Retinoid Chemoprevention: Who Can Benefit?

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Published online: 14 April 2015
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Abstract Acute promyelocytic leukemia (APL) is a treatment success story. From a highly deadly disease, it was turned into a highly curable disease by the introduction of differentiation-induction therapy with all-*trans* retinoic acid (ATRA) in the 1990s. During the last quarter of the century, ATRA and other retinoids were used for the treatment and prevention of other cancers and even other diseases. The results were less spectacular, but nevertheless important. Progress has been made toward understanding the mechanism of action of retinoids in different physiological and pathological contexts. For some diseases, specific genetic backgrounds were found to confer responsiveness to retinoid therapy. Therapies that include retinoids and other modalities are very diverse and used both for combined targeting of multiple pathways and for diminishing toxicity.

Keywords Retinoid · Chemoprevention · Retinoic acid signaling

Introduction

Retinoids

Vitamin A is a class of lipid-soluble essential micronutrients for all vertebrates [1]. “Vitamin A” is a generic term for all the

retinoids with the biological activity of all-*trans* retinol [2]. There are two main forms of dietary vitamin A: vitamin A (retinyl esters and retinol) and provitamin A carotenoids (beta-carotene, alpha-carotene, and beta-cryptoxanthin). Dietary precursors serve as substrates for the biosynthesis of two metabolites of vitamin A: 11-*cis*-retinal, essential for vision and all-*trans* retinoic acid (ATRA), essential for cell differentiation, cell survival, and the regulation of gene transcription. Retinol, retinal, and ATRA may be further metabolically modified, generating a myriad of retinoids with biological activities that are still to be fully elucidated. Besides the natural retinoids, structural analogs have also been synthesized. While natural products are metabolites/metabolizable, the synthetic ones typically are not.

Retinoic Acid

ATRA is the prototype for retinoid therapy and chemoprevention. It is an important developmental morphogen with pleiotropic actions. One of the most studied effects of retinoic acid in embryogenesis is the specification of the anterior–posterior axis and left–right patterning [3]. During development, retinoic acid gradients are controlled by the retinoic acid levels in the precursor cells and the expression of retinoic acid synthesizing (retinol dehydrogenase and aldehyde dehydrogenase) as well as degradation enzymes (Cyp26) [4–7]. Retinoic acid is important during developmental differentiation in many systems including hematologic and immune systems, eye, head, and heart [3]. Retinoic acid has well known chemopreventive effects in a variety of cancers [8–11]. It is also a teratogen [12–14]. Adults are also susceptible to side effects of excessive amounts of retinoic acid. For example, the adult hippocampus is very sensitive to extreme amounts of retinoic acid, as reviewed in [14], and differentiation syndrome (retinoic acid syndrome) is a life threatening

This article is part of the Topical Collection on *Cancer Chemoprevention*

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pathological sequela of high doses of therapeutic retinoic acid, as reviewed in [15]. Many of the effects of extreme doses of retinoic acid are systemic, affecting many cell types and their interactions.

Mechanisms of Action of Retinoids

Although initially the biological effects of retinoic acid were rationalized to be due to cognate nuclear receptor-dependent direct regulation of transcription exclusively, accumulating evidence points out to a more complex reality. The mechanism of action of retinoic acid can now be categorized as nuclear and extranuclear. Furthermore, the nuclear effects could be categorized as transcriptional and epigenetic. The extranuclear effects can be categorized as plasma membrane derived or cytosolic.

Transcriptional Regulation

Retinoic acid and its metabolites, including its isomerization product, 9-*cis*-RA, and many synthetic retinoids are ligands for two classes of ligand-activated transcription factors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) [16, 17]. RARs and RXRs present each three subtypes: alpha, beta, and gamma encoded by different genes [18]. RARs and RXRs are members of the superfamily of steroid thyroid hormone receptors. They can form homo- and hetero-dimers. They bind DNA at hexad consensus sequences, AGGTCA, that occur in repeated (direct repeat, DR) or palindromic configurations separated by several nucleotides [18, 19], although exceptions are known [20]. 9-*cis*-retinoic acid is capable of binding to the nuclear receptors and may be a major ligand of the RXRs. 13-*cis*-retinoic acid is effectively used as a therapeutic agent for dermatological diseases. It is present in human plasma and does not have high-affinity for RAR or RXR but localizes predominantly to the nuclei [21]. Numerous selective ligands have been synthesized with varying receptor specificity and biological responses.

RARs were reported to be phosphorylated, and the phosphorylation has functional consequences. For example, blocking the S77 RAR α phosphorylation contributes to Xeroderma pigmentosum pathology [22]. Retinoic acid induced neuronal differentiation requires RAR γ phosphorylation [23]. RXR α is phosphorylated in response to UV radiations and As₂O₃ [24]. S22 RXR α and S369 RAR α phosphorylation are essential for cell cycle control and transcriptional regulation of target genes in F9 cells [25, 26].

Some genotypes of retinoid receptors, identified at the level of SNPs, are known to be risk factors for neoplasms or to affect response to retinoid chemoprevention and therapy. For example, the SNP rs3118570 in RXR α is correlated with

increased risk for developing head and neck cancer, but the carriers of this genotype were shown to also benefit from retinoid chemoprevention [27].

Epigenetic Regulation

Recent research shows that retinoic acid induces profound epigenetic changes that are able to affect transcription other than by RAR and RXR direct transcriptional regulation by binding to cognate response elements. The RAR proteins are directly involved in inducing those changes by interacting with proteins of the transcription complex able to either place or remove epigenetic marks on histones or on DNA, recently reviewed by Gudas and collaborators [28].

Recent studies showed that inhibitors of lysine-specific demethylase 1 (LSD1) restored ATRA responsiveness in non-APL acute myelogenous leukemia (AML). LSD1 inhibition increased H3K4 (Me2) and expression of RA-dependent myeloid-differentiation-associated genes [29••]. Methylation of CpG islands at the promoter region of the HOXA gene cluster is increased in leukemia patients and leukemia cell lines, especially in T cell leukemia and B cell leukemia. In normal cells, the same region is unmethylated. In AML, ALL, and CML patients in complete remission and in RA-treated HL-60 and K562 cells, the methylation levels are downregulated [30].

Understanding epigenetic regulation induced by retinoids in more details is likely to improve therapies that seem promising, such as combination therapies including retinoids and valproic acid (currently recruiting patients clinical trials NCT00867672, NCT00995332, for example) or retinoids and LSD1 inhibitors such as tranilcypromine (currently recruiting patients clinical trial NCT02261779).

Plasma Membrane-Derived Signaling

Although ATRA binds both the RAR and RXR classes of receptors, it is found in higher concentrations outside the nucleus than inside, in contrast with 13-*cis*-retinoic acid which does not have high affinity for RAR or RXR but localizes predominantly to the nuclei [21]. More recently, it was shown that RAR α is present in the plasma membrane in a membrane lipid raft containing components of a MAPK signaling axis [31••]. Retinoic acid induces RAR α to complex with the G protein α Q and to activate p38MAPK [31••]. Additionally, p38MAPK further phosphorylates the nuclear pool of retinoic receptors, influencing their transcriptional activity [32].

Cytosolic Signaling

Retinoic acid elicits biological response through cytosolic signaling as well as the canonical interaction with RARs or RXRs. We and others have shown that the mechanism of

action of RA-induced cell differentiation involves prolonged, hyperactive MAPK signaling and SFK signaling [33•, 34–39]. This presents the possibility of new targets of intervention for differentiation-induction therapy. Early studies demonstrating that MAPK involvement exploited HL-60 cells as an *in vitro* experimental model susceptible to molecular manipulation. The cells were derived from the blood of a leukemia patient from whom the leukemic cells capable of self-renewal were isolated. *In vitro* systems provide potent experimental means of gaining initial mechanistic insight for translation to *in vivo* clinical application. HL-60 is a myeloblastic leukemia cell line that is lineage uncommitted and capable of myeloid or monocytic differentiation in response to different agents. HL-60 is one of the standard NCI-60 cell lines, used for example in drug testing. It has been extensively used as a model for pharmacologically induced differentiation. HL-60 cells undergo myeloid differentiation with G0/G1 growth arrest, when treated with RA. This process requires sustained activation of MAPK signaling [40], and involves a cascade of signaling regulatory events including Src family kinases (Fgr and Lyn), p38, PI3K, c-Cbl, SLP-76, VAV1, CK2, KSR, as well as the IRF-1 transcription factor [33•, 41–44]. These are implicated as parts of a large signaling complex (signalsome) with RA-upregulated expression of its components. For a number of these putative signalsome components, their ability to drive differentiation has been directly demonstrated. For example, during RA-induced differentiation, ectopic expression of Raf [37, 45], Cbl [41], SLP-76 partnered with c-FMS [42], interferon regulatory factor 1 (IRF-1) [43], and aryl hydrocarbon receptor (AhR) [46] have been shown to enhance MAPK signal activation, in particular Raf activation, and to promote RA-induced differentiation and G0-arrest. Inclusion of IRF-1 as a functionally significant component of the signalsome was an unanticipated finding, but the interferon and RA pathways are known to cooperate [47], and this may be a novel part of the mechanism. In the case of AhR, which is a classical cognate transcription factor that binds XRE sites of target gene promoters, we found that RA-induced differentiation was dependent on its RA-induced upregulation [46]. We found that overexpression of AhR enhanced RA-induced differentiation. Furthermore, AhR upregulation caused Oct4 downregulation. Oct4 is a Yamanaka–Thomson transcription factor that confers pluripotency in iPS, and it is the one thus far found essential for driving reversion to stem cells from differentiated cells. RA thus induces formation of a putative signalsome in which a Raf/Mek/Erk axis is imbedded amidst adaptors, kinases, GEF, and transcription factors. These signalsome components are part of the mechanistic underpinnings of the prolonged hyperactive MAPK signal that drives cell differentiation. They are potential targets for augmenting RA effects as well as indicators of cellular response to RA.

Retinoids in Treatment and Chemoprevention

Chemoprevention is the use of drugs, food, or food supplements to prevent disease. The term includes but it is not limited to cancer. Chemoprevention by dietary factors can have a significant effect on the population by reducing cancer incidence in normal populations and relapse in former patients. A goal of cancer chemoprevention is to reverse or block the process of neoplastic transformation. In this regard, it can have several facets. In one paradigm, neoplastic transformations due to environment, for example, are constantly occurring but are reversed or lost and hence do not result in tumors. Thus, reversing the transformed phenotype is a form of chemoprevention, as is prevention of relapses [27]. In another perspective, processes directing stem-like cells, including those that seed to cause metastatic spread in a nurturing niche, to lose their self-renewal capability and assume a differentiated phenotype can also be considered chemopreventive. And any means of reversing the transformed phenotype or retarding tumorigenesis are in the broad sense all chemopreventive. Both of these instances provide motivation for trying to understand how a dietary factor can reverse the neoplastic phenotype, thereby providing early prevention. Such mechanistic insights are needed to find ways of enhancing this chemopreventive process. Frequently, mechanistic insights from therapeutic approaches provide valuable information for future chemoprevention modalities. Although there are many instances of retinoic acid chemopreventive effects, there are also enigmatic instances where it has had the opposite effect [48, 49].

Retinoids are used for treatment of a variety of conditions. The most successful is the differentiation therapy for APL. Currently, a number of ongoing clinical trials are using this differentiation modality in treating a large number of conditions. Some clinical trials have been withdrawn, such as NCT00003564 for glioma. However, this points to the need for detailed fuller understanding of the mechanism of action of retinoids to enhance their therapeutic and chemopreventive benefits.

Acute Myelogenous Leukemia

APL (AML3), a subtype of AML, is the success story for ATRA treatment [50]. About 25 years ago, APL was a fatal disease for most patients. Introduced in the late 1980s and early 1990s [50, 51], ATRA therapy induced high remission rates that were much higher remission rates achievable by chemotherapy alone, but the remission was not durable, and the relapsed APL- blasts were resistant to ATRA [52]. In the hope of alleviating relapses, combination therapy of ATRA and chemotherapy was used [52]. A recent study analyzing the dataset derived from the North American Intergroup Study INT0129 calculated for the first time the estimated duration of

ATRA needed after chemotherapy to eliminate the leukemic stem population to a year [53]. This study authored by top experts in the field points out that ATRA can eliminate the cancer stem population by inducing differentiation of the blasts and modulating the cell cycle of the cancer stem cells. Prior to this, it was thought that ATRA could overcome a block in differentiation but did not eliminate the leukemic clone. In patients with relapsed APL, ATRA and arsenic trioxide was found to be effective [54]. Some experts therefore advocate a front line therapy of ATRA and arsenic trioxide without chemotherapy from the initial diagnosis for patients with low or intermediate risk APL [55–57]. In elderly APL patients or patients not qualifying for chemotherapy or arsenic trioxide therapy due to concurrent disease, ATRA as single therapy was proved to be effective in both induction and maintenance of remission [58, 59]. In one such study, the regimen was ATRA (45 mg/m²/day) for 21 days, then 1 week every 2 weeks (pulsed ATRA) [58]. An earlier study established that a single oral ATRA dose of 15 mg/m² led to a plasma concentration of 1 µM, a concentration sufficient to induce APL blast differentiation [60]. However, a practical problem encountered clinically is that plasma levels of ATRA drop after prolonged treatment. Liposomal ATRA was shown to better maintain plasma levels of ATRA and induce a more durable remission as a single agent [61, 62].

The traditional inclusion criteria for ATRA treatment were the RAR fusion proteins characteristic of APL. More recently, however, the use of ATRA treatment is being expanded to other AMLs.

Adult patients with AML and NPM1 mutations who are ineligible for intensive chemotherapy, especially elderly patients, might benefit from chemotherapy with cytarabine, etoposide, and retinoic acid. A clinical trial of this type that is recruiting patients is NCT01237808. Another clinical trial, NCT00892190 “Study of Dasatinib and All-Trans Retinoic Acid for Relapsed/Refractory and/or Elderly Patients with Acute Myelogenous Leukemia (AML) or Myelodysplastic Syndrome (MDS)”, is also recruiting patients. We and others have provided evidence that combination therapy of retinoic acid plus SFK inhibitors targets critical cytosolic signaling components, and this could be of therapeutic benefit [33•, 63]. Another proposed combination therapy with ATRA in non-APL AML patients uses ATRA with valproic acid. In this case, there are two patient recruiting clinical trials, NCT00867672 and NCT00995332, being conducted in Germany. We had shown that valproic acid with retinoic acid enhances RA-induced differentiation and cell cycle arrest by upregulating AhR and consequently downregulating Oct4 in an AML cell line model, HL-60 [46]. Also, in non-APL AML subtypes, tranylcypromine (TCP), an irreversible monoamine-oxidase (MAO) and lysin-specific demethylase (LSD) inhibitor that also inhibits LSD1 (a histone H3 lysine 4 demethylase), induces blast cell differentiation and leukemic

stem cell exhaustion in vitro and in vivo with RA [29••, 64]. A related clinical trial, NCT02261779 is recruiting patients.

The phase II clinical trial NCT00326170 was an interventional UT MD Anderson Cancer Center study for patients with refractory or relapsed MDS and AML. 5-azacytidine (5-aza) and valproic acid were coadministered with ATRA. The hypothesis was that cotreatment of a DNA hypomethylating agent along with a histone deacetylase inhibitor plus ATRA will restore ATRA responsiveness in resistant cells. The response rate was 42 % and the median remission was 26 weeks [65].

The success of treating some AML cases with ATRA-5-azacytidine combination therapy increased efforts to determine the mechanism of action underlying this effect. Olfactomedin 4 (OLFM4 also known as GCSF-stimulated clone 1, hGC-1, or GW112) was found to be one of the targets regulated by this combination therapy [66]. Using non-APL- AML patient samples and the HL-60 cell line, it was found that the OLFM4 gene has a DR5 site in its promoter that is negatively regulated by methylation to which the RARα/RXRα heterodimer binds [66]. A downstream effect of OLFM4 is the inhibition of cap-dependent mRNA protein initiation. By overexpression and knock-down studies, it was shown that OLFM4 expression enhances differentiation and apoptosis of AML cells [66]. OLFM4 is likely not the only target of ATRA in RA-responsive AML—including non-APL subtypes of AML. Nevertheless, it is a prototype that encourages the search for a fuller understanding of the mechanism of action of ATRA in successful treatment regimens so that combination therapy with ATRA can be extended to other cancer types. However, for colorectal [67, 68], endometrial adenocarcinoma [69], solid gastric, pancreatic, and head and neck [68] cancers, high level of OLFM4 is a negative prognostic factor. For prostate cancer, the deletion of OLFM4 gene was reported to be associated with tumor progression [70]. This further points to the role of gene regulation for optimal levels of protein expression and tissue specificity. Moreover, OLFM4, being RA regulated, points to the need of understanding in greater detail the RA-induced proteomic changes in different tissues. This is needed in order to expand and optimize RA chemoprevention and treatment applications.

Neuroblastoma

In children with high-risk neuroblastoma, 13-*cis*-RA plus immunotherapy with anti-GD2 is the standard treatment for minimal residual disease [71]. In chemoresistant osteomedullary neuroblastoma, 13-*cis*-RA with anti-GD2 antibody and granulocyte-macrophage colony-stimulating factor are clinically effective by augmenting the antineoplastic potency of the myeloid effectors [72]. Patients with neuroblastoma have

an increased risk of secondary neoplasia, especially acute myeloid leukemia/myelodysplastic syndrome [73]. The fact that those malignancies are responsive to retinoids and the fact that a history of neuroblastoma predisposes to AML or myelodysplastic syndrome points to the fact that the etiology involves possibly a disruption in RA signaling pathways, and that those pathways and retinoid chemoprevention (alone or in combination with other agents) needs to be further investigated by detailed mechanistic studies.

Breast Cancer

An interesting characteristic of retinoid use for breast cancer management is the wide variety of the mechanisms of action proposed and the discrepancy between a large number of pre-clinical trials and very limited clinical trials for retinoid and breast. On clinicaltrials.gov site, there are just two clinical trials listed plus those completed and with no results listed. Additionally, there are some clinical trials on chemoprevention conferred by retinoids and breast is included among other sites studies, studies done both in the USA and in Italy [74]. A recent review assessed the current understanding for retinoid chemoprevention and therapy for breast cancer [75].

Cervical Cancer

As reviewed by Helm et al. [76], various retinoids (natural: 9-*cis*-RA, 13-*cis*-RA, ATRA, and synthetic: *N*-(4-hydroxyphenyl)retinamide) were tested. Different doses, routes, and intervals of administration tested were not significantly effective in causing regression or prevent progression of invasive cervical carcinoma. *N*-(4-hydroxyphenyl)retinamide caused a worse outcome (absence of desired effects in a small sample set) for the patients in the treatment group than in placebo, and the study was terminated early [77].

Lung Cancer

An interventional study was conducted under the phase II clinical trial NCT00062010. Patients with recurrent small cell lung cancer were treated with IFN α , isotretinoin, and paclitaxel. The overall response percentage was 8.8 %. The median survival was 6.2 months and the median progression-free survival was 2 months. Participants were recruited from Eastern Cooperative Oncology Group member institutions between February 24, 2004 and August 22, 2007. The rationale for adding the retinoid was to reduce resistance to paclitaxel.

In another clinical trial, NCT00601796, conducted at the H. Lee Moffitt Cancer Center and Research Institute, ATRA (150 mg/m²/day given orally three times a day for three consecutive days in two courses, during week 1 and week 9) was added to cyclophosphamide and a vaccine regimen in order to

enhance dendritic differentiation. The vaccine was made using irradiated allogeneic lung adenocarcinoma cells and K562 cell line transfected with hCD40L and hGM-CSF. The hypothesis was that by recruiting and activating the dendritic cells, the treatment would induce tumor regression in refractory lung adenocarcinoma. The median progression-free survival was 1.7 months and the median overall survival was 7.9 months. There was no radiologic tumor regression achieved [78].

Hepatocellular Carcinoma

TAC-101, a synthetic retinoid, administrated 20 mg/day orally led to disease stabilization and prolonged median survival (19.2 months) in patients with advanced hepatocellular carcinoma [79].

Head and Neck Cancer

The first line of treatment for head and neck squamous cell carcinoma (HNSCC) is surgery and/or radiation therapy. Within 5 years, however, 10–25 % of the treated patients develop recurrence or second primary tumors. Common sites for second primary tumor in the HNSCC survivors are the esophagus and the lung [80, 81]. 13-*cis*-retinoic acid was tested as a chemopreventive agent to prevent both recurrent and second primary tumors. Daily doses of 50–100 mg 13-*cis*-RA/m²/day for 12 months prevented second primary tumors but had no effect on recurrence in patients treated for HNSCC [82, 83]. The 13-*cis*-RA-dependent side effects (skin dryness, cheilitis, hypertriglyceridemia, and conjunctivitis) at those doses were very severe [82]. After the completion of the 13-*cis*-retinoic acid administration, its chemopreventive effect against second primary tumors diminishes in time [83]. A randomized, placebo-controlled trial of daily low-dose (30 mg/m²/day) 13-*cis*-RA administrated for 3 years followed by 4 years of observation was conducted in a population of survivors of HNSCC treated for early stage disease [84]. In this group of population, overall, 31 % developed SPT/recurrence in the 13-*cis*-RA vs 35 % in the placebo. However, the SNP rs3118570 in RXR α is correlated with both increased risk for developing head and neck cancer, and benefit from retinoid chemoprevention. This genotype represented 71 % of the population in the placebo group. Additionally, patients with certain genotypes of Janus kinase 2 (JAK2), matrix metalloproteinase 3 (MMP3), RAD-54-like (RAD54L), and cell division cycle 25 homolog C (CDC25C) were 1.85–3.57 times more likely to develop an SPT/recurrence, compared with patients who had variant containing genotypes JAK2:rs188724 and CDC25C:rs6896428 being predictors for 13-*cis*-response as well [27]. Likewise, the SNPs rs739442, rs4962225, rs7035940, and rs7874234 in TSC1 and PI3KCD:rs4129341 and PTEN:rs1234221 confer high-risk of SPT/high-benefit for 13-*cis*-RA chemoprevention [85].

Alzheimer's Disease

ATRA, 9-*cis*-RA, and 13-*cis*-RA are promising agents for prevention of Alzheimer's disease. The mechanism likely involves RAR- and RXR-dependent regulation of apolipoprotein E [86]. The synthetic retinoid acitretin is a promising drug for the treatment of Alzheimer's disease by its ability to enhance the nonamyloidogenic amyloid precursor protein (APP) processing [87].

Dermatological Conditions

In dermatological conditions, retinoids have a long history of applications. A detailed systematic description of those applications is beyond the scope of this review. Here, we present a few examples in order to give a sense of the diversity of the retinoid applications. Xeroderma pigmentosum is a rare genetic disorder. One of its characteristics is a mutated TFIIH transcription factor. This leads to impaired RAR α phosphorylation and therefore impaired ligand-dependent control of the activation of the hormone-responsive genes [22]. Anti-psoriatic synthetic retinoid tazarotene, AGN 190168/ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)-ethynyl] nicotinate, is a RAR β and RAR γ agonist which upregulates tazarotene-induced gene 2 (TIG2) protein in psoriatic lesions toward the levels found in normal skin [88]. Tazarotene-induced TIG3 seems to suppress inflammatory angiogenesis by downregulating PlGF expression, in psoriatic lesions [89]. Randomized, double-blind, vehicle-controlled, parallel-group studies led at 39 centers in the USA and Canada found that foam containing 0.1 % tazarotene significantly reduced the number and severity of acne lesions after 3 months [90]. Adapalene is a synthetic retinoid that diminishes the number of lesions [91]. Currently, a clinical trial (NCT02173054) is recruiting acne patients. In certain cases, retinoids are useful in treating mycosis fungoides [92]. Chronic eczema is treated with 9-*cis*-RA, and one of the mechanisms involved is an increase of FoxP3 cells in the skin [93]. In dissecting cellulitis, topical 13-*cis*-RA is the most effective drug to induce remission [94]. The synthetic retinoid acitretin is effective in treating psoriasis [95, 96], although its mechanism of action is not fully understood. Effects on metabolism were proposed [97–99].

Conclusions

Recent research has demonstrated that multiple mechanisms account for retinoid chemopreventive and therapeutic effects. Despite the common perception that retinoid therapy is successful just in the case of APL and skin diseases, recent

evidence shows that retinoids are valuable therapeutic and chemopreventive agents. Progress in the detailed mechanism of action of retinoids is likely to extend the benefits of retinoids and to develop more specific and less invasive means of chemoprevention and therapy. Finding the biomarkers of enhanced response or resistance is very important toward achieving personalized medicine. A very important point is made by Formelli [74] regarding the interdependence of the time of administration of retinoids, diet, retinoid serum levels, and effects. It is of interest that he points out that in cohorts of people taking oral retinoids after dinner, the serum levels are much higher and this effect is especially prevalent in populations habitually consuming important quantities of olive oil. This is not unexpected as retinoids are lipid-soluble; however, it is an often ignored detail in retinoid clinical trials. Additionally, we and others have reported that different oils have different effects on cell proliferation or differentiation, endpoints often targeted by retinoid chemoprevention [100–104]. This points to the need of future studies of retinoid cellular and systemic effects and mechanism of action where other relevant factors such as diet are carefully controlled. On the other hand, there are recent efforts being made to use diet and dietary supplements for ameliorating the retinoid-induced side effects [105]. Detailed mechanistic insight of retinoid effects will likely reveal points of further modulation by dietary or pharmacological agents for enhanced chemopreventive/therapeutic benefit. There are many other conditions in which retinoids have a potential to be chemopreventive; the examples given here are meant to raise awareness of the complexity of this field and to point out that despite a common perception that the field is mature and understood, there are many benefits of retinoids, many struggles in understanding the balance between benefits and risks, and a wide variety of biological processes modulated by retinoids, encompassing but clearly not limited to the retinoid receptors.

We regret it was not possible to cite all of the important work that has been reported in this broad and active field and apologize for omissions.

Acknowledgments We gratefully acknowledge partial support from the National Institutes of Health (NIH) CA033505 (Yen), CA152870 (Yen), 1U54 CA143876 (Shuler), and New York State Stem Cell Science (NYSTEM) (Yen).

Compliance with Ethics Guidelines

Conflict of Interest Rodica P. Bunaciu and Andrew Yen declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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