Published in final edited form as:
Exp Cell Res. 2014 May 15; 324(1): 1–12. doi:10.1016/j.yexcr.2014.03.018.

Retinoid receptor signaling and autophagy in acute promyelocytic leukemia

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Abstract

Retinoids are a family of signaling molecules derived from Vitamin A with well established roles in cellular differentiation. Physiologically active retinoids mediate transcriptional effects on cells through interactions with retinoic acid (RARs) and retinoid-X (RXR) receptors. Chromosomal translocations involving the *RARα* gene, which lead to impaired retinoid signaling, are implicated in acute promyelocytic leukemia (APL). All-*trans*-retinoic acid (ATRA), alone and in combination with arsenic trioxide (ATO), restores differentiation in APL cells and promotes degradation of the abnormal oncogenic fusion protein through several proteolytic mechanisms. RARα fusion-protein elimination is emerging as critical to obtaining sustained remission and long-term cure in APL. Autophagy is a degradative cellular pathway involved in protein turnover. Both ATRA and ATO also induce autophagy in APL cells. Enhancing autophagy may therefore be of therapeutic benefit in resistant APL and could broaden the application of differentiation therapy to other cancers. Here we discuss retinoid signaling in hematopoiesis, leukemogenesis, and APL treatment. We highlight autophagy as a potential important regulator in anti-leukemic strategies.

Keywords

AML; APL; Arsenic Trioxide; ATRA; Autophagy; Differentiation; Hematopoiesis; PML-RARα; Retinoid

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Disclosures: No competing financial interests exist.

Introduction

Retinoids are an important class of signaling molecules related to Vitamin A (retinol). Mammals lack the biosynthetic machinery to synthesize retinoids, therefore the primary sources of vitamin A are plant-derived carotenoids and animal food sources in the form of retinyl esters. Over the last two decades the molecular basis of the diverse roles of retinoids in the regulation of cellular differentiation and metabolism has emerged [1]. Furthermore, retinoids remain the backbone of therapy for acute promyelocytic leumekia (APL) and hold promise for the prevention and treatment of some solid malignancies [2–4] (Figure 1). In this review we will summarize recent advances in understanding retinoid signaling pathways and how defects in retinoid signaling are implicated in leukemogenesis. We will highlight mechanistic links between retinoid signaling and the cellular process of autophagy and explore how these links may be exploited in future therapies for APL.

Retinoid uptake, metabolism and transcriptional roles

The intestinal absorption and metabolism of food-derived retinoids has been reviewed in detail recently [5]. Briefly, dietary retinoids in the form of unesterified retinol derived from β-carotene or retinyl esters from animal sources are transported in the blood by the serum retinol binding protein (RBP4). Interestingly, RBP4 has emerged as a potential regulator of insulin sensitivity, providing a direct mechanistic link between dietary retinoids and metabolic regulation [5,6,7]. In the eye, RBP4-bound retinol is delivered to target cells via the cell membrane-associated ‘stimulated by retinoic acid 6’ (STRA6) protein. However STRA6 is not required for vitamin A homeostasis in all tissues, but plays complex signaling roles in diverse tissue types[89,10]. Retinol can be esterified by lecithin-retinol acyl transferase (LRAT) for storage [11,12]. Retinyl esters are mobilized from storage by hydrolases to yield retinol, which is converted via retinaldehyde to the active physiological form, all-*trans*-retinoic acid (ATRA), by retinaldehyde dehyrogenases. The transcriptional actions of retinoids are mediated by the retinoic acid (RARs) and rexinoid (RXRs) receptors. There are three isoforms of the RAR and RXR subtypes, each encoded by a unique gene: RARα(NR1B1), RARβ(NR1B2), RARγ(NR1B3) and RXRα(NR2B1), RXRβ(NR2B2), RXRγ (NR2B3). The RARs and RXRs are members of the nuclear receptor family of ligand dependent transcription factors [13,14] which preferentially interact with specific retinoic acid response elements, RAREs, in the promoter and/or enhancer regions of retinoid regulated genes [15]. Whereas ATRA is the predominant physiological agonist for RARs [16–18], there is evidence that 9-*cis*-RA is an RXR agonist [19,20] (Figure 1). While there is evidence that both RARs and RXRs can form multimeric complexes with diverse nuclear receptor partners [21,22], the major transcriptional effects of retinoids are mediated by RXR-RAR heterodimers [23]. In the absence of agonist, the RXR-RAR heterodimer functions to repress transcription by recruiting histone deacetylase (HDAC) complexes via the NCoR (NCoR1) and SMRT (NCoR2) corepressors [24–28]. Conversely, in the presence of agonist, the RXR-RAR complex recruits multiple, enzymatically diverse transcriptional coregulators [29], including histone lysine acetyl transferases [30] and the mediator complex [31], which cooperate in the transcriptional activation of retinoid target genes (Figure 3). In the last decade, agonist induced repressors of RAR function have been identified. For example, PRAME (Preferentially Expressed Antigen in Melanoma) is recruited to ATRA activated RARs and

prevents the recruitment of the transcriptional activation complex [32]. Recruitment of RIP140-HDAC and polycomb complexes to RARs attenuates ATRA induced transcription [33–35]. Thus in normal cells, retinoid receptor complexes transition through cycles of transcriptional activation and repression.

Retinoid Signaling in Hematopoiesis

The importance of retinoid signaling in the regulation of embryonic development and stem cell differentiation is well established [36–39]. A role for retinoids in the regulation of hematopoiesis has also emerged. Hematopoiesis is dependent upon rare multipotent cells termed hematopoietic stem cells (HSCs), which reside within a specialized bone marrow stem cell niche. HSCs possess the ability to both self-renew and, through a series of hierarchical events, produce terminally differentiated progeny [40,41]. Early epidemiologic observations that vitamin A deficient (VAD) human populations suffered impaired blood cell production prompted further investigation into the role of retinoid signaling in this process [42]. More recent mechanistic insights have revealed important roles for retinoids in HSC homeostasis [43,44] and in myeloid [45,] and lymphoid [46, 47] lineage development.

As hematopoiesis is required throughout life, the retention and maintenance of optimal HSC function is essential. HSCs express high levels of the retinaldehyde dehydrogenase 1 (ALDH1a1) enzyme, indicating a potentially important role for retinoid signaling in these cells [48]. Interestingly, the nuclear receptors RARα and RARγ appear to have opposing effects on HSC fate, as observed in knockout and over-expression studies by Purton and colleagues [43,44]. RARγ signaling enhances self-renewal, whereas RARα signaling promotes HSC proliferation and differentiation, with a decreased repopulation capacity [43,44].

Exogenous retinoid treatment of myeloid cells in culture leads to changes in the gene expression of known myeloid associated transcription factors, some of which harbor RAREs in their promoter regions, such as CCAAT enhancer binding protein ε (*CEBPε*), and others which likely represent secondary response genes, such as *PU.1* [49,50]. However, mice with specific homozygous knockouts of either the RARα or RARγ nuclear receptors fail to show a robust hematopoietic defect. While a combined $RAR\alpha^{-/-}$ $RAR\gamma^{-/-}$ genotype is embryonically lethal, extracted fetal liver cells contain a predominant granulocyte population similar to that of wild type mice [51,52]. This suggests that the granulopoietic effects of retinoid signaling are likely to be more complex than those predicted from a linear ligand-receptor model. Possible receptor co-operation/compensation and extra-nuclear retinoid-mediated events remain to be fully elucidated. Animals subjected to a vitamin A deficient (VAD) diet from conception display impaired embryonic erythropoiesis with reduced GATA binding protein 2 (GATA-2) transcript levels [45]. Mature cellular retinol-binding protein type I (CRBP1)-deficient mice have low endogenous vitamin A stores and when further subjected to a VAD diet, develop severe vitamin A (retinol) deficiency. This is associated with an abnormal expansion of neutrophils in the blood and extra-medullary tissues, with a high proportion of immature granulocytes, indicating a role for endogenous retinoids in terminal myeloid differentiation [51]. As we will discuss further, this finding is definitively supported by the myeloid differentiation block observed in RARα-fusion leukemias.

Retinoids and leukemia

Acute Myeloid Leukemia (AML) is a type of cancer characterized by the malignant proliferation of immature white blood cell precursors in the bone marrow and peripheral blood [53]. The discovery of cytogenetic, molecular and epigenetic aberrations in AML patients has underscored the complex heterogeneity of this disease and has generated classification systems now used for prognostication [54,55]. A common phenotypic abnormality in all cases of AML is a differentiation block in which malignant hematopoietic precursors are unable to differentiate into functional granulocytes. Overcoming this differentiation block pharmacologically is the basis of differentiation therapy [56].

Acute promyelocytic leukemia (APL) represents approximately 10% of all cases of AML. It is distinguished by a potentially lethal coagulation defect at presentation, characteristic cellular morphology, and chromosomal translocations that lead to oncogenic fusions of the RARα locus on chromosome 17q21 [57–59]. Since APL associated translocations were first identified in 1990, nine unique translocations have been identified, each involving the same genomic location within the second intron of the *RARα* gene (Figure 2) [57,58,60–67]. Thus, all APL associated gene fusions contain the DNA and ligand binding domains (LBD) of the RARα protein.

Current understanding of the mechanisms underlying gene translocations in leukemogenesis remains incomplete. However, gene fusions have also been identified in solid tumors, most notably associated with prostate cancer [68]. Interestingly, the induction of *Tmprss2* fusions in prostate cancer is dependent on androgen receptor (AR) induced intra- and inter-chromosomal interactions and inaccurate double strand DNA break repair [69–71]. The AR is a nuclear receptor structurally and functionally related to the RARs and RXRs. Therefore, the insights obtained in prostate cancer may have some relevance to the induction of gene translocations involving the *RARα* locus in APL. In this context it is interesting to note that genomewide chromatin immunoprecipitation assay data [72] indicates RARα protein recruitment proximal to the common breakpoint within the *RARα* locus that is characteristic of APL (Figure 2B). Future studies should address a potential role for retinoid induced transcriptional events in the formation of chromosomal abnormalities involving the *RARα* locus.

RARα-fusion genes all share some functional characteristics, including the ability to homodimerize and antagonize retinoid signaling in a dominant-negative manner through associating with transcriptional repressor complexes [73,74]. The most common RARα fusion partner, involved in over 95% of cases, is the promyelocytic leukemia (PML) gene [75]. The resultant PML-RARα fusion protein binds to RAREs on DNA with high affinity and recruits repressive complexes that block the transcription of RARα target genes necessary for myeloid differentiation [76]. PML-RARα binding at target promoters also recruits histone-deacetylases (HDACs), histone lysine methyltransferases (KMTs), and DNA methyltransferases (DNMTs) that together result in repressive conformational chromatin changes, further blocking gene transcription [77,78] (Figure 3).

Retinoids in Treatment of Leukemia

Following pre-clinical observations in the early 1980s that ATRA promoted the granulocytic differentiation of promyelocytic leukemia cells *in vitro*, the first clinical trial of ATRA in the treatment of APL was conducted [79,80]. Twenty-four patients (16 newly-diagnosed and 8 treatment-refractory) received treatment with single-agent ATRA, 23 of whom achieved a complete hematologic remission (CR) [81]. Subsequent trials confirmed the efficacy of ATRA in remission induction and maintenance when compared with standard AML chemotherapy regimens. ATRA has been used in combination with optimized chemotherapy for APL treatment since 1990, transforming the prognosis of this aggressive condition, with CR rates of up to 95%, good second remission rates and 5 year disease-free survival (DFS) rates of up to 74% [82,83]. However, the use of ATRA in non-APL AML has shown conflicting therapeutic benefit [84–87] and further clinical trials of retinoid combination therapies are underway (Table 1, Figure 1).

The specificity of ATRA for APL lies in its effect on the RARα-fusion oncoprotein. Pharmacologic doses of ATRA induce a conformational change in PML-RARα, resulting in the dissociation of HDACs and DNMTs [73]. This allows the recruitment of co-activator complexes and restoration of retinoid target gene transcription, including the transcription of those genes involved in granulocytic differentiation [88] (Figure 3). ATRA also induces the degradation of the PML-RARα oncoprotein itself through several non-overlapping, co-operating proteolytic mechanisms (Figure 4). Upon ATRA treatment, caspase-3 targets a cleavage site within the α-helix of PML, leaving an intact RARα moiety of the PML-RARα protein [89]. ATRA activates the ubiquitin/proteasome system (UPS) through the phosphorylation of serine-873 on PML-RARα [90] and also via the binding of SUG-1 to the RARα transactivation domain [91]. Both caspase inhibitors and proteasomal inhibitors interfere with PML-RARα degradation [92]. Most recently, the cellular process of autophagy has been proposed as another important pathway in the catabolism of the PML-RARα oncoprotein. Ablain and colleagues have reported that synthetic retinoids capable of re-activating transcription in PML-RARα positive leukemic cells but with no effects on PML-RAR protein levels, successfully induce differentiation but fail to eliminate the leukemia-initiating activity of transplanted clones *in vivo* [93]. This establishes two distinct mechanisms of therapeutic action exerted by ATRA (Figure 4) and emphasizes the importance of oncoprotein proteolysis in achieving long-term cure. This is the subject of a recent comprehensive review by Dos Santos and colleagues [94].

The majority of patients receiving ATRA monotherapy will eventually relapse. This may result from incomplete clearance of leukemia initiating cells (LICs) residing in the bone marrow or from the proliferation of ligand binding domain (LBD)-mutated leukemic clones naturally selected during ATRA induction therapy [88,94]. A proportion of PML-RARα fusion APL patients are clinically resistant even to initial ATRA therapy. This may result from genetic mutations in the LBD of the RARα moiety that induce conformational changes in the protein, interfering with co-repressor release and reducing the affinity of ATRA binding [95]. Increased intracellular catabolism of ATRA, leading to decreased nuclear bioavailabilty, is another possible mechanism of acquired resistance of particular concern in patients treated with ATRA over a long duration [96]. Despite retaining functional RARα

LBDs, certain X-RARα APL subtypes - particularly PLZF-RARα and STAT5b-RARα fusions, also display a clinical ATRA resistance [88]. One proposed explanation for this is that the NCoR/SMRT co-repressor complex interacts with very high affinity such that even pharmacologic doses of ATRA cannot induce transcriptional de-repression [97].

Arsenic Trioxide (ATO) was first reported to have an anti-leukemic effect on APL cells in 1996 [98]. It has since proved to be a valuable first line therapy for the condition, attaining single-agent cure rates of 70% and >90% when administered in combination with ATRA [99,100]. A recently published clinical trial reported non-inferior outcomes with an ATRA/ATO regimen compared to the now conventional ATRA/anthracycline-based regimen in low and intermediate risk disease, facilitating the potential unprecedented treatment of an acute leukemia without toxic chemotherapy [101]. Improved outcomes have also been observed with the inclusion of ATO in the treatment of relapsed or resistant disease, and ATO now forms the backbone of salvage regimens [83]. Phenotypically ATO induces partial differentiation of APL cells at low doses and apoptotic cell death at higher concentrations [102]. ATO promotes degradation of the PML-RARα fusion oncoprotein with the effect of depleting LICs, resulting in long-term disease remission [90] (Figure 4). Interestingly, ATO potently induces autophagy in cultured leukemic cells and both pharmacological and genetic autophagy inhibition reverses the anti-leukemic effect of ATO [103].

Autophagy

Autophagy is a ubiquitous cellular pathway involved in protein turnover [104]. Cytoplasmic material tagged for autophagic degradation is sequestered within double-membrane vesicles known as ‘autophagosomes’. These vesicles then traffic to and fuse with lysosomes, where their contents are degraded by resident hydrolases to yield reusable monomers [105] (Figure 4). Initial studies in the yeast *Saccharomyces cerevisiae* identified a family of ‘AuTophaGy-related’ (ATG) proteins that form the core molecular machinery of autophagy [106]. These proteins are hierarchically recruited to regulate each step in the autophagic process: autophagosome initiation, elongation, maturation, docking, fusion and degradation. The mechanisms underpinning autophagy have been reviewed excellently elsewhere [107,108].

The serine/threonine kinase mammalian target of rapmaycin (mTOR) serves as a cytoplasmic master negative regulator of autophagy, phosphorylating and inactivating proteins of the ULK1-ULK2-ATG13-FIP200 complex, an essential component in autophagy induction [107,109]. mTOR integrates signals from multiple cellular signaling and nutrient-sensing pathways and activates autophagy in times of cellular stress or growth factor deprivation. Several mTOR inhibitors have been developed and are in clinical use, with the proven effect of autophagy induction (e.g. rapamycin, everolimus, temsirolimus) [107,110]. Autophagy can also be induced in an mTOR-independent manner via (i) the direct activation of ULK1 by adenosine monophosphate-activated protein kinase (AMPK) and (ii) the lowering of cytosolic myo-inositol 1,4,5-triphosphate $^{(IP3)}$ levels – as is observed with pharmacologic lithium treatment [111]. The basic helix-loop-helix (bHLH) leucine zipper transcription factor EB (TFEB) has recently been identified as a transcriptional regulator of autophagy, entering the nucleus upon autophagy initiation to activate the transcription of at

least 17 autophagy-related genes [112]. Other transcription factors known to activate autophagy gene expression include NF-κB, E2F1, HIF-1α, and FOXO3 [107,113,114].

Mammalian cell differentiation often involves structural remodeling and requires tight control of protein turnover. Autophagy is observed in murine oocytes within 4 hours of fertilization, with deletion of the essential autophagy gene *ATG5* resulting in embryonic lethality prior to implantation[115]. Autophagy has also been observed in human embryonic stem cells (hESCs) induced to differentiate in culture [116]. As recently reviewed by Guan and colleagues, autophagy is important in maintaining hematopoietic stem cell (HSC) quiescence and is highly active during hematoopoietic cell differentiation [41]. GATA-1, a master regulator of hematopoiesis, directly activates the transcription of the *ATG8* gene family [117]. The elimination of mitochondria in developing reticulocytes is mediated by mitophagy, with severe functional red cell defects resulting due to loss of the autophagy genes ULK1, ATG7 or BNIPL3 [118]. Specific roles for autophagy have also been identified in lymphocyte, monocyte-macrophage, and plasma cell differentiation [119,120].

Defective autophagy has been linked to tumour development in several cancer models. *Beclin1* is often mono-allelically deleted in solid tumour malignancies [121]. Tumours with constitutive activation of the PI3K-AKT signaling pathway display decreased autophagy [122]. FIP200, a member of the ULK1-ULK2-ATG13-FIP200 autophagy initiation complex (Figure 4), is required for the maintenance of fetal HSCs, with conditional knockout in murine study leading to severe anemia and perinatal lethality [123]. Further *in vivo* study has shown that the conditional knockdown of ATG7 in murine HSCs results in a severe myeloproliferative syndrome with dysplastic features and early death [124]. Autophagy interacts at multiple levels with apoptotic signaling pathways and may itself contribute to programmed cell death in cancer cells [125]. Thus, promoting autophagy represents a novel avenue of cancer therapeutics for both solid and hematologic malignancies.

Autophagy in APL cell differentiation

Human myeloid leukemic cell lines and human primary monocytes treated with ATRA in culture display increased levels of autophagy [126–128]. Studies on the NB4 human PML-RARα positive APL cell line have shown that this ATRA-induced autophagy is necessary for the successful granulocytic differentiation of malignant cells [127–129]. Autophagy has specifically been linked to the breakdown of the PML-RARα oncoprotein [127,128]. The molecular mechanisms through which ATRA induces autophagy are poorly understood and will hopefully be explored in future studies. Isakson and colleagues proposed that the activation of autophagy is mTOR-dependent and showed that mTOR inhibition with rapamycin increases autophagy and promotes PML-RARα degradation [128]. As a transcriptional regulator, ATRA may also promote autophagy at a nuclear level through the activation of transcription factors known to up-regulate the autophagic process [130]. ATRA may also have a biologic role in autophagosome maturation through the redistribution of a cation-dependent mannose-6-phosphate receptor (CIMPR) to the developing autophagosome, leading to vesicle acidification. This effect is thought to be independent of nuclear hormone receptors and instead to be mediated by direct binding of ATRA to CIMPR [131]. A recent study indicates that ATO induces autophagy via the induction of

MEK/ERK signaling independently of the mTOR autophagy-signaling axis [103]. A similar role for autophagy in oncoprotein elimination has been shown in BCR-ABL fusion chronic myeloid leukemia (CML), where imatinib has the dual effects of inhibiting the tyrosine kinase activity of BCR-ABL and inducing its autophagic degradation [132]. It is worth noting that autophagy was not shown to have a role in the turnover of the AML1-ETO fusion oncoprotein, instead enhancing the survival of malignant cells in culture [133]. Thus it is possible that autophagy is involved in the breakdown of large, aggregate forming oncogenic fusion proteins such as PML-RARα and BCR-ABL. Further research will clarify this point.

Conclusions

Chromosomal translocations in hematopoietic cells involving the *RARα* gene lead to impaired retinoid signaling and a differentiation block, causing APL. In nine documented *RARα* fusion variants, the translocation within the *RARα* gene occurs at the same genomic location, proximal to RARα binding in NB4 cells (Figure 2). While the potential role for retinoid nuclear receptors in the initiation of chromosomal translocations involving the *RARα* gene remains to be confirmed, such a mechanistic link has been identified between AR signaling and chromosomal translocations in prostate cancer [69–71].

While pharmacologic doses of ATRA can restore retinoid-induced transcription and myeloid differentiation in APL, the elimination of RARα oncogenic fusion proteins is critical for sustained remissions and long-term cure [93]. This proteolysis is likely to be one of the integral mechanisms through which ATRA and ATO exert their clinically proven anti-leukemic efficacy in APL [101] (Figure 4). Autophagy is the predominant cellular pathway utilized in the disposal of large aggregate-prone proteins and is likely to be critical for RARα fusion protein degradation. Both ATRA and ATO induce autophagy through mechanisms which have not been fully elucidated. Pharmacologic induction or enhancement of autophagy in combination with established therapies ATRA and ATO may provide a means of overcoming treatment resistance in APL, either through improved oncoprotein elimination or through driving the cellular remodeling necessary for cellular differentiation. This may also hold promise to broaden the application of differentiation therapy to non-APL fusion leukemias.

Acknowledgments

We thank the members of the Gudas Laboratory, Weill Cornell Medical College, NY and Cork Cancer Research Centre, University College Cork, Ireland for critical discussion. We are grateful to Dr. Michelle Nyhan for help in editing. Dr. Nina Orfali is funded by the Haematology Education and Research Trust (H.E.R.O), Cork, Ireland with unrestricted educational supporting bursaries from Pfizer, Merck Sharp & Dohme, Bristol Myers Squibb, Novartis and Amgen. We gratefully acknowledge the support of the University of Nottingham, Weill Cornell, and NIHRO1CA43796 to LJG.

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Highlights

- Normal and aberrant retinoid signaling in hematopoiesis and leukemia is reviewed
- We suggest a novel role for RARα in the development of *X-RARα* gene fusions in APL
- ATRA therapy in APL activates transcription and promotes onco-protein degradation
- Autophagy may be involved in both onco-protein degradation and differentiation
- Pharmacologic autophagy induction may potentiate ATRA's therapeutic effects

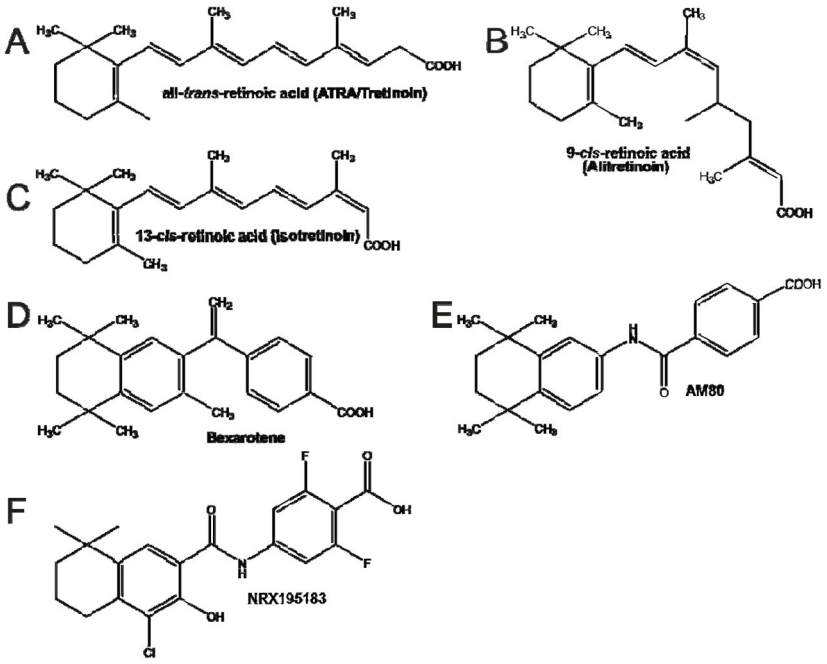

Figure 1. Physiological and clinically relevant retinoids

Chemical structures for retinoids are provided for comparison. (A) all-*trans*-retinoic acid (ATRA/Tretinoin), B. 9-*cis*-retinoic acid (Alitretinoin), C. 13-*cis*-retinoic acid (Isotretinoin), D. bexarotene (Targretin™), E. AM80 (Tamibarotene) and F. NRX195183.

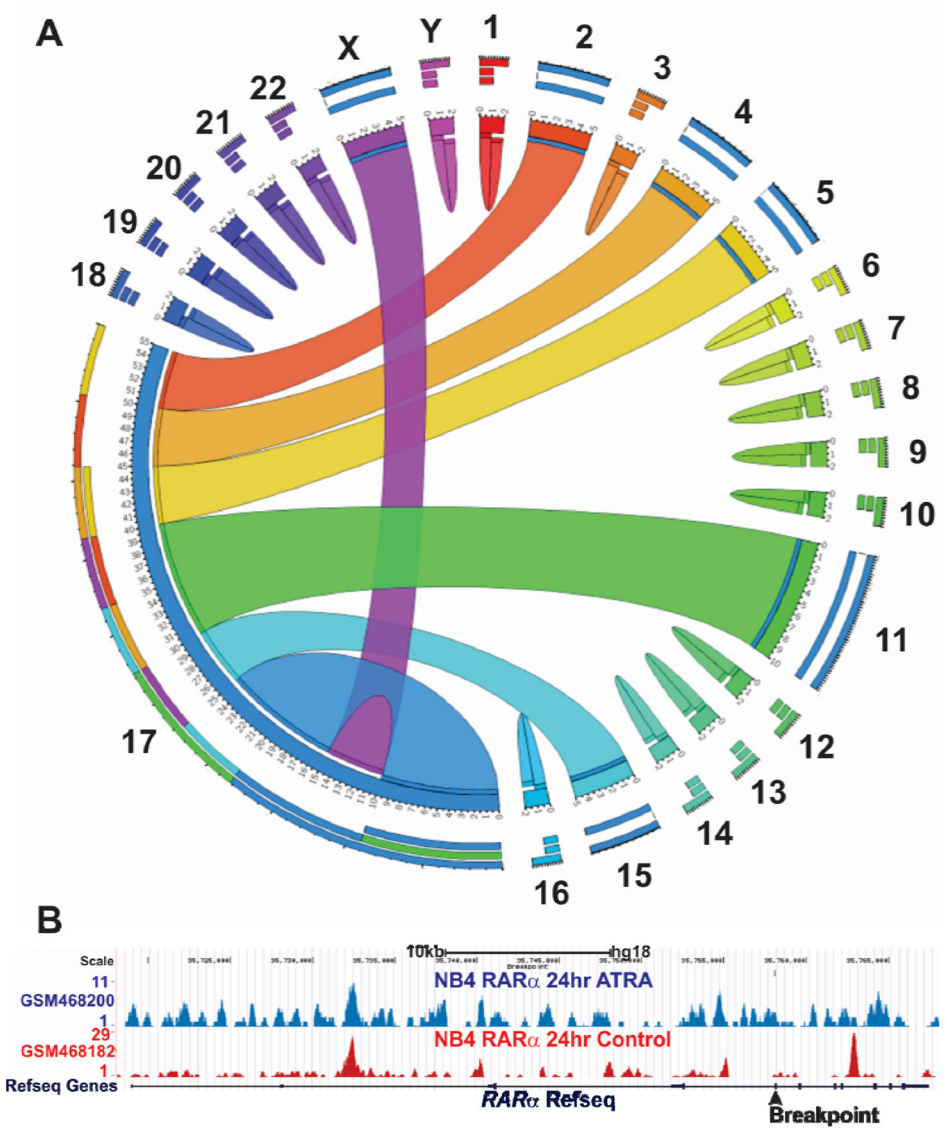

Figure 2. RARα fusion proteins associated with acute promyelocytic leukemia (APL)

A. Circos (www.circos.ca) diagram depicting chromosomal origins of loci implicated in APL associated gene translocations. The *RARα* locus (chromosom me 17) is implicated in translocations involving the *PML*(Chr15), *PLZF/ZBTB16*(Chr11), *NuMA*(Chr11), *NPM*(Chr5), *STAT5b*(Chr17), *PRKAR1A*(Chr17), *FIP1L1*(Chr4), *BCOR*(ChrX) and *OBFC2A/NABP1*(Chr2). **B.** Chromatin immuno-precipitation coupled with next generation sequencing (ChIPseq) has been conducted to analyze the genomewide distribution of RARα in the absence (GSM468182) and presence of ATRA (GSM468200) in NB4 APL cells [72]. Peaks indicate genomic regions where chromatin is associated with RARα binding. ATRA increases RARα association with the *RARα* locus (blue peaks). The chromosomal location of the common breakpoint present in all RARα fusion genes is indicated (▲).

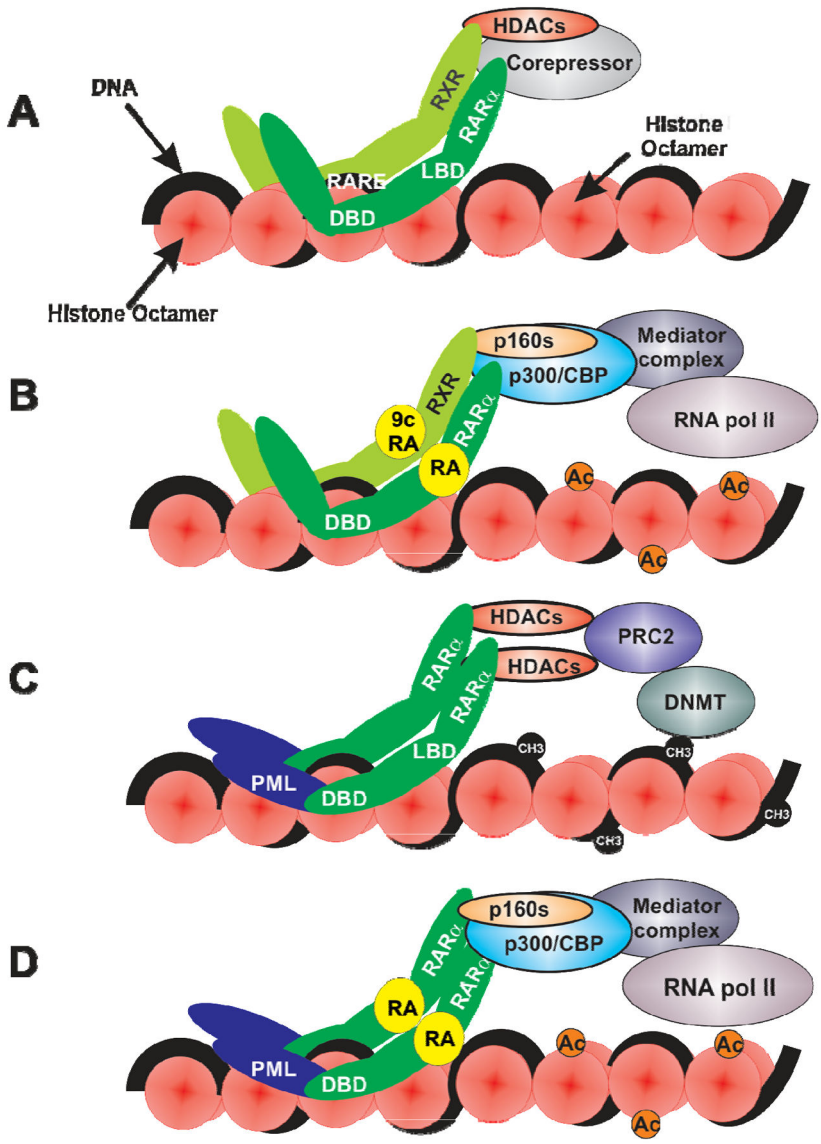

Figure 3.

A. RXR-RAR heterodimers bind to retinoic acid response elements (RAREs) via their DNA binding domains (DBD). In the absence of all-*trans*-retinoic acid (ATRA), RXR-RARs are believed to recruit corepressors (SMRT/NCoR) and histone deacetylases (HDACs) which function to temporarily repress transcription. B. In the presence of ATRA and 9-cis-retinoic acid, HDAC-corepressor complexes dissociate and enabling the recruitment of the p160, p300/CBP and mediator coactivators which increase histone acetylation (Ac) which in turn facilitate the transcriptional actions of RNA polymerase II. C. The PML-RARα fusion protein forms homodimers resulting in a stochiometric increase in HDAC-corepressor recruitment to RAREs which can facilitate the association of DNA methyltransferases resulting in the epigenetic silencing of retinoid response genes. D. Therapeutic ATRA concentrations are believed to promote differentiation in part via the restoration of retinoid target genes transcription.

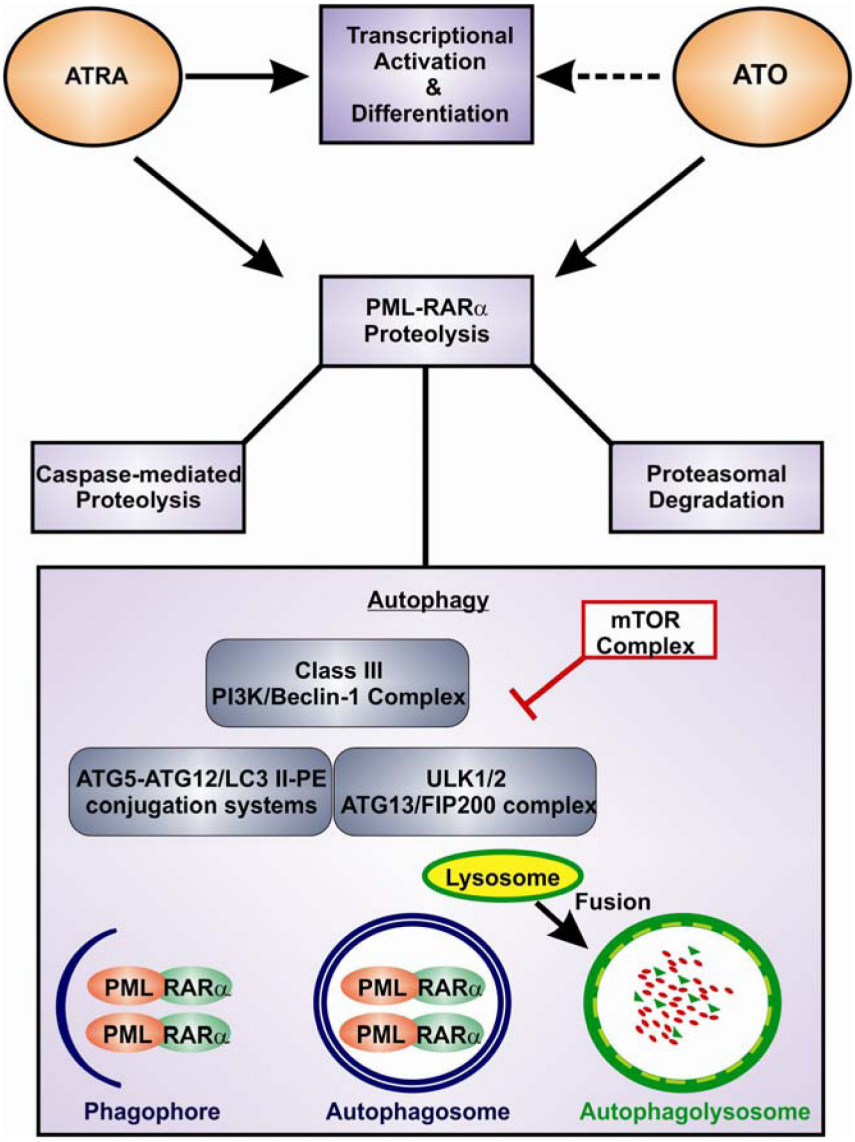

Figure 4. Therapeutic mechanisms of ATRA and Arsenic Trioxide (ATO) in PML-RARα positive acute promyelocytic leukemia (APL)

Therapeutic concentrations of ATRA de-repress transcription and lead to the myeloid differentiation of APL cells with a transient hematologic remission of the disease. ATO also encourages partial differentiation. Both agents have a distinct action of promoting PML-RARα proteolysis through co-operating mechanisms, caspase-mediated degradation, proteasomal degradation and autophagy. Autophagy is a catabolic process capable of digesting large proteins aggregates, using the lysosomal machinery. Upon autophagy activation, the ULK1/2 kinase complex recruits the Class III PI3K/Beclin-1 complex and series of conjugation reactions involving the complex's ATG5, ATG7, ATG10, ATG12 and ATG8/LC3 ensue. This initiates formation of a double membrane known as the 'phagophore'. As this expands it becomes an enclosed double membraned vesicle known as an 'autophagosome'. This sequesters aggregated proteins for degradation – such as PML-RARα. Lysosomal fusion with autophagosomes results in the proteolysis of their contents.

mTOR is a negative regulator of autophagy as it phosphorylates and suppresses the ULK1/2 kinase complex. AMPK (not shown) is an activator of the ULK kinase complex.

Table 1

ATRA combination therapies reported in ClinicalTrials.gov (January 2014).

Grants.gov ID	AML Subtype	Drug Combination	Phase
NCT00136461	AML, MDS	**ATRA** and Bryostatin 1	II
NCT00892190	Refractory AML	Dasatinib, **ATRA**	I
NCT00867672	AML	Decitabine, VPA, **ATRA**	II
NCT01575691	AML, MDS	5-aza-cytidine, VPA, **ATRA**	1
NCT00151255	AML	**ATRA,** Cytarabine, Idarubicin Mitoxantrone, Etoposide	III
NCT00151242	AML	Cytarabine, Idarubicin, Etoposide, **ATRA,** Pegfilgrastim	II/III
NCT00326170	AML	5-Azacytidine, VPA, **ATRA**	II
NCT01237808	AML (NPM1 mutation)	Cytarabine, **ATRA,** Etoposide	III
NCT00143975	Refractory AML	Cytarabine, Mitoxantrone, Gemtuzumab-Ozogamicin, **ATRA**	II
NCT00175812	AML	Theophyllin, **ATRA,** VPA	I/II
NCT00339196	AML, MDS	**ATRA,** VPA	II
NCT00049582	AML, CML, MDS	Decitabine	I
NCT01161550	Refractory AML	G-CSF, Cladribine, Cytarabine, **ATRA,** Midostaurin	I
NCT00995332	AML	Cytarabine, **ATRA,** VPA	I
NCT00146120	AML	Idarubicin, Cytosin-Arabinosid, Etoposide, **ATRA**	III
NCT00893399	AML (with NPM1 mutation)	**ATRA,** Standard chemotherapy Gemtuzumab-Ozogamicin	III
NCT01369368	AML relapse after allo-transplantation	**ATRA,** 5-azacitidine, VPA, hydroxurea and eventually donor leukocyte infusions.	I/II
NCT00615784	AML	**Bexarotene**	II
NCT01020539	AML, MDS, Juvenile myelomoncytic leukemia	Fludarabine, Busulfan, Graft-versus-host disease (GVHD) Prophylaxis, Gemtuzumab Ozogamicin, Anti-Thymocyte Globulin**, Isotretinoin**	I
NCT00003405	AML	Interferon α, Amifostine trihydrate, bromodeoxyuridine, cytarabine, idarubicin, idoxuridine, **isotretinoin**, mitoxantrone hydrochloride	II
NCT00482833	AML	As_2O_3, Idarubicin, mercaptopurine, methotrexate, **ATRA**	III
NCT00217412	Refractory cancers	SAHA, **ATRA**	I
NCT00003619	AML, MDS, ALL,	Topotecan, fludarabine, cytarabine, and filgrastim followed by peripheral stem cell transplantation or **Isotretinoin**	I/II
NCT00006239	AML, MDS	Sodium phenylbutyrate, **Tretinoin**	I
NCT00866073	AML	Decitabine	II
NCT01987297	AML	**ATRA**+ As_2O_3, **ATRA**+chemo	IV
NCT00413166	APL	**ATRA**, As_2O_3, Idarubicin	II
NCT01409161	APL	**ATRA**, As_2O_3, Gemtuzumab ozogamicin, Methylprednisolone	I
NCT00903422	AML, MDS	Eltrombopag olamine, platelet transfusions, mild chemo, cytokines, VPA, **ATRA**, ESAs or G-CSF	I
NCT00528450	APL	As_2O_3, Idarubicin, **Tretinoin**	II
NCT00002701	APL	Busulfan, cyclophosphamide, cytarabine, etoposide, idarubicin, mercaptopurine, methotrexate, mitoxantrone hydrochloride, thioguanine, **tretinoin**, allogeneic/ autologous bone marrow transplantation, radiation therapy	III
NCT00465933	APL	**ATRA,** Idarubicin	IV
NCT00675870	APL	NRX 195183 synthetic retinoid	II

Grants.gov ID	AML Subtype	Drug Combination	Phase
NCT00180128	APL	**ATRA,** idarubicin, mitoxantrone, daunorubicin, cytarabine	IV
NCT01404949	APL	**Tretinoin,** As_2O_3,	II
NCT01226303	APL	**ATRA,** Idarubicin	III
NCT00520208	APL (relapsed/refractory)	**Tamibarotene (AM80)**	II
NCT00985530	APL	**Tamibarotene**, As_2O_3	I
NCT00504764	APL (relapsed)	As_2O_3, **ATRA**, Autologous/Allogenic transplantation, As_2O_3	IV
NCT00196768	APL (relapsed)		IV