Editorial

Retinoids Induced Cancer Stem Cell Differentiation and Apoptosis for Cancer Therapies

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Abstract

Retinoids show great potential in various kinds of cancer chemotherapy due to its ability to induce signals for cell differentiation or death, as well as inhibit cancer stem cell proliferation. This paper summarized the recent progress of retinoids induced cancer stem cell differentiation and apoptosis in cancer therapy field, with the highlighted novel retinoid named WYC-209 in our lab, which could inhibit the tumor stem cell and malignant melanoma tumors with high efficacy and little toxicity.

Keywords: Retinoids, cancer stem cell, differentiation, apoptosis.

The discovery of retinoic acid receptors (RARs) from early research elucidates how vitamin A (Figure 1(A)) regulates mammalian physiology including spermatogenesis, fertilization, pregnancy maintenance, morphogenesis and organogenesis. Then studies indicate the main metabolized active form of Vitamin A, all-trans retinoic acid (ATRA, Figure 1(B)), played important roles in certain DNA activation and protein expression regulations [1]. Accordingly,

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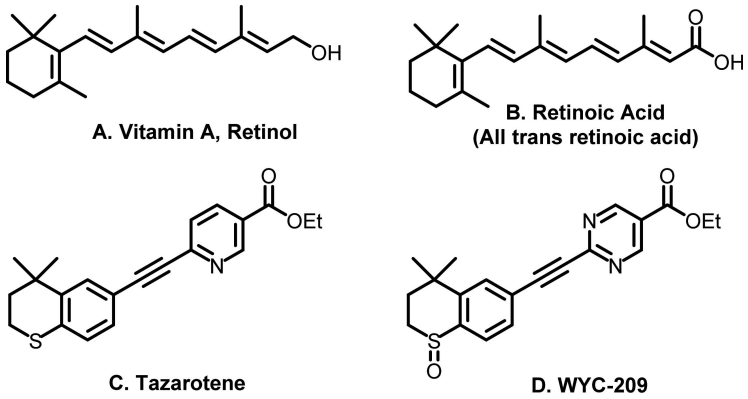

Figure 1 Structures of several retinoids.

retinoids firstly occurred as naturally low-molecular weight, fat-soluble unsaturated isoprenoids, including retinaldehyde, 9-*cis* retinoic acid and 13-*cis* retinoic acid et al., which also played essential roles as *all-trans* retinoic acid (ATRA) in various aspects of human vision, immunity, as well as fetal cell proliferations [2]. Follow this rationale, scientists designed and developed more synthetic retinoic acid analogues, such as tazarotene (Figure 1(C)), tamibarotene and bexarotene, which could be used for regulating retinoic acid receptors (RARs), and largely broadened the range of retinoids. Since retinoids mainly influence cell growth, differentiation, and death, the deregulation of RAR signaling pathways with retinoids has being linked to tumorigenesis therapies [3].

The discovery of cellular heterogeneity and functional hierarchy within various tumor tissues implies highly pluralisms existing in a population of cells, while further evidences prove it seems only a small fraction of the cancer cells, named cancer stem cells (CSCs) [4, 5] or tumor-repopulation cells (TRCs) [6–8], owned the unlimited self-renewal and hence tumorigenesis characters. Bonnet and Dick firstly isolated a subpopulation of leukemia cells that expressed surface marker CD34 but not CD38 in 1997 [9]. They established that the $CD34^{+}/CD38^{-}$ subpopulation is capable of initiating tumors in NOD/SCID mice that were histologically similar to the donor. The first evidence of a solid tumor cancer stem-like cell followed in 2002 with the discovery of a clonogenic, sphere-forming cell isolated and characterized from adult human brain gliomas [10]. Cancer stem cells isolated from adult human gliomas were shown to induce tumors that resembled the parent tumor when grafted into intracranial nude mouse models [11].

However, the identification of stem-cell-like cancer cells through conventional methods that depends on stem cell markers was often so reliable. In 2012, Wang et al. developed a mechanical method for selecting tumorigenic cells by culturing single cancer cells in fibrin matrices of 100 Pa in stiffness [6]. When cultured within these gels, primary human cancer cells or single cancer cells from mouse or human cancer cell lines grew within a few days into individual round colonies that resembled embryonic stem cell colonies. Subcutaneous or intravenous injection of 10 or 100 fibrin-cultured cells in syngeneic or severe combined immunodeficiency mice led to the formation of solid tumors at the site of injection or at the distant lung organ much more efficiently than control cancer cells selected using conventional surface marker methods or cultured on conventional rigid dishes or on soft gels. Remarkably, as few as ten such cells were able to survive and form tumors in the lungs of wild-type non-syngeneic mice. Further research data suggest that 3D soft-fibrin-matrix-mediated cell softening, H3K9 demethylation and Sox2 gene expression are essential in regulating the so called Tumor-repopulating cells (TRCs) [7, 8].

Retinoic Acid in Cancer Differentiation Therapy

RA is a key regulator of multiple differentiation pathways through activation of RA-responsive transcription factors in embryo development and tissue formation based on the differentiation function. So they were natural ideas for scientist that if people could induce differentiation strategy towards the cancer stem cells, and cause their self-renewal ability lost through reprogramed transcriptional regulations. It could be an exciting method for cancer therapies [1, 2, 12]. As the consequence, retinoids induced differentiation cancer therapy method is always an attracting topic in cancer treatments. The notable success has been widely recognized, which the treatment of acute promyelocytic leukemia (APL), a condition that is now highly curable by the combination of retinoic acid (RA) and arsenic [13, 14].

Current understanding of acute promyelocytic leukemia (APL) pathogenesis depicts the dual roles of transcriptional silencing and promyelocytic leukemia protein (PML) nuclear body disorganization. Retinoic acid receptor-α (RARα) is a retinoic acid (RA)-responsive transcription factor that, when bound to its retinoid X receptor (RXR) co-receptor, binds specific sequences, known as retinoic acid response elements (RAREs)224. The fusion protein PML–RARα blocks basal transcription of a number of targets, notably those involved in differentiation [14].

RA induced differentiation therapy strategy are also explored in primary solid tumors, though the situations of most solid tumors are considerably much more complex than that of APL [15]. The expression of the sodium/iodide cotransporter (SLC5A5) is often lost in thyroid cancer. However, RA treatment can rescue the SLC5A5 expression through transcriptional reactivation of the initially silenced gene. This strategy permits subsequent radioactive iodine treatment, which selectively accumulates in normal cells or transformed thyroid cells expressing SLC5A5. Moreover, the RA therapy approach did not reverse the iodine resistance of thyroid cancers according to related clinical trial results. The therapeutic potential of retinoids is based on their key role in the regulation of cell differentiation, growth, and apoptosis, which provides a basis for their use both in cancer therapy and chemoprevention [15].

Retinoic Acid Induced Cancer Stem Cell Apoptosis

Besides the differentiation function, the regulation of RARs by retinoic acid (RA) often results in proliferation inhibition. For more effectively abrogating the growth and metastasis of malignant tumors, scientists developed lots of novel synthetic retinoid. With the same purpose, a novel synthetic retinoid library was established via a parallel synthesis manner in house, and drug screening was carried out using the developed 3D B16-F1 TRC models [16]. Among these new probes, a wonderful drug candidate namely WYC-209 (Figure 1(D)), which inhibits proliferation of malignant murine melanoma tumor-repopulating cells (TRCs), known to resist the conventional chemotherapeutic drug treatment, including *cis*-platin and doxorubicin. In contrast with the failed first-line therapy drugs such as doxorubicin and cisplatin, WYC-209 showed effective in efficiently inducing apoptosis of melanoma TRCs with an IC_{50} of 0.19 μM in a dose-dependent manner. Moreover, WYC-209 inhibits proliferation of TRCs of human melanoma, lung cancer, ovarian cancer, and breast cancer in culture at similar level. The treated TRCs fail to resume growth even after the drug washout treatment accordingly. WYC-209 also works well during the in vivo evaluation, since it could abrogate round 88% of lung metastases of melanoma TRCs in immune-competent wild-type C57BL/6 mice at 0.22 mg kg^{-1} without showing apparent toxicity.

The possible mechanism of WYC-209 is to regulate self-renewing gene Sox2 and master differentiation gene Mitf in B16 cells. We found that Sox2 expression decreased by ˜50% when TRCs were treated with the 10 μM compound, but no change was observed in Sox2 when TRCs were treated

with 0.1 or 1 μM compound. Mitf expression did not change when the cells were treated with these compounds at 0.1–10 μM for 24 h. These results suggest that WYC-209 did not induce TRC differentiation. WYC-209 induces TRC apoptosis and pretreating the TRCs with caspase 3 inhibitor or depleting caspase 3 with siRNAs substantially rescues growth of TRCs from WYC-209 inhibition, suggesting that WYC-209 induces TRCs apoptosis primarily via the caspase 3 pathway, while the exact mechanism for the finding that WYC-209 has different impacts on the cells at 1 μM and at 10 μM is still on the way. Our findings demonstrate the promise of the new retinoid WYC-209 in treating malignant melanoma tumors with high efficacy and little toxicity.

Challenges and Perspective

Retinoids show great potential in various kinds of cancer chemotherapy due to its ability to induce signals for cell differentiation or death, as well as inhibit cancer stem cell proliferation; in the meanwhile, novel retinoid drug are developed and the cancer retinoid treatment strategy has made big progress in the clinics, especially in leukemia therapy area. However, the drug resistance towards retinoids also emerges in their clinic usage process. Though the retinoid drug resistances are commonly categorized as either acquired drug resistance or intrinsic drug resistance, the phenotypic features of these resistances are usually based on similar mechanisms which are closely related to or combined in both of the above types [5, 17–19].

ATRA in combination with different antitumor drugs could be a solution for avoiding the drug resistance problem. The combination of retinoid drugs with tyrosine kinase inhibitors, HER2 inhibitors, taxoids as well as other chemotherapeutic drugs have been tried in various clinical treatments. These research demonstrated additive or synergistic effects in anti-cancer activities of retinoid drugs. The mechanisms by which the compounds demonstrate their noticeable synergistic effects depend on the tumor categories and the driven cell types. For example, when ATRA was combined with sorafenib, a multi-kinase inhibitor, there was an obvious increase in gap junctional intercellular communication and connexin expression levels, which was associated with the observed increase in sorafenib-related cell growth inhibition and apoptosis induction [20]. When the retinoid drug resistance problems are well controlled, the retinoid drug therapy towards both leukemia and solid tumors through the cancer stem cell differentiation or induced apoptosis pathway could be a more important choice in clinics.

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Biography

Xin Cao received his B.S. and Ph.D degrees in Pharmaceutical Science and Medicinal Chemistry in China Pharmaceutical University, China. Dr. Cao pursued his post-doctoral trainings in Shanghai Institute of Organic Chemistry and Harvard University in 2009 and 2013 respectively. Dr. Cao became a YIPA member of China Academy of Sciences in 2017, and he promoted to be the PI in Zhongshan Hospital Institute of Clinical Science, Fudan University Shanghai Medical College in 2018.