clearance in association with ATO-induced PML/RARA degradation by a mechanism that appeared to be uncoupled from the observed cell differentiation (Nasr *et al.*, 2008; Shao *et al.*, 1998). In addition, ATO has been reported to cause increased proliferation of LICs in a chronic myelogenous mouse model, hence sensitizing otherwise therapy-insensitive leukemic cells to Ara-C-based treatment (Ito *et al.*, 2008; Ito *et al.*, 2009).

The proapoptotic activity of ATO is not specific for APL cells (Akao *et al.*, 1998; Bachleitner-Hofmann *et al.*, 2001; Ishitsuka *et al.*, 1998; Perkins *et al.*, 2000; Rousselot *et al.*, 1999; Wang *et al.*, 1996; Zhang *et al.*, 1998; Zheng *et al.*, 1999), although non-APL tumor cells have been shown to be less sensitive to this drug (Huang *et al.*, 1999). ATO induces apoptosis by downregulation of the antiapoptotic protein Bcl-2, leading to a disturbance in the regulated balance between pro- and antiapoptotic proteins (Akao *et al.*, 1998; Chen *et al.*, 1996; Zhang *et al.*, 1998). In addition, ATO increases radioactive oxygen species (ROS) production in malignant cells. As a consequence, this drug leads to disruption of the mitochondrial membrane potential, followed by cytochrome c release, caspase activation and subsequent apoptotic cell death (Jing *et al.*, 1999).

4.3 The synergy between ATRA and ATO

While ATRA and ATO on their own are known to be effective in curing APL, it is also becoming increasingly clear that treatment regiments based on a combination of the two drugs leads to a quicker clinical remission, a more effective clearance of leukemic cells and a significantly longer period of relapse free survival (Estey *et al.*, 2006; Hu *et al.*, 2009; Shen *et al.*, 2004; Wang *et al.*, 2004). This synergistic effect may result due to the ability of both these drugs to cause PML/RARA degradation, a parameter that appears to be critical for the success of APL therapy. In addition, the combined effect of ATO and ATRA may also result due to the ability of the two agents to act on separate targets, both of which are important for disease remission. For example, ATO may be effective in eradicating self-renewable LICs through stimulated PML/RARA degradation, while ATRA represents a more effective differentiating agent, and hence may lead to a more complete clearance of undifferentiated APL cells.

5. Therapy-induced degradation of PML/RARA

ATRA and ATO-induced therapy of APL may be connected to the ability of these drugs to induce PML/RARA catabolism (Fig. 2.). In agreement with this, reduced PML/RARA expression can be observed in both ATRA and ATO-treated cells, and the two drugs synergize both for their ability to induce oncoprotein degradation as well as for their capacity to promote clinical remission (Hu *et al.*, 2009; Nasr *et al.*, 2008; Shen *et al.*, 2004). An important role of protein degradation for effective APL therapy is also supported by experiments in mice. For example, treatment of an APL mouse model with the proteasome inhibitor bortezomid led to reduced degradation of PML/RARA and concomitant resistance to ATRA and ATO-based therapy (Nasr *et al.*, 2008). In addition, PML/RARA mutated in critical SUMOylation target sites, were found to be more resistant to ATO-mediated degradation compared to unmodified PML/RARA (Lallemand-Breitenbach *et al.*, 2001; Lallemand-Breitenbach *et al.*, 2008).

In addition to proteasome-dependent degradation induced by ATRA and ATO, PML/RARA has also been shown to be amenable for degradation by the lysosome-dependent degradation pathway autophagy (Isakson *et al.*, 2010; Klionsky, 2007). This degradation mechanism appears to play a major role both for basal turnover as well as for therapy-induced catabolism of PML/RARA. Indeed, pharmacological inhibitors of autophagy were found to completely prevent ATRA and ATO-stimulated degradation of PML/RARA expressed in the APL cell line NB4 (Isakson *et al.*, 2010). In contrast to proteasome-dependent degradation, autophagy-mediated proteolyses of PML/RARA appears to be independent of a direct interaction between the drugs and the target protein. Instead, ATRA and ATO seem to stimulate autophagy in APL cells primarily through a mechanism that involves the mammalian target of rapamycin (mTOR) and Unc-51-like kinase 1 (ULK1) (Bøe & Simonsen, 2010; Isakson *et al.*, 2010). Furthermore, PML/RARA is highly aggregation prone and therefore a good substrate for this degradation pathway (Isakson *et al.*, 2010; Lallemand-Breitenbach *et al.*, 2001). Aggregates of PML/RARA may form during the process of protein synthesis. In agreement with this, synthesis of PML/RARA has been shown to be associated with endoplasmatic reticulum stress, a feature indicative of aberrant folding during protein synthesis (Khan *et al.*, 2004).

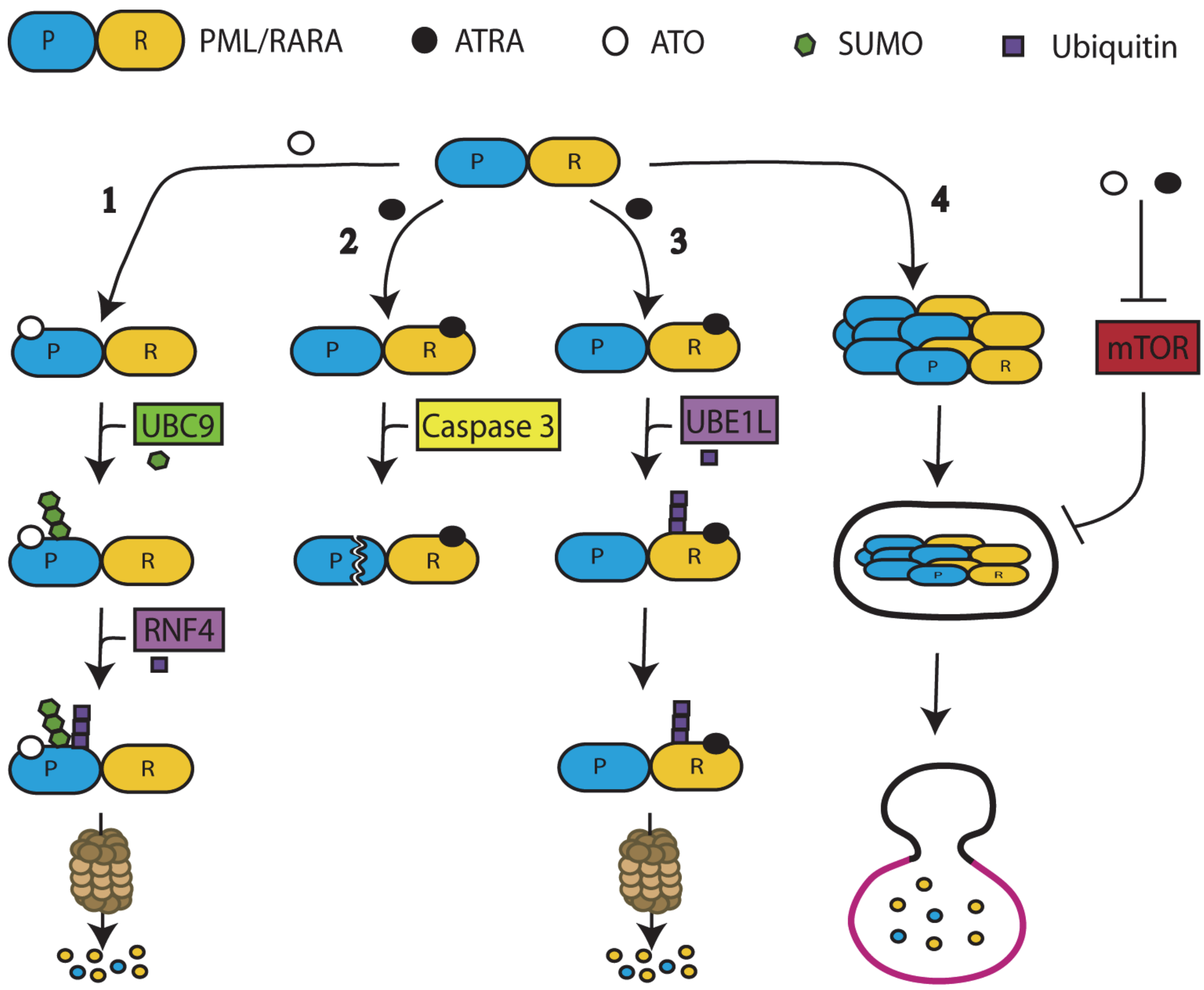

Fig. 2. Schematic overview of the four main ATRA and ATO-mediated PML/RARA degradation pathways: 1. ATO-induced proteasome-dependent degradation, 2. ATRA-induced caspase cleavage, 3. ATRA-induced proteasome-dependent degradation, 4. ATRA/ATO-induced autophagy-mediated degradation.

Two different types of proteases have also been implicated in PML/RARA proteolysis. First, PML/RARA has been shown to be susceptible to a caspase 3-like activity expressed in APL cells and that becomes induced by the presence of ATRA (Nervi *et al.*, 1998). The second protease shown to be involved is neutrophil elastase, a myeloid specific serine protease that is maximally expressed in promyelocytes (Lane & Ley, 2003). The contribution of this protease to APL development is unclear since one study showed enhanced penetrance of PML/RARA in a neutrophil elastase defective mice (Lane & Ley, 2003), while another demonstrated decreased tumorigenesis in a mouse model expressing a neutrophil elastase cleavage defective PML/RARA protein (Uy *et al.*, 2010).

PML turnover has also been shown to be regulated by a pathway that involves direct phosphorylation by the casein kinase 2 (CK2) and subsequent ubiquitin-mediated degradation, a mechanism that was proposed to cause decreased PML tumor suppressor activity in lung cancer (Scaglioni *et al.*, 2006). However, the significance of CK2-mediated PML phosphorylation in PML/RARA degradation and APL pathogenesis has not been elucidated.

6. The mechanism of APL therapy resistance

The second most common translocation associated with APL, the t(11;17) translocation that expresses PLZF/RARA fusion instead of PML/RARA, is generally insensitive to ATRA and ATO-based therapy (Chen *et al.*, 1993; Licht *et al.*, 1995). The poor response of these patients to ATO add support to studies showing that this drug primarily target PML, which is absent in PLZF/RARA. In the case of the poor response to ATRA, on the other hand, the underlying mechanism has been hypothesized to be due to enhanced co-repressor activity conferred by the PLZF moiety of the PLZF-RARA fusion (Grignani *et al.*, 1998; He *et al.*, 1998; Lin *et al.*, 1998). However, the notion that PLZF/RARA is irresponsive to ATRA stimulation has been contradicted in more recent studies demonstrating ATRA-induced gene expression and differentiation also in PLZF/RARA expressing APL cells (Nasr *et al.*, 2008; Petti *et al.*, 2002; Rice *et al.*, 2009). Thus, further work is needed in order to fully understand the mechanism underlying the insensitivity of PLZF/RARA positive APL cells to ATRA.

Resistance to ATRA-mediated therapy is also seen in APL patients that have relapsed following the first clinical remission. Such acquired resistance may be caused by a number of different physiological factors, including increased catabolism, reduced cellular uptake, or increased cytoplasmic sequestration of the therapeutic drugs (Freemantle *et al.*, 2003; Gallagher, 2002). In addition, *in vitro* cell culture experiments, using the APL cell line NB4, have revealed mutations within the PML/RARA gene of subclones with acquired resistance to ATRA. Interestingly, several of these mutations were found clustered at/or near the ligand binding domain of RARA leading to defects in ATRA binding. Since these mutants generally retain their capacity to form complex with RXR and to bind DNA, they have been suggested to act as dominant inhibitors of wild type RARA (Duprez *et al.*, 2000; Kitamura *et al.*, 1997; Nason-Burchenal *et al.*, 1998; Rosenauer *et al.*, 1996; Shao *et al.*, 1997). Mutations in PML/RARA have also been identified in a subset of ATRA-relapsed patients, and these mutations were found to be variably associated with inactivation of ATRA binding (Ding *et al.*, 1998; Gallagher *et al.*, 2006; Imaizumi *et al.*, 1998; Marasca *et al.*, 1999; Takayama *et al.*, 2001; Zhou *et al.*, 2002). Interestingly, one study identified mutations within the intact PML locus of APL patients with ATRA-resistance and poor prognosis (Gurrieri *et al.*, 2004).

Recently, PML/RARA mutations have also been discovered in two APL cases with poor response to ATO (Goto *et al.*, 2011). In both cases, the mutations were located within the second B-box motif of the PML protein. Since the amino acids affected by these mutations were close to a cysteine-rich region, previously proposed to bind ATO (Jeanne *et al.*, 2010), the authors of this paper hypothesized that these mutations may affect interactions between this drug and PML/RARA. Alternatively, the mutated protein may have defects in oligomerization, since the B-box domains are known to function in PML multimerization. Combined, the PML/RARA mutations that have been identified in ATRA and/or ATO–resistant APL cells support the notion that these drugs interact with separate moieties of the fusion protein to induce clinical remission.

7. Perspectives

During the past 30 years, APL has progressed from a deadly disease to a highly curable malignancy. In addition, the advances that have been made in understanding the pathology and cure of APL at the molecular level have led to the emergence of a highly attractive model disease for the development of targeted cancer therapy. For example, the case of APL clearly demonstrates the therapeutic effectiveness of targeting a defined oncoprotein, and since recurrent translocations and expression of fusion oncoproteins similar to that of PML/RARA is a common trait also among other types of cancers (including leukemias and sarcomas), a large number of malignancies, in addition to APL, may benefit from similar targeted therapies. Thus, it will be important to continue identifying therapeutic concepts that contribute to the success of APL therapy and to modulate these concepts for treatment of other cancers.

Since both ATRA and ATO have been shown to exert their therapeutic effects through interactions with specific regions of the PML/RARA oncoprotein, it may be assumed that these drugs will be effective only against APL. However, one should also keep in mind that the ability of ATRA and ATO to mediate cure of APL is regarded as a rather fortuitous discovery and not merely as a result of rational therapeutic design. For this reason, these drugs are likely to have other yet unidentified cellular targets, beside the APL-associated fusion portion, that are important for effective treatment. Evidence for this comes from one of the studies mentioned above showing that both ATRA and ATO-stimulated autophagic degradation of PML/RARA through a mTOR-dependent pathway that does not seem to involve direct interactions between drugs and the oncoprotein (Isakson *et al.*, 2010). In addition, it is also becoming increasingly clear that ATO has the potential to cure a subset of cancers that don't express PML/RARA. For example, induced clearance of LICs has been demonstrated both in PML/RARA positive as well as PML/RARA negative leukemic cells (Ito *et al.*, 2008; Nasr *et al.*, 2008). Furthermore, a phase II clinical study was recently published that showed promising results of using ATO in combination with interferon alpha and zidovudine for treatment of patients with chronic adult T cell leukemia (Kchour *et al.*, 2009), and finally, this drug was found to sensitize glucocorticoid-resistant acute lymphoblastic leukemia cells to dexamethasone (Bornhauser *et al.*, 2007). Thus, it is likely that APL for many years to come will continue to represent an important model disease for targeted and non-targeted effects of ATRA and ATO, while increased understanding of the molecular pathways involved may lead to discoveries of new therapies that are applicable for other types of cancers.

8. Acknowledgements

The work in our laboratory is funded by the Research Council of Norway, The Norwegian Cancer society and South-Eastern Norway Regional Health Authority.

9. References

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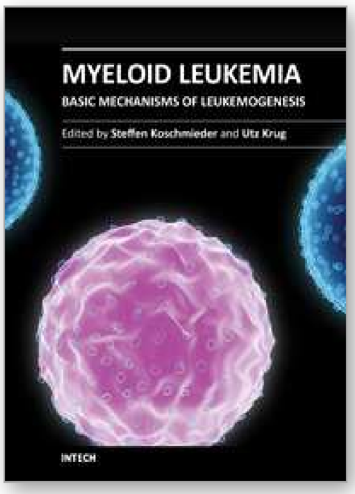

Myeloid Leukemia - Basic Mechanisms of Leukemogenesis

Edited by Dr Steffen Koschmieder

ISBN 978-953-307-789-5
Hard cover, 484 pages
Publisher InTech
Published online 14, December, 2011
Published in print edition December, 2011

The current book comprises a series of chapters from experts in the field of myeloid cell biology and myeloid leukemia pathogenesis. It is meant to provide reviews about current knowledge in the area of basic science of acute (AML) and chronic myeloid leukemia (CML) as well as original publications covering specific aspects of these important diseases. Covering the specifics of leukemia biology and pathogenesis by authors from different parts of the World, including America, Europe, Africa, and Asia, this book provides a colorful view on research activities in this field around the globe.