Clinical Cancer Research

Molecular Pathways: Current Role and Future Directions of the Retinoic Acid Pathway in Cancer Prevention and Treatment

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Abstract

Retinoids and their naturally metabolized and synthetic products (e.g., all-*trans* retinoic acid, 13-*cis* retinoic acid, bexarotene) induce differentiation in various cell types. Retinoids exert their actions mainly through binding to the nuclear retinoic acid receptors (α, β, γ), which are transcriptional and homeostatic regulators with functions that are often compromised early in neoplastic transformation. The retinoids have been investigated extensively for their use in cancer prevention and treatment. Success has been achieved with their use in the treatment of subtypes of leukemia harboring chromosomal translocations. Promising results have been observed in the breast cancer prevention setting, where fenretinide prevention trials have provided a strong rationale for further investigation in young women at high risk for breast cancer. Ongoing phase III randomized trials investigating retinoids in combination with chemotherapy in non–small cell lung cancer aim to definitively characterize the role of retinoids in this tumor type. The limited treatment success observed to date in the prevention and treatment of solid tumors may relate to the frequent epigenetic silencing of *RARβ*. Robust evaluation of *RARβ* and downstream genes may permit optimized use of retinoids in the solid tumor arena. *Clin Cancer Res; 19(7); 1651–9. ©2013 AACR.*

Background

Vitamin A is derived from animal and plant food sources and has critical functions in many aspects of human biology. Its natural derivatives and metabolized products (retinoids) such as β-carotene, retinol, retinal, isotetrinoin, all-*trans* retinoic acid (ATRA), 9-*cis* retinoic acid, and 13-*cis* retinoic acid have important roles in cell differentiation, growth, and apoptosis (1). Synthetic retinoids are also available and include bexarotene and fenretinide. In clinical practice, retinoids have a wide range of dermatologic indications including for psoriasis, acneiform, and keratinization disorders (2). Systemic retinoids are approved by the U.S. Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma (3) and acute promyelocytic leukemia (APL; refs. 4, 5). However, the chemopreventive and therapeutic effects of retinoids in solid tumors remain controversial. Therefore, an overview of the research to date and future directions in this area is the focus of this review.

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Retinoic acid and the retinoic acid receptor pathway

Retinoic acids (RA) exert their functions through their specific receptors. The 2 distinct classes of receptors are retinoic acid receptors (RAR) and retinoic X receptors (RXR). Each class contains 3 different subtypes—α, β, and γ (6). ATRA and fenretinide can bind specifically to RARS, 13-*cis* RA and bexarotene only to RXRS, and 9-*cis* RA to RARS or RXRS (refs. 1, 5; Table 1). The expression of these receptors is regulated by the receptors themselves, other nuclear receptors such as ERα, or by other subtypes in the same family (5, 7). Upon the binding of ligands, RARs and RXRs form heterodimers and function as ligand-dependent transcription factors to activate their downstream effectors by binding to the retinoic acid response elements (RARE) located in the 5′-region of RA downstream genes (5). The above model of RAR or RXR function via binding to RARE is considered the RA classical or genomic pathway. Activation of the classical pathway will trigger cell differentiation, cell arrest, and eventual apoptosis (8).

The function of RA and its receptors involves not only the classical pathway but also multiple other important pathways. RAs have been shown to regulate *NF-κB* (9), *IFN-γ* (10), *TGF-β* (11), *VEGF* (12), mitogen-activated protein kinase (*MAPK*; ref. 13), and chromatin remodeling (14). Furthermore, RARs and RXRs can form heterodimers with other types of receptors, including the estrogen receptor-α (ERα; refs. 7, 15), AP-1 receptor (16), peroxisome proliferator-activated receptor (PPAR; ref. 17), liver X receptors (LXR; refs. 18, 19), and vitamin D receptor (VDR; ref. 20; Fig. 1). When RARs/RXRs heterodimerize with these

Table 1. Select clinical trials evaluating retinoids in solid tumors

Retinoid	Other names	Target	Clinical trial setting	Dose and schedule (ref.)	Study outcome	Biomarker evaluation
ATRA	Tretinoin	RAR	Advanced NSCLC Phase II randomized ($n = 107$)	20 mg/m^2/d commencing 1 wk pre-paclitaxel/cisplatin every 3 wk (50)	RR (55.8% vs. 25.4%) and median PFS (8.9 vs. 6 months) favored the ATRA arm Phase III study pending	No significant association between RAR-β2 expression and response rate detected ($n = 60$)
			Metastatic breast cancer Phase II single arm ($n = 17$)	45 mg/m^2/d for 4 d commencing 2 d preweekly paclitaxel (PMID 20596747)	Clinical benefit rate of 76.4% Note that the majority of patients had not received prior paclitaxel	
13-*cis* RA	Isotretinoin Roaccutane Accutane	RXR	Primary prevention: H+N cancer	Induction phase: high dose (1.5 mg/kg) for 3 mo; maintenance phase: low dose (0.5 mg/kg/d) vs. β-carotene (30 mg/d) for 9 mo (30)	Induction phase ($n = 66$): RR 55%; maintenance phase ($n = 53$): response or stable disease 92% ($n = 22$, isotretinoin) vs. 45% ($n = 13$, β-carotene)	
			Advanced solid tumors Phase I ($n = 13$)	1 mg/kg twice daily 3 wk of 4 with MS-275 (69)	Antitumor activity observed and recommended phase II dose determined	
			Metastatic breast cancer Phase II randomized ($n = 99$)	1 mg/kg/d + tamoxifen 20 mg/m^2 vs. tamoxifen alone vs. tamoxifen + IFN-α-2a 3 MU 3 times weekly IM (62)	No significant difference in RR or overall survival between the 3 arms	
9-*cis* RA	Alitretinoin	RAR RXR	Metastatic breast cancer Phase I ($n = 12$)	70 mg/m^2/d + 20 mg/d tamoxifen (PMID 11352969)	Antitumor activity observed and recommended phase II dose determined	
Fenretinide	4-OH Phenylretinamide	RAR	Primary prevention: women at high risk of breast cancer Randomized double-blind 2 × 2 design ($n = 235$)	Tamoxifen 5 mg/d vs. fenretinide 200 mg/d vs. the combination vs. placebo (58)	Low-dose tamoxifen plus fenretinide did not reduce breast cancer events vs. placebo; numerical reduction in annual odds of breast cancer observed with both single-agent tamoxifen and fenretinide	
			Secondary prevention: early breast cancer Phase III randomized ($n = 2,867$)	200 mg/d oral for 5 y with 3 d off every month vs. observation (55)	No difference in rates of breast cancer in overall population, but 35% reduction in events in premenopausal women in unplanned exploratory analysis	Baseline IGF-I/mammographic density, as well as change in mammographic density did not predict breast cancer events

(Continued on the following page)

Table 1. Select clinical trials evaluating retinoids in solid tumors (Cont'd)

Retinoid	Other names	Target	Clinical trial setting	Dose and schedule (ref.)	Study outcome	Biomarker evaluation
Bexarotene		RXR	Chemotherapy-naïve advanced NSCLC Phase III randomized ($n = 623$)	400 mg/m^2/d every 4 wk with cisplatin/vinorelbine (52)	No survival benefit for addition of bexarotene to chemotherapy	Grade 3/4 hypertriglyceridemia associated with longer median survival in an unplanned subgroup analysis
			Metastatic breast cancer Phase II single arm ($n = 148$)	200 mg/m^2 oral daily (61)	Clinical benefit rate (complete response, partial response, and stable disease >6 mo) of ~20%	

Abbreviations: H+N, head and neck; IM, intramuscular; MS-275, entinostat; MU, million units; PFS, progression-free survival; RR, response rate.

receptors, they are involved in regulating their partner receptor's pathways, referred to as nonclassical or nongenomic pathways (5). Interestingly, these pathways often regulate processes that have functions opposite to the classical pathway. For example, a study has shown that RA activation of the PPARβ/δ pathway resulted in upregulation of prosurvival genes (17), contrary to the known differentiation function of RARs and RXRs in response to RA. The function of RAs, which involves nongenomic pathways, may provide opportunities for cancer cells to develop resistance to RA treatment, discussed later in this review. Another important function of RARA is the regulation of stem cell differentiation (11). RAs target stem cells via both genomic and nongenomic pathways such as the Notch pathway and inflammation (10, 11). In summary, RAs and their receptors play important roles as regulators of critical processes in cells.

Retinoids and cancer

The retinoids have been investigated extensively for the prevention and treatment of cancer, predominantly because of their ability to induce cellular differentiation and arrest proliferation. RA-regulated tumor suppressor genes, when expressed, can inhibit tumor growth (21). Among the 3 RARs, RARβ has been well known for its tumor-suppressive effects in epithelial cells (5, 8, 22). Exogenous expression of the RARβ gene can cause RA-dependent and -independent apoptosis and growth arrest (23). RARβ-induced growth arrest and apoptosis is mediated through RARα (24). As RA ligand-bound RARα binds to the RARE on the *RARβ* promoter, multiple activator proteins assemble at the site and result in the upregulation of the *RARβ* gene (5). The expression of RARβ results in the transactivation and expression of a number of its target genes that mediate cell differentiation and death (5, 6, 8). The ability of ATRA to initiate differentiation of promyelocytic leukemic cells to granulocytes is the basis of the dramatic success of retinoic acid therapy for acute promyelocytic leukemia harboring the *RAR/PML* translocation (4) and confirms the important role of RARβ in tumor growth inhibition. It is also becoming increasingly clear that *RARβ* expression is lost early in carcinogenesis or is epigenetically silenced (25) in many solid tumors, providing an opportunity for novel treatment strategies to be investigated using retinoids together with epigenetic modifiers that promote reexpression of silenced genes, described further below.

Clinical–Translational Advances

The retinoids have an established role in the treatment of certain hematologic malignancies, with FDA approval for use in cutaneous T-cell lymphoma and APL. Bexarotene (an RXR-selective retinoid or rexinoid) is associated with an overall response rate of approximately 50% in patients with refractory advanced-stage mycosis fungoides, a cutaneous T-cell lymphoma (3). ATRA, a synthetic retinoid, exhibited improvements in disease-free and overall survival when compared with chemotherapy alone in APL, with long-term

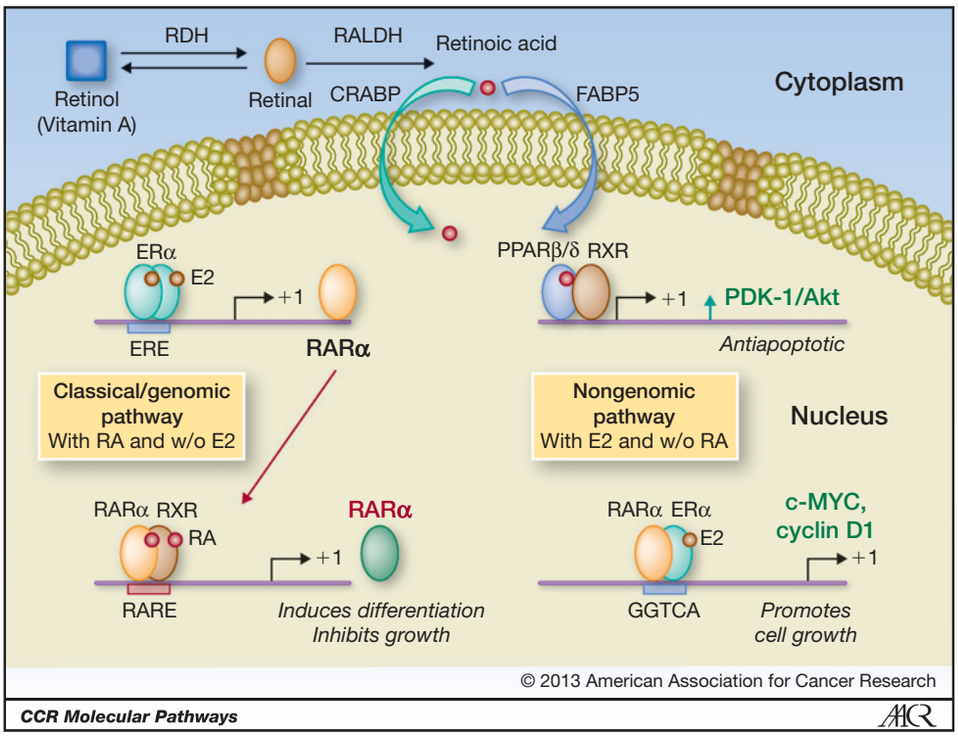

Figure 1. The RARs and their action. In a series of enzymatic steps, vitamin A (retinol) is metabolized through the oxidizing action of retinaldehyde (RDH) to retinal, and by retinaldehyde dehydrogenase (RALDH), to RA. RA has 3 different isomers: all-*trans*, 9-*cis*, and 13-*cis* RA. RA is transported to the nucleus by the protein cellular RA–binding protein (CRABP) and delivered to the RARα. RARα heterodimerizes with and binds to RARE present most often in gene promoters. In the classical pathway of RA action, RA binds to dimers of RARα and RXRs (α, β, or γ) to induce expression of its downstream target genes, including *RARβ*. Upon activation, RARβ can regulate its own expression and that of its downstream genes, the function of which is mainly to inhibit cell growth. Alternatively, RA can be bound and transported to the nucleus by other factors such as FABP5. This delivers RA to other nonclassical receptors such as PPARβ/δ and ERα which activate nongenomic pathways such as PDK-1/Akt or the ERα pathway. Contrary to the differentiation functions attributed to the classical pathway, the nongenomic pathways exert strong antiapoptotic and proliferative effects on cancer cells. It is believed that the classical and nongenomic pathways are controlled by the relative abundance of their own ligands. RA has a stronger affinity for RARs than for the other receptors, and the classical pathway plays a dominant role over the nongenomic pathways. Thus, if RA is present with other ligands such as estrogen, signaling through the classical pathway is preferred to result in cell differentiation and growth inhibition.

remissions occurring in almost 70% of cases (4). The success of retinoids in treating this disease relates to the underlying chromosomal translocation and production of the PML/RARα fusion protein and the ability of retinoids to induce differentiation and inhibition of cell growth in this setting (26, 27). Clinical trials investigating the role of retinoids in the prevention and treatment of solid tumors will now be outlined with a focus on cancers of the upper aerodigestive tract (oropharyngeal and lung) and breast (Table 1).

Head and neck cancer

Premalignant oropharyngeal lesions have been shown to express low levels of RARβ, and it has been hypothesized that restoration of expression could reinstate normal growth and differentiation patterns. Indeed, *RARβ* mRNA expression was induced with retinoid therapy in specimens of oral mucosa available before and after 13-*cis* RA ($n = 39$). The levels of *RARβ* mRNA increased in the specimens from 18 of the 22 patients who had responses to 13-*cis* RA and in 8 of the 17 specimens from the patients without responses ($P = 0.04$), suggesting *RARβ* mRNA as a biomarker of response to therapy (28). An early randomized trial compared the use of 13-*cis* RA with placebo in patients with premalignant oral leukoplakia, with a dramatic decrease in the size of the lesions observed in 67% and 10% of patients, respectively. Unfortunately, relapse occurred in the majority of patients within a few months (29). In a follow-up trial, a lower dose of 13-*cis* RA was significantly more active against leukoplakia than β-carotene and was well tolerated (30). A Cochrane review has subsequently concluded, however, that there is not sufficient evidence currently to support the use of any agent to prevent the progression of oral leukoplakia to oropharyngeal cancer (31). The retinoids have also been evaluated in patients with a diagnosis of localized head and neck cancer after completion of surgery or radiation therapy with little promise overall (32). Because evidence supporting RA's nongenomic action, such as through inhibiting jun N-terminal kinase (*JNK*) phosphorylation or inhibiting the transactivation potential of NF-κB has been reported in head and neck cancer, this mode of action could have contributed to its limited success in treatment of this type of cancer (9, 33, 34).

Lung cancer

A number of studies have also investigated the role of single-agent retinoids in lung cancer prevention in patients at high risk for lung cancer (primary chemoprevention; refs. 35–39), in those with existing premalignant changes in bronchial epithelium or sputum (secondary chemoprevention; refs. 40–42), and in those with a history of lung cancer (tertiary chemoprevention; refs. 43–45). These studies have not indicated a benefit with use of retinoids in these settings, and indeed an increased risk of lung cancer was observed in smokers in some studies (36, 38). Interestingly, one study was designed to investigate whether either of 2 retinoid-based regimens could reverse *RARβ* expression loss in former smokers. A statistically significant restoration of *RARβ* expression and reduction of metaplasia were found in the 9-*cis* RA group when compared with placebo (46). A recent study revealed a dual growth-promoting and repressive role for *RARβ2* in lung cancer cells, which may help explain the inconsistent results observed in clinical trials (47).

On the basis of preclinical observations of the ability of retinoids to enhance chemotherapy-induced cytotoxicity (48, 49), clinical studies have combined retinoids with chemotherapy in the treatment of lung cancer. A randomized phase II study of paclitaxel and cisplatin with or without ATRA was conducted in patients with advanced non–small cell lung cancer (NSCLC, $n = 107$; ref. 50). Both response rate (55.8% vs. 25.4%) and median progression-free survival (8.9 vs. 6 months) favored the arm incorporating ATRA. An association between *RAR-β2* expression and response rate was investigated, but no significant association was identified, perhaps due to the small numbers of tumor samples that expressed the gene (10%, $n = 6$; ref. 50). On the basis of the promising clinical results, a phase III trial is now in the planning stages and aims to evaluate the benefit of *RARβ2* and *RARα* expression as a response biomarker.

In contrast with these results, a phase III trial of bexarotene in combination with chemotherapy yielded disappointing results despite promising single-agent and phase II data (51). Cisplatin and vinorelbine with or without bexarotene were administered to 623 patients with chemotherapy-naïve advanced NSCLCs. There was no difference in survival (the primary study endpoint) between the arms (52).

Breast cancer

Fenretinide has been extensively studied in breast cancer prevention trials. Supportive preclinical studies revealed its inhibition of mammary carcinogenesis in animal models (53), and the selective accumulation of fenretinide in human breast tissue has been documented (54). The role of fenretinide in reducing contralateral or second ipsilateral breast cancer in patients with early breast cancer ($n = 2,867$) revealed no significant difference in these endpoints at 8-year follow-up (55). However, an unplanned exploratory analysis indicated a 35% reduction in events in premenopausal women, with a trend toward a detrimental effect being observed in postmenopausal women. These results have prompted a phase III primary prevention trial in premenopausal women at high risk for breast cancer (56).

Efforts to improve on these results have also included a biomarker trial of fenretinide and low-dose tamoxifen in premenopausal women at high risk of breast cancer. Tamoxifen is an approved agent for breast cancer prevention in high-risk individuals. Despite promising preclinical data supporting the combination (57) and its favorable effects on plasma insulin-like growth factor (IGF)-I levels and mammographic density in this clinical trial, the combination of low-dose tamoxifen plus fenretinide did not reduce breast cancer events compared with placebo. A numerical reduction in the annual odds of breast cancer was observed with both single-agent tamoxifen and fenretinide, supporting ongoing investigation of fenretinide in the breast cancer prevention setting (58).

Clinical trials investigating the retinoids as a single agent in metastatic breast cancer have been disappointing. In a phase II trial investigating single-agent 13-*cis* RA in metastatic breast cancer that was refractory to treatment, no objective responses were observed (59). ATRA administration as a single agent yielded a clinical benefit rate of 26.8% (60). A phase II trial of oral bexarotene ($n = 148$) in metastatic breast cancer reported a clinical benefit rate of approximately 20% with minimal toxicity observed (61).

Binding of RARs throughout the genome is highly coincident with ERα binding in an ER-dependent manner at ER-binding sites, potentially by maintaining ER–cofactor interactions. These findings suggest that RARs, acting in a nongenomic manner, can cooperate with ERα for effective transcriptional activity in breast cancer cells (7, 15). On the basis of the known interaction between the RARs and the ERα pathways, a clinical trial was conducted in patients with hormone-responsive metastatic breast cancer that investigated the addition of hormonal therapy to retinoids. No benefit to the combination therapy was observed at 8-year follow-up (62). Finally, a phase II single-arm trial of ATRA plus paclitaxel was conducted in patients with metastatic breast cancer ($n = 17$). Partial response was observed in 3 patients (17.6%) and stable disease in 10 patients (58.8%), with a clinical benefit rate of 76.4%. Although these results appear promising, they are comparable with historical reports with paclitaxel alone (63).

Potential Mechanisms of Resistance

Although pharmacologic doses of retinoids have proved effective in the treatment of hematologic malignancies (64), clinical trials in the prevention and treatment setting in a number of solid tumors, including lung cancer and breast cancer, have failed to show significant benefit to date (51, 63). The lack of a robust biomarker of response to therapy is one reason for this failure. In addition, a number of potential mechanisms of resistance to these therapies have been proposed.

Epigenetics

In solid tumors, *RARβ* gene expression is frequently lost in primary tumors and their metastasis compared with adjacent noncancerous tissues (65, 66). This provides a possible explanation as to why treatment using RAs in solid

tumors such as breast cancer have previously failed. Our laboratory and others have provided extensive evidence that *RARβ* is silenced in breast cancer by epigenetic modification including both methylation at the promoter region of the gene and a compacted chromatin structure (25, 67). Epigenetically silenced *RARβ* has been shown to be reexpressed in the presence of DNA methyltransferase inhibitors (DNMT) and histone deacetylase (HDAC) inhibitors in *RARβ2*-silent breast cancer cells (67). Treatment with an HDAC inhibitor combined with 9-*cis* RA resulted in regression of prostate and breast cancer xenografts (67, 68). It is possible that the addition of epigenetic modifiers to RA-based therapy will be needed to reactivate *RARβ* in *RARβ*-silent tumors to accomplish significant growth inhibition (Fig. 2). With this in mind, a number of clinical trials have incorporated epigenetic modifiers with retinoids in an attempt to improve the outcomes observed with single-agent retinoids, and thereby potentially overcome resistance. Entinostat (MS-275), a HDAC inhibitor, has been combined with 13-*cis* RA in a phase I trial in patients with advanced solid tumors and lymphomas. The combination was reasonably well=tolerated and a recommended phase II dose was identified for future studies (69). A single-arm phase II study has also been reported that investigated the efficacy of 5-azacitidine (DNMT inhibitor), valproic acid (HDAC inhibitor), and ATRA in patients with hematologic malignancies (70). The best responses to this combination of agents included 14 complete responses and 3 partial responses to therapy. It is important to note that in the clinical trials described to date, the RARβ status of the tumors was not assessed before therapy. Tumors that do not express the *RARβ* receptor are unlikely to respond to RA treatment. The efficacy of retinoids may be further enhanced with the addition of cytotoxic agents to the combination of retinoids and HDAC inhibitors, perhaps by debilitating several critical interacting pathways that the cancer cell depends on for continued growth and proliferation. We have shown that the combination of the HDAC inhibitor entinostat ATRA and low-dose chemotherapy yielded the greatest inhibition of tumor cell growth *in vitro* and in human tumor breast cancer xenografts (71).

Cancer stem cells

Another potential mechanism of resistance to retinoids in solid malignancies is the presence of cancer stem cells. Many studies have attempted to target cancer stem cells with differentiation treatments including RAs (72, 73). Our laboratory has found that treatment of tumors using the HDAC inhibitor ATRA and low-dose doxorubicin not only results in striking tumor regression but also significantly reduces the number of cancer stem cells (unpublished data). Therefore, HDAC treatment may induce differentiation in the stem-like tumor cells, which may circumvent resistance to standard chemotherapy or ATRA treatment

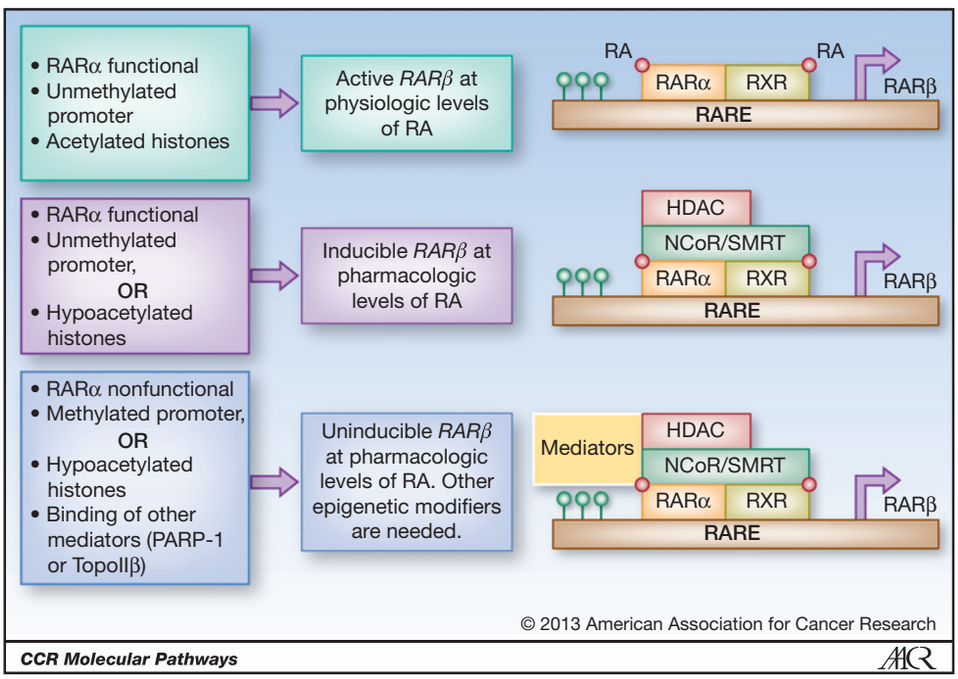

Figure 2. Mechanism of activation of RARβ, an important downstream effector of the RA pathway, in cancer growth inhibition. Under conditions where RARα is functional and the RARβ promoter is not epigenetically silenced, physiologic levels of RA can activate RARβ expression. A small number of solid tumors display this phenotype. Under less ideal conditions in which the RARβ promoter is hypoacetylated, pharmacologic doses of RA are needed to activate RARβ. In the majority of solid tumor types, the RARβ promoter is methylated and/or the histones are significantly deacetylated. In this case, treatment with pharmacologic doses of RA is not sufficient to overcome the repressive effect of epigenetic silencing. Epigenetic-modifying drugs such as DNA methytransferases or HDAC inhibitors are needed to release the epigenetic stress and activate the RARβ gene. NCOR, nuclear receptor corepressor 1; SMRT, silencing mediator for retinoid and thyroid receptors.

alone. Further studies are needed to delineate the role of RA in targeting these cells that are generally deemed treatment resistant.

Other potential mechanisms of resistance

Cancer cells may silence or repress RARβ by mechanisms other than epigenetic modulation to initiate and promote their growth and resist treatment with RA. A number of alternative mechanisms have been proposed, including the loss of coactivators (74), increased RA metabolism (75), decreased RA availability (76), and impaired RARα signaling (77). For example, studies have shown that AF2 coactivators of the RAR–thyroid hormone receptor complex are often lost in human lung cancer (74). The loss of AF-2 cofactors results in low levels of transcribed RARβ, suggesting an important function of these cofactors in mediating *RARβ* expression. Another study showed that impaired RARα function failed to facilitate changes in the chromatin structure of *RARβ* necessary for *RARβ* activation, implicating a critical role for RARα in controlling *RARβ* expression.

Potential mechanisms of resistance that are independent of RARβ have also been suggested. Aberrant p53 expression, for example, has been associated with 13-*cis* RA resistance in the clinic (78); RA, it appears, can promote intrinsic transactivation of p53 (79). It is also possible that cross-talk between the RAR and ER in breast cancer can create opportunities for cancer cells to bypass pathways inhibited by targeted therapies such as RA or hormonal therapies (7, 19).

Conclusions and Future Directions

In summary, retinoids have been investigated extensively for their use in solid tumor cancer prevention and treatment. Promising results have been observed in the breast cancer prevention setting, where fenretinide prevention trials have provided a strong rationale for a new trial in young women at high risk for breast cancer. Clinically relevant outcomes have also been observed with the use of retinoids combined with chemotherapy in NSCLC, prompting the development of confirmatory phase III randomized trials. Further delineation of the mechanisms of action and resistance of retinoids in solid tumors may provide the rationale for future studies and result in clinical benefit for patients. Ongoing and future studies that combine retinoids with epigenetic modifiers, such as the HDAC inhibitors, as well as standard cytotoxic agents, tyrosine kinase inhibitors, and other novel agents are more likely to yield clinically relevant outcomes than observed with single-agent therapy. Novel RA metabolism blocking agents (RAMBA) are also undergoing investigation at this time (80). Finally, clinical trialists should be encouraged to incorporate correlative endpoints in their studies to identify accurate biomarkers of response to retinoid therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R. Connolly, N.K. Nguyen, S. Sukumar
Development of methodology: N.K. Nguyen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N.K. Nguyen
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Connolly, N.K. Nguyen
Writing, review, and/or revision of the manuscript: R. Connolly, N.K. Nguyen, S. Sukumar
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Connolly, N.K. Nguyen
Study supervision: R. Connolly, S. Sukumar

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References

1. Theodosiou M, Laudet V, Schubert M. From carrot to clinic: an overview of the retinoic acid signaling pathway. Cell Mol Life Sci 2010;67:1423–45.
2. Orfanos CE, Zouboulis CC, Almond-Roesler B, Geilen CC. Current use and future potential role of retinoids in dermatology. Drugs 1997;53:358–88.
3. Duvic M, Hymes K, Heald P, Breneman D, Martin AG, Myskowski P, et al. Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous T-cell lymphoma: multinational phase II-III trial results. J Clin Oncol 2001;19:2456–71.
4. Tallman MS, Andersen JW, Schiffer CA, Appelbaum FR, Feusner JH, Ogden A, et al. All-trans-retinoic acid in acute promyelocytic leukemia. N Engl J Med 1997;337:1021–8.
5. Bushue N, Wan YJ. Retinoid pathway and cancer therapeutics. Adv Drug Deliv Rev 2010;62:1285–98.
6. Alvarez S, Germain P, Alvarez R, Rodriguez-Barrios F, Gronemeyer H, de Lera AR. Structure, function and modulation of retinoic acid receptor beta, a tumor suppressor. Int J Biochem Cell Biol 2007;39:1406–15.
7. Ross-Innes CS, Stark R, Holmes KA, Schmidt D, Spyrou C, Russell R, et al. Cooperative interaction between retinoic acid receptor-alpha and estrogen receptor in breast cancer. Genes Dev 2010;24:171–82.
8. Tang XH, Gudas LJ. Retinoids, retinoic acid receptors, and cancer. Annu Rev Pathol 2011;6:345–64.
9. Cras A, Politis B, Balitrand N, Darsin-Bettinger D, Boelle PY, Cassinat B, et al. Bexarotene via CBP/p300 induces suppression of NF-kappaB-dependent cell growth and invasion in thyroid cancer. Clin Cancer Res 2012;18:442–53.
10. Papi A, Guarnieri T, Storci G, Santini D, Ceccarelli C, Taffurelli M, et al. Nuclear receptors agonists exert opposing effects on the inflammation dependent survival of breast cancer stem cells. Cell Death Differ 2012;19:1208–19.
11. Ying M, Wang S, Sang Y, Sun P, Lal B, Goodwin CR, et al. Regulation of glioblastoma stem cells by retinoic acid: role for Notch pathway inhibition. Oncogene 2011;30:3454–67.
12. Lu TY, Li WC, Chen RY, Fan QX, Wang LX, Wang RL, et al. Inhibition effects of all trans-retinoic acid on the growth and angiogenesis of

esophageal squamous cell carcinoma in nude mice. Chinese Med J 2011;124:2708–14.
13. Piskunov A, Rochette-Egly C. A retinoic acid receptor RARalpha pool present in membrane lipid rafts forms complexes with G protein alphaQ to activate p38MAPK. Oncogene 2012;31:3333–45.
14. Dilworth FJ, Chambon P. Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription. Oncogene 2001;20:3047–54.
15. Hua S, Kittler R, White KP. Genomic antagonism between retinoic acid and estrogen signaling in breast cancer. Cell 2009;137:1259–71.
16. Lefebvre P, Martin PJ, Flajollet S, Dedieu S, Billaut X, Lefebvre B. Transcriptional activities of retinoic acid receptors. Vitam Horm 2005;70:199–264.
17. Schug TT, Berry DC, Shaw NS, Travis SN, Noy N. Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. Cell 2007;129:723–33.
18. Wan YJ, An D, Cai Y, Repa JJ, Hung-Po Chen T, Flores M, et al. Hepatocyte-specific mutation establishes retinoid X receptor alpha as a heterodimeric integrator of multiple physiological processes in the liver. Mol Cell Biol 2000;20:4436–44.
19. Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, Mangelsdorf DJ. LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 1995;9:1033–45.
20. Wang Q, Lee D, Sysounthone V, Chandraratna RAS, Christakos S, Korah R, et al. 1,25-dihydroxyvitamin D3 and retinoic acid analogues induce differentiation in breast cancer cells with function- and cell-specific additive effects. Breast Cancer Res Treat 2001;67:157–68.
21. Houle B, Rochette-Egly C, Bradley WE. Tumor-suppressive effect of the retinoic acid receptor beta in human epidermoid lung cancer cells. Proc Natl Acad Sci U S A 1993;90:985–9.
22. Freemantle SJ, Spinella MJ, Dmitrovsky E. Retinoids in cancer therapy and chemoprevention: promise meets resistance. Oncogene 2003;22:7305–15.
23. Liu Y, Lee MO, Wang HG, Li Y, Hashimoto Y, Klaus M, et al. Retinoic acid receptor beta mediates the growth-inhibitory effect of retinoic acid by promoting apoptosis in human breast cancer cells. Mol Cell Biol 1996;16:1138–49.
24. Chambon P. A decade of molecular biology of retinoic acid receptors. FASEB J 1996;10:940–54.
25. Sirchia SM, Ferguson AT, Sironi E, Subramanyan S, Orlandi R, Sukumar S, et al. Evidence of epigenetic changes affecting the chromatin state of the retinoic acid receptor beta2 promoter in breast cancer cells. Oncogene 2000;19:1556–63.
26. Catalano A, Dawson MA, Somana K, Opat S, Schwarer A, Campbell LJ, et al. The PRKAR1A gene is fused to RARA in a new variant acute promyelocytic leukemia. Blood 2007;110:4073–6.
27. Pandolfi PP. Oncogenes and tumor suppressors in the molecular pathogenesis of acute promyelocytic leukemia. Hum Mol Genet 2001;10:769–75.
28. Lotan R, Xu XC, Lippman SM, Ro JY, Lee JS, Lee JJ, et al. Suppression of retinoic acid receptor-beta in premalignant oral lesions and its up-regulation by isotretinoin. N Engl Med 1995;332:1405–10.
29. Hong WK, Endicott J, Itri LM, Doos W, Batsakis JG, Bell R, et al. 13-cis-retinoic acid in the treatment of oral leukoplakia. N Engl J Med 1986;315:1501–5.
30. Lippman SM, Batsakis JG, Toth BB, Weber RS, Lee JJ, Martin JW, et al. Comparison of low-dose isotretinoin with beta carotene to prevent oral carcinogenesis. N Engl J Med 1993;328:15–20.
31. Lodi G, Sardella A, Bez C, Demarosi F, Carrassi A. Interventions for treating oral leukoplakia. Cochrane Database Syst Rev 2006;CD001829.
32. Khuri FR, Lee JJ, Lippman SM, Kim ES, Cooper JS, Benner SE, et al. Randomized phase III trial of low-dose isotretinoin for prevention of second primary tumors in stage I and II head and neck cancer patients. J Natl Cancer Inst 2006;98:441–50.
33. Lee HY, Sueoka N, Hong WK, Mangelsdorf DJ, Claret FX, Kurie JM. All-trans-retinoic acid inhibits Jun N-terminal kinase by increasing dual-specificity phosphatase activity. Mol Cell Biol 1999;19:1973–80.
34. Sun SY, Yue P, Chandraratna RA, Tesfaigzi Y, Hong WK, Lotan R. Dual mechanisms of action of the retinoid CD437: nuclear retinoic acid receptor-mediated suppression of squamous differentiation and receptor-independent induction of apoptosis in UMSCC22B human head and neck squamous cell carcinoma cells. Mol Pharmacol 2000;58:508–14.
35. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N Engl J Med 1994;330:1029–35.
36. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996;334:1150–5.
37. Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. J Natl Cancer Inst 1999;91:2102–6.
38. Virtamo J, Pietinen P, Huttunen JK, Korhonen P, Malila N, Virtanen MJ, et al. Incidence of cancer and mortality following alpha-tocopherol and beta-carotene supplementation: a postintervention follow-up. JAMA 2003;290:476–85.
39. Goodman GE, Thornquist MD, Balmes J, Cullen MR, Meyskens FL Jr, Omenn GS, et al. The Beta-Carotene and Retinol Efficacy Trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping beta-carotene and retinol supplements. J Natl Cancer Inst 2004;96:1743–50.
40. Lee JS, Lippman SM, Benner SE, Lee JJ, Ro JY, Lukeman JM, et al. Randomized placebo-controlled trial of isotretinoin in chemoprevention of bronchial squamous metaplasia. J Clin Oncol 1994;12:937–45.
41. Kurie JM, Lee JS, Khuri FR, Mao L, Morice RC, Lee JJ, et al. N-(4-hydroxyphenyl)retinamide in the chemoprevention of squamous metaplasia and dysplasia of the bronchial epithelium. Clin Cancer Res 2000;6:2973–9.
42. Kelly K, Kittelson J, Franklin WA, Kennedy TC, Klein CE, Keith RL, et al. A randomized phase II chemoprevention trial of 13-CIS retinoic acid with or without alpha tocopherol or observation in subjects at high risk for lung cancer. Cancer Prev Res (Phila) 2009;2:440–9.
43. Lippman SM, Lee JJ, Karp DD, Vokes EE, Benner SE, Goodman GE, et al. Randomized phase III intergroup trial of isotretinoin to prevent second primary tumors in stage I non-small-cell lung cancer. J Natl Cancer Inst 2001;93:605–18.
44. van Zandwijk N, Dalesio O, Pastorino U, de Vries N, van Tinteren H. EUROSCAN, a randomized trial of vitamin A and N-acetylcysteine in patients with head and neck cancer or lung cancer. For the EUropean Organization for Research and Treatment of Cancer Head and Neck and Lung Cancer Cooperative Groups. J Natl Cancer Inst 2000;92:977–86.
45. Pastorino U, Infante M, Maioli M, Chiesa G, Buyse M, Firket P, et al. Adjuvant treatment of stage I lung cancer with high-dose vitamin A. J Clin Oncol 1993;11:1216–22.
46. Kurie JM, Lotan R, Lee JJ, Lee JS, Morice RC, Liu DD, et al. Treatment of former smokers with 9-cis-retinoic acid reverses loss of retinoic acid receptor-beta expression in the bronchial epithelium: results from a randomized placebo-controlled trial. J Natl Cancer Inst 2003;95:206–14.
47. Pappas JJ, Toulouse A, Basik M, Levesque L, Bradley WE. Knockdown of RARB2 identifies a dual role in cancer. Genes Chromosomes Cancer 2011;50:700–14.
48. Lin LM, Li BX, Xiao JB, Lin DH, Yang BF. Synergistic effect of all-trans-retinoic acid and arsenic trioxide on growth inhibition and apoptosis in human hepatoma, breast cancer, and lung cancer cells in vitro. World J Gastroenterol 2005;11:5633–7.
49. Kucukzeybek Y, Gul MK, Cengiz E, Erten C, Karaca B, Gorumlu G, et al. Enhancement of docetaxel-induced cytotoxicity and apoptosis by all-trans retinoic acid (ATRA) through downregulation of survivin (BIRC5), MCL-1 and LTbeta-R in hormone- and drug resistant prostate cancer cell line, DU-145. J Exp Clin Cancer Res 2008;27:37.
50. Arrieta O, Gonzalez-De la Rosa CH, Arechaga-Ocampo E, Villanueva-Rodriguez G, Ceron-Lizarraga TL, Martinez-Barrera L, et al. Randomized phase II trial of All-trans-retinoic acid with chemotherapy based on paclitaxel and cisplatin as first-line treatment in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28:3463–71.

51. Khuri FR, Rigas JR, Figlin RA, Gralla RJ, Shin DM, Munden R, et al. Multi-institutional phase I/II trial of oral bexarotene in combination with cisplatin and vinorelbine in previously untreated patients with advanced non-small-cell lung cancer. J Clin Oncol 2001;19:2626–37.
52. Ramlau R, Zatloukal P, Jassem J, Schwarzenberger P, Orlov SV, Gottfried M, et al. Randomized phase III trial comparing bexarotene (L1069–49)/cisplatin/vinorelbine with cisplatin/vinorelbine in chemotherapy-naive patients with advanced or metastatic non-small-cell lung cancer: SPIRIT I. J Clin Oncol 2008;26:1886–92.
53. Moon RC, Thompson HJ, Becci PJ, Grubbs CJ, Gander RJ, Newton DL, et al. N-(4-Hydroxyphenyl)retinamide, a new retinoid for prevention of breast cancer in the rat. Cancer Res 1979;39:1339–46.
54. Mehta RG, Moon RC, Hawthorne M, Formelli F, Costa A. Distribution of fenretinide in the mammary gland of breast cancer patients. Eur J Cancer 1991;27:138–41.
55. Veronesi U, De Palo G, Marubini E, Costa A, Formelli F, Mariani L, et al. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. J Natl Cancer Inst 1999;91:1847–56.
56. Bonanni B, Lazzeroni M. Retinoids and breast cancer prevention. Recent Results Cancer Res 2009;181:77–82.
57. Ratko TA, Detrisac CJ, Dinger NM, Thomas CF, Kelloff GJ, Moon RC. Chemopreventive efficacy of combined retinoid and tamoxifen treatment following surgical excision of a primary mammary cancer in female rats. Cancer Res 1989;49:4472–6.
58. Decensi A, Robertson C, Guerrieri-Gonzaga A, Serrano D, Cazzaniga M, Mora S, et al. Randomized double-blind 2 $\times$ 2 trial of low-dose tamoxifen and fenretinide for breast cancer prevention in high-risk premenopausal women. J Clin Oncol 2009;27:3749–56.
59. Cassidy J, Lippman M, Lacroix A, Peck G. Phase II trial of 13-cis-retinoic acid in metastatic breast cancer. Eur J Cancer Clin Oncol 1982;18:925–8.
60. Sutton LM, Warmuth MA, Petros WP, Winer EP. Pharmacokinetics and clinical impact of all-trans retinoic acid in metastatic breast cancer: a phase II trial. Cancer Chemother Pharmacol 1997;40:335–41.
61. Esteva FJ, Glaspy J, Baidas S, Laufman L, Hutchins L, Dickler M, et al. Multicenter phase II study of oral bexarotene for patients with metastatic breast cancer. J Clin Oncol 2003;21:999–1006.
62. Chiesa MD, Passalacqua R, Michiara M, Franciosi V, Di Costanzo F, Bisagni G, et al. Tamoxifen vs tamoxifen plus 13-cis-retinoic acid vs tamoxifen plus interferon alpha-2a as first-line endocrine treatments in advanced breast cancer: updated results of a phase II, prospective, randomised multicentre trial. Acta Bio-Medica 2007;78:204–9.
63. Bryan M, Pulte ED, Toomey KC, Pliner L, Pavlick AC, Saunders T, et al. A pilot phase II trial of all-trans retinoic acid (Vesanoid) and paclitaxel (Taxol) in patients with recurrent or metastatic breast cancer. Invest N Drugs 2011;29:1482–7.
64. Fenaux P, Wang ZZ, Degos L. Treatment of acute promyelocytic leukemia by retinoids. Curr Pics Microbiol and Immunol 2007;313:101–28.
65. Widschwendter M, Berger J, Daxenbichler G, Muller-Holzner E, Widschwendter A, Mayr A, et al. Loss of retinoic acid receptor beta expression in breast cancer and morphologically normal adjacent tissue but not in the normal breast tissue distant from the cancer. Cancer Res 1997;57:4158–61.
66. Mehrotra J, Vali M, McVeigh M, Kominsky SL, Fackler MJ, Lahti-Domenici J, et al. Very high frequency of hypermethylated genes in breast cancer metastasis to the bone, brain, and lung. Clin Cancer Res 2004;10:3104–9.
67. Sirchia SM, Ren M, Pili R, Sironi E, Somenzi G, Ghidoni R, et al. Endogenous reactivation of the RARbeta2 tumor suppressor gene epigenetically silenced in breast cancer. Cancer Res 2002;62:2455–61.
68. Qian DZ, Ren M, Wei Y, Wang X, van de Geijn F, Rasmussen C, et al. In vivo imaging of retinoic acid receptor beta2 transcriptional activation by the histone deacetylase inhibitor MS-275 in retinoid-resistant prostate cancer cells. The Prostate 2005;64:20–8.
69. Pili R, Salumbides B, Zhao M, Altiok S, Qian D, Zwiebel J, et al. Phase I study of the histone deacetylase inhibitor entinostat in combination with 13-cis retinoic acid in patients with solid tumours. Br J Cancer 2012;106:77–84.
70. Raffoux E, Cras A, Recher C, Boelle PY, de Labarthe A, Turlure P, et al. Phase 2 clinical trial of 5-azacitidine, valproic acid, and all-trans retinoic acid in patients with high-risk acute myeloid leukemia or myelodysplastic syndrome. Oncotarget 2010;1:34–42.
71. Nguyen N, Korangath P, Sabnis G, Brodie A, Ordentlich P, Stearns V, et al. A combination of HDAC inhibitor entinostat (MS-275), all trans retinoic acid (ATRA) and low dose doxorubicin causes regression of established xenografts of triple negative breast cancer [abstract]. In: Proceedings of the 101st Annual Meeting at American Association for Cancer Research; 2010 Apr 17–21, Washington, DC. Philadelphia (PA): AACR; 2010. Abstract nr 5593.
72. Lee ER, Murdoch FE, Fritsch MK. High histone acetylation and decreased polycomb repressive complex 2 member levels regulate gene specific transcriptional changes during early embryonic stem cell differentiation induced by retinoic acid. Stem Cells 2007;25:2191–9.
73. Gillespie RF, Gudas LJ. Retinoid regulated association of transcriptional co-regulators and the polycomb group protein SUZ12 with the retinoic acid response elements of Hoxa1, RARbeta(2), and Cyp26A1 in F9 embryonal carcinoma cells. J Mol Biol 2007;372:298–316.
74. Moghal N, Neel BG. Evidence for impaired retinoic acid receptor-thyroid hormone receptor AF-2 cofactor activity in human lung cancer. Mol Cell Biol 1995;15:3945–59.
75. White JA, Beckett-Jones B, Guo YD, Dilworth FJ, Bonasoro J, Jones G, et al. cDNA cloning of human retinoic acid-metabolizing enzyme (hP450RAI) identifies a novel family of cytochromes P450. J Biol Chem 1997;272:18538–41.
76. McPherson LA, Woodfield GW, Weigel RJ. AP2 transcription factors regulate expression of CRABPII in hormone responsive breast carcinoma. J Surg Res 2007;138:71–8.
77. Ren M, Pozzi S, Bistulfi G, Somenzi G, Rossetti S, Sacchi N. Impaired retinoic acid (RA) signal leads to RARbeta2 epigenetic silencing and RA resistance. Mol Cell Biol 2005;25:10591–603.
78. Lippman SM, Shin DM, Lee JJ, Batsakis JG, Lotan R, Tainsky MA, et al. p53 and retinoid chemoprevention of oral carcinogenesis. Cancer Res 1995;55:16–9.
79. Duong V, Rochette-Egly C. The molecular physiology of nuclear retinoic acid receptors. From health to disease. Biochim Biophys Acta 2011;18:1023–31.
80. Njar VC, Gediya L, Purushottamachar P, Chopra P, Vasaitis TS, Khandelwal A, et al. Retinoic acid metabolism blocking agents (RAMBAs) for treatment of cancer and dermatological diseases. Bioorganic Med Chem 2006;14:4323–40.