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Original Research

Retinoids offer new and promising cancer therapeutic avenues

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All trans-retinoic acid (ATRA) as well as several key retinoids including tamibarotene, acyclic retinoid (ACR), and WYC-209, have made a major progress in both pre-clinical cancer therapeutics as well as in the clinical setting regarding the treatment of leukemia and solid tumors via their important impacts on cancer stem cell differentiation or apoptosis. ATRA exerts its antitumor activity by binding to retinoic acid receptors, which in turn specifically bind to DNA as a heterodimer with the retinoid X receptors, at promoter regions known as retinoic acid response elements. The impressive new studies and clinical achievements with retinoids as key pre-clinical research tools and antitumor agents, are summarized and discussed in the current paper. The ongoing clinical trial of tamibarotene, which is the first agent targeting super-enhancers-containing cancers, could provide a new treatment modality for acute myeloid leukemia patients. A recent clinical study for evaluation of the preventive effects of ACR on second primary hepatocellular carcinoma (HCC) demonstrated that the oral administration of ACR for 12 months, significantly reduced HCC recurrence. WYC-209 strongly inhibited cell proliferation of different tumor repopulating cells (TRCs), a highly tumorigenic subpopulation of mouse melanoma cells, and also blocked $>$ 80% of B16 TRCs' lung metastases in wild-type C57BL/6 mice, without any apparent toxicity. These remarkable findings reveal that retinoids constitute a promising class of antitumor agents for the treatment of both hematological malignancies and solid tumors.

Keywords

Retinoids; cancer therapy; cancer stem cells; differentiation; apoptosis

1. Introduction

All-trans retinoic acid (ATRA, Fig. 1A), the main metabolite of vitamin A1, plays important roles in various gene activation and regulation of protein expression through RAR-RXR transcriptional functions [1]. ATRA acts by binding to the retinoic acid receptor (RAR), which typically binds DNA as a heterodimer with the retinoid X receptor (RXR) in well-defined promoter regions knowns as retinoic acid response elements (RAREs) [2, 3]. Since ATRA could effectively influence cell differentiation, growth, and apoptosis, deregulation of RAR signaling pathways with ATRA emerging as a cancer therapeutic strategy. Accordingly, scientists designed and synthesized various retinoic acid analogues, which are termed retinoids, largely broadening the medical applications of RA based on their robust RARs regulatory and modulatory functions [4, 5].

Recently, ATRA as well as several excellent retinoic acid derivatives, including tamibarotene (Fig. 1B), acyclic retinoid (ACR) (Fig. 1C), and WYC-209 (Fig. 1D), demonstrated promising therapeutic activities towards leukemia and various solid tumors, hence offering a novel cancer therapeutic avenue (Table 1).

2. All-trans retinoic acid acts as an effective differentiating agent in acute promyelocytic leukemia (APL) therapy

ATRA is an important key regulator of cell differentiation through activation of RA-responsive transcriptional factors during embryonic development and tissue formation [13]. Thus, the retinoid-induced differentiation of cancer cells as a treatment strategy is highly attractive, while enormous success has been made in the area of acute promyelocytic leukemia (APL) therapy with the introduction of the efficacious combination of retinoic acid (RA) and arsenic trioxide known as the ATRA-ATO protocol [6, 7].

Retinoic acid receptor α (RARα) is an RA-responsive transcription factor which heterodimerizes with the retinoid X receptor (RXR) coreceptor, thus binding to retinoic acid response elements (RAREs) in the promoters of various RA-responsive genes. However, the pathogenic fusion protein PML-RARα exerts dual roles of transcriptional silencing and promyelocytic leukemia protein (PML) nuclear body disorganization, since this fusion protein, PML-RARα has a dominant negative effect on RA signaling; the latter results in the blockade of differentiation by recruiting abnormal transcription factors and histone-modifying enzymes to critical genes, which are notably those involved in clonal cell expansion process in leukemia [7]. It should be noted that in approximately 95% of APL patients, the retinoic acid receptor-α (RARA) gene which resides on chromosome 17, is involved

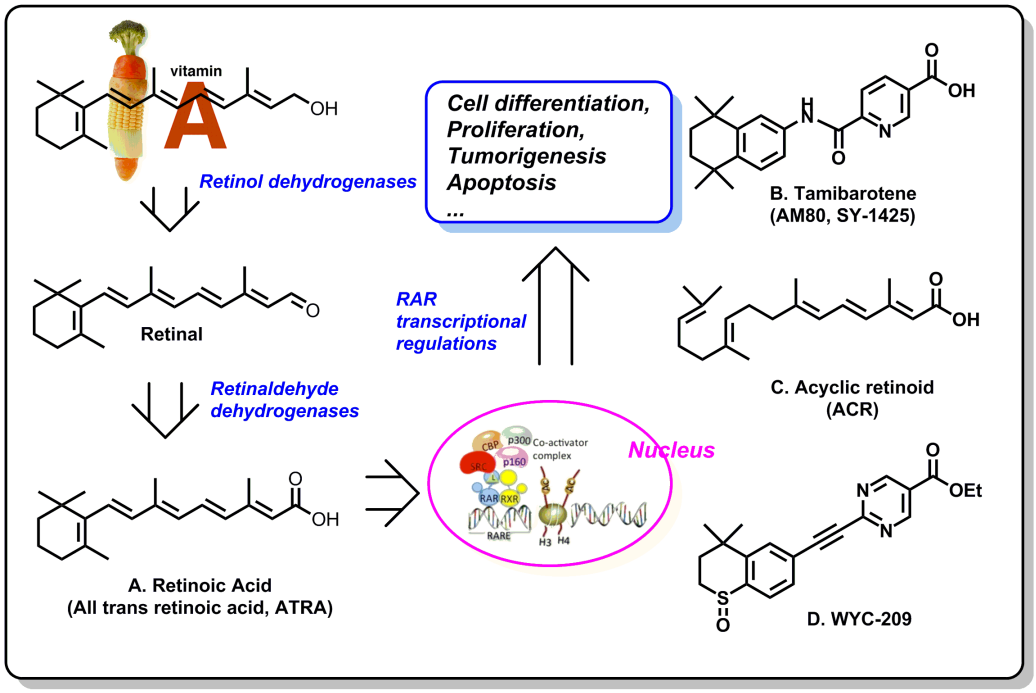

Figure 1. Vitamin A metabolic pathway and important retinoids in advanced cancer research and therapy. Vitamin A is converted to retinal via oxidation by retinol dehydrogenases; while retinal is converted to ATRA via further oxidation by retinaldehyde dehydrogenases. The important retinoids in advanced cancer research and therapy include ATRA, tamibarotene, acyclic retinoid, and WYC-209.

in a reciprocal translocation with the promyelocytic leukemia gene (PML) residing on chromosome 15. This translocation is known as t(15;17)(q24;q21) [14, 15]. Fortunately however, when ATRA concentrations were increased via exogenous administration, ATRA was found to bind to the PML-RARα fusion protein, overcoming the inhibitory effects of the latter, thus transactivating the expression of target genes. This resulted in restoration of normal leukemic cell differentiation, thus rendering APL a highly treatable disease.

3. Tamibarotene operates as the first super-enhancer targeting drug in the treatment of acute myeloid leukemia (AML)

Tamibarotene, also known as Am80 or SY-1425, is an orally active, synthetic retinoid, which was developed to overcome ATRA drug resistance. Structurally, tamibarotene is a dicarboxylic acid monoamide resulting from the condensation of the carboxyl group of terephthalic acid with the amino group of 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-amine [16, 17]. Tamibarotene is a potent and selective RARα agonist which appears to be better tolerated than ATRA in APL treatment.

It is well established that gene enhancers play a key role in regulation of gene expression. Accordingly, a study driven by scientists from Syros Pharmaceuticals characterized the enhancer landscape of 66 AML patients, hence identifying 6 novel subgroups and their associated regulatory loci [8]. These subgroups were defined by their super-enhancer (SE) maps, orthogonal to somatic cell mutations, and are associated with distinct leukemic cell states. Thus, this novel study employed the SE landscape of primary human AML to elucidate transcriptional circuitry and to identify novel cancer vulnerabilities. A subset of patients was found to have an SE at the RARα locus, which is predictive of response to treatment with SY-1425, a potent and selective RARα agonist, in preclinical models, forming the rationale for its clinical investigation in biomarker-selected patients. Based on these novel SE maps, a Phase II clinical trial with tamibarotene was initiated to assess its safety and efficacy in combination with 5-azacytidine, a standard-of-care therapy, in genomically defined subsets of AML patients, including those with relapsed or refractory AML. To the best of our knowledge, this clinical trial is the first entry which targets these novel SE maps for human cancer treatment [8, 9].

Initial data from the ongoing Phase II study suggest that and a combination of tamibarotene with 5-azacytidine displayed high response rates and rapid onset of responses in biomarker-positive, newly diagnosed AML patients, who were not suitable candidates for standard chemotherapy. These preliminary findings also suggest that screening for particular SEs can identify AML patients who might benefit from treatment with tamibarotene [8].

4. Acyclic retinoid prevents hepatocellular carcinoma via targeting MYCN-positive liver cancer stem cells

ACR, also known as Peretinoin, is a synthetic and orally available vitamin A-like compound, with potential antineoplastic and chemopreventive activities [18, 19, 20]. ACR could bind to, and activate the RAR nuclear receptors, which in turn recruit coactivator nuclear factors and promote, along with other transcriptional factor complexes, the transcriptional transactivation of target genes. As a consequence, ACR could modulate the expression of genes involved in the regulation of cell proliferation, differentiation, and apoptosis of both normal and tumor cells.

Table 1. Retinoids in recent cancer treatments and advanced cancer research

Retinoids	Company	Targets	Stage	Cancer Indications	Side effects
Tretinoin (ATRA)	Triax Pharm, etc	pan-RAR/RXR agonist	Launched	Acute promyelocytic leukemia (APL)	Liver dysfunction, heart rhythm abnormalities, intestinal toxicity, etc [6, 7]
Tamibarotene	Nippon Shinyaku, Syros Pharma	RARα/β agonist	Launched; Phase II, NCT02807558 (US)	Recurrent acute promyelocytic leukemia (APL) Acute Myeloid Leukemia (AML)	Side effects were similar but milder than those of ATRA [8, 9]
Peretinoin (Acyclic retinoid)	Kowa Pharm	pan-RAR/RXR agonist	Phase III, NCT01640808 (US)	Hepatic Neoplasm Malignant Recurrent	Side effects were similar but milder than those of ATRA [10, 11]
WYC-209	Baiyu Pharm	RAR modulator	Pre-clinical	Melanoma metastases, hepatocellular carcinoma (HCC)	Unclear [12]

Hepatocellular carcinoma (HCC) is a highly malignant cancer with significant recurrence rates [21, 22]. In order to evaluate ACR's potential in HCC therapy, a placebo-controlled clinical study for evaluation of the preventive effects of ACR on second primary HCC was initiated. The patients who were free of HCC after surgical resection or percutaneous treatment of the primary liver tumors were selected. Remarkably, this study demonstrated that oral administration of ACR for 12 months, significantly reduced post HCC recurrence [23]. In this respect, it was reported that ACR prevents HCC recurrence via targeting MYCN-positive liver cancer stem cells. Through a genome-wide transcriptional screening, it was found that ACR could selectively suppress the expression of MYCN, a key member of the MYC family of transcription factors [24]. High-content single cell imaging analysis as well as flow cytometric analysis revealed that ACR could selectively inhibit the MYCN+ cluster of stem cells from cultured heterogeneous HCC cells. Based on subsequent functional experiments, cell-cycle progression, cell proliferation, colony formation, as well as the activation of caspase 8, were substantially inhibited after silencing of MYCN gene expression. These remarkable findings bring new insights into retinoid-based therapeutics and prevention of HCC [24].

5. Retinoid WYC-209 induces apoptosis of tumor repopulating cells (TRCs)

Regulation of RARs by RA often results in inhibition of cellular proliferation. To overcome the emerging drug resistance to RA, and more effectively abrogate the growth and metastasis of malignant tumors, various novel synthetic retinoids were developed in the past decades [25, 26]. With the same purpose, a novel synthetic retinoid library was established in our lab; we thus explored the ability of these compounds to block tumor cell proliferation using the developed 3D B16-F1 TRC model, which is known to display resistance to conventional chemotherapeutic drugs including doxorubicin and cisplatin [27, 28]. Among these new retinoid compounds, a potent drug candidate namely WYC-209 (Fig. 1D) was discovered, which strongly inhibited cell proliferation of different TRCs from various murine and human cancer cell lines with IC_{50} values below 1 μM. Upon *in vivo* testing, WYC-209 abrogated more than 80% of B16 TRCs' lung metastases in wild-type C57BL/6 mice without any apparent toxicity [12].

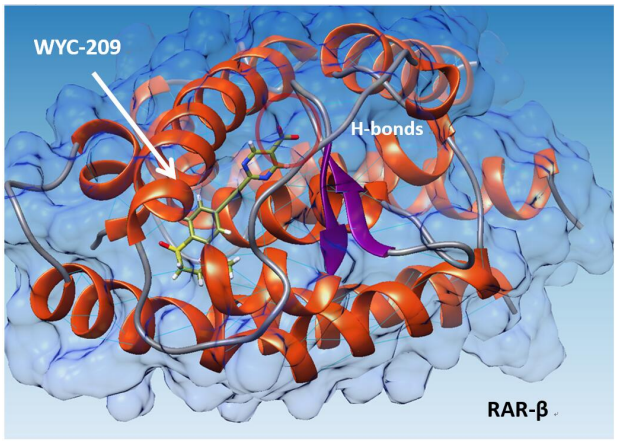

Figure 2. The docking mode of WYC-209 on RARβ (PDB:1XAP, Gold Suite 5.0). The double H-bonds formed between the oxygen atoms of the carboxyl group of WYC-209 and Arg269/Ser280 of RARβ, may play key roles in functional modulation of RARα/β activity.

However, the exact mechanism underlying WYC-209-induced apoptosis of TRCs remains unclear at this stage (Fig. 2). The docking research of WYC-209 to the RARβ ATRA binding pocket (PDB:1XAP) [29], illustrated that there were two H-bonds formed between the oxygen atoms of WYC-209 carboxyl group and Arg269/Ser280 of RARβ, and these interactions may play key roles in the induction of TRCs apoptosis via RARβ-bound WYC-209. Interestingly, the expression of self-renewing Sox2 gene and the master differentiation gene Mitf in B16 TRCs' were found to be regulated by different concentrations of WYC-209. Subsequently, a caspase 3 inhibitor experiment rescued the TRCs' growth from

WYC-209 inhibition, while depletion of caspase 3 with siRNAs achieved similar results. These findings may suggest that WYC-209 induces TRCs apoptosis primarily via caspase 3 activation [12]. This research line of the promising association between the expression changes in Sox2 and/or Mitf and the activation of caspase 3, is currently under intense investigation.

6. Conclusion

In summary, the retinoids have made a remarkable progress in cancer research as well as in the clinical oncology setting regarding the treatment of leukemia and solid tumors via their important impacts on cancer stem cell through differentiation or apoptotic functions [30, 31, 32]. For AML treatment, though drug resistance towards ATRA emerges in the clinic, tamibarotene appears to be better tolerated and is thus being evaluated for its capacity to surmount clinical resistance to ATRA without inflicting significant untoward toxicity. Furthermore, recent novel findings suggest that genomic screening for particular SE can identify AML patients who might benefit from tamibarotene treatment.

As to HCC treatment, the clinical study with ACR demonstrated that oral administration of ACR significantly reduced the incidence of post-therapeutic HCC recurrence. Whereas for melanoma treatment, low doses of WYC-209 could effectively abrogate lung metastases of melanoma TRCs in immune-competent wild-type C57BL/6 mice, whereas no apparent toxicity was observed. Taken collectively, these findings reveal that retinoids constitute a promising therapeutic treatment avenue for both hematological and solid tumors.

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Conflicts of interest

The authors have no conflicts of interest, including specific financial interests or relationship and affiliations relevant to the subject matter or materials discussed in the manuscript.

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