Chapter

Retinoids in Treatment of Colorectal Cancer

Caroline O.B. Facey and Bruce M. Boman

Abstract

Retinoids are vitamin A metabolites best known for their role in embryonic development. Indeed, retinoid acid (RA) signaling plays a key role in regulating the development of the embryo body-plan by controlling embryonic stem cells (SCs). Retinoids function through their ability to induce cellular differentiation. Mutations in RA signaling pathway genes occur in most human cancers. The classic example is the chromosomal translocation involving RA receptor alpha in acute promyelocytic leukemia (APL). Because all-trans retinoic acid (ATRA) is a highly effective and often curative treatment for APL patients, determining if retinoids are efficacious for other cancer types is imperative. We review the current research on retinoids in colorectal cancer (CRC) and provide bioinformatics analyses of RA signaling. Our results show that most RA pathway genes are overexpressed and often mutated in CRC. Moreover, aberrant expression of many RA signaling proteins predicts decreased CRC patient survival. We also review aldehyde dehydrogenase (ALDH) expression in CRC because ALDH is a key enzyme in RA signaling, which regulates colonic SCs. Further investigation of RA signaling mechanisms that regulate colon SCs and how dysregulation contributes to the SC overpopulation that drives CRC growth should provide insight into strategies for designing new SC-targeted therapies for CRC.

Keywords: retinoic acid, stem cells, colon cancer, adenomatous polyposis coli, aldehyde dehydrogenase

1. Introduction

Our goal herein is to review current research findings on retinoids in colorectal cancer (CRC), and to provide an update from our bioinformatics analysis of RA signaling components in CRC. Retinoic acid (RA) is currently being used in the treatment of specific types of human cancers [1]. The classic example is use of ATRA as first line treatment for acute promyelocytic leukemia (APL). RA therapy has also been shown to improve survival in patients with neuroblastoma [2–4]. Additionally, RA-based agents have been evaluated for clinical anti-cancer activity in breast cancer and in lung cancer [5]. In this review, we discuss the anti-cancer activity of retinoids using *in vitro* and *in vivo* models of CRC, and the use of ATRA as a differentiation agent in SC research [4, 6–8].

A strong rationale to investigate RA signaling in oncology research is that ATRA is an effective drug used to treat APL patients. Indeed, ATRA effectively induces APL cells to terminally differentiate into neutrophils [9–11]. Current treatment regimens for APL also include arsenic in combination with ATRA because the combination provides a synergic drug response that cures the majority of APL patients, who

would otherwise be facing a highly fatal illness. The precise mechanism involved in triggering APL cells have been extensively studied with the hope of understanding how it can be applied to trigger differentiation in other cancer types. What appears to be the basis for clinical success in treating APL is that the RA/arsenic combination not only induces terminal differentiation, but it also abrogates self-renewal of APL SCs [12]. Thus, future retinoid-based treatments for other cancers will likely necessitate drug combinations that incorporate a RA signaling differentiation therapy and a SC-targeting therapy that inhibits cancer SC self-renewal.

2. Key components of the retinoic acid signaling pathway

To understand how the RA signaling pathway is altered in cancer and to provide a basis for designing retinoid-based treatment approaches to cancer, we provide a brief description of the key components in the RA signaling pathway. The reader is referred to Das et al. [13] for more detailed information. Listed below are the main proteins essential to proper functioning of the RA signaling pathway. A simplified schematic of the RA signaling pathway is illustrated in **Figure 1**.

2.1 STRA6 (stimulated by retinoic acid 6)

STRA6 is a cell surface protein that functions as a receptor to accept all-trans retinol from the extracellular retinol-binding protein RBP4 and to transport retinol across the cell membrane. STRA6 removes the retinol from RBP4 and transfers it to RBP1 in the cytoplasm. STRA6 does not transport RA.

2.2 LRAT (lecithin retinol acyltransferase)

LRAT is an enzyme that converts retinol to all-trans retinyl esters, which is a storage form of vitamin A. LRAT also functions to enhance cellular uptake of retinol by STRA6, which contributes to the activation of the RA signaling cascade.

2.3 RDHs (retinol dehydrogenases)

RDHs are a family of dehydrogenase enzymes involved in the conversion of retinol to retinaldehyde by catalyzing the oxidation of cis-isomers of retinol, including 11-cis-, 9-cis-, and 13-cis-retinol in an NAD-dependent manner. This family of short-chain dehydrogenases/reductases functions to catalyze the final step in the biosynthesis of 11-cis retinaldehyde.

2.4 DHRS3 (retinaldehyde reductase-3)

DHRS3 is an oxidoreductase that catalyzes the oxidation/reduction of all-trans-retinal to all-trans-retinol in the presence of NADPH. DHRS3 is essential for preventing the formation of excess RA during embryonic development.

2.5 ADHs (alcohol dehydrogenases)

ADHs are a family of alcohol dehydrogenases involved in retinoid metabolism via conversion of retinol to retinaldehyde by catalyzing the NAD-dependent oxidation of all-trans-retinol and its derivatives such as all-trans-4-hydroxyretinol. These enzymes metabolize a wide variety of substrates, including ethanol, retinol,

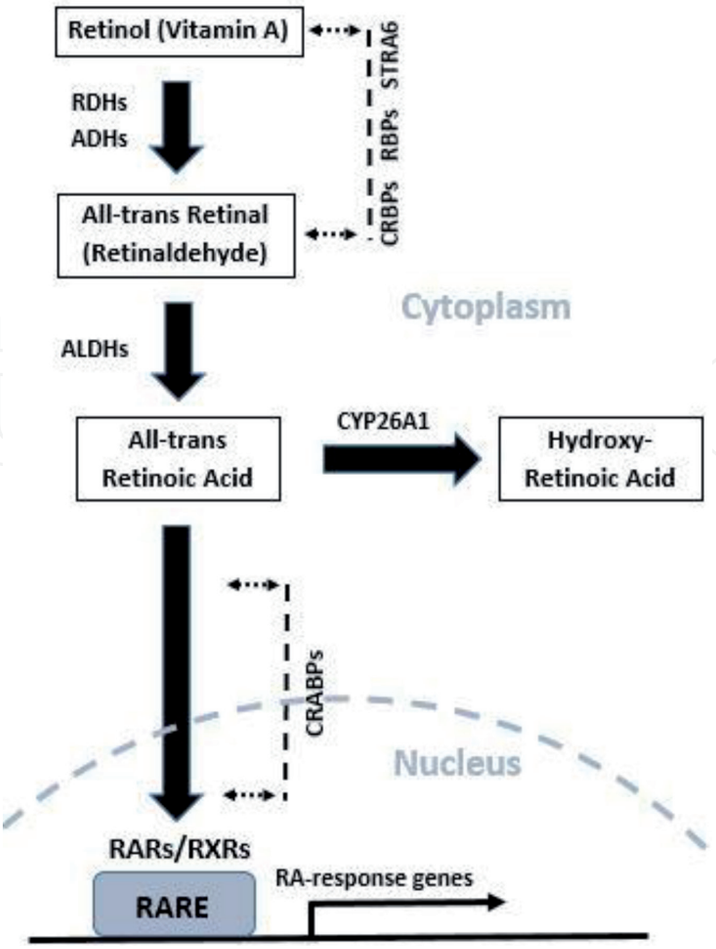

Figure 1.
This figure illustrates a simplified schematic of the RA signaling pathway, which plays a key role in embryogenesis and adult tissue homeostasis. The cell surface protein STRA6 accepts all-trans retinol from the extracellular milieu to transfer it across the cell membrane into the cytoplasm. STRA6 does not transport retinoic acid. After transfer or diffusion into cytoplasm, the internalized free retinol is bound to CRBP or is oxidized to retinal by retinol dehydrogenases (RDH) or alcohol dehydrogenases (ADH) and eventually to form all-trans retinoic acid (ATRA) by aldehyde dehydrogenases (ALDHs). ATRA then binds to cellular retinoic acid-binding proteins (CRABPs), which transfers ATRA to the nucleus. Once localized in the nucleus, ATRA serves as a ligand for binding to retinoid X receptors (RXRs) and retinoic acid receptors (RARs). Once ATRA travels to the nucleus, it binds RARs to induce the transcription of retinoid-responsive genes. Specifically, bound ATRA (or other ligands such as 9-cis) induces formation of a heterodimer (RA:RAR:RXR) in a complex at retinoic acid receptor elements on DNA, which then is able to induce transcription of RA-response genes. Thus, the RAR:RXR heterodimer acts as the main transcription factor in the classical RA signaling pathway. Nonetheless, the rate of formation of the RA:RAR:RXR complex, is still affected by other intracellular RA binding proteins such as CRABPs, which can sequester RA in the cytosol and limit the amount of RA available for binding to RARs. CRABPs can also facilitate RA degradation by directing RA to CYP26A1 RA-degrading enzymes. STRA6 = stimulated by retinoic acid 6, RDHs = retinol dehydrogenases, ADHs = alcohol dehydrogenases, RBPs = retinol binding proteins, ALDHs = aldehyde dehydrogenases, CRABPs = cellular retinoic acid binding proteins, CYP26A1 = cytochrome p450 family 26 subfamily a member 1, RARE = retinoic acid response element; RXRs = retinoid X receptors, RARs = retinoic acid receptors.

aliphatic alcohols, hydroxysteroids, and products of lipid peroxidation. ADHs consist of several homo- and heterodimers of alpha, beta, and gamma subunits, which plays major roles in ethanol catabolism. For example, three genes encoding alpha, beta, and gamma subunits of ADH1 are tandemly organized in a genomic segment as a gene cluster.

2.6 RBPs (retinol-binding proteins)

RBP1 (Retinol Binding Protein 1) and RBP2 (Retinol Binding Protein 2) are cytoplasmic retinol-binding proteins, which contribute to retinol uptake, storage,

and retinoid homeostasis. Specifically, RBP1 is the carrier protein for transport of retinol from the liver storage site to peripheral tissue. RBP2 also plays an important role in the uptake and intracellular transport of retinol, which is necessary for intracellular metabolism of vitamin A.

2.7 ALDHs (aldehyde dehydrogenases)

ALDHs are cytoplasmic enzymes that convert/oxidize retinaldehyde to RA. ALDHs are the enzymes that function after the alcohol dehydrogenase step in the RA signaling pathway. Nineteen ALDH isoforms encoded by 19 different genes exist in humans with as many orthologs in the mouse plus some alternatively spliced transcriptional variants. Through its role in retinol metabolism, ALDHs play a major role in the regulation of responses to RA.

2.8 CRABPs (cellular retinoic acid-binding proteins)

CRABP1 (Cellular Retinoic Acid Binding Protein 1) and CRABP2 (Cellular Retinoic Acid Binding Protein 2) are paralogous genes that encode cellular RA binding proteins. These proteins transport RA to the nucleus and function to regulate the access of RA to the nuclear RA receptors. Specifically, CRABPs are cytosol-to-nuclear shuttling proteins, which facilitate RA binding to its cognate receptor complex and nuclear transfer. These activities in the retinoid signaling pathway play an important role in RA-mediated differentiation and proliferation processes. CRABPs are structurally similar to the cellular retinol-binding proteins, but CRABPs only bind RA, which contributes to RA-directed differentiation in epithelial tissue. Diseases associated with CRABPs include embryonal carcinomas.

2.9 CYP26A1 (cytochrome P450 family 26 subfamily A member 1)

CYP26A1 is a cytochrome P450 monooxygenase that plays a key role in the metabolism of ATRA. The cytochrome P450 superfamily contains 57 members that are monooxygenase enzymes which catalyze many processes including drug metabolism and synthesis of cholesterol, steroids, and various lipids. CYP26A1 acts on ATRA by catalyzing the hydroxylation of carbon hydrogen bonds of ATRA. This includes both 4-hydroxylation and 18-hydroxylation activities. It has little activity toward 9-cis and 13-cis RA ligands. By regulating intracellular concentrations of RA, CYP26A1 can control RA signaling mediated gene expression in both embryonic and adult tissues. There are two alternatively spliced transcript variants of CYP26A1 that encode the different isoforms. This enzyme regulates the cellular level of RA which in turn regulates gene expression in both embryonic and adult tissues. Diseases associated with CYP26A1 include embryonal carcinoma and APL.

2.10 Retinoid X receptors (RXRs) and retinoic acid receptors (RARs)

The proteins encoded by RARs (*RARA, RARB, RARG*) and RXR (*RXRA, RXRB, RXRG*) genes are classified as members of the steroid and thyroid hormone receptor superfamily of transcriptional regulators. Various receptor isoforms can result from differential splicing of RA receptor genes and alternate promoter usage. RXRs and RARs are nuclear receptors that are central to retinoid acid (RA) signaling through their role in RA-mediated gene activation in response to their ligands ATRA or 9-cis retinoic acid. The 9-cis RA ligand has a high affinity for RXRs. These receptors are localized to cytoplasm and sub-nuclear compartments where they can bind RA to activate cellular signaling by forming homodimers or heterodimers. These dimers

primarily act as transcription factors via binding to the retinoic acid response elements (RARE) made of tandem 5'-AGGTCA-3′ sites known as DR1-DR5. When the ligand is absent, RXRA/RARB forms a multiprotein complex containing transcription co-repressors that can induce histone deacetylation, chromatin condensation and transcriptional suppression. When the ligand is present, it induces the co-repressors to dissociate from the receptors and co-activators are recruited which leads to transcriptional activation. Moreover, depending on the RARE DNA element condition, the heterodimer can act as a transcriptional repressor or transcriptional activator. For example, the heterodimer can act as a repressor on the DR1 element and as an activator on the DR5 element. RA receptors can also dimerize with thyroid hormone, and vitamin D receptors, which increases their DNA binding and transcriptional effects on their respective response elements. RA signaling regulates gene expression in various biological processes such as embryonic morphogenesis, granulocytopoiesis, and skeletal growth. It also plays an essential role in mediating the antiproliferative effects of RA by inducing cellular differentiation and apoptosis. In oncology, translocations between RARA and other loci are associated with the development of APL.

Now that we have briefly covered the key components in the RA signaling pathway that are critical to its proper function, we will discuss alterations of this pathway that occur in CRC.

3. Studies on alterations of retinoic acid signaling in CRC

Many studies have been done to identify mechanisms that explain how RA resistance occurs in solid tumors. Indeed, CRCs have been shown to lose the ability to produce ATRA and fail to growth inhibit or differentiate in response to treatment with ATRA [14–16]. Retinoic acid resistance appears to arise spontaneously in human cancers. To assess how alterations in RA signaling components effect response to RA ligands, we performed a literature search. Most of the published studies discussed below used *in vitro* experiments on CRC cell lines and analysis of human CRC tissues.

In a study by Jette et al. [16], seven CRC cell lines were evaluated for retinol dehydrogenase (RDH) enzymatic activity. They found CRC cells have decreased conversion of retinol into RA compared to normal cells. This inhibition of RDH expression appeared to be due to loss of adenomatous polyposis coli (APC) function. Interestingly, reintroduction of *wild-type APC* into an *APC*-mutant CRC cell line (HT29) increased expression of DHRS9 (RDHL) but not RDH5. Transfection of *wild-type APC* also increased production of RA. This study indicates intracellular crosstalk occurs between WNT signaling and RA signaling pathways.

Another study by Park et al. [14] examined the ability of retinol to inhibit the growth of CRC cell lines. They observed that some CRC cells are ATRA-sensitive (HCT-15) and other cells are ATRA-resistant (HCT-116, SW620, and WiDR). They also found that retinol inhibited the growth of both ATRA-sensitive and ATRA-resistant CRC cells through a RA receptor-independent mechanism.

Other studies by Shelton et al. [17] evaluated for over-expression of CYP26A1 enzymes that could lead to increased ATRA degradation. Indeed, CYP26A1 was upregulated in *APC*–deficient CRC tissues which provides a mechanism that might explain how increased WNT-signaling might be tied to impaired RA-signaling function in ATRA-resistant cells.

Lecithin retinol acyltransferase (LRAT), which esterifies retinol to retinyl esters, has also been evaluated by Cheng et al. [18]. Indeed, the LRAT gene promoter was hypermethylated in CRC cell lines and neoplasms compared to normal tissue [18]. A decrease in LRAT expression due to hypermethylation could lower availability of retinoids and reduce intracellular storage of retinol.

Additionally, several studies have investigated whether RA receptors are intact in CRC cells [19]. We discuss below a few studies that reported loss of RAR in CRC cells. In one study by Moison et al. [20], epigenetic changes appeared to lead to loss of RARB expression in HCT116 cells from DNA hypermethylation [20]. Interestingly, a DNA methylation inhibitor is able to restore RARB expression [21]. In a second study by Nicke et al. [22], the RA-resistant LoVo CRC line was induced to over-express RARB, which produced responsiveness to ATRA resulting in growth inhibition. A third study by Lee et al. [23] had similar results. They observed that ATRA treatment of RA-sensitive and RA-resistant CRC lines induced *RARA* expression in all cell lines, but ATRA only increased RARB expression in lines that were sensitive to RA. The DLD-1 RA resistant cells acquired sensitivity to ATRA when RARB was over-expressed. Additional studies that examine RA resistance due to alterations in RARs have also been reported [23, 24].

Finally, a recent study by Kropotova et al. [15] used RT-PCR to measure expression patterns of genes involved in ATRA biosynthesis. They evaluated normal human colorectal tissues, primary carcinomas, and cancer cell lines. Expression of most genes involved in ATRA synthesis was altered in CRC tumors and colorectal cell lines. Moreover, the expression of several genes, particularly ADH isoforms ADH2 and ADH3, showed decreased gene expression in adenomas when compared to more advanced carcinomas.

Overall, the studies on CRC discussed above show that RA signaling components become altered at many levels along the pathway. This includes: (i) loss of RAR expression that impairs RA response and gene transcription; (ii) decreased ability to enzymatically synthesize ATRA; (iii) LRAT alterations that impair retinoid storage; (iv) enhanced degradation of ATRA via CYP26A1. Many of these alterations appear to be a consequence of the mutations, such as *APC*, that drive CRC development [1, 25]. Thus, as CRC progresses, tumor cells develop resistance to ATRA by losing their ability to produce and respond to it, as well as, by causing its degradation.

4. Animal model studies

In addition to the studies on RA signaling in cell lines and CRC tissues discussed above, other important investigations have been done using animal models. Many of these studies were done using azoxymethane (AOM) or 1,2-dimethylhydrazine (DMH) to induce colonic neoplasms in rats to investigate the anti-tumor effects of retinoids [26]. An early study by Stopera and Bird [27] found that ATRA treatment reduced the number of AOM-induced aberrant crypt foci (ACF), a precursor to CRCs. Two studies [28, 29] using the DMH-induced colon carcinogenesis model indicated that vitamin A dietary supplementation may diminish ACF formation. Other studies by Wargovich et al. [30, 31] reported that 13-cis-retinoic acid (13-cRA), 9-cis-retinoic acid, and the synthetic Vitamin A derivative 4-hydroxy-phenretinamide (4-HPR) diminished AOM-induced ACF in rats. An interesting study by Zheng et al. [32] screened thirteen retinoids for prevention of ACF. They found that two retinoids, 9-cis-retinoic acid and 4-HPR, reduced both colonic ACF and tumor formation. In another study by Zheng et al. [33], 2-(carboxyphenyl)retinamide (2-CPR) was evaluated because it prevents ACF. However, they found that this synthetic retinoid analogue increased the number of colon tumors. Thus, these studies on rats show that ATRA, retinol, 9-cis-retinoic acid, 4-HPR, 13-cRA, and 2-CPR can inhibit the formation of carcinogen-induced ACF. However, only 9-cis-retinoic acid and 4-HPR were

shown to reduce colonic tumor formation, and 2-CPR actually increased the number of colon tumors in this rat model.

Several other animal studies to evaluate the effect of retinoids have employed the *Apc*$^{Min/+}$ mouse model. Experiments using this model are important because these mice develop intestinal tumors due to *Apc* mutations and *APC* is a driver mutation for CRC growth in humans.

A study of *Apc*$^{Min/+}$ mice by Volate et al. [34] showed that retinoic receptors including *Rara, Rarb, Rxrb, Rxrg* were all expressed in *Apc*$^{Min/+}$ adenomas. However, in AOM-treated *Apc*$^{Min/+}$ mice, *Rxra* was selectively downregulated in intestinal tumors. Therefore, these findings indicate that *Rxra* downregulation occurs early in CRC carcinogenesis and is not dependent on *Apc* mutations and beta-catenin.

Another study by Mollersen et al. [35] administered ATRA to *Apc*$^{Min/+}$ mice and discovered that ATRA treatment failed to prevent tumor formation. Three studies were then performed that gave results which provide mechanisms that helps explain this unexpected ATRA resistance.

One line of investigation focused on C-Terminal Binding Protein 1 (CTBP1), which has been reported to inactivate retinoid dehydrogenase RDH [36]. Examination of adenomas from *Apc*$^{Min/+}$ mice and familial adenomatous polyposis coli (FAP) patients showed an increased expression of CTBP1. Because CTBP1 decreases RDH levels, upregulated CTBP1 will lead to lower ATRA levels in tumors [37].

In another study on *Apc*$^{Min/+}$ mice, Shelton et al. [17] analyzed expression levels of CYP26A1, the major RA catabolic enzyme. They found that CYP26A1 expression was increased in tumors from *Apc*$^{Min/+}$ mice, and in tumors from FAP patients. They also determined that CYP26A1 is a TCF4 target gene which explains why CYP26A1 expression is increased due to upregulated WNT signaling in *APC* mutant tissues. An increase in CYP26A1 would lead to increased ATRA degradation, which provides a mechanism that helps explain why ATRA treatment failed to prevent tumor development in *Apc*$^{Min/+}$ mice.

A recent innovative study by Penny et al. [38] involved treating *Apc*$^{Min/+}$ mice with the CYP26a inhibitor Liarozole. Administration of Liarozole to *Apc*$^{Min/+}$ mice increased endogenous RA signaling (presumably by blocking ATRA metabolism) and effectively reduced intestinal adenoma numbers in these *Apc* mutant mice. We also found that treatment of human CRC cells with Liarozole decreased proliferation, sphere formation and size of the ALDH+ stem cell population [39]. This suggests that Liarozole might decrease tumor stem cell numbers in *APC* mutant tissues.

Thus, the above discussed animal model studies have provided valuable information on how the retinoid pathway might be targeted in designing treatment approaches for human CRC patients. The studies using chemical carcinogen models show that different retinoid drugs have different activities against colon tumors. The studies using the *Apc*$^{Min/+}$ model reveal it might be an effective screen for other retinoid drugs that have anti-tumor activity against *APC* mutant tissues. Perhaps a reasonable place to start would be to screen other agents for their ability to inhibit specific cellular processes upregulated in tumors that lower endogenous ATRA levels and decrease RA signaling.

5. Clinical studies

There have been an increasing number of clinical trials done on solid tumors using retinoids. However, our search of trials listed www.clinicaltrials.gov does not show any trials on CRCs using retinoids, Tretinoin or Liarozole. There were several

trials listed for breast, lung, prostate, pancreatic, renal, cervical, brain, skin, and several hematologic malignancies. Given the pre-clinical data discussed above, it seems like it would be reasonable to develop a retinoid-based trial for CRC.

6. Prospect for retinoid-based, stem cell-targeted therapies for CRC

We have been interested in the role of RA signaling in regulation of colonic SCs and how dysregulation of RA signaling may contribute to CRC development for several reasons: (i) RA regulates embryonic SCs during development [40] and WNT signaling, another key developmental pathway, has an opposing effect on embryonic SCs [41]. The idea that the mechanisms that regulate embryonic SCs are the same mechanisms that become dysregulated in the SC etiology of cancer [42] is intriguing because some scientists view cancer as aberrant organogenesis [43] and metastases as aberrant morphogenesis [44]. (ii) *APC* mutations occur in most CRCs (nearly 90%) during CRC development and *APC* mutation leads to constitutively activated WNT signaling. (iii) *APC* mutations that drive CRC development appear to do so by causing SC overpopulation [45]. (iv) ALDH, a key component in RA signaling, marks colonic SCs and tracks SC overpopulation during CRC development.

Indeed, our research team [39, 46–49] and others [50–52] have been using ALDH as a marker to identify and isolate SCs from patient tissues for several years. ALDH not only marks colonic SCs, but ALDH+ cells also have SC properties of self-renewal, drug resistance, and cell differentiation potential [53]. For example, ALDH+ cells possess self-renewing ability as shown by sphere-forming ability *in vitro* and tumor-initiating ability in mice [46, 51, 52, 54]. The drug resistance property of ALDH+ SCs comes from aldehyde dehydrogenase's enzymatic function, which is the cell's natural detoxification mechanism [50, 55, 56]. The ability of ALDH+ cells to differentiate comes from ALDH's functional role in the RA signaling pathway [5, 13, 57–59]. Moreover, we examined ALDH+ cells from colon tissues and observed that retinoid receptors RXR and RAR are selectively expressed in ALDH+ cells [39], which indicates that RA signaling mainly occurs via ALDH+ SCs. That RA signaling primarily occurs in ALDH+ stem cells provides a mechanism for selective treatment of SCs using RA analogues.

ATRA is commonly used as a differentiating agent in SC research. For example, we found that treatment of ALDH+ cancer SCs (CSCs) with ATRA inhibits cell proliferation, reduces SC proliferation, sphere formation, and SC population size, as well as enhances SC differentiation [39, 47]. Others have shown that retinoids decrease proliferation of ALDH+ SCs and, conversely, that inhibitors of ALDH increase proliferation of ALDH+ SCs [4, 6–8]. Because ALDH is key to retinoid acid (RA) signaling and retinoids are well known to promote differentiation of SCs [4], it follows that having ALDH in a SC provides the capacity for it to differentiate in response to retinoids.

Since *APC* mutations are known to increase WNT signaling in CRC, this raises the question: does increased WNT signaling lead to decreased retinoid signaling? Indeed, previous studies have implicated a role for APC in regulating RA biosynthesis and that *APC* mutations may lead to aberrant RA signaling [16, 36]. Notably, studies show that appropriately regulated WNT signaling is necessary for RA to induce neuronal differentiation of embryonic SCs [60]. Furthermore, not only does WNT suppress retinoid signaling, but conversely, increased RA signaling diminishes the ability of WNT signaling to block retinoid induction of the neural differentiation of SCs [61, 62]. That WNT signaling must be downregulated for neural differentiation to be inducible by RA treatment helps explain how *APC* mutation and increased WNT signaling might prevent maturation of ALDH+ colonic SCs in CRC development. Thus, it appears that *APC* mutations may alter the ability of

ALDH+ SCs to differentiate in response to retinoids, which would lead to expansion of the ALDH+ SC population size in CRC [39, 46].

7. Bioinformatics analysis of retinoid signaling components in CRC

We extended our study of RA signaling in CRC herein by using bioinformatics to analyze expression and mutation of RA signaling genes in CRCs and identify RA pathway genes that predict CRC patient survival. We found that most genes in the RA pathway are overexpressed and many are mutated in CRC (**Figure 2**). This is consonant with our previous result showing that RAR, RXR and other RA signaling proteins are overexpressed in CRC, which parallels overpopulation of ALDH-positive SCs that occurs during CRC tumorigenesis [39, 46]. Moreover, we found that aberrant expression of many RA signaling proteins (10 of 27) predicted ($p < 0.05$) decreased survival of CRC patients (**Figure 3**). We refer the reader to the meta-analysis by Chen et al. [63] which reveals that increased ALDH also indicates a poor prognosis in CRC patients. These updated findings provide insight into the complexity of RA signaling mechanisms and how RA signaling, when dysregulated, contributes to the development of CRC.

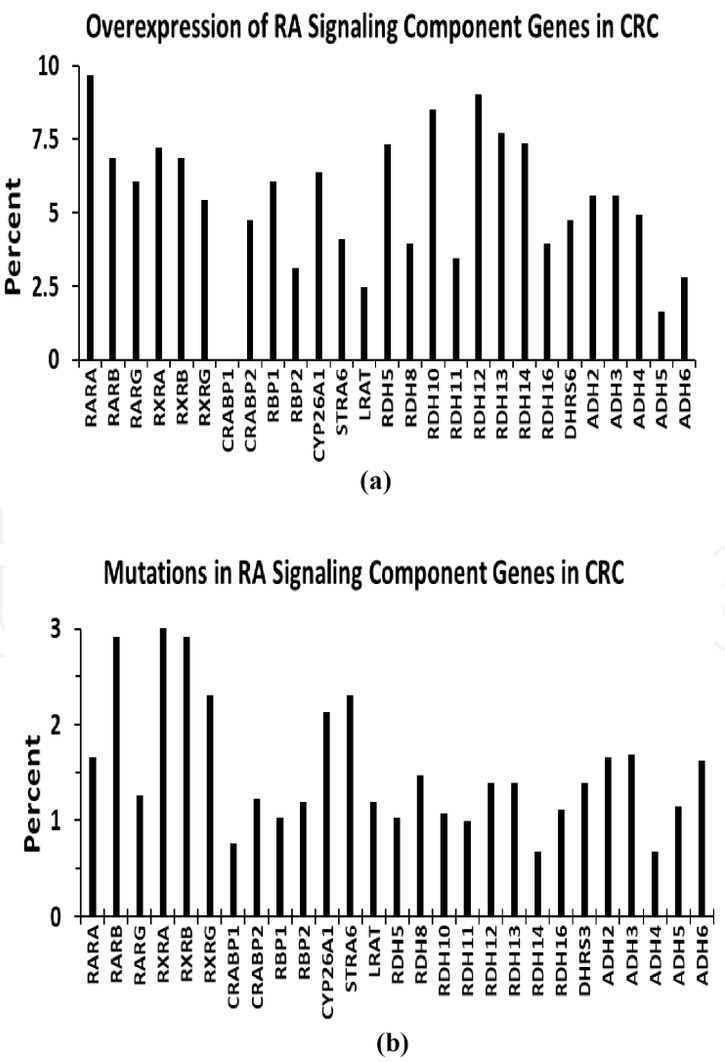

Figure 2.
Bioinformatics analyses of RA signaling genes in CRC, including overexpression (a) and mutations (b). Bioinformatics data derived from cosmic catalog of somatic mutations in cancer (https://cancer.sanger.ac.uk/cosmic).

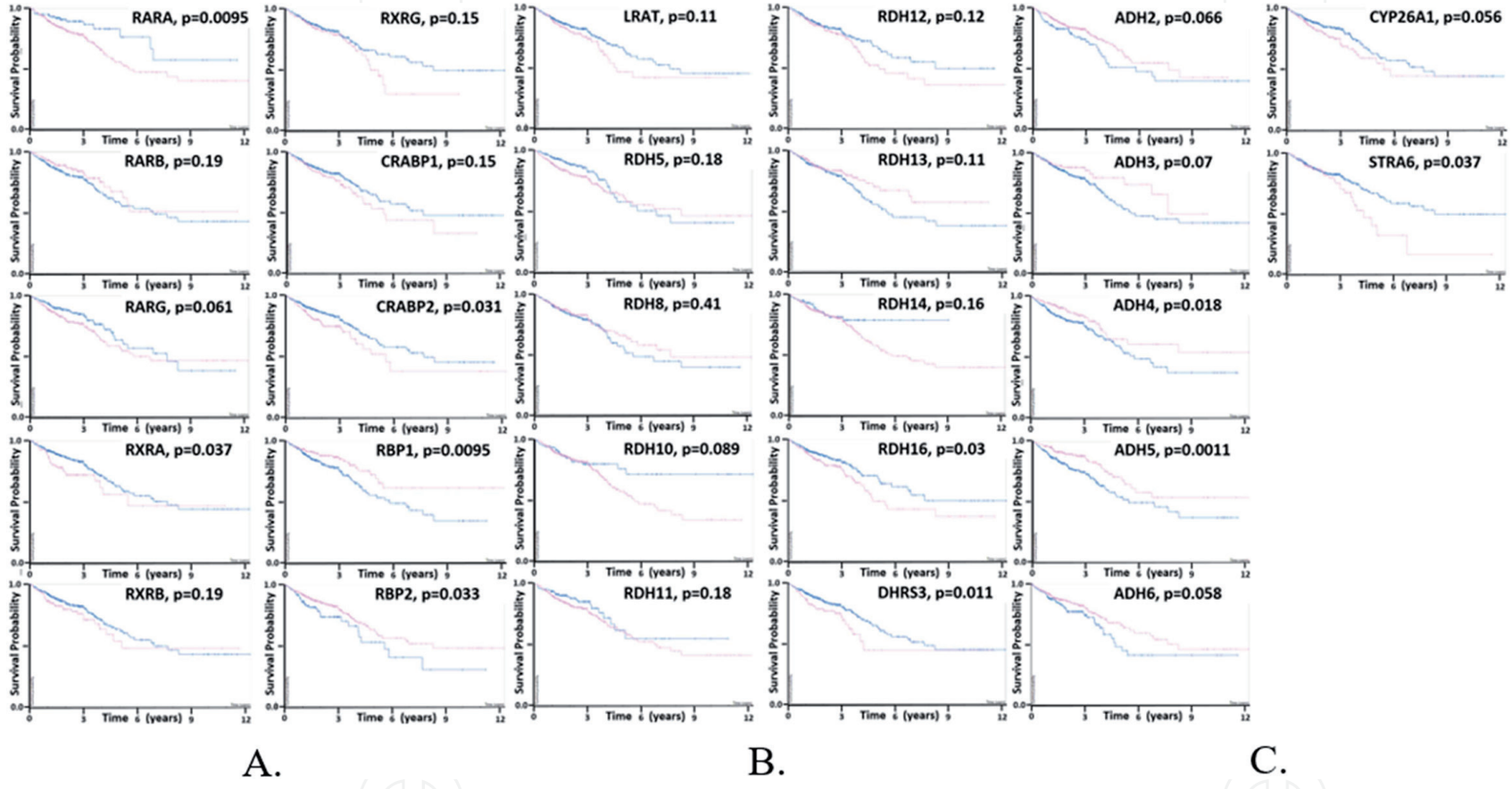

Figure 3.
Kaplan-Meier survival analysis of RA signaling genes that predict ($p < 0.05$) CRC patient survival. (A) shows survival curves for RARs (retinoic acid receptors), RXRs (retinoid X receptors), CRABPs (cellular retinoic acid binding proteins), and RBPs (retinol binding proteins). (B) shows survival curves for LRAT (lecithin retinol acyltransferase), RDHs (retinol dehydrogenases), and DHRS3 (retinaldehyde reductase-3). (B) shows survival curves for CYP26A1 (cytochrome P450 family 26 subfamily A member 1), STRA6 (stimulated by retinoic acid 6), and ADHs (alcohol dehydrogenases). Curves reflect low (blue) and high (red) gene expression. Y axis = survival probability (0.0–1.0). X axis = time (years 0–12). Bioinformatics data derived from the human protein atlas (https://www.proteinatlas.org).

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DOI: http://dx.doi.org/10.5772/intechopen.93699

CRC Cell line	APC mutation	Microsatellite instability	Beta-catenin mutation	RARA mutation	RARB mutation	RARG mutation	RXRA mutation	RXRB mutation	RXRG mutation
HT-29	Yes	Stable	No	No	No	No	No	No	No
SW480	Yes	Stabe	No	Yes	No	No	No	No	Yes
HCT116	Yes	High	Yes	Yes	No	No	No	No	No
LoVo	Yes	Low	Yes	No	No	No	Yes	No	No
DiFi	Yes	Stabe	Yes	No	No	No	No	No	No
SW48	Yes	High	Yes	Yes	No	No	No	No	No
SW1116	Yes	Stabe	No	No	No	No	Yes	No	No
COLO320	Yes	Stabe	No	No	No	No	No	No	No
LIM1863	Yes	Stabe	Yes	?	?	?	?	?	?
DLD-1	Yes	Stabe	No	?	?	?	?	?	?
RKO	No	High	No	No	No	No	Yes	No	Yes

Analysis done using the COSMIC database on human somatic mutations in cancer (https://cancer.sanger.ac.uk/cosmic).

Table 1.
Bioinformatics results on RXR and RAR mutations in CRC cell lines.

8. Conclusion and future perspectives

Our results indicate that RA signaling, when dysregulated, plays a major role in the SC origin of CRC. Overall, our review provides a strong rationale for future exploration of retinoid therapies for CRC in precision oncology. A few clues gleaned from our review are as follows: (i) drug screens using CRC cell lines (**Table 1**) and knockout of RA-signaling genes in human CRC cells might identify which retinoid drugs are active against cells with specific mutations; (ii) $Apc^{Min/+}$ mice may be useful to identify additional retinoid agents that are active against *Apc* mutant tissues; (iii) strategies for designing retinoid-based CRC therapies will likely need to incorporate retinoids into drug combination regimens; (4) CRCs will likely need to be genotyped to determine the status of RA signaling genes when administering RA-based treatments to CRC patients. Finally, continued discovery of the mechanisms that explain how RA signaling regulate normal colon SCs and how dysregulation of RA signaling in cancer SCs drive CRC growth should provide insight into how new SC-targeted therapies might be designed for CRC.

8.1 Materials and methods

The bioinformatics analysis on overexpression and mutation of RA signaling component genes in CRCs was done through the COSMIC website (cancer.sanger.ac.uk/cosmic). Bioinformatics analysis to identify RA signaling genes that predict CRC patient survival was done through The Human Protein Atlas (https://www.proteinatlas.org).

Conflict of interest

The authors do not have any conflicts of interest.

Funding

This study was supported in part by the Lisa Dean Moseley Foundation (BB), Cancer B*Ware Foundation (BB), and Cawley Center for Translational Cancer Research Fund (BB, CF).

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