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 preclinical 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 preclinical 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 retinoid 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
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   | RARa/b 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 IC50 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].

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 the carboxyl group of WYC-209 and Arg269/Ser280 of RARβ, may play key roles in functional modulation of RARα/β activity.

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.
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 relationships relevant to the subject matter or materials discussed in the manuscript.

References

[1] De Luca LM. Retinoids and their receptors in differentiation, embryogenesis and neoplasia. *FASEB Journal*, 1991; 5(14): 2924-2933.
[2] Altucci L, Leibowitz MD, Ogilvie KM, de Lera AR, Gronemeyer H. RAR and RXR modulation in cancer and metabolic disease. *Nature Reviews Drug Discovery*, 2007; 6(10): 793-810.
[3] Dominguez M, Alvarez S, de Lera AR. Natural and structure-based RXR ligand scaffolds and their functions. *Current Topics in Medicinal Chemistry*, 2017; 17(6): 631-662.
[4] Hansen LA, Sigman CC, Andreoula F, Ross SA, Kelloff GJ, De Luca LM. Retinoids in chemoprevention and differentiation therapy. *Carcinogenesis*, 2000; 21(7): 1271-1279.
[5] Hanahan D, Weinberg, RA. Hallmarks of cancer: the next generation. *Cell*, 2011; 144(5): 646-674.
[6] Tallman MS, Andersen JW, Schiffer CA, Appelbaum FR, Feusner JH, Ogden A, Shepherd L, Willman C, Bloomfield CD, Wiernik PH. All-trans-retinoic acid in acute promyelocytic leukemia. *New England Journal of Medicine*, 1997; 337(15): 1021-1028.
[7] Wei S, Kozono S, Kats L, Nechama M, Li W, Guarniero J, Luo M, You MH, Yao Y, Kondo A, Hu H, Bozkurt G, Moerke NJ, Cao S, Reschke M, Chen CH, Rego EM, Lo-Coco F, Cantley LC, Lee TH, Wu H, Zhang Y, Pandolfi PP, Zhou XZ, Lu KP. Active Pin1 is a key target of all-trans retinoic acid in acute promyelocytic leukemia and breast cancer. *Nature Medicine*, 2015; 21(5): 457-466.
[8] McKeown MR, Corees MR, Eaton ML, Fiore C, Lee E, Lopez JT, Chen MW, Smith D, Chan SM, Koenig JL, Austgen K, Gaugenthinger MG, Orlando DA, Lovén J, Fritz CC, Majeri R. Supershaker Analysis Defines Novel Epigenomic Subtypes of Non-APL AML. Including an ARAr0 Dependency Targetable by SV-1425, a Potent and Selective ARAr0 Agonist. *Cancer Discovery*, 2017; 7(10): 1136-1153.
[9] Ledford H. Cancer researchers seek to harness mysterious DNA super-enhancers. *Nature*, 2018; 564(7735): 173-174.
[10] Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, Tanaka T, Tsurumi K, Okuno M, Tomita E, Nakamura T, Kojima T. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. *New England Journal of Medicine*, 1996; 334(24): 1561-1567.
[11] Kada N, Suzuki T, Aizawa K, Matsumura T, Ishibashi N, Suzuki N, Takeda N, Munemasa Y, Sawai D, Ishikawa T, Nagai R. Acyclic retinoid inhibits neoinitima formation through retinoic acid receptor beta-induced apoptosis. *Arteriosclerosis Thrombosis and Vascular Biology*, 2007; 27(7): 1535-1541.
[12] Chen JW, Cao X, An QL, Zhang Y, Li K, Yao WT, Shi FC, Pan YF, Jia Q, Zhou WW, Yang F, Wei FX, Wang N, Yu B. Inhibition of cancer stem cell like cells by a synthetic retinoid. *Nature Communications*, 2018; 9(1): 1406.
[13] de Thé H. Differentiation therapy revisited. *Nature Reviews Cancer*, 2018; 18(11): 117-127.
[14] Sanz MA, Fenaux P, Tallman MS, Estey EH, Löwenberg B, Naoe T, Lengfelder E, Döhner H, Burnett AK, Chen SJ, Mathews V, Iland H, Rego E, Kantarjian H, Adès L, Avissis G, Montesinos P, Platzerbecker U, Ravandi F, Russell NH, Lo-Coco F. Management of acute promyelocytic leukemia: updated recommendations from an expert panel of the european leukemia net. *Blood*, 2019; 133(15): 1630-1643.
[15] Iland H. Curative strategies in APL. *Seminars in Hematology*, 2019; 56(2): 131-138.
[16] Kagechika H, Kawachi E, Hashimoto Y, Himi T, Shudo K. Retinobenzoic acids. 1. Structure-activity relationships of aromatic amides with retinoidal activity. *Journal of Medicinal Chemistry*, 1988; 31(11): 2182-2192.
[17] Tamura K, Kagechika H, Hashimoto Y, Shudo K, Ohsugi K, Ide H. Synthetic retinoids, retinobenzoic acids, Am80, Am580 and Ch55 regulate morphogenesis in chick limb bud. *Cell Differentiation and Development*, 1990; 32(1): 17-26.
[18] Ono T, Tamaoka T, Yuasa Y. Reaction of alpha-(phenylsulfinyl)acetonitrile with aldehydes and ketones to gamma-hydroxyalkenenitriles and syntheses of terpenoids. *Journal of the American Chemical Society*, 1984; 106(25): 7890-7893.
[19] Shafritz DA. Synthetic retinoids for the secondary prevention of hepatocellular carcinoma. *New England Journal of Medicine*, 1996; 334(24): 1600-1601.
[20] Decensi A, Costa A. Polyprenoic acid in hepatocellular carcinoma. *New England Journal of Medicine*, 1996; 335(7): 1461-1462.
[21] Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nature Reviews Gastroenterology & Hepatology*, 2019; 16(10): 589-604.
[22] de’ Angels N, Landi F, Carra MC, Azoulay D. Managements of recurrent hepatocellular carcinoma after liver transplantation: A systematic review. *World Journal of Gastroenterology*, 2015; 21(39): 11185-11198.
[23] Shirakami Y, Sakai H, Shimizu M. Retinoid roles in blocking hepatocellular carcinoma. *Hepatobiliary Surgery and Nutrition*, 2015; 4(4): 222-228.
Prevention of hepatocellular carcinoma by targeting MYCN-positive liver cancer stem cells with acyclic retinoid. *Proceedings of the National Academy of Sciences*, 2018; 115(19): 4969-4974.

Lehmann-Che J, Bally C, de Thé H. Therapy resistance in APL. *New England Journal of Medicine*, 2014; 371(12): 1171-1172.

Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. *Nature Reviews Cancer*, 2013; 13(10): 714-726.

Liu J, Tan Y, Zhang H, Zhang Y, Xu P, Chen J, Poh YC, Tang K, Wang N, Huang B. Soft fibrin gels promote selection and growth of tumorigenic cells. *Nature Materials*, 2012; 11(8): 734-741.

Tan Y, Tajiik A, Chen J, Jia Q, Chowdhury F, Wang L, Chen J, Zhang S, Hong Y, Yi H, Wu DC, Zhang Y, Wei F, Poh YC, Seong J, Singh R, Lin LJ, Doğanay S, Li Y, Jia H, Ha T, Wang Y, Huang B, Wang N. Matrix softness regulates plasticity of tumour-repopulating cells via H3K9 demethylation and Sox2 expression. *Nature Communications*, 2014; 5(9): 4619.

Germain P, Kammerer S, Perez E, Peluso-Ilitis C, Tortolani D, Zusi FC, Starrett J, Lapointe P, Daris JP, Marinier A, De Lera AR, Rochel N, Gronemeyer H. Rational design of RAR-selective ligands revealed by RARbeta crystal structure. *Embo Reports*, 2004; 5: 877-882.

Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Medicine*, 1997; 3(7): 730-737.

Visvader JE, Lindeman GJ. Cancer stem cells in solid tumors: accumulating evidence and unresolved questions. *Nature Reviews Cancer*, 2008; 8(10): 755-768.

Cao X. Retinoids Induced Cancer Stem Cell Differentiation and Apoptosis for Cancer Therapies. *Molecular and Cellular Therapy*, 2019; 7: 1-8.