

# Natural and synthetic retinoids in preclinical colorectal cancer models

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Colorectal cancer (CRC) remains a leading cause of cancer-related morbidity and mortality worldwide. Although targeted therapy in combination with chemotherapy in CRC prolongs the overall survival of patients with metastatic disease, acquired resistance and relapse hinder their clinical benefits. Moreover, patients with some specific genetic profile are unlikely to benefit from targeted therapy, suggesting the need for safe and effective treatment strategies. Retinoids, comprising of natural and synthetic analogs, are a class of chemical compounds that regulate cellular proliferation, differentiation, and cell death. Retinoids have been used in the clinic for several leukemias and solid tumors, either as single agents or in combination therapy. Furthermore, retinoids have shown potent chemotherapeutic and chemopreventive properties in different cancer models, including CRC. In this review, we summarize the major preclinical findings in CRC in which natural and synthetic retinoids showed promising antitumor activities and stress

on the proposed mechanisms of action. Understanding of the retinoids' antitumor mechanisms would provide insights to support and warrant their development in the management of CRC. *Anti-Cancer Drugs* 30:655–669 Copyright © 2019 Wolters Kluwer Health, Inc. All rights reserved.

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## Introduction

Colorectal cancer (CRC) represents a global health threat owing to its high incidence, mortality rates, and morbidity [1]. Being the third most commonly diagnosed cancer and the third cause of cancer-related death in both men and women, CRC was estimated in 2018 to account for ~51 000 deaths in the USA [2].

Like most solid tumors, the clinical progression of CRC follows four stages, from small adenomas to large carcinomas and metastasis. The progression of CRC has long been studied and was initially described as a model of the adenoma–carcinoma sequence, where somatic mutations in oncogenes and tumor suppressor genes occur in a multistep manner [3].

The transformation from normal intestinal epithelium to adenocarcinoma is governed by a series of aberrant genetic and epigenetic alterations in regulatory genes such as *p53*, *RAS*, and DNA mismatch repair, as well as the splicing of regulators of the colorectal epithelium homeostasis [4,5]. One of the most frequent and early mutations in CRC affects the tumor suppressor *adenomatous polyposis coli* (*APC*) gene, resulting in the loss of APC and the constitutive activation of the oncogenic Wnt/ $\beta$ -catenin signaling pathway [6]. CRC is also characterized by microsatellite and/or chromosomal instability, which results in its heterogeneity. Epigenetic processes have

also been implicated in CRC progression [7]. In fact, the DNA methylation status, which was recently reviewed in different stages and categories of CRC, was shown to be a marker for diagnosis and prognosis [8].

## Clinical management of colorectal cancer

Standard treatment of CRC is multidisciplinary and may include surgery, radiation, and chemotherapy. Despite recent advances in treatment strategies for CRC, the 5-year survival rate is still relatively low (65%) [9]. This underlies the molecular heterogeneity among CRC subtypes that affects the treatment regimen and the prognosis of patients, even when diagnosed with the same disease stage. Thus, efficient chemotherapy, in neoadjuvant or adjuvant settings, requires a deeper understanding of genetic and epigenetic aberrations in CRC initiation and progression. Accordingly, diagnosis, prognosis, and targeted therapy can be optimized. The use of combinatory treatment for CRC was reviewed in an attempt to lower the toxicity of high doses and to avoid the risk of patient relapse owing to resistance to single drugs [10].

## Retinoids as therapeutic agents

Retinoids are a class of chemical compounds that have been extensively studied for their role as tumor-suppressive agents owing to their control of cell proliferation, differentiation, and cell death, in embryonic

development as well as in adult life [11]. Retinoids comprise both natural and synthetic analogs of vitamin A (retinol) (Figs 1 and 2). All-*trans* retinoic acid (ATRA) and 13-*cis* retinoic acid (13-*cis*RA) are the active metabolites of retinol and are considered natural retinoids [12]. The physiological relevance of the other retinoic acid (RA) isomer, 9-*cis* retinoic acid (9-*cis*RA), remains controversial, with conflicting studies regarding its presence in animal tissues [13]. More studies focused on ATRA as it was shown to display major pleiotropic effects in cellular proliferation, differentiation, and cell death [14]. This latter natural retinoid emerged in 1981 as a cytodifferentiating agent and is still being used as a treatment regimen in combination with other drugs for patients with acute promyelocytic leukemia (APL) to date [15]. In addition, few studies investigated the role of retinoids in liver diseases [16], diabetes [17], dermatological [18], and neurodegenerative disorders [19]. Retinoids are also used as therapeutic options for some retinal degenerative diseases [20]. However, adverse effects [12] and resistance to treatment [14,21] often hindered the use of

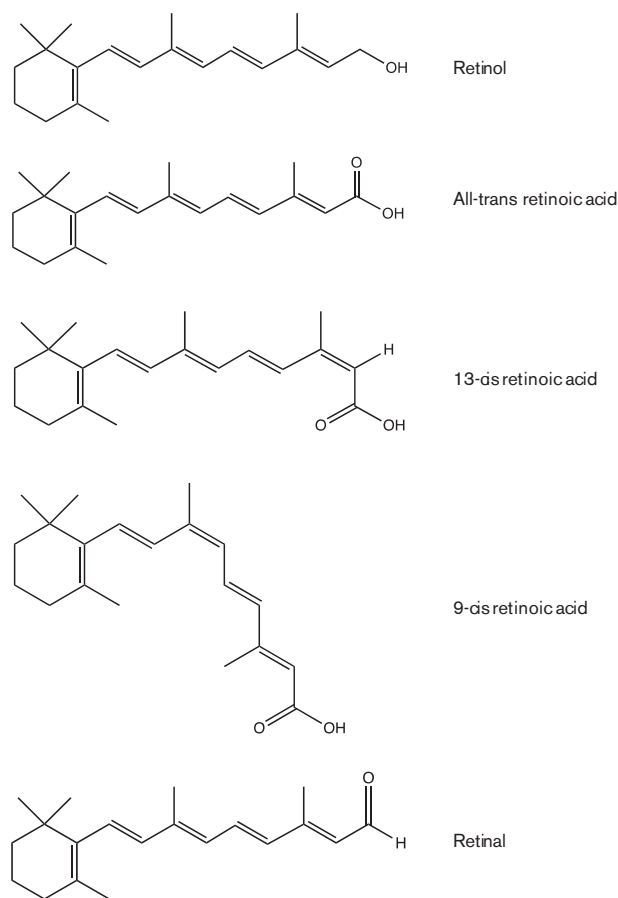
natural retinoids in clinical trials, and as such they failed to achieve their primary end point. For instance, ATRA failed phase-II clinical trials in patients with metastatic breast cancer [14,21]. Other retinoids, such as 9-*cis*RA and 13-*cis*RA also failed testing in breast cancer clinical trials [12]. Furthermore, a clinical trial was conducted in 22 adult patients having solid tumors, three of which experienced CRC. None of the patients showed an objective response [22].

#### Mechanism of action of retinoid receptors

The pharmacological activities of retinoids are primarily mediated by members of two distinct classes of receptors which belong to the steroid/thyroid hormone nuclear receptors family, retinoic acid receptors (RARs) and retinoid X receptors (RXRs), with each comprising of three different isoforms –  $\alpha$ ,  $\beta$ , and  $\gamma$  [23]. The involvement of the retinoid receptors in physiological development, disease, and treatment/prevention, as well as the function of their ligands, was reviewed elsewhere [14,24,25]. Being a nuclear receptor, the ligand-activated complex RAR heterodimerizes with RXRs and acts as a transcription factor by binding to RA responsive elements in retinoid-responsive genes, initiating their transcription [12,14]. Interestingly, in the absence of ligands, RAR/RXR heterodimers function as transcriptional repressors owing to their high affinity to corepressors. Conversely, the presence of ligands alters the RAR/RXR heterodimer interactions with corepressors and increases their affinity to coactivator proteins [26,27]. ATRA and 13-*cis*RA are pan-RAR agonists as they can activate all RAR isoforms with high efficiency, whereas 9-*cis*RA can bind to both RARs and RXRs and activate RAR/RXR heterodimers [12].

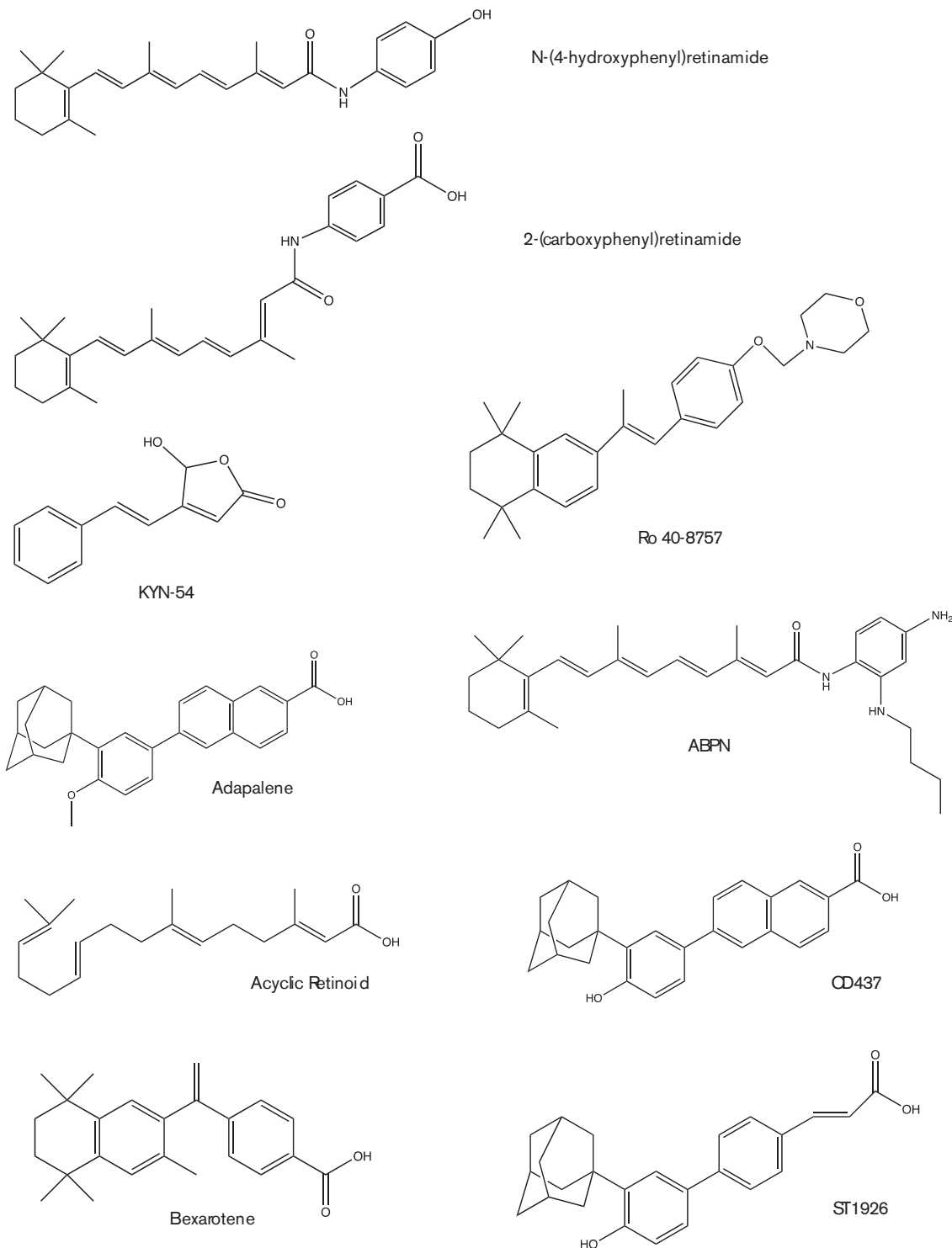
The ability of retinoid receptors to regulate complex cellular mechanisms is owing to their capacity to heterodimerize with many other nuclear receptors, widening the range of regulatory ligands and target genes. RXR is a promiscuous receptor that can dimerize with the thyroid hormone receptor, vitamin D receptor, liver X receptor, farnesoid X receptor, peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), and others [28]. These heterodimers result in distinct physiological responses affecting cell proliferation and cell death, metabolism, inflammation, blood coagulation, fatty acid transport, and biosynthesis, among other functions. For instance, RXRs are involved in the repression of one of the most oncogenic pathways in CRC, the Wnt/ $\beta$ -catenin signaling pathway.  $\beta$ -Catenin is considered oncogenic in colon tumors, and its activity is regulated by the tumor suppressor APC. RXR $\alpha$  is shown to interact with  $\beta$ -catenin, and treatment with multiple RXR agonists, leads to the degradation of  $\beta$ -catenin through an APC-independent mechanism [29]. On the contrary, RXRs are activated in colonocytes upon treatment with the chemopreventive n-3 polyunsaturated fatty acids that reduce colonocytes proliferation [30].

Fig. 1



Chemical structures of natural retinoids.

Fig. 2



Chemical structures of synthetic retinoids.

RXR $\alpha$  induction is also associated with aberrant crypt foci and cell growth inhibition by  $\beta$ -ionone [31], and with the suppression of colonic tumor formation by the

synthetic RXR retinoid, bexarotene [32]. Similar studies have showed the involvement of RXR $\gamma$  in limiting colon tumor invasiveness upon treating with spironolactone,

**Table 1 Antitumor activities of natural retinoids in colorectal cancer cells**

Natural retinoids	Colorectal cancer cells	Antitumor mechanisms	References
All-trans retinoic acid	SW480	Inhibition of cell growth, urokinase secretion, and PKC stimulation induced by diglycerides	Kahl-Rainer <i>et al.</i> [49]
	Moser and HT-29 DLD-1, HT-29, WiDr, HCT-15, and Colo201	Cell growth inhibition; upregulation of fibronectin and CEA Morphological and biochemical changes indicative of apoptosis (membrane shrinkage, chromatin condensation, and DNA cleavage), induction of RAR $\beta$ only in sensitive cells, and acquired sensitivity in resistant cells upon RAR $\beta$ overexpression	Reynolds <i>et al.</i> [50] Lee <i>et al.</i> [51]
	Caco-2	Activation of insulin-like growth factor binding protein 6; deregulation of the IGF-II autocrine loop	Kim <i>et al.</i> [52]
	IEC6 normal intestinal epithelial cells; DLD1, Caco2-BBE, HT29, and RKO	In rat cells: inhibition of proliferation, G <sub>1</sub> /S cell-cycle arrest, and decreased levels of KLF5. No significant effect of ATRA on low KLF5-expressing RKO cells; growth inhibition in high KLF5-expressing DLD1, Caco2-BBE, and HT29 proliferation	Chanchevalap <i>et al.</i> [53]
	Lovo and SW1116	Growth suppression; increased expression of the tumor suppressor gene <i>XAF-1</i> ; enhanced growth suppression with <i>XAF-1</i> overexpression	Wang <i>et al.</i> [54]
	HCT-116, Caco-2, and SW480	Induction of CysLT <sub>2</sub> R through RAR $\alpha$ ; differentiation induction shown by an increase of MUC-2 and alkaline phosphatase	Bengtsson <i>et al.</i> [55]
	Colo 201	Growth inhibition; increase in the production of CEA; differentiation induction; increase in cell adhesion	Nakagawa <i>et al.</i> [56]
	HCT116	Epigenetic activation of E-cadherin; induction of mesenchymal-to-epithelial transition	Woo and Jang [57]
	HCT8-R1	Decreased invasiveness by up-regulating the function of the complex E-cadherin/catenin that is a suppressor of invasion	Vermeulen <i>et al.</i> [58]
	DLD-1 and CHC-Y1 CHC-Y1, DLD-1, HT-29, BM314, CaR-1, and WiDr	Decreased invasiveness by down-regulating matrilysin Invasion suppression through matrilysin inhibition	Yamamoto <i>et al.</i> [59] Adachi <i>et al.</i> [60]
	HCT-15 DLD-1	Growth inhibition and apoptosis Synergy with DNA methylation inhibitors, reduced clonogenicity, and activation of RAR $\beta$	Zhao <i>et al.</i> [61] Cote <i>et al.</i> [62]
	HT-29 and HCT116 HT-29 and SW480	Decrease in COX-2 and C/EBP expression Resistance of both cell lines to ATRA alone; synergistic inhibition of proliferation and induction of apoptosis by combination with COX-2 inhibitors	Eisinger <i>et al.</i> [45] Liu <i>et al.</i> [63]
	HT29 LoVo	Cytotoxic effects of hyperthermia amplified G <sub>0</sub> /G <sub>1</sub> cell-cycle arrest; decrease of cell survival; combinations with 5-fluorouracil and mitomycin C, induction of apoptosis and inhibition of survivin	Callari <i>et al.</i> [64] Wei <i>et al.</i> [65]
	CT-26	Decrease in cell viability and induction of apoptosis equally by free and nanoparticle-encapsulated ATRA; inhibition of tumor cell invasion more effectively by encapsulated ATRA than by free ATRA	Park <i>et al.</i> [66]
	CT-26	Equal inhibition of cell growth by naked ATRA and nanoparticle-encapsulated ATRA; when combined with nanoparticle-incorporated Paclitaxel, enhanced inhibition of tumor cell proliferation and invasion	Hong <i>et al.</i> [67]
9-cis retinoic acid (9-cisRA)	SW480, APC <sup>MIN</sup> adenoma-derived intestinal organoids, and organoids derived from the villin-creERT2: APC <sup>ox/lox</sup> ; KrasG12D <sup>+/lox</sup> ; Trp53 <sup>lox/lox</sup> murine colon cancer model	Induction of HOXA5 and epithelial differentiation phenotype	Ordonez-Moran <i>et al.</i> [68]
	RKO	Decreased invasion by reducing the expression and activity of MLCK by the ERK/MAPK signaling pathway	Zuo <i>et al.</i> [69]
	Caco-2	Increased MHC1 expression	Bhattacharya <i>et al.</i> [70]
	Caco-2	Reduction in the activity of $\beta$ -catenin-LEF/TCF signaling pathway	Easwaran <i>et al.</i> [71]
	Caco-2	Cell growth inhibition, apoptosis induction, and decrease in the expression levels of COX-2 and c-Jun synergistically with ciglitazone	Yamazaki <i>et al.</i> [35]
	CHC-Y1, DLD-1, HT-29, BM314, CaR-1, and WiDr APC[+/-] 1638N COL colon epithelial cell line (origin: histologically normal colon)	Reduction of matrilysin expression and suppression of invasion Chemopreventive activities, growth arrest, and decrease in anchorage-independent colony-forming ability	Adachi <i>et al.</i> [60] Katdare <i>et al.</i> [72]
	LOVO, CC-531, and SW-403	Enhancement of antiproliferative and proapoptotic effects of tamoxifen	Herold <i>et al.</i> [73]
	HT-29	Increase in apoptosis and inhibition of COX-2 through the activation of PPAR $\gamma$ ; synergistic effects with ciglitazone	Wang and Frucht [74]
	SW480	Induction of differentiation through HOXA5 increased expression	Ordonez-Moran <i>et al.</i> [68]
	13-cis retinoic acid (13-cisRA)	Caco-2	Growth inhibition
CHC-Y1, DLD-1, HT-29, BM314, CaR-1, and WiDr		Reduction of matrilysin expression and suppression of invasion	Adachi <i>et al.</i> [60]

Table 1 (continued)

Natural retinoids	Colorectal cancer cells	Antitumor mechanisms	References
Retinol	SW480	Inhibition of cell growth, urokinase secretion, and PKC stimulation induced by diglycerides	Kahl-Rainer <i>et al.</i> [49]
	HCT-15, HCT116, SW620, and WIDR	Growth inhibition independent of RAR signaling pathway and without apoptosis, necrosis, and differentiation induction	Park <i>et al.</i> [76]
Retinal	HT29	Induction of HOXA5 and repression of the stem cell markers prominin 1 and ALDH1	Ordóñez-Moran <i>et al.</i> [68]

ALDH1, aldehyde dehydrogenase 1; ATRA, all-trans retinoic acid; CDH1, e-cadherin; CEA, carcinoembryonic antigen; C/EBP, CCAAT/enhancer binding protein; COX-2, cyclooxygenase-2; CTNNB1,  $\beta$ -catenin, LEF/TCF lymphoid enhancer factor/T-cell factor; CysLT<sub>2</sub>, cysteinyl leukotriene receptor 2; DLD1, D-lactate dehydrogenase [cytochrome] 1; ERK, extracellular-signal-regulated kinase; FN1, fibronectin; IGFBP6, insulin-like growth factor binding protein 6; HOXA5, homeobox protein Hox-A5; IGF-II, insulin-like growth factor 2; KLF, Kruppel-like factor; MAPK, mitogen activated protein kinase; MHC1, major histocompatibility complex I; MLCK, myosin light chain kinase; MMP7, matrix metalloproteinase 7 or matrilysin; MUC-2, mucin-2; PKC, protein kinase C; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; PROM1, prominin 1; RAR, retinoid acid receptor; RAR $\alpha$ , retinoid acid receptor  $\alpha$ ; RAR $\beta$ , retinoid acid receptor  $\beta$ ; XAF-1, XIAP-associated factor 1.

and its crucial role in the antitumor functions of this diuretic drug on the colon [33].

RXRs also heterodimerize with PPAR, and the heterodimer has proven to exert crucial antitumor activities. It was demonstrated, using in-vitro and xenograft colorectal tumor models, that the combination of RXR and PPAR $\gamma$  agonists, bexarotene and rosiglitazone, respectively, causes growth inhibition and also decreases the expression of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2), and increases the expression of the differentiation marker, carcinoembryonic antigen (CEA) [34]. In a similar study, when RXR $\alpha$  is activated, by inhibition of its phosphorylation, 9-*cis*RA and ciglitazone, RXR and PPAR $\gamma$  agonists, respectively, synergistically inhibited cell growth, induced apoptosis, and decreased the expression levels of COX-2 and c-Jun [35]. The same properties of cell growth inhibition and decreasing the expression of COX-2 are observed when RXR and PPAR $\gamma$  are synergistically activated by the rexinoid 6-OH-11-O-hydroxyphenantrene (IIF) in combination with ciglitazone or pioglitazone, both of which are PPAR $\gamma$  agonists [36].

In accordance with the previous studies, RXR $\alpha$  was found to be downregulated in azoxymethane (AOM)-induced APC<sup>Min/+</sup> mouse intestinal tumors [37]. Interestingly, this downregulation is owing to the promoter methylation, and the reactivation of RXR $\alpha$  through a decrease in CpG methylation by green tea is crucial for the inhibition of intestinal tumorigenesis in this mouse model [37]. The importance of RXRs in CRC is also confirmed by the downregulation of the three RXR isoforms in rats and humans CRC [38]. A genetic study also associated two single nucleotide polymorphisms in RXR $\alpha$  gene with a high risk of CRC and a high risk of cancers with microsatellite instability [39].

In contrast, one study points out to an oncogenic property of RXR $\alpha$ , where it inhibits the expression of miR-193a that acts as an important tumor suppressor in CRC [40]. Specifically, miR-193a inhibits the expression of the oncogene *Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)*, affecting growth, and of the serine protease urokinase-type plasminogen activator involved in the

degradation of the extracellular matrix, affecting invasiveness. RXR $\alpha$  binds directly to the miR-193a promoter and inhibits its expression during transformation [40].

## Retinoids in colorectal cancer

### Natural retinoids in cancer treatment

Aberrant or deregulated retinoid signaling pathway is shown to be involved in several tumors and constitutes an important target for cancer therapy [14,41]. In particular, APL is characterized by the chromosomal translocation between the RAR $\alpha$  gene on chromosome 17 and the promyelocytic leukemia (PML) gene on chromosome 15, and the formation of the fusion oncoprotein, PML-RAR $\alpha$ , which has been linked to the etiology of this aggressive leukemia [42]. The degradation of PML-RAR $\alpha$  is crucial for a long-term cure of APL. In solid tumors, loss of RARs in skin is associated with a high risk of premalignant progression [43]. In colorectal adenomas and carcinomas, the retinoid signaling pathway is deregulated. Two genes in particular, encoding for the enzymes retinol dehydrogenase 5 and retinol dehydrogenase-like, both involved in the biosynthesis of RA, were shown to be downregulated in neoplastic colon [44]. Colon tumor cells that lack these two enzymes fail to convert retinol to its active metabolite, ATRA. The induction of the colon tumor suppressor APC in these cells increases the expression of the enzymes and consequently the synthesis of ATRA, suggesting a RA-mediated program of differentiation [44]. When APC is mutated in CRC cells, the increase in COX-2 expression is owing to the lack of RA biosynthesis, and ATRA treatment significantly reduces the expression of COX-2 [45].

The emergence of retinoids as therapeutic agents is further supported by their specificity to targeting cancer cells as shown by imaging studies in xenograft models that used near-infrared-labeled retinoid agents to detect human cancers and visualize drug redistribution within the body [46]. These studies showed that unbound retinoids, but not the free dye, specifically bind to the human tumor cells and are internalized, where they permit the imaging of human cancer xenografts. The complexity of cross-talk between retinoid signaling and several other pathways involved in colorectal tumorigenesis has been

Table 2 Antitumor activities of natural retinoids in in-vivo colorectal cancer models

Natural retinoids	Colorectal animal models	Dose, duration, and mode of administration	Antitumor mechanisms	References
All-trans retinoic acid	Chemical carcinogenesis, male Sprague-Dawley rats	75 or 150 mg/kg in the diet for 4 weeks; oral	Reduction of aberrant crypt foci; decrease in aberrant crypt foci expressing <i>myc</i> -specific mRNA and protein	Stopera <i>et al.</i> [77]
	Chemical carcinogenesis, male Wistar rats	50 mg/kg, daily for 8 weeks; oral	Decreased dimethylhydrazine-induced tumors; reduced expression of the proliferating cell nuclear antigen and the nucleolar organizer region-associated protein; increased expression of RAR	Wei <i>et al.</i> [78]
9- <i>cis</i> retinoic acid (9- <i>cis</i> RA)	LoVo xenograft, female BALB/c nude mice	15 mg/kg, daily for 5 days; intraperitoneal	Growth suppression; induction of the tumor suppressor gene <i>XAF-1</i> ; growth suppression increased with the overexpression of <i>XAF-1</i>	Wang <i>et al.</i> [54]
	BM314 and WDr xenograft, female CB7/ICR SCID Jcl mice	10 or 20 mg/kg every other day for 3 weeks; oral	Reduced invasion; no significant effect on tumor growth	Adachi <i>et al.</i> [60]
13- <i>cis</i> retinoic acid (13- <i>cis</i> RA)	Zebrafish embryos	Embryo incubation with 900 nmol/l for 1 h then treatment with 20 nmol/l for 1 h, <i>APC</i> mutant embryo incubation with 1 $\mu$ mol/l for 2 h	Reduced expression of COX-2 and C/EBP- $\beta$ in <i>APC</i> mutant zebrafish; downregulation of $\beta$ -catenin and its downstream pro-proliferative signaling events	Eisinger <i>et al.</i> [45,79]
	Chemical carcinogenesis, male Wistar rats	0.75 or 1.5 mg/kg, daily for 45 weeks; subcutaneous	Prevention of malignant growth formation and progression	Narahara <i>et al.</i> [80]
Retinol	HT29 xenograft, male Nu/Nu BALB-c mice	400 nmol/animal; daily for 17 days; intraperitoneal	Tumor growth reduction by ATRA; synergistic effects of ATRA when administered with difluoromethylornithine and/or colon mitosis inhibitor	Paulsen JE, Lutzow-Holm [81]
	Chemical carcinogenesis, male F344 Rats	190 or 390 mg/kg in the diet for 5 weeks; oral	Inhibition of aberrant crypt foci progression	Wargovich <i>et al.</i> [82]
Retinol	Transgenic <i>APC</i> <sup>Min</sup> mice, male and female C57BL/6J and patient-derived xenografts, male and female nude mice	2.5 mg/kg every other day for 8 weeks; intraperitoneal	Decrease of adenoma number and size; more differentiated phenotype	Ordonez-Moran <i>et al.</i> [68]
	Transgenic Villin creERT2: <i>APC</i> <sup>lox/lox</sup> mice, male and female nude mice	2.5 mg/kg every other day for 6 weeks; intraperitoneal	Inhibition of tumor initiation after induction of <i>APC</i> deletion; normalized cell phenotypes	Ordonez-Moran <i>et al.</i> [68]
Retinol	SW480 xenograft, male and female nude mice	2.5 mg/kg every other day for 6 weeks; intraperitoneal	Inhibition of metastasis	Penny <i>et al.</i> [83]
	<i>Apc</i> <sup>Min/+</sup> male and female mice	40 ppm liarazole or 8 ppm talarazole in 4 IU/g of vitamin A base diet	Restoration of ATRA levels; attenuation of inflammation and tumor burden by regulatory T-cell induction	Bhattacharya <i>et al.</i> [70]
Retinol	Chemical carcinogenesis, female C57BL/6J mice	200 $\mu$ g either twice a week or every other day for 9 weeks; intraperitoneal	Reduction in tumor burden that is CD8 <sup>+</sup> T-cell dependent	Adachi <i>et al.</i> [60]
	BM314 and WDr xenograft, female CB7/ICR SCID Jcl mice	10 mg/kg every other day for 3 weeks; oral	Reduction of matrilysin expression and suppression of invasion without affecting tumor growth	Wargovich <i>et al.</i> [82,84], Zheng <i>et al.</i> [85]
Retinol	Chemical carcinogenesis, male F344 Rats	190 or 390 mg/kg in the diet for 3 weeks; oral	Inhibition of aberrant crypt foci formation and inhibition of their outgrowth into multiple crypt clusters	Adachi <i>et al.</i> [60]
	Chemical carcinogenesis, BALB/c male and female mice	Retinol-supplemented diet containing retinyl acetate (5000 IU/kg) for 7 weeks	Inhibition of aberrant crypt foci progression	Wargovich <i>et al.</i> [82]
Retinol	Chemical carcinogenesis, BALB/c male and female mice	Retinol-supplemented diet containing retinyl acetate (5000 IU/kg) for 7 weeks	Inhibition of colitis and colorectal cancer development	Okayasu <i>et al.</i> [86]

APC, adenomatous polyposis coli; ATRA, all-trans retinoic acid; C/EBP- $\beta$ , CCAAT/enhancer binding protein; COX-2, cyclooxygenase-2; CTNNB1,  $\beta$ -catenin; ILU, international unit; MMP7, matrix metalloproteinase 7 or matrilysin; RAR, retinoic acid receptor; *XAF-1*, XIAP-associated factor 1.

recently elegantly depicted in a review by Applegate and Lane [47]. For the purpose of this review, we will tackle CRC preclinical studies that involved the use of natural and synthetic retinoids in the treatment and/or prevention of CRC, and summarize their major findings.

### Preclinical efficacy of natural retinoids in the treatment of colorectal cancer

Although repeated measurements in patients with CRC showed no major association of carotenoid and retinol serum levels with risk of CRC [48], the natural retinoids, ATRA, 9-*cis*RA, and 13-*cis*RA, have been extensively studied for their chemopreventive and therapeutic properties against this type of cancer. Chemopreventive and therapeutic properties of natural retinoids in CRC are summarized next and highlighted in Table 1 for the in-vitro studies and in Table 2 for the in-vivo studies.

#### All-trans retinoic acid

One of the earliest studies on the effect of ATRA on CRC was conducted *in vivo*, using Sprague-Dawley rats, and showed chemopreventive properties in reducing the formation of aberrant crypt foci, known to be one of the earliest events in this cancer [77]. ATRA inhibited at nanomolar concentrations CRC cell growth induced by diglycerides with long-chain fatty acid residues and blocked protein kinase C-induced stimulation of diglycerides in SW480 cell line [49]. ATRA was also shown to suppress invasiveness of CRC cells by down-modulating matrilysin [59,60], up-regulating the E-cadherin/catenin complex [58], through epigenetics-related unknown mechanisms [57], and more recently, by inhibiting the expression and activation of myosin light chain kinase by ERK/MAPK signaling pathway [69].

With the development of DNA methylation inhibitors, ATRA in combination with 5-aza-2'-deoxycytidine synergistically abrogated the growth of CRC cells. Although the mechanisms of action remain elusive, it was postulated that the increased expression of *RARβ*, a tumor suppressor that is epigenetically silenced during CRC evolution, may play a role in the observed synergy [62]. In line with these findings, ATRA induced *RARβ* expression in sensitive cells, whereas overexpression of *RARβ* in ATRA-resistant cells restored their sensitivity, clearly showing the role of *RARβ* as a direct predictor of ATRA responsiveness [51]. In several chemical carcinogenesis CRC mouse models, ATRA decreased malignant tumor formation and progression [78,80,81], inhibited the formation of aberrant crypt foci and their evolution [82], and increased the expression of RARs [78]. Data gathered from in-vitro studies revealed that ATRA inhibited the proliferation of many CRC cell lines and increased CEA production [50,56]. ATRA has also been shown to act on CRC cells synergistically with celecoxib, a selective COX-2 inhibitor that has been reported to reduce the incidence of CRC and to inhibit proliferation [63]. ATRA

is also used to sensitize CRC cells to other treatments. In fact, the hyperthermia cytotoxic effects are amplified if cells are treated with ATRA [64]. ATRA increases as well the sensitivity of CRC to several drugs, particularly to cell-cycle-specific agents like 5-fluorouracil (5-FU) and mitomycin C, and acts synergistically with these agents to promote apoptosis, possibly through the inhibition of survivin gene expression [65]. Treatment with ATRA significantly inhibits the invasion of CRC cells by reducing the expression and activity of the myosin light chain kinase [69].

Although the mechanisms behind ATRA-induced growth inhibition in CRC are not well defined [64], numerous studies highlight the involvement of multiple signaling pathways that are either activated or shut down. For example, ATRA activates insulin-like growth factor binding protein-6 in Caco-2 cells, which is in part responsible for the antiproliferative effects of ATRA as it deregulates the insulin-like growth factor-II autocrine loop in these CRC cells [52]. Another study revealed that Kruppel-like factor (KLF), a mitogenic transcription factor, might be a potential target of ATRA. In fact, ATRA-induced growth inhibition of the normal rat intestinal epithelial cell line IEC6 was correlated with decreased levels of KLF5, and constitutive expression of KLF5 abolishes ATRA inhibitory effects on these cells [53]. Furthermore, Wang *et al.* [54] showed that ATRA increases the expression of XIAP-associated factor 1 (*XAF-1*), a tumor suppressor gene, through interferon regulatory factor 1-mediated transcriptional regulation, in Lovo and SW1116 cells, and in BALB/c nude mice. Further in-vitro and in-vivo characterizations of ATRA-mediated growth inhibition revealed a decrease in COX-2 and CCAAT/enhancer binding protein expression levels [45], downregulation of the oncogenic  $\beta$ -catenin signaling pathway [79],  $G_0/G_1$  cell-cycle arrest [65], induction of cysteinyl leukotriene receptor 2 through *RARα*, and differentiation [55]. To further elucidate the antitumor mechanisms of ATRA, a recent exhaustive proteomic study performed on HCT-15 CRC cells treated with ATRA showed deregulation in 13 expressed proteins, involved in several cell functions such as cell structure, translation, post-translation modifications, and phospholipid metabolism [61].

Concomitant with the rise of nanomedicine to overcome low bioavailability of drugs, particularly hydrophobic ones [87], attempts to evaluate the preclinical efficacy of nanoparticle-encapsulated ATRA were conducted by two separate groups. Park *et al.* [66] showed that nanoparticle encapsulation of ATRA had similar effect on cell viability of CRC cells; however, enhanced inhibition of tumor invasion was more evident by the nanoparticle formulation versus the naked ATRA. In tandem with these findings, nanoparticle formulation of combined ATRA and paclitaxel proved to be superior to either one alone, and enhanced inhibition of tumor cell growth and invasion

Table 3 Antitumor activities of synthetic retinoids in in-vitro and in-vivo colorectal cancer models

Synthetic retinoids	Colorectal cancer models	Dose, duration, and mode of administration <i>in vivo</i>	Antitumor mechanisms	References
Fenretinide (HPR)	HT-29 and HCT-15	-	Growth inhibition by inhibition of prostaglandins synthesis; reduced COX-2	Merritt <i>et al.</i> [91]
	DLD-1, COLO-205, and CACO-2	-	Enhanced growth inhibition when combined with tamoxifen	Ziv <i>et al.</i> [92]
	SW480, SW620, HCT116, HT29, DLD-1, and HCT-15	-	Increased expression of DR5 by the induction of the transcription factor CHOP; enhanced apoptosis when combined with TRAIL, caspase activation	Kouhara <i>et al.</i> [88]
	LoVo and HT-29	-	Synergistic effects when combined with safinolol	Maurer <i>et al.</i> [93]
	HT-29	-	Apoptosis; increased dihydrospingolipids and sphinganine production	Wang <i>et al.</i> [94]
	Chemical carcinogenesis, male F344 Rats	390 or 780 mg/kg in the diet for 5 weeks; oral	Reduced aberrant crypt foci formation; chemopreventive effects	Wang <i>et al.</i> [46], Wargovich <i>et al.</i> [84], Zheng <i>et al.</i> [85,95]
2-C-PR: N-(2-carboxyphenyl) retinamide	<i>Apc<sup>Min/+</sup></i> male and female mice	20 mg/kg in the diet for 14 weeks together with a high fat diet	Reduced tumor burden and angiogenesis by the inhibition of tumor-associated macrophages M2 polarization	Dong <i>et al.</i> [96]
	Chemical carcinogenesis, male F344 rats	315 mg/kg in the diet for 5 weeks; oral	Chemopreventive effects on aberrant crypt foci formation; tumor-promoter effects once tumors are formed	Zheng <i>et al.</i> [85,95]
	Chemical carcinogenesis, male F344 rats	315 ppm in the diet for 10 weeks; oral	Chemopreventive effects on the total number, multiplicity, and size of aberrant crypt foci; tumor-promoter effects on precursor lesions	Zhi <i>et al.</i> [97]
KYN-54: 2(5H)-Furanone	Chemical carcinogenesis, male F344 rats	100 or 200 ppm in the diet for 20 weeks; oral	Chemopreventive effects; decreased activity of the proliferation marker ODC; reduced formation of aberrant crypt foci	Kawamori <i>et al.</i> [98,99]
Arotinoid Ro 40-8757	HT-29, HT-29-5-FU resistant, and Caco-2	-	Growth inhibition in cell lines sensitive or resistant to 5-fluorouracil	Louvet <i>et al.</i> [100]
Adapalene	HT-29, HT-29-5-FU resistant, HT29, HT29-S-B6, and Caco-2	-	Growth inhibition in cell lines; additive effects when combined with 5-fluorouracil and IFN- $\alpha$ in HT29	Louvet <i>et al.</i> [101]
ABPN	HCT116, HT29, and DLD-1	-	Induction of apoptosis; inhibition of DNA synthesis; more potent than Ocker <i>et al.</i> [102]	Ocker <i>et al.</i> [102]
IIF-6-OH-11-O-hydroxyphenanthrene	HT-29 and CACO-2	-	Growth inhibition and apoptosis; PARP, caspase-3, and caspase-8 cleavage; decreased cancer cell invasion through downregulation of (MMP1, 2 and 3; reduced AP-1 activity; greater potency than 4-HPR	Um <i>et al.</i> [103]
	Multidrug-resistant LoVo	-	Strong antiproliferative effects; induction of differentiation	Bartolini <i>et al.</i> [104]
	HT-29 and LoVo	-	Growth inhibition, apoptosis induction, reduction of P-glycoprotein; more effective than ATRA	Bartolini <i>et al.</i> [105]
	HCT116 and SW480	-	Enhanced growth inhibition and decreased invasion when combined with the HDAC inhibitor, sodium valproate; inhibition of MMP2 and MMP9 activity	Papi <i>et al.</i> [106]
Acyclic retinoid	HCT116 and SW480	-	Growth inhibition; G1-cell-cycle arrest, apoptosis; cyclin D1 inhibition; increased transcription of <i>p21</i> and differentiation markers such as alkaline phosphatase and E-cadherin	Suzui <i>et al.</i> [107]
Bexarotene	Moser cell line and Moser xenograft mouse model	50 mg/kg once daily for 6 weeks; oral	Enhanced growth inhibitory effects when combined with the PPAR $\gamma$ agonist rosiglitazone; similar effects observed <i>in vivo</i>	Ceserio <i>et al.</i> [34]
	C57BL/6J- <i>APC<sup>Min/+</sup></i> male and female mice	30, 60, or 200 ppm in the diet for 80 days; oral	Inhibition of polyp formation and colonic tumor formation, reduced expression of cell nuclear antigen, cyclin D1, and COX-2, increased RXR $\alpha$ levels	Janakiram <i>et al.</i> [32]
	Chemical carcinogenesis, male F344 Rats	50 or 100 ppm in the diet for 40 weeks; oral	Reduction in adenocarcinoma formation; enhanced effect when combined with ER antagonist raloxifene	Janakiram <i>et al.</i> [108]
CD437	HCT116	-	Growth inhibition through direct binding and inhibition of POLA1	Han <i>et al.</i> [109]
ST1926	HCT116, HCT116 <i>p53<sup>-/-</sup></i> , HCT116 <i>p21<sup>-/-</sup></i> , HT29, and LoVo	-	Growth inhibition, S-phase arrest, dissipation of mitochondrial membrane dissipation, apoptosis induction, and DNA damage in p53 and p21-independent manner; implication of POLA1 in ST1926 resistance	Abdel-Samad <i>et al.</i> [110]
	HT29 xenograft, female CD-1 nude mice	15 mg/kg, daily for 3 weeks; oral	Inhibition of tumor volume and reduction of tumor volume doubling time	Abdel-Samad <i>et al.</i> [110]

ABC1, p-glycoprotein; AP-1, activator protein 1; Casp-3, caspase-3; Casp-8, caspase-8; CCND1, cyclin D1; CDKN1A p21, CDH1 e-cadherin; CHOP C/EBP, homologous protein; COX-2, cyclooxygenase-2; DR5, death receptor 5; ER, estrogen receptor; HDAC, histone deacetylase; IFN- $\alpha$ , interferon- $\alpha$ ; MMP1, matrix metalloproteinase 1; MMP2, matrix metalloproteinase 2; MMP3, matrix metalloproteinase 3; MMP9, matrix metalloproteinase 9; ODC, ornithine decarboxylase; p53, tumor suppressor P5; PARP, poly(ADP-Ribose) polymerase; POLA1, DNA polymerase  $\alpha$ ; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; RXR $\alpha$ , retinoid X receptor  $\alpha$ ; TRAIL, TNF-related apoptosis-inducing ligand.

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[67]. Finally, ATRA treatment of organoids derived from several genetically modified mouse models of CRC induced homeobox protein Hox-A5 (HOXA5) expression and epithelial differentiation [68]. In the transgenic *APC*<sup>MIN</sup> mouse model and in patient-derived xenografts, ATRA decreased the number and size of adenomas and induced epithelial differentiation [68]. Similarly, ATRA treatment after induction of *APC* deletion in the transgenic Villin creERT2:*APC*<sup>lox/lox</sup> mouse model inhibited tumor initiation and normalized cell phenotypes [68]. Finally, ATRA treatment of xenografts of human CRC cells in immunocompromised mice suppressed tumor growth [54], reduced invasion [60], and inhibited metastasis [68]. Data gathered from multiple preclinical studies clearly support the role of ATRA as a potent CRC chemopreventive agent. With regards to tumor growth and progression, conflicting data were observed and may be explained by the selected mouse model, used dose, duration, or even the mode of administration of ATRA. Future studies should also take into consideration the pharmacological properties of ATRA, by assessing its pharmacokinetic and pharmacodynamic properties in mice owing to its hydrophobicity and instability.

More recently, patients with familial adenomatous polyposis, *APC*<sup>Min/+</sup> mice, and mice with colitis-associated CRC were all shown to have significant deficiencies in colonic ATRA owing to its enhanced catabolism by ATRA-catabolizing enzyme CYP26A1 [70]. Expectedly, pharmacological blockade of CYP26A1 restored ATRA levels and reduced tumor inflammation and burden in *APC*<sup>Min/+</sup> mice [83]. ATRA supplementation reduced tumor burden by two proposed immune-related mechanisms: one that links CD8<sup>+</sup> T-cell activation with an upregulation of the major histocompatibility complex I in tumor cells [70], and the other by induction of regulatory T cells by the lamina propria dendritic cells instead of the inflammatory T helper 17 cells [88]. These findings clearly show an unprecedented link between the immune system activation and ATRA supplementation, and warrant more research in this area, particularly when assessing immunotherapeutic drugs for CRC.

#### Other natural retinoids

Fewer studies tackled the effects of other natural retinoids than ATRA on the development and progression of CRC in-vitro and in-vivo studies. Using murine models, 9-*cis*RA and 13-*cis*RA were both shown to suppress invasiveness by reducing matrilysin expression without affecting tumor growth [60]. Other studies have in contrast shown that 9-*cis*RA inhibits aberrant crypt foci formation and outgrowth into multiple crypt clusters in F344 rats [82,84,85]. In F344 rats, 13-*cis*RA and retinol were observed to both inhibit progression of aberrant crypt foci [82]. More recently, a retinol-supplemented diet was shown to prevent colitis and CRC development in BALB/c mice [86]. Mechanistically, 9-*cis*RA was shown

to reduce in Caco-2 cells the activity of  $\beta$ -catenin-LEF/TCF signaling pathway [71] and, synergistically with ciglitazone, to inhibit cell growth, induce apoptosis, and decrease the expression levels of COX-2 and c-Jun [35]. Other studies highlighted the beneficial effects of 9-*cis*RA on the apoptotic and anti-proliferative properties of ciglitazone and tamoxifen [73,74]. 9-*cis*RA also increased the expression of HOXA5, thus inducing SW480 cell differentiation [68]. In the 1638N cell line, derived from a histologically normal colon, 9-*cis*RA exhibited chemopreventive activities, by inducing growth arrest and decreasing the anchorage-independent colony-forming ability [72]. Both 9-*cis*RA and 13-*cis*RA were shown to suppress invasion by reducing matrilysin expression on a diverse panel of human CRC cell lines [60]. 13-*cis*RA, along with retinol, was shown to inhibit growth [49,75], independently of RAR signaling pathway and without inducing apoptosis, necrosis, or differentiation [76]. Finally, a lone study highlighted the differentiation ability of retinal by inducing HOXA5 expression and repressing the stem cell markers prominin 1 and aldehyde dehydrogenase 1 [68].

#### Preclinical efficacy of synthetic retinoids in the treatment of colorectal cancer

To overcome ATRA resistance and its toxic adverse effects, synthetic retinoids were developed and were shown to enhance specificity and reduce toxicity [89,90]. Preclinical studies on the use of synthetic retinoids in targeting CRC are summarized in Table 3.

#### *N*-(4-hydroxyphenyl)retinamide

The very early synthetic retinoid that gained much attention was 4-(hydroxyphenyl)retinamide (HPR or fenretinide) [111]. It is the most studied retinoid in breast cancer chemoprevention clinical trials because of its selective accumulation in breast tissues and favorable pharmacokinetics [112].

4-HPR exhibits a favorable hormonal profile in premenopausal women at risk for breast cancer, reinforcing its administration for prevention of breast cancer during premenopause [113]. In CRC, HPR was shown to reduce the proliferation of several human CRC cell lines, possibly through the inhibition of prostaglandins synthesis and the reduction in COX-2 transcript levels [91]. HPR was also shown to act through other molecular mechanisms such as the induction of the transcription factor CCAAT/enhancer binding protein homologous protein and the consequent upregulation of the apoptotic death receptor 5 expression in HPR-treated CRC cells [88]. HPR also affected the ceramide pathway where it increased the levels of dihydrosphingolipids and sphinganine, leading to apoptosis in HT-29 human CRC cells [94]. Interestingly, HPR was shown to display superior antitumor activities when combined with the widely used selective estrogen receptor (ER) modulator, tamoxifen, in the treatment of

ER-positive breast cancer [92]. Furthermore, in human CRC cells lacking ER, HPR in combination with tamoxifen inhibited their proliferation through unknown mechanisms [92]. Combination treatment of HPR with other compounds such as safinol [93], TRAIL [88], or modulators of ceramide metabolism, namely, dimethylsphingosine, enhanced the antitumor efficacy of HPR. The chemopreventive properties of HPR were evaluated in a preclinical rat model of chemical carcinogenesis [114]. HPR reduced aberrant crypt foci formation and prevented AOM-induced tumors in this latter model [84,85,115], and selectively inhibited tumor-associated macrophages M2 polarization, therefore blocking angiogenesis and tumorigenesis in the *Apc<sup>+/-min</sup>* mouse model [96].

### **2-(carboxyphenyl)retinamide**

2-(carboxyphenyl)retinamide (2-CPR) has controversial antitumor effects compared with other retinoids. Oral administration of 2-CPR for 5 weeks in a chemopreventive chemical carcinogenesis rat model was shown to significantly reduce aberrant crypt foci formation by 58% [85]. However, 2-CPR failed to alleviate AOM-induced CRC and resulted in doubling the tumor yield [95], clearly suggesting that 2-CPR acts as a chemopreventive agent, but not as a chemotherapeutic one. When compared with HPR and 9-*cis*RA, 2-CPR reduced the mitotic activity but failed to induce apoptosis in adenomas like the former retinoids [95]. These controversial findings were also validated in another study where it was shown that 2-CPR was indeed effective in reducing the total number, multiplicity, and size of aberrant crypt foci, but promoted mitosis of precursor lesions through increasing the proliferating cell nuclear antigen index [97].

### **Retinoidal butenolide, furanone**

5-hydroxy-4-(2-phenyl-(E)ethenyl)-2(5H)-furanone (KYN-54 or furanone) is a synthesized retinoidal butenolide that was shown to be endowed with chemopreventive activities against AOM-induced aberrant crypt foci formation [98]. The effect of furanone on CRC was studied in a chemical carcinogenesis rat model where it inhibited intestinal tumor development after AOM initiation and decreased the activity of the proliferating marker colonic ornithine decarboxylase [98]. KYN-54 also suppressed the formation of AOM-induced aberrant crypt foci [99].

### **Arotinoid Ro 40–8757**

With the emergence of chemotherapy-resistant tumors in the clinical settings, development of effective treatments that can overcome resistant CRC to the commonly used chemotherapeutics namely, 5-FU, becomes a necessity. The arotinoid Ro 40–8757 is a synthetic retinoid and a member of the polyaromatic derivatives of vitamin A that is endowed with potent antitumor activities against ATRA and/or 5-FU-resistant human CRC cell lines [100]. In addition to its potent chemotherapeutic role as a single agent, combination treatments of Ro 40–8757 with 5-FU

and/or interferon- $\alpha$  [101] demonstrated enhanced activities. The mechanism of action of Ro 40–8757 growth inhibitory effects remains unknown, but it was shown to induce a significant accumulation of the active dephosphorylated form of retinoblastoma with no changes at the transcript levels [101].

### **Naphtoic acid derivative, adapalene**

The synthetic retinoid CD271, 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphtoic acid, also called adapalene, is a naphthoic acid derivative with retinoid-like biologic properties and belongs to the third generation of retinoids. Adapalene is used in the clinical treatment of acne [116]. This latter synthetic retinoid induced apoptosis and caused a rapid decrease in DNA synthesis and proved to be more potent than the natural retinoid 9-*cis*RA [102].

### **The ATRA-derivative, ABPN**

Although HPR proved to be a potential chemotherapeutic and chemopreventive agent, several synthetic retinoids showed greater potency, in particular, the ATRA-derivative 4-amino-2-(butyrylamino)phenyl(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6,8-nonatetraenoate, also called ABPN or CBG41. Early drug screening in HCT116 cells showed that ABPN was the most potent among 85 other synthesized retinoid derivatives [103]. ABPN showed greater potency than HPR in inducing apoptosis and inhibiting the growth of ATRA-resistant HCT116, HT-29, and DLD-1 cell lines. Treatment with ABPN resulted in PARP, caspase-3, and caspase-8 cleavage, reduced activator protein-1 activity, and limited cancer cell invasion probably by downregulating matrix metalloproteinases (MMP) 1, 2, and 3 *in vitro* [103]. Interestingly, ABPN was able to activate the three RAR isoforms with the same potency as ATRA.

### **Polyprenoic acid or acyclic retinoid**

Another synthetic retinoid that showed promising results in inhibiting CRC cell growth is 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid, also known as polyprenoic acid or the acyclic retinoid (ACR). ACR induced G<sub>1</sub>-phase cell-cycle arrest and apoptosis in HCT116 and SW480 cells and decreased cyclin D<sub>1</sub> protein levels. Treatment with ACR increased p21 protein levels and the differentiation markers alkaline phosphatase and E-cadherin in HCT116 cells independently of p53 and p27 [107]. Interestingly, ACR was shown to prevent the recurrence of hepatoma after surgical resection of primary tumors in patients with hepatocellular carcinoma [117] but was never tested in CRC setting.

### **The rexinoid 6-OH-11-O-hydroxyphenanthrene**

The synthetic retinoid 6-OH-11-O-hydroxyphenanthrene, also called IIF, is a rexinoid tested on Caco-2 and HT-29 CRC cell lines and was shown to exert strong

antiproliferative effects and to induce cellular differentiation [104]. The same team conducted a study on LoVo cells and their counterparts that developed multidrug resistance (LoVo/MDR) and found that IIF is more effective than ATRA in inhibiting cell growth and inducing apoptosis, particularly in the LoVo/MDR cell line [105]. The proapoptotic properties of the rexinoid IIF were then confirmed by another study that pointed out to a greater growth inhibition of HT-29 and LoVo cells by IIF when combined with the histone deacetylase inhibitor, sodium valproate [106]. Combination treatments inhibited MMP2 and MMP9 expression and activity and increased the expression of their inhibitors [106].

### The rexinoid bexarotene

Among the few synthetic retinoids that were tested *in vivo* for their chemotherapeutic potencies was targretin or 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl) ethenyl] benzoic acid, also known as bexarotene or LGD-1069. Bexarotene belongs to the third-generation retinoids and is an RXR agonist. Bexarotene is used in the treatment of cutaneous T-cell lymphoma and non-small-cell lung cancer and has been tested in clinical trials of several cancers including breast and prostate [118]. Early studies revealed that treatment with bexarotene as a single agent had little to no effect on the growth of human CRC *in vitro* and *in vivo*; however, enhanced growth inhibitory effects were achieved when combining bexarotene with the PPAR $\gamma$  agonist, rosiglitazone [34]. Mechanistically, the latter combination treatment decreased COX-2 expression and PGE2 synthesis, whereas it increased the expression of the differentiation marker human CEA [34]. The chemopreventive efficacy of bexarotene was then evaluated in two studies where it was shown to inhibit polyp and colonic tumor formation in *APC<sup>Min/+</sup>* mice [32] and adenocarcinoma formation in a rat model of chemical carcinogenesis [108]. Furthermore, enhanced effects were observed when bexarotene was combined with the ER antagonist, raloxifene [108]. Bexarotene was also shown to reduce the expression levels of cyclin D<sub>1</sub> and COX-2, while increasing RXR $\alpha$  levels [32].

### The adamantyl retinoid CD437

6-[3-(1-Adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid, also known as AHPN or CD437, is among one of the most studied synthetic retinoids in multiple cancer models including hematologic and solid tumors [119–121]. CD437 selectively binds to RAR $\gamma$ , although it can bind RAR $\alpha$  and RAR $\beta$  but with low affinities [122,123]. The effects of CD437 on CRC were not elucidated until recently, where Han *et al.* [109] characterized the DNA damaging effects of CD437 on HCT116 cells. Through cutting-edge genomic technology, the group used CRISPR/Cas9 editing tool to introduce mutations in DNA polymerase  $\alpha$  (POLA1) and showed that the mutant cell lines are resistant to CD437, which led them

to hypothesize that CD437 may be modulating the activity of POLA1 [109]. Indeed, *in-vitro* primer extension assay showed that CD437 inhibited the activity of wild-type DNA POLA1 at nanomolar concentrations [109]. Finally, their claim regarding direct binding interaction was further supported by the increase in total fluorescence intensity and anisotropy of CD437 in the presence of increasing concentration of POLA1 [109]. The authors discuss their results in light of the approved POLA1 inhibitor, aphidicolin, which is toxic at working concentrations and reversibly binds POLA1, claiming that CD437 might be a better candidate to inhibit POLA1 through its selective nonreversible targeting of cancer but not of normal cells [109].

### The adamantyl retinoid ST1926

A prominent molecule belonging to the family of synthetic retinoids is the adamantyl retinoid ST1926 or E-4-(4'-hydroxy-3'-adamantyl biphenyl-4-yl) acrylic acid [124], a CD437 analog [125]. ST1926 is synthesized from CD437 through a three-step sequence where the naphthalene ring in CD437 is replaced with a styrene moiety in ST1926 [124]. ST1926 was shown to be endowed with potent antitumor activities in several *in-vitro* and *in-vivo* cancer models, independently of RAR and *p53* signaling pathways [126–131], and displayed a favorable pharmacokinetic profile when compared with CD437 [125]. Furthermore, ST1926 can be administered orally, while achieving micromolar concentrations in human and mouse plasma [127,132]. ST1926 was shown to induce growth inhibition in a panel of human CRC cell lines independently of *p53* and *p21* status and to reduce the tumor volume and the doubling time in a xenograft mouse model [110]. ST1926-induced growth inhibitory effects resulted in apoptosis, S-phase cell-cycle arrest, dissipation of mitochondrial potential, and early DNA damage [110]. Furthermore, resistance to ST1926 resulted in cross-resistance to CD437 and POLA1 mutations – in tandem with Han *et al.* [109]. Similarly to CD437, ST1926 inhibited the activity of POLA1 in a concentration-dependent manner [110]. Of interest, ST1926 was tested in phase I clinical trials in patients with ovarian carcinoma but was subsequently halted owing to its limited bioavailability as micromolar concentrations dropped shortly to submicromolar levels [132]. We have recently reported a novel nanoparticle formulation of ST1926 that may improve its bioavailability, and showed that it is effective in reducing the tumor burden even at four-fold lower the effective concentration [129]. We hope with these findings to warrant the clinical development of ST1926 nanoparticles or repurpose its clinical use for tumors that overexpress POLA1 [110].

### Conclusion

Development of targeted therapy in combination with chemotherapy regimens has significantly led to improvements in the treatment of CRC. However, the overall

effect of such treatments in the management of metastatic disease remains partially insufficient, necessitating the advancement of more efficient therapies. The retinoid family of naturally occurring and synthetic analogs of vitamin A is implicated in several physiologically vital processes such as the regulation of cell proliferation, cell death, and differentiation in embryonic development and in adult life [11].

### Expert commentary

Retinoids have gained attention given their chemopreventive and therapeutic properties in different types of tumors, including CRC. Both natural and synthetic retinoids proved to be strong agents in preventing CRC initiation, reducing metastasis, and inducing differentiation in multiple preclinical studies. Despite promising results, retinoids never reached clinical trials for patients with CRC; we try to address some of the reasons. Retinoids are notorious for their adverse effects in clinical trials: their systemic administration results in severe skin disorders, hepatotoxicity, and altered serum lipid profiles [133]. In addition, pregnant women can never receive retinoid-based treatments owing to their teratogenic effects, whereas long exposure to retinoids results in chronic skeletal toxicities and developmental defects in children [133]. The failure of retinoids in reaching their primary end point in clinical trials for other types of tumors may partially account for retinoids never being used in CRC management. ATRA failed phase-II clinical trials in patients with metastatic breast cancer [14,21]. Other retinoids, such as 9-*cis*RA, 13-*cis*RA, HPR, bexarotene, and retinyl palmitate equally failed testing in breast cancer clinical trials [12]. However, one can argue that the failure of retinoids in reaching a desirable clinical outcome may be owing to acquired resistance of patients after receiving established treatment regimens and not responding, which brings into perspective the importance of treating early stages of the disease.

A pertinent problem that may delay advancing retinoids to clinical trials is their poorly understood mechanism of action, which seems to be pleiotropic rather than specific, especially in the case of natural retinoids. Retinol was suggested to have nongenomic effects by interfering with pathways involving phosphatidylinositol 3-kinase and  $\beta$ -catenin, highly affecting cancer progression [47]. Only until recently, the molecular targets of two synthetic retinoids, CD437 and ST1926, were identified [109,110]. Throughout this review, we summarized the proposed anticancer effects of natural and synthetic retinoids, as a more thorough understanding of how retinoids mediate their anticancer effects is needed to overcome resistance and relapse of patients in clinical settings.

Preclinical studies involving the use of retinoids in CRC resulted in conflicting data, which may be explained by the selected mouse model. In the case of genetically engineered CRC mouse models, it is widely established

that inherent differences exist in colon and rectum composition between mice and humans and that can account to a certain extent for the observed conflicting results. In addition, xenografts are established by injecting CRC cell lines in mice lacking a functional immune system. It was recently shown that CD8<sup>+</sup> T-cell and regulatory T-cell activation is increased with ATRA treatment, which clearly suggests a role for the immune system in mediating or enhancing ATRA's mechanisms of action [70]. Lack of immune system in immunodeficient mice has been a major hurdle in studying the interactions between the immune system and tumors cells. To overcome this limitation, substantial efforts have been directed toward the development of humanized mouse models, which contain a functional human immune system, and will allow researchers to address questions pertinent to CRC progression in the context of an immune response. So far, these mice models failed to fully reconstitute the frequencies of immune cells seen in humans owing to low maturation and differentiation in the presence of mouse cytokines. This remains to be improved with gene editing tools such as the knock-in of human genes encoding for cytokines into their respective mouse loci, which was shown to improve the maturation, differentiation, and function of myeloid and natural killer cells [134].

Equally important is the retinoids' dose, duration of treatment, and the mode of administration, all of which do not seem to be consistent in the literature. Given the hydrophobic properties of retinoids in general, it is advisable to assess the pharmacokinetic and pharmacodynamics properties in mice, which may provide an indication to how the drug will be tolerated in humans.

In summary, our review shows that retinoids may hold promise in CRC management, especially if used in combination treatment or in early or chemopreventive settings, calling for further development of retinoids in CRC management.

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### Conflicts of interest

There are no conflicts of interest.

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