Vitamin A and its Derivatives- Retinoic Acid and Retinoid Pharmacology

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Abstract

Retinol (Vitamin A) and its derivative retinoic acid (RA) are essential in the control of epithelial cell growth and cellular differentiation. Retinoid is indispensable in vision and RA inhibits the growth of some malignant cells. RA has also striking effects on pattern formation in developing and regenerating limbs, and also a potent morphogen in chick limb bud. Retinoic acid (RA) proved therapeutic benefits in cancer prevention, in skin diseases and in acute promyelocytic leukemia (APL). The elucidation of the molecular basis of vitamin A and its retinoid pharmacology emerged as paradigm for the connection between RA and its structure of RA receptors [RAR], oncogenic pml/RARa as constitutive transcriptional repressor or that block myeloid differentiation at promyelocytic phenotype, and the molecular model of retinoic acid action in a specific APL. A molecular model is further revised. As an approach to APL treatment, one possible action of retinoic acid (RA), A consensus sequence (TCAGGTCA motif) has been postulated for thyroid hormone (TRE) and retinoic acid responsive element (RARE)-containing in the promoter region of target genes. High dose of RA-RARE-PML/RARa complexes intracellular localization appears to relieve repressors from DNA-bound receptor, including the dissociation of corepressor complexes N-CoR, SMRT and HDACs from PML-RARa or partially PML-RARa/RXR. Also release PML/RARa mediated transcription repression. This transcriptional derepression occurs at RARa target gene promoter. Consequentially, PML-RARa chimera converted receptor from a repressor to a RA-dependent activator of transcription. The resulting pml-RARA oncoprotein proteolytic degradation occurs through the autophagy-lysosome pathway and the ubiquitin SUMO-proteasome system (UPS) as well as caspase 3, or lysosomal protease (cathepsin D) enzyme or/and E1-like ubiquitin-activating enzyme(UBEIL) induction. Accordingly the expression level of PML-RARA downregulated. PML protein relocates into the wild-type nuclear body (PML-NB) configuration or a truncated PML-RARa fusion fragment detected or/and the wild-type RAR upregulated. An effect is to relieve the blockade of pml/RARA-mediated RA dependent promyelocytic differentiation and retinoic acid (9-cis RA, ATRA, Am80) in APL therapy (See figure by Zhu G, January 1991, revised in 2012). Here, RA can overcome the transcriptional repressor activity of pml/RARA The oncogenic pml/RARA uncover a pathogenic role in leukemogenesis of APL through blocking promyelocytic differentiation. This oncogenic receptor derivative pml/RARA chimera is locked in their “off” regular mode thereby constitutively repressing transcription of target genes or key enzymes (such as AP-1, PTEN, DAPK2, UP1, p21WAF/CCKN1A) that are critical for differentiation of hematopoietic cells. This is first described in eukaryotes.

Keywords: Vitamin A; Retinoic acid and retinoid pharmacology; Gene transcription; Molecular model of RA

Introduction

In vivo, the fat soluble Vitamin A (retinol) can be reversibly metabolised to the aldehyde (retinal) which can in turn, be further oxidised in a non-reversible manner to retinoic acid (RA). Enzymes that oxidize retinol to retinaldehyde belong to two classes: the cytosolic alcohol dehydrogenases (ADHs) belonging to the medium-chain dehydrogenases/reductase family; and microsomal short-chain dehydrogenases/reductases (retinol dehydrogenases, RDHs [1]. The next step in RA synthesis is the oxidation of retinaldehyde to RA, which is carried out by three retinaldehyde dehydrogenases (RALDHs): RALDH1, RALDH2 and RALDH3 [1,2]. The orange pigment of carrots (beta-carotene) can be represented as two connected retinyl groups, which are used in the body to contribute to vitamin A levels [3]. The physiological and biological actions of this class of substances centre on vision, embryonic development and production, cellular growth and differentiation, skin health, and maintenance of immune function.

Initial studies had focused on vitamin A deficiency and its major consequences: night blindness and Xerophthalmia. Fridericia and Holm [4] investigated the influence of dietary A in the rhodopsin of the retina. Clearly, the rats lacking the fat-soluble vitamin A had a defect in the function of visual purple. Yudkin [5] achieved one of the earliest identifications of vitamin A as a component of the retina. Subsequently, Wald [6] determined the amount of vitamin A present in pig retinas. Wald G [7,8] was well established the visual cycle: light decomposed rhodopsin to retinal and opsin. Retinal could either recombine with opsin to reform rhodopsin...
or it converted to free retinol. Retinol could reform rhodopsin, but only in the presence of the RPE (Kuhne). The further structure and metabolism of retinoids implicated that retinaldehyde was the visual pigment.

**Biochemistry of Vitamin A**

There is now a well-developed medicinal chemistry of RA (Figure 2) [9]. The group of pharmacologically used retinoids include vitamin A (all-trans retinol), tretinoin (all-trans retinoic acid), isotretinoin (13-cis retinoic acid) and altretinoin (9-cis retinoic acid). The monoaromatic retinoids include acitretin and etretinate. The third generation polyaromatic retinoids include bexarotene and tazarotene. In view of this broad spectrum of pharmacological activity, these substances provide useful to treat multifactorial dermatological disorders and other hematological disorders such as acute promyelocytic leukemias (APL) (Figure 1 & 2) [8,10-12].

**Function of Vitamin A and its Physiological Role**

**Vision**

Vitamin A is needed by the eye retina, 11-cis-retinal (a derivative of vitamin A) is bound to the protein “opsin” to form rhodopsin (visual purple) in rods cells [8], the molecule necessary for both low light (scotopic vision). As light enters the eye, the 11-cis-retinal is isomerized to all-trans retinal in photoreceptor cells of the retina. This isomerization induces a nervous signal (a type of G regulatory protein) along the optic nerve to the visual center of the brain. After separating from opsin, the all-trans-
retinal is recycled and converted back to the 11-cis-retinal form via a series of enzymatic reactions. The all-trans-retinal dissociates from opsin in a series of steps called photo-bleaching. The final stage is conversion of 11-cis-retinal to retinal, which is transported to RPE. In the retina, 11-cis retinal is converted to 11-cis retinol, then 11-cis retinol is oxidized to 11-cis-retinal via RDH5 with possible RDH11 and RDH11 [1]. This represent each RDH for the roles in the visual cycle (Figure 3).

**Embryonic Development**

More recent, vitamin A and its metabolites play a key importance in embryo morphogenesis, development and differentiation in normal tissues. Retinoic acid (RA) is lipophilic molecule that act as ligand for nuclear RA receptors (RARs), converting them from transcriptional repressor to activators [2,11,12] in RA signaling pathway. It has been demonstrated that retinoic acid was identified as a morphogen (teratogen) responsible for the determination of the orientation of the limb outgrowth in chicken [13,14] and its retinoic acid receptors (RARs) appear at early stage of human embryonic development in certain types of tissues [15]. Vitamin A play a role in the differentiation of this cerebral nerve system in Xenopus laevi. The other molecules that interact with RA are FGF-8, Cdx and Hox genes, all participating in the development of various structures within fetus. For instance, this molecule plays an important role in hindbrain development. Both too little or too much vitamin A results in the embryonic defect in the central nervous system, various abnormalities in head and neck, the heart, the limb, and the urogenital system [15]. With an accumulation of these malformations, an individual can be diagnosed with DeGeorge syndrome [2].

**Dermatology**

Vitamin A, in the retinoic acid form, plays an important role in maintaining normal skin health through differentiating keratinocytes (immature skin cells) into immature epidermal cells. In earlier studies, Frazier and Hu (1931) [16] made the observation that both hypovitaminosis A and hypervitaminosis A provokes epithelial alterations together with decreased keratinization and hair loss. At present, 13-cis retinoic acid (isotretinoin) in clinical used to acne treatment. The mechanism was shown to reducing secretion of the sebaceous glands, triggering NGAL (neutrophil gelatinase-associated lipocalin) and other gene expression and selectively inducing apoptosis [17]. But precise action of retinoid therapeutic agents in dermatological diseases are being researched.

**Hematopoiesis**

vitamin A is important for the regulation of hematopoietic stem cell dormancy [18]. Mice maintained on a vitamin A-free diet loss HSCs (hematopoietic stem cells), showing a disrupted re-entry into dormancy after exposure to inflammatory stress stimuli. This condition highlights the impact of dietary vitamin A on the regulation of cell-cycle mediated stem cell plasticity [19]. In vitro, all-trans retinoic acid (ATRA) stimulates at least two-fold the clonal growth of normal human CFU-GM and early erythroid precursor BFU-E [20]. Cis-RA stimulates clonal growth of some myeloid leukemia cells. In suspension culture, there was an increase in cell number at day 5 in the presence of RA in half of 31 samples, which suggest that RA may play a role in the proliferation and survival of certain leukemia clones in vitro [21,22].

In contrast to the enhancement of normal hematopoietic proliferation, RA (10-6 - 10-9 mol/l) is capable of inducing differentiation of the F9 mouse teratocarcinoma, HL-60 cells [23,24] and some blasts from patients with promyelocytic leukemia [25]. Maximum HL-60 differentiation (90% of cells) occurs after a 6 day exposure to 10-6mol/l retinoic acid. Further in vitro studies found that retinoic acid induced differentiation of leukemia blast cells in only 2 of 21 patients with AML, both of these patients had promyelocytic variant [24]. These data suggest that retinoids may induce maturation of promyelocytes. Retinoic acid also inhibits the proliferation of other dermatological malignant cells (Myger, 1975; Peck, 1975).

**Maintenance of Immune Homeostasis**

There is a link between retinoid and immune homeostasis. RA is crucial for maintaining homeostasis at the intestinal barrier and equilibrating immunity and tolerance. de Mendonca Oliveira LM and colleagues [25] have in detail illustrated the impact of retinoic acid on immune cells and inflammatory diseases. After the absorption and metabolism of vitamin A and its precursor (β-carotene) into RA by alcohol dehydrogenase (ADH) and retinal dehydrogenase (RALDH) in CD103+ DC cells in gut, RA plays an important role in mucosal immune response by promoting differentiation of Foxp3+ inducible regulatory T (Treg) cell and immunoglobulin(Ig) A production. In this process, RA promote dendritic cells to express CD103 and to produce RA. Vitamin A and zinc deficiency (VAD) lead to a decrease of serum IgA. Oral administration of RA in VAD mice can efficiently be reestablishing IgA production. These effects are mediated by an increase of the early B cell factor 1(EBF1) and paired box protein-5(pan-5) transcription factors, which are critical for B cell development. RA accelerates the maturation of human B cells and their differentiation into antibody-secreting plasma cells.

In addition, RA induces the homing of innate immune cells, such as innate lymphoid cells (ILCs) besides regulatory and effector T and B cells, to the gut. Among three ILCs, ILC3 depend on the transcription factor retinoic acid receptor-related orphan nuclear receptor gamma (RORγt) and secrete IL-17 and IL-22. During infections, RA can induce the production of proinflammatory cytokines by dendritic cells (DCs), promoting the generation of effector T cells and restoring the balance of Th17/Treg cells in the GALT (gut-associated lymphoid tissue), and the protection of the mucosa. Moreover, vitamin A is capable of inducing the IL-6-driven induction of proinflammatory TNF(α) 17 cells, promoting anti-inflammatory T reg cells differentiation, regulating the balance between pro- and anti-inflammatory immunity [26,27].
Retinoid Acids in MDS Treatment

The geometric isomer of the naturally occurring retinoic acid is 13-cis retinoic acid (13-CRA). Based on in vitro and in vivo antineoplastic activity, this agent has entered clinical trials for a variety of neoplasms including MDS. Retinoic acid is one of the biological inducers of differentiation that has been preliminarily tested in patients with preleukemia. Myelodysplastic syndrome (MDS) are a group of hematopoietic disorders characterized by ui- or multilineage maturation defects of the bone marrow [28]. Differentiation induction therapy is used in MDS to improve this maturation defects and induce a multilineage clinical response in a subgroup of MDS patients.13-CRA may have moderate effect on 20-30% of patients with MDS [29]. A various of combination therapy with 13-cis RA and growth factors G-CSF or erythropoietin (EPO) improve impaired cytokine secretion (IL-1beta, IL-6, IL-8) from monocytes [30]. In a prospective multicenter study, EPO-beta-ATRA [31] or EPO-13-cis RA [32] combination appears to erythroid response reaching about 36%-60% of therapeutic efficacy in anemia of low/intermediate risk MDS (LDMDS) (marrow blasts < 10% or excluding RARB). More data analysis, erythroid response maintained an independent positive impact on survival, particularly in non-RARE patients in the first 3 years from diagnosis (90% survival in EPO responders compared to 50% of non-responders) [33]. Zhu [34] successfully conducted a CR patient with refractory anemia with multilineage megaloblastic dysplasia following traditional medicine and erythropoiesis-stimulating agent vitamin B12 and folate growth factor. His peripheral parameters presented pancytopenia (hemoglobin 59g/l, red blood cell count 1.9x1012/l, leukocyte count 2.6x109/l, platelet value 11.8x109/l).

He remained well over 10 years. While another MDS had its unequivocal evidence of disease progression in response to phytohemagglutinin (PHA), inducing the generation of interleukin-2, accelerating the number recovery of CFU-S and initiating DNA synthesis of cells. She had 2.5% blast plus promyelocytes in ~70% cellular marrow before beginning PHA, and 20.7% blast plus promyelocytes in a 90% cellular marrow after ten days (total dosage 250mg) of PHA. Venditt et al [35] conduct that 23 patients with high-risk myelodysplastic syndrome (HRMDS) were treated with a 10 days course of oral ATRA (45mg/m2) and subcutaneous low-dose cytosine arabinoside (LDARAc) given at the dose of 20mg twice a day. In all cases (RAEB9, RAEB9 and CMML4) [36] bone marrow blasts infiltration was greater than 10% (12-30%). Overall, 5(23%) of 22 patients achieved complete responder and 2(9%) as partial responders. The overall median survival was 8 months (range 1-27months), whereas the median survival of responders was 16months (8-27months), the median duration of response was 11months (2-21months). It seems that the combination of ATRA and LDARA-c may be effective in approximately 30% of HRMDS patients [35].

Valproic acid (VPA) has been used as an anticovulsant for decades. VPA is a potent inhibitor of histone deacetylases (HDAC). It can modify the structure of chromatin allowing recruitment of transcription factors to restore epigenetically suppressed genes. VPA has been shown to posses antiproliferative activity and to overcome the differentiation block in leukemia blast cells [37]. Some clinical trials with VPA monotherapy or in combination with ATRA have been reported in MDS. In a piloty study of Kuendgen and colleagues [38- 40] patients with MDS or AML secondary to MDS were treated with VPA monotherapy or with ATRA later resulting in a 44% of response rate. In the follow-up study of 43 patients, an even higher response rate of 52% was observed in those low-risk MDS patients, while for the patients with excess blasts (RAEB) and CMML response rates were 6% and 0% respectively, which implicate the difference of MDS subtypes. In another trials, Siitonen et al [41] reported that according to IWG criteria,3 patients(16%) of 19 MDS responded to treatment following VPA,13-cis RA and 1,25(OH)2D3 combination. All the responses were hematological improvement. One patient responded to the treatment with an increase in platelet value from 67x109/l to 105x109/l. His peripheral blood and bone marrow blast cells decreased from 4% to 0% and from 19% to 7%, respectively. Furthermore, the disease remained stable in 11 patients but progressed in 5 during treatment. This is encouraging results.

| Number of patients | Drug protocol | Dose Schedule | Response | Median Survival | Authors |
|--------------------|---------------|---------------|----------|----------------|---------|
| 15                 | 13-cis retinoic acid | 20-120mg/m2 day | 5PR | Gold et al (1983) |
| 18                 | 13-CRA | 1-2mg/kg twice a day | 2PR | Greenberg et al (1995) |
| 10                 | 13-CRA | 2-4mg/kg | 2PR | Roberto et al (1996) |
| 24                 | 13-CRA | 100mg/day | 2CR, 3PR, 1MPR | Bessis et al (1993) |
| 66                 | 2/15-CRA alone | 1CR, 4PR, 2MPR, 2MPR, 2PR | 1CR, 4PR, 2MPR, 2MPR, 2PR | Bessis et al (1993) |
| 21/13-CRA+betaxiol | 100mg/day | 1CR, 4PR, 2MPR, 2PR | 1CR, 4PR, 2MPR, 2PR | Bessis et al (1993) |
| 13                 | 13-CRA | 100mg/day | 1CR, 4PR, 2MPR, 2PR | Bessis et al (1993) |
| 13                 | 13-CRA+LDARAc | 100mg/day | 1CR, 4PR, 2MPR, 2PR | Bessis et al (1993) |
| 13                 | 13-CRA+LDARAc | 50-100mg twice a day | 1CR, 4PR, 2MPR, 2PR | Bessis et al (1993) |
| 13                 | 13-CRA+LDARAc | 30-60mg twice a day | 1CR, 4PR, 2MPR, 2PR | Bessis et al (1993) |
| 13                 | ATRA alone | 100mg/kg | 1CR, 4PR, 2MPR, 2PR | Bessis et al (1993) |
| 13                 | ATRA+G-CSF | 100mg/kg | 1CR, 4PR, 2MPR, 2PR | Bessis et al (1993) |
| 3                  | 6-CRA alone | 6mg/kg | 1CR, 4PR, 2MPR, 2PR | Venditt et al (2002) |
| 19                 | 6-CRA alone | 6mg/kg | 1CR, 4PR, 2MPR, 2PR | Venditt et al (2002) |
| 6                 | 13-CRA |仅有1%的骨髓内皮细胞 | 1CR, 4PR, 2MPR, 2PR | Venditt et al (2002) |
| 60                 | 13-CRA +ATRA | 10mg/kg/day | 1CR, 4PR, 2MPR, 2PR | Venditt et al (2002) |
| 19                 | 13-CRA+LDARAc | 100mg/kg | 1CR, 4PR, 2MPR, 2PR | Venditt et al (2002) |

Table 1: Results of Retinoic acid therapy in MDS.
A series of these studies are summarized in (Table 1). While some patients experienced improvement in peripheral blood counts, complete responses were reported in only a small proportion of these studies [42-43]. The sole exception was a patient who presented with 29% marrow blasts and 90% abnormal metaphases with 13-cis RA. He obtained a complete clinical and cytogenetic remission therapy [44-49]. This clinical response to 13-cis RA drug was due to in vivo growth inhibition of malignant monocytoid clone [50]. Continued follow-up of this study in this field will be of interest [51-54] (Table 1).

Retinoic Acids in Skin Disease

Vitamin A is necessary for normal epithelial cell differentiation and maturation [55-57]. Retinoids influence on skin keratocyte proliferation, epidermal differentiation and keratinisation. Those retinoids including natural and chemically synthesized vitamin A derivatives are common used as systemic and topical treatment of various skin disorders. At present there have well developed three generations: the naturally occurring retinoids (all-trans retinol, Retinoin, Isotretinoin, Alitretinoin) the monoaromatic retinoid and the polyaromatic retinoid derivatives [58].

Table 2: Results of 13-cis RA in severe acne treatment.

| Number of patients | Dose of 13-cis RA | A-Dose-Response | Authors |
|--------------------|------------------|-----------------|---------|
| 10                 | 0.5 mg/kg/day    | 13/14 CR complete clearing | Peck et al (1979) |
| 10                 | 1.0 mg/kg/day    | 11/14 with 75% improvement | Farrell et al (1980) |
| 10                 | 1.5 mg/kg/day    | 10/14 complete clearing | Jones et al (1968) |
| 10                 | 2.0 mg/kg/day    | 10/14 complete clearing | Plewig et al (1969) |
| 10                 | 2.5 mg/kg/day    | 10/14 complete clearing | Rapini et al (1970) |

Table 2: Results of 13-cis RA in severe acne treatment.

Isotretinoin is an orally active retinoic acid derivative for the treatment of acne (papulo- pustular, nodulo-cystic, conglobata) [59], since it shows an excellent efficacy against severe refractory nodulocystic acne. Peck’s [60] original observation in 1978-79 of the effects of 13-cis RA in cystic acne has been well supported. In double-blind studies using small doses of 13-cis RA regimen, Farrell [61] in 15 patients, Jones [62] in 76 patients, Plewig [63] in 79 patients and Rapini [64] 150 patients reporting have confirmed this results. A summary of limited review on 365 affected persons are presented in (Table 2). The drug action involves an inhibition of sebum excretion rate (SER) in sebaceous glands and production rate of free fatty acids [60,61,65-68] through trigerring NGAL (neutrophil gelatinase-associated lipocalin) expression [17] normalise follicular keratinisation [69] and the decrease in colonisation of propionibacterium acnes and associated inflammation in skin surface microflora [70]. This response, mediated by toll-like-receptor 2 (TLR2), is increased in acne patients due to high expression of TLR2 [71] (Table 2).

Figure 4: An advanced squamous cell carcinoma of skin before(left) and after(right) isotretinoin [57].

Encouraging results have also been used 13-cis RA in small numbers of patients with rosacea, Gram-negative folliculitis, Darier’s disease, ichthyosis and pityriasis rubra pilaris [72,73]. In the treatment of rosacea, isotretinoin led to a significant reduction of erythema, papules and pustules in several studies [72,73]. During treatment of rosacea, 13-cis RA act as a potent anti-inflammatory and sebum-suppressive agent. Long-lasting remission can be reported for first patient over 12 months [72]. The use of low dose isotretinoin (0.15-0.3 mg/kg bw daily) showed high efficacy and was well tolerated. Isotretinoin is only partially effective in psoriasis, in contrast etretinate which is effective in psoriasis but ineffective in severe acne. Promising, some trials have reported with isotretinoin in patients with squamous and basal cell carcinomas [74,75] cutaneous T-cell lymphoma [56] recurrent malignant glioma [76] malignant eccrine poroma [77] and keratoacanthomas [78,79] and xeroderma pigmentosum with squamous cell carcinoma [79]. In literature, there were at least 10 CR patients with squamous cell carcinoma (SCC). Skroza et al [74]
reported a CR patient with well-differentiated SCC following the daily dosage of 0.5mg/kg/day for 5 months. Dring 1-year follow up, he remained all in normal range. Using combination chemotherapy and isotretinoin for 4 months, Zaman [80] reported a complete clinical remission of tumors in a case of 15 year old female of xeroderma pigmentosum with SCC. Another collection of four SCC of skin obtained CR through isotretinoin at daily dose of 1mg/kg/day twice a day for 4 months (Figure 4) [57]. The mechanism may involve the modification of epidermal growth factor receptor (EGFR) and certain protein kinase. At present, it has clearly known the results that amplified (50-fold EGF receptor in SCC relative to normal skin keratinocytes) or mutant EGFR is oncogenic in origin of some SCC [81]. This oncogenic receptor EGFRvIII has also been found in malignant glioma and invasive breast carcinoma [82-89]. Zhu [90] conduct a short CR using chemotherapy and topical 5% Fu of retinoic acid ointment in a 75-year old patient with SCC. She had a 8x5cm rodent ulcer in her left ear and facial area. A shrinkage of irregular and harden marginal valgus converted to flat and superficial red and scar noted after one month treatment. These findings suggest that retinoids may be effective and well-tolerated therapy for advanced epidermoid SCCs in some studies [91-95] (Figure 4).

**ATRA in patients with gastric cancer (GC)**

Recently [96], two cohorts of group presented ATRA trials on patients with GC. Jin et al presented a better benefits of gastric dysplasia with omeprazole and sucralfate and the addition of ATRA (68% vs 37%) compared to patients treated with omeprazole and sucralfate alone. Hu et al also showed that ATRA significantly prolong overall survival following the combination of conventional chemotherapy. ATRA anticancer mechanisms of action against GC cells included cell cycle blocking and differentiation initiation(p21WAF1/CIP1 induction, decreased ERK/MAPK pathway), decreased expression of HER2 oncogenic receptor in patient’s gastric mucosa, apoptosis initiation and inhibiting CSC(cancer stem cell) properties such as tumorspheres formation and patient derived xenografts(PDX) growth in mice. In GC cells, CD44+ stem/progenitor cells and a high ALDH (aldehyde dehydrogenase, R-ALDH, ALDH1A1 and ALDH1A3) activity could be considered as putative targets to inhibit tumor growth, to overcome resistance to cancer therapy and to improve GC prognosis.

**The Structure of Retinoic Acid Receptors, Molecular Basis of Retinoic Acid Action and the RAR Gene Transcription**

**RARs structure**

The retinoic acid receptors (RAR) belong to the large family of ligand responsive gene regulatory proteins that includes receptors for steroid and thyroid hormones [97]. There are three retinoic acid receptors (RAR), RARα, RARβ and RARγ which are conserved throughout vertebrates encoded by their different receptor (chr 17q21, chr 3p24 and chr 12q13) gene, respectively. The RARs contain 462 amino acids (aa) [98,99] RARB consists of 455aa [100] and RARG contains 454aa [101] respectively. The RAR is a type of nuclear receptor which act as a transcription factor that is activated by both all-trans RA and 9-cis RA. The RARs have different functions and may activate distinct target genes. The RARs is expressed in a wide variety of different hematopoietic cells [98,99] the RARβ in a variety of epithelial cells [100] and the RARγ in differentiation of squamous epithelia and human skin tissue [101,102].

All RARs contain a variable N-terminal region(A/B), a highly conserved cysteine-rich central domain(C) responsible for the DNA binding activity, and a relatively well-conserved C-terminal half(E) functionally its role in ligand binding and nuclear translocation. These three main domain are separated by a hinge region(D) [12,97,102].The central DNA binding domain (88-153aa) exhibits an array of cysteine residues compatible with the formation of two so-called zinc fingers Miller,1985. Each of them a zinc atom tetrahedrically coordinated to four cysteine and each of the hypothetical zinc finger is encoded by a separate exon of the receptor gene (Figure 5) Zinc finger 1, 88-108aa, Zinc finger 2, 124-148aa [96-103]. The N-terminal zinc finger of the DNA binding domain confers hormone responsiveness to HREs, determining target gene specificity and responsible for functional discrimination between HREs whereas the C-terminal finger contains the sugar-phosphamide backbone of the flanking sequences [103,104] (Figure 5).

**The molecular basis of retinoic acid action and the RAR gene transcription**

Retinoic acid (RA) is a lipophilic signal molecule which is able to induce acute and direct activation of the expression of specific genes supports its molecular model of action that resembles that of steroid hormones [105]. The cellular retinoic acid-binding protein (CRABP) may be involved in this transfer [9,10]. In the nucleus, RA receptors (RAR) function as a heterodimer with retinoid X receptors (RXRs) [106-109]. RAR/RXR can bind to DNA motif at RA-response elements (RAREs, also HRE) in the...
regulatory sequences of target genes in the absence of ligand, thereby interacting with multiple protein complexes that include co-repressors N-CoR [110] SMRT [111] and histone deacetylases (HDACs), and maintaining gene repression. Here, RAREs consist of a direct repeat of a core hexameric sequence 5’(A/G)G(G/T)TCA-3’ [112] or of the more relaxed 5’-(A/G)G(G/T) (G/T)(G/C)A-3’ motif, separated by 1,2,5 bp [113]. A corepressor represses expression of genes by binding to and activating a repressor transcription factor, the repressor in turn bind to target gene’s operator including RARE sequence, then blocking transcription of that gene (see corepressor-wikipedia). Transcriptional regulation thus drives from the binding of hormone-receptor complexes to RARE sites on target DNA [12,97,103]. In the presence of RA(all-trans RA, 9-cis RA),binding of the RA ligand to RAR alter the conformation of the RAR, a conformational change in the DNA-bound receptor leads to the release of co-repressor complexes associated with the RAR/RXR dimer and the recruitment of co-activator complexes.

These induce chromatin remodeling and facilitate assembly of the transcription pre-initiation complex including RNA polymerase II (Pol II) [114], TATA-binding protein (TBP) and TBP-associated factors (TAFs) [2,12,103,115,116] (Figure 6). Subsequently, transcription of target genes is initiated. This also represent ligand-dependent transcriptional activation which mediated by nuclear receptors. Like thyroid hormone receptor (THR) [117,118] retinoic acid act as ligand for RARs, converting RARa from transcriptional repressor to activators [2,12,119-122]. Numerous RAR target genes after RA induction have been identified including genes within retinoid pathway, such as RARB, Crbp1/2 (Rbp1/2), Crabp1/2 and CYP26a1. And also, several members of HOX gene family, including HOXa1, HOXb1, HOXb4 and HOXd4, and other genes Tshz1 and Cdx1 [123] the function of which has been demonstrated in vivo in the normal roles of retinoids in patterning vertebrate embryogenesis, early neurogenesis, cell growth and differentiation (Figure 6).

**Figure 6:** Retinoid receptor-dependent gene regulation [116] & (b): Gene regulation by retinoic acid signalling [2].

**Molecular Model of the Gene Regulation of Retinoic Acid Action in APL.**

Acute promyelocytic leukemia (APL) is a clonal expansion of promyelocytic precursors. Retinoic acid (RA) (initial 13-cis RA, later ATRA and tamibarotene) plus chemotherapy is currently the standard of care [124-131]. APL has a very good prognosis, with long-term survival rates up to near 70%-90% [132]. Molecular analysis has uncovered the facts that approximately 98% of APL, RARa translocates and fuses with the PML gene on chromosome 15 [133-136]. The resulting RAR chimeric genes encode pml/RARA fusion protein, which is specifically expressed in the promyelocytic lineage [20]. In addition to oncogenic receptor derivative pml/RARA [108,137-139] the translocation involves oncogenic TBL1XR1-RARB [140] and NUP98/RARG [141] and oncogenic PML-RARG [142] which share high homolog (90%) of three RAR family that were also detected in APL rare cases.

Most studies have shown in APL that oncogenic pml/RARa act as constitutive transcriptional repressor that blocks neutrophil differentiation at the promyelocyte stage. Without its ligand, retinoic acid (RA), PML-RARA functions as a constitutive transcriptional repressor of RARE-containing target genes, abnormally associating NcoR/HDACs complex and blocking hematopoietic differentiation. In the presence of pharmacological concentration of RA (about 350ng/ml), RA induce the corepressors
NCoR/HDACs dissociation from PML-RARA, thereby activates transcription and stimulates differentiation [11,12,108,139]. In vitro by using a dominant negative RAR construct transfected with interleukin 3 (IL-3)-dependent multipotent hematopoietic cell line (FDCP mix A4) and normal mouse bone marrow cells, GM-CSF-induced neutrophil differentiation was blocked at the promyelocyte stage. The blocked promyelocytes could be induced to terminally differentiate into neutrophils with supraphysiological concentration of ATRA [143]. Similarly, overexpression of normal RARa transduced cells displayed promyelocyte-like morphology in semisolid culture, and immature RARa transduced cells differentiate into mature granulocytes under high dose of RA(10-6M) [144]. Moreover, mutation of the N-CoR binding site abolishes the ability of PML-RARa to block differentiation [145,146]. Therefore, ectopic expression of RAR fusion protein in hematopoietic precursor cells blocks their ability to undergo terminal differentiation via recruiting nuclear corepressor N-CoR/histone deactylase complex and histone methyltransferase SUV39H1 [147]. In vivo, transgenic mice expressing PML-RARA fusion can disrupt normal hematopoiesis, give sufficient time, develop acute leukemia with a differentiation block at the promyelocytic stage that closely mimics human APL (APL-like syndrome, even in its response to RA in many studies. These results are conclusive in vivo evidence that PML/RARa is indeed oncogenic, and oncogenic pml/RARa is etiology of APL pathogenesis [148-150]. This also represents a steroid receptor in tumorigenesis (Figure 7).

Moreover, in Rousselot's group experiments, HL-60 cells transfected with 15-30ug of PML-RARa fusion in culture show no features of granulocytic differentiation after 7 days of incubation with 10-7, 10-6 uM RA (5.5-9.5% of differentiated cells by the NBT test). At 5ug of PML-RARa plasmid concentration, the blockage of RA-dependent myeloid differentiation could be overcome with high doses(10-6M) of RA (99% of differentiated cells by NBT test) (Figure 7) [151]. The results clearly indicate that PML-RARa mediated transcriptional repression, as well as PML-RARa oncprotein blocks RA-mediate promyelocyte differentiation. (Figure 8).

By using Xenopus oocyte system to uniquely the comparison of the transcriptional properties of RAR and PML-RAR is due to the lack of endogenous nuclear receptors and the opportunity to evaluate the role of chromatin in transcriptional regulation. The
results shown in Figure 8 demonstrated that, indeed, PML-RARA is a stronger transcriptional repressor that is able to impose its silencing effect on chromatin state even in the absence of RXR. Only pharmacological concentration of RA,pml/RARA become transcriptional activator function [139], (Figure 9).

In vitro experiments, ATRA induce pml-RARA itself cleavage into a 85-97kd delta PML-RARA product (a truncated pml/RARA form) in RA sensitive NB4 [152-156] (Figure 10). Delta PML-Rara is not formed in ATRA differentiation resistant NB4 subclones [152,155] which indicate the loss of PML/RARa may be directly linked to ATRA-induced differentiation [152,155].This induction of of PML-RARa cleavage and degradation by RA(ATRA,9-cis RA,Am80) involve the proteasome-dependent [152-154] and caspase mediated pathway [155] or independent of proteasome and caspase cleavage[156] and possibly ubiquitin-activating enzyme El-like(UBEIL) induction in NB4 cells. This is reason that proteasome inhibitor MG-132 and caspase inhibitor ZVAD do not block ATRA-induced pml/RARa cleavage and differentiation whereas this delta pml-RARA is blocked by RARA itself antagonist Ro-41-5253 [156].The proteasome-dependent pml/RARA degradation, by using proteasome inhibitor lactacystin test, allows APL cells to differentiation by relieving the differentiation block [153]. These data suggest a set of multiple molecular mechanisms for restoration by RA induced myeloid differentiation in APL cells. (Figure 10).

Next we further examine the pml/RARa three region functions,in vitro deletion of the RARa DNA binding domain decreased the ability of pml/RARa to inhibit vitD3 and TGF-induced the myeloid precursor U937 and TF-1 cell differentiation [145]. This is also supported by functional analysis of DNA binding domain mutation in vitro. The RARa zinc finger is a sequencespecific DNA binding through which RARa contacts the RA target genes. Moreover, deletion of PML coiled-coil region also blocked the differentiation capacity of TF-1 cells [145]. The coiled-coil region directs the formation of pml/RARa homodimers tightly interact with the N-CoR/HDACs complex, so that transcriptional de-repression cannot occur at RARA target gene promoter even if the presence of ATRA [RA resistant, 12,157]. In vitro, using established subclones of NB4 resistant to both ATRA and 9-cis RA, they were
significantly less able to stimulate transcription of a RARE driven CAT-reporter gene induction by ATRA and showed altered DNA binding activity on a RARE [158]. In the resistant cases, mut PML stabilizes PML-RARA [159]. PML-RARA with ligand-binding domain (LBD) mutation, ligand RA binding with LBD is impaired. These results have clearly shown that PML protein dimerization and RARA DNA binding domain are indispensable for the myeloid precursors differentiation which was blocked by PML/RARA and eventually leukemic transformation.

In accordance, the pml/RARA/RXR target genes is found to block differentiation by constitutively silencing a set of RA-responsive genes in the control of hematopoietic precursor cells. Five major transcription factors, Ap-1 [160] C/EBP epsilon [161,162] Pu.1/PML-RARA degradation [172]. myeloid genes in APL, linking a novel autophagy mechanism of pml/RARA fusion, as measured by the Gr-1 and Mac-1 expression [171]. Thus, pml/RARA represses PAPK2/PU.1-mediated transcription of its reversal by provoking differentiation. Overexpression of PU.1 overcomes the differentiation block in SCa 1+/Lin- HSC with transduction of PML/RARA, inducing a maturation block. Silencing of PU.1 in the adult hematopoietic tissue produces dysfunctional stem cells and impaires granulopoiesis by inducing a maturation block. Overexpression of PU.1 overcomes the differentiation block in Sca 1+/Lin- HSC with transduction of PML/RARA fusion, as measured by the Gr-1 and Mac-1 expression [171]. Thus, pml/RARA represses PAPK2/PU.1-mediated transcription of myeloid genes in APL, linking a novel autophagy mechanism of pml/RARA degradation [172].

Phosphatase and Tensin homolog (PTEN) is a protein and lipid phosphatase, which plays a pivotal dual role in tumor suppression and self-renewal of hematopoietic stem cells as its promoting exhaustion of normal hematopoietic stem cells (HSCs) and generation of leukemia-initiating cells (LICs) [166,167]. PTEN expression is downregulated in APL, while ATRA treatment increases PTEN levels by inducing PU.1 transcriptional activity via pml/RARA degradation, allowing the binding of PU.1 in PTEN promoter, in turn promotes PTEN nuclear re-location and decreases expression of the PTEN target Aurora A kinases. Therefore, PTEN is one of the primary target genes of oncogenic pml/RARA in APL.

Importantly, restoring DAPK2 expression in PU.1 knockdown APL cells partially rescued neutrophil differentiation [168]. In addition, DAPK2 interacts with other cyclin-dependent kinase inhibitors such as p15INK4b and p21WAF1/CIP, which is needed for the cell-cycle arrest in terminal differentiation of neutrophils. Moreover, DAPK2 can bind and activate the key autophagy gene beclin-1 [169]. DAPK phosphorylates beclin 1 on Thr 119 located at a crucial position within its BH3 domain and thus promotes the dissociation of beclin 1 from BCL-XL inhibitor and induction of autophagy [169]. Here, beclin 1 was initially identified as a BCL-2-binding protein, which is part of a class I1I3K (phosphatidylinositol-3-kinase) multiprotein complex that participate in autophagosome nucleation. Death-associated protein kinase (DAPK1) is a calcium/calmodulin (CaM) serine/threonine kinase for mediator of cell death [170]. PU.1, an ETS transcription factor known to regulate myeloid differentiation. Silencing of PU.1 in the adult hematopoietic tissue produces dysfunctional stem cells and impaires granulopoiesis by inducing a maturation block. Overexpression of PU.1 overcomes the differentiation block in Sca 1+/Lin- HSC with transduction of PML/RARA fusion, as measured by the Gr-1 and Mac-1 expression [171]. Thus, pml/RARA represses PAPK2/PU.1-mediated transcription of myeloid genes in APL, linking a novel autophagy mechanism of pml/RARA degradation [172].

Figure 11: Molecular model of the gene regulation of retinoic acid (RA) action (George Zhu, January 1991, revised in 2012, further revised in 2018 and in this paper). Schematic alignment of the receptor protein. The two highly conserved regions, identified as the putative DNA-binding (C) and hormone-binding (E), a hinge region (D) and the non-conserved variable NH2-terminus (A/B) as described above. CAT:CAAT box, CCAAT-enhancer binding proteins (or C/EBPs); GC:GC box; TATA:TATA box. Note: In APL cells, pml/RARA fusion point is located in the first 60 amino acids from the N-terminus (A/B) of RARA [12,182].
The elucidation of the molecular basis of retinoic acid and retinoid pharmacology in APL has been illustrated in several publications [157,173-176] the detail molecular model of gene regulation had also been proposed by Zhu in 1990s [12,177,178]. As an approach to APL treatment, one possible the action of retinoic acid, A consensus sequence (TCAGGTTCA motif) has been postulated for thyroid hormone (TRE) and retinoic acid responsive element (RARE) -containing in the promoter region of target genes [112]. High dose of RA-RARE-PML/RARα complexes in intracellular localization appears to relieve repressors from DNA-bound receptor [11,12,117,145,158,179] including the dissociation of corepressor complexes N-CoR, SMRT and HDACs from PML- RARα or partially PML-RARα/RXR [11,12,146,157,179]. Also release PML/RARα -mediated RA dependent repression [175]. This transcriptional derepression occurs at RARα target gene promoter [12,157,180]. Consequentially, PML-RARα chimera converted receptor from a repressor to a RA-dependent activator specific binding protein, possibly the true retinoid receptor. Jpn J Cancer Res 79(4): 473-483.

This is first described in eukaryotes (Figure 11). The elucidation of the molecular basis of retinoic acid and retinoid pharmacology in APL has been illustrated in several publications [157,173-176] the detail molecular model of gene regulation had also been proposed by Zhu in 1990s [12,177,178]. As an approach to APL treatment, one possible the action of retinoic acid, A consensus sequence (TCAGGTTCA motif) has been postulated for thyroid hormone (TRE) and retinoic acid responsive element (RARE) -containing in the promoter region of target genes [112]. High dose of RA-RARE-PML/RARα complexes in intracellular localization appears to relieve repressors from DNA-bound receptor [11,12,117,145,158,179] including the dissociation of corepressor complexes N-CoR, SMRT and HDACs from PML- RARα or partially PML-RARα/RXR [11,12,146,157,179]. Also release PML/RARα -mediated RA dependent repression [175]. This transcriptional derepression occurs at RARα target gene promoter [12,157,160]. Co-activator complexes containing histone acetyltransferase (e.g. p300/CBP) are recruited. The resulting pml-RARα oncoprotein proteolytic degradation occurs through the autophagy-lysosome pathway [172] and the ubiquitin SUMO-proteasome system (UPS) [152] as well as caspase 3 [155] or lysosomal protease (cathepsin D) enzyme or/and El-like ubiquitin-activating enzyme (UBE1L) induction [181]. An effect is to relieve the blockade of pml/RARα-mediated RA dependent promyelocytic differentiation and retinoic acid (9-cid RA, ATRA, Am80) in APL therapy Zhu G, March 1990- January 1991, revised in 2012). Here, RA can overcome the transcriptional repressor activity of pml/RARα [11,12,156,176,182]. The oncogenic pml/RARα uncover a pathogenic role in leukemogenesis of APL through blocking promyelocytic differentiation. This oncogenic receptor derivative pml/RARα chimera is locked in their “off” regular mode thereby constitutively repressing transcription of target genes or key enzymes (such as AP-1, PTEN, DAPK2, UP1, p21WAF/CKN1A) [160-165] that are critical for differentiation of hematopoietic cells. This is first described in eukaryotes (Figure 11).

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