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Tumor-suppressive activity of retinoic acid receptor- β in cancer

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Abstract

Retinoids, a group of structural and functional analogs of vitamin A, are known to regulate a large number of essential biological processes and to suppress carcinogenesis. The effects of retinoids are mainly mediated by nuclear retinoid receptors, which include retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Each receptor has three subtypes (α , β , and γ) and each subtype has different isoforms. Retinoic acid receptor- β (RAR- β) has four isoforms that have different affinities to retinoids and different biological functions. Loss of expression of RAR- β_2 during cancer development is associated with tumorigenesis and retinoid resistance; induction of its expression, on the other hand, can suppress carcinogenesis. Expression of another isoform, RAR- β_4 , is increased in various types of cancer. RAR- β_4 transgenic mice develop hyperplasia and neoplasia in various tissues, and induction of RAR- β_4 expression increases the growth of tumor cells that do not express RAR- β_2 . Future studies will focus on molecular pathways involving RAR- β_2 and the role of RAR- β_4 in cancer development.

Keywords

Retinoids; RAR- β_2 ; RAR- β_4 ; biomarker; methylation; tumorigenesis

1. Introduction

Retinoids, a group of structural and functional analogs of vitamin A, regulate several essential biological processes [1,2]. Pharmacologically, they have been recognized as important in modulating cell growth, differentiation, and apoptosis and in suppressing carcinogenesis in a broad range of tissue types in vitro, in animal models, and in clinical trials [see reviews in 3,4]. The ability of all-trans retinoic acid (RA) to induce the differentiation of acute promyelocytic leukemia (PML) cells into granulocytes is the basis for its therapeutic activity, and it was its effectiveness in this regard that led the U.S. Food and Drug Administration to approve RA as a therapeutic agent for PML. Promising results of retinoid studies in vitro and in vivo led to retinoid compounds being tested in a battery of clinical prevention and treatment trials being carried out all over the world. In clinical chemoprevention trials, retinoids showed activity in patients with oral leukoplakia [5,6], cervical dysplasia [7], bronchial metaplasia [8], actinic keratosis [9], and second primary tumors in the aerodigestive tract [10,11]. However, findings from several other studies indicated that retinoids were not effective and could even be harmful [12–15]. Large randomized trials conducted in Europe and the United States showed that

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moderate doses of natural or synthetic vitamin A compounds were ineffective in reversing premalignancy or in suppressing recurrence of primary tumors [12,13]. Thus, the use of retinoids as chemopreventive agents in human cancers has gone from a zenith in the 1990s to something of a nadir nowadays. Current research efforts focus mainly on the molecular mechanisms underlying the actions of retinoids [16–21] and on use of retinoid X receptor-selective or retinoid receptor-independent retinoids in the prevention of human cancers [22–25].

The effects of retinoids are mediated mainly by two classes of nuclear retinoid receptors: retinoic acid receptors (RARs) and retinoid X receptors (RXRs), both of which are members of the steroid hormone receptor superfamily. Each receptor has three subtypes (α , β , and γ) and each subtype has different isoforms [26,27]. In humans, mRNA for RAR- α is expressed in most tissues; RAR- β expression is prevalent in neural tissues but hardly detectable in skin; and RAR- γ is expressed predominantly in the skin [26,27]. As for the RXRs, RXR- β is found in nearly all tissues; RXR- α is abundantly expressed in the liver, kidney, spleen, and skin; and RXR- γ expression seems to be restricted to mostly muscle and brain [26,27]. Changes in the expression of these receptors have been thought to cause neoplasia and malignant transformation in human cells [see reviews in ref. 3,4]. For example, chromosomal translocations that fuse the PML gene to the RAR- α gene form the chimeric genes PML-RAR α and RAR α -PML during the malignant transformation of PML [28], and recent evidence suggests that these fused genes are causally linked to the pathogenesis of the disease [29–31]. The RAR- β gene is rearranged in human hepatocellular carcinoma as a result of insertion of a hepatitis B virus sequence [32], although the importance of the rearrangement in causing hepatocellular carcinoma warrants further investigation. However, most alterations in nuclear retinoid receptors result from the reduction or loss of their expression in various premalignant and malignant tissues and cells. For example, loss of RAR- γ expression has been noted in skin cancer or premalignant lesions [33], but loss of RAR- β_2 expression is common in a wide variety of cancers [3,4]. Because much of the work published to date has focused on role of RAR- β_2 in various cancers, the focus of this review is on the molecular role of RAR- β in regulating cell growth and differentiation and in suppressing carcinogenesis.

2. Loss of RAR- β_2 expression as a biomarker in solid tumors

Since the 1990s, loss of the expression of nuclear retinoid receptors, including RAR- β_2 , in various cancer cell lines has been detected by northern blotting or reverse transcriptase-polymerase chain reaction techniques [34–36]. Our group was the first to analyze the expression of transcripts of these receptors in formalin-fixed and paraffin-embedded tissues by using in situ hybridization [37]. Afterwards, many studies have demonstrated that among these receptors, loss of RAR- β_2 is the most common and the loss is progressive in premalignant and malignant tissues and cells (Table 1), including those of the head and neck [38,39], breast [40,41], lung [42,43], esophagus [44,45], pancreas [46], cervix [47], and prostate [48,49]. The observations that RAR- β_2 was upregulated in patients after treatment with 13-cis RA and that increased expression of RAR- β_2 correlated with clinical response [39] suggest that RAR- β_2 has an important role in suppressing carcinogenesis. After these findings were published, the methylation status of the RAR- β_2 gene promoter was used as a biomarker for the early detection of malignancy or as an intermediate end-point marker to monitor the efficacy of chemoprevention agents in clinical trials [50–60].

Methylation of the RAR- β_2 gene promoter, along with methylation of other gene promoters, has been evaluated as a biomarker of breast cancer risk [50,51]. In one study, methylation of the RAR- β_2 gene promoter occurred in 32% of benign breast samples from patients with cancer but in only 9% of similar samples from patients without breast cancer [50]. In another study, random periareolar fine needle aspiration samples from the mammary glands showed

methylation of the RAR- β_2 gene promoter in 69% of primary breast cancers tested, and methylation was positively associated with increasing cytologic abnormality in those samples [51]. Hypermethylation of the RAR- β_2 gene promoter is also an important factor in predicting early recurrence of lung cancer and may be useful as a prognostic marker in non-small cell lung cancer (NSCLC) as well, although the clinical implications of these findings need further investigation [52]. Methylation analysis of the RAR- β_2 gene promoter can also aid in the diagnosis of primary lung cancer in bronchial aspirate samples [53] or in serum DNA [54].

In chemoprevention studies, RAR- β_2 has often been used as an intermediate end-point biomarker in clinical trials of various retinoids [39,43,57,58,61]. However, evidence from preclinical studies suggests that RAR- β_2 may also be useful for monitoring the efficacy of other agents, such as inhibitors of the epidermal growth factor receptor (EGFR) and β -cryptoxanthin [59,60].

3. Molecular mechanisms responsible for loss of RAR- β_2 expression

The mechanisms underlying the loss of RAR- β_2 gene expression are not fully understood [reviewed in 3,4]. Early studies showed that loss of RAR- β_2 expression in some forms of cancer (e.g., NSCLC) resulted from chromosome 3p deletion [62]), but in others (e.g., head and neck cancer) neither homozygous deletions nor gene rearrangements have been found [34]. Since those findings were published, other studies have shown that transcriptional deregulation can silence RAR- β_2 expression through decreased levels of co-activators, the presence of co-repressors, or epigenetic mechanisms such as histone deacetylation [63–68]. Expression of RAR- β_2 also depends on the cellular level of retinoids because this receptor is itself an RA-inducible gene. Indeed, RAR- β_2 expression is selectively reduced in several organs during vitamin A-deficient states and is enhanced by RA, as demonstrated in studies of rats [69,70]. In humans, some premalignant tissues are deficient in vitamin A because of reduced uptake of vitamin A from serum or abnormally elevated catabolism of intracellular retinoids [71,72]. We found that the binding of an anti-RA antibody to tissue sections of premalignant lesions was much lower than the binding to normal oral mucosa, suggesting that the lesions had lower levels of retinoids [72]. Lack of RAR- α expression may also contribute to lost RAR- β_2 expression. RAR- α regulates RAR- β_2 transcription by mediating dynamic changes of RAR- β_2 chromatin in the presence and absence of RA. Interfering with RA signaling by silencing RAR- α exacerbates the repression of chromatin for RAR- β_2 and leads to RAR- β_2 transcriptional silencing. RAR- β_2 silencing is also associated with resistance to the growth-inhibitory effect of RA, indicating that RAR- β_2 silencing and RA resistance result from impaired integration of RA signaling at RAR- β_2 chromatin [73].

Nevertheless, many recent studies have shown that the diminished expression of RAR- β_2 in the development of different human cancers results from epigenetic silencing by methylation of cytosine-phospho-guanosine (CpG) islands in the promoter region of the gene [50–56]. Moreover, treatment with 5-aza-2-deoxycytidine, a DNA demethylation agent, was able to induce RAR- β expression in various cancer cell lines [74–77], further supporting the notion that RAR- β_2 is a tumor suppressor gene. Cigarette smoke causes morphologic changes and the loss of RAR- β_2 expression in lung tissues of animals [78], and cigarette smoke and the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone have been shown to induce the methylation of the RAR- β_2 gene promoter in murine lung cancer [79]. Our own studies similarly showed that benzo[a]pyrene diol epoxide (BPDE), a carcinogen present in tobacco smoke and environmental pollution, and bile acid, a tumor promoter in the gastrointestinal tract, suppressed RAR- β_2 expression in premalignant and malignant esophageal cells and that BPDE induced the methylation of the RAR- β_2 gene promoter [19,80]. Notably, epigallocatechin gallate, a chemopreventive agent extracted from the tea plant, has been shown to bind to DNA methyltransferases and reactivate RAR- β_2 expression in esophageal cancer

cells [81]. Thus, as discussed in section 2, methylation of the RAR- β_2 gene promoter is being evaluated as a biomarker for the early detection or prediction of prognosis for various forms of cancer in humans.

In patients with NSCLC, hypermethylation of the RAR- β gene promoter has different effects on the development of second primary lung cancers (SPLCs) depending on smoking status [82]. In one study, Kim et al [82] showed that current smokers developed more SPLCs when the RAR- β gene promoter was unmethylated than when it was hypermethylated. In contrast, the development of SPLCs in former smokers was higher in patients with hypermethylated than unmethylated RAR- β . This group suggested that in current smokers, the continuous high oxygen tension and free radicals induce apoptosis, which could inhibit the development of SPLC. However, another explanation is that overexpression of RAR- β_4 may be important in the development of SPLCs in active smokers. Three observations prompted this idea: First, expression of both RAR- β_2 and RAR- β_4 is controlled by the same RAR- β promoter (P2; details are given below); second, methylation of the RAR- β gene promoter shuts down the expression of both isoforms; and third, RAR- β_2 expression is lost but RAR- β_4 expression is increased in various forms of cancer, including lung [83]. Collectively, these observations support the idea that hypermethylation of the RAR- β gene promoter may have a protective effect in tumors in which RAR- β_4 is overexpressed. Previous studies have also revealed that exposure to tobacco smoke or BPDE suppresses RAR- β_2 expression through methylation of the RAR- β gene (19,79,80). This may be true for active smokers, but unmethylated RAR- β could trigger RAR- β_4 expression in various cancers [84–86], although the hypothesis that tobacco smoke can increase RAR- β_4 expression requires further testing. In contrast, hypermethylation of the RAR- β gene reduces RAR- β_2 expression, thereby losing any RAR- β_2 -protective effects in the suppression of tumorigenesis; therefore nonsmokers or former smokers with hypermethylated RAR- β tend to develop more SPLCs.

In contrast to previous evidence of the importance of RAR- β_2 in suppressing cancer development, our own studies showed that expression of RAR- β in patients with early-stage NSCLC was unexpectedly associated with very poor prognosis [87]. Overexpression of RAR- β correlated with the increased expression of cyclooxygenase-2 (COX-2), an enzyme known to be present at elevated levels in progressive carcinogenesis and a marker of poor prognosis in various malignancies [88], and with increased levels of telomerase [89]. These findings were unexpected, as they contradict the concept of RAR- β_2 functioning as a tumor suppressor gene. In seeking possible mechanisms for this effect, we recently found that reduced expression of RAR- β_2 correlated with increased RAR- β_4 expression in esophageal cancer tissues [84]. This finding may help to clarify this apparent discrepancy between studies, because the in situ hybridization technique we used in the NSCLC studies cannot distinguish among RAR- β isoforms (β_1 , β_2 , and β_4). Given the finding that the ratio of RAR- β_4 to RAR- β_2 expression in human lung and breast cancer cell lines is higher than that in normal cells [83–86] and that reduced RAR- β_2 expression correlated with increased RAR- β_4 expression [84], we speculate that the in situ hybridization–detected RAR- β mRNA in NSCLC tissues is the RAR- β_4 isoform, which has oncogenic effects on cells. This speculation, however, requires further investigation.

4. RAR- β isoforms

Differences in the actions of P1 and P2, the two known RAR- β promoters (P1 initiates transcription of isoforms 1 and 3, and P2 initiates transcription of isoforms 2 and 4), and alternative splicing [26,27,90,91] give rise to four major isoforms of RAR- β in mice (β_1 , β_2 , β_3 , and β_4) and three in humans (β_1 , β_2 , and β_4). Additional isoforms (e.g., RAR- β_5 and RAR- β_1') have been identified in human cancer cells [92,93]. Briefly, RAR- β_1 is a fetal isoform that may be a master developmental gene in humans; it is also expressed in small-cell lung cancer [94]. In one study involving transgenic mice, RAR- β_1 was found to have unique tumor

suppressor activity that could not be entirely compensated by the overexpression of RAR- β_2 and the suppression of RAR- β_4 [95]. RAR- β_2 is the most abundant and the major RA-inducible isoform, and thus the term RAR- β in the literature usually refers to the RAR- β_2 isoform. RAR- β_4 is generated by alternative splicing from the same primary transcripts as those generating RAR- β_2 and is initiated by the CUG codon [90]. The cDNA sequence of RAR- β_4 in regions B to F is identical to that of the same regions of RAR- β_2 . The A region of RAR- β_4 —only 4 amino acids long—is much shorter than that of RAR- β_2 [90] (Fig. 1). Moreover, this non-AUG codon seems to be relatively inefficient, resulting in alternative use of an internal methionine codon at +448 bp that yields an RAR- β isoform in which all amino acid sequences at the N-terminal of the second finger of the DNA binding domain are truncated. Because the full-length and truncated RAR- β_4 proteins retain the ability to heterodimerize with RXR- α and to interact with transcription cofactors but lack the DNA-binding capacity to regulate gene expression [85,90,96], RAR- β_4 and the truncated RAR- β_4 may act structurally as a dominant-negative form of RAR- β_2 [96]. In addition, RAR- β_4 has an elevated K_d value for RA binding and cannot inhibit AP-1 activity, as RAR- β_2 can [97]. Little is known as yet about the most recently discovered isoforms, RAR- β_5 and RAR- β_1' . RAR- β_5 is expressed in human breast cancer cells, and its detection correlates with the RA resistance of estrogen-negative breast cancer cells [92]. RAR- β_1' has antitumor activity in lung cancer [93]. In summary, the various RAR- β isoforms in humans have different affinities to RA (at least with regard to RAR- β_2 and RAR- β_4) and different biological functions (e.g., the RAR- β_2 protein is a tumor suppressor whereas RAR- β_4 has oncogenic properties).

The etiology of RAR- β_4 upregulation in cancer cells remains unknown. We speculate that altered expression of microRNAs (miRNA) in cancer tissues may be responsible. miRNA is a class of naturally occurring small noncoding RNA, 18 to 22 nucleotides long, that posttranslationally silences gene expression through binding to complementary target mRNAs, thereby degrading these mRNAs or inhibiting their being translated into protein [see reviews in 98]. This discovery has greatly broadened our understanding of gene regulation mechanisms. Although comprehensive knowledge of the specific mRNA targets of miRNAs is lacking [98,99], bioinformatics analyses may predict their mRNA targets; for example, miR-16, miR-128, and miR-30e can target the RAR- β gene [98]. Further investigation of this exciting discovery is warranted.

5. Role of RAR- β_2 and RAR- β_4 in human carcinogenesis

To date, several convincing lines of evidence have shown correlations between loss of RAR- β_2 expression and increased carcinogenesis in humans, but only a few studies have linked RAR- β_4 expression with cancer development. With respect to RAR- β_2 , lung carcinoma cells expressing transfected RAR- β_2 are less tumorigenic in nude mice than the vector-control-transfected cells [100], and transgenic mice expressing antisense RAR- β_2 develop lung cancer [101]. Transfection with an RAR- β_2 expression vector can suppress the growth of various cancer cell lines and restore the sensitivity of those cells to RA treatment [see reviews in 3,4]. Knocking out RAR- β_2 by homologous recombination in F9 mouse teratocarcinoma cells resulted in the loss of RA-associated growth arrest and changes in cell morphology and differentiation [102]. Our own findings have shown that induction of RAR- β_2 expression in esophageal cancer cells suppressed tumor cell growth and colony formation and induced apoptosis [18]. Others have shown that RAR- β_2 also suppressed breast cancer cell metastasis in a mouse xenograft model [103]. In another study, loss of RAR- β_2 expression in patients with neuroblastoma correlated with poor prognosis [104]. Also, esophageal, lung, and breast cancer cell lines that do not express RAR- β_2 are resistant to retinoid treatment [35,44,73]. Finally, in one study of oral dysplastic tissues, half of the cells cultured from those tissues had become immortalized, as indicated by loss of RAR- β_2 and p16 expression, mutations in p53, and induction of hTERT mRNA [105]. Treating these cells with 5-aza-2-deoxycytidine reversed

one of the immortal phenotypes and led to re-expression of both RAR- β_2 and p16 [106]. A subsequent study by the same group showed that only the loss of RAR- β and p16 was responsible for the immortalization of oral dysplastic cells [107].

Expression of RAR- β_4 , on the other hand, was found to be increased in esophageal cancer tissues and the increase was associated with reduced expression of RAR- β_2 [84]. Pharmacologic concentrations of RA have been shown to increase RAR- β_4 expression in esophageal cancer cell lines (our unpublished data) and in breast cancer cells (86). Several other studies have shown that hyperplasia and neoplasia developed spontaneously in various tissues in RAR- β_4 transgenic mice [83] and that induction of RAR- β_4 expression enhanced the growth of cancer cells that do not express RAR- β_2 (our unpublished data). As noted earlier in this review, RAR- β_4 has an elevated K_d value for RA binding and cannot inhibit AP-1 activity, as does RAR- β_2 [97]. Collectively, these findings suggest that RAR- β_4 either may be an oncogene or may have oncogenic effects. Further investigation of the effects of RAR- β_4 on tumor cell growth, tumorigenesis, and gene expression will clarify whether RAR- β_4 is a dominant-negative form of RAR- β_2 or an oncogene. Such knowledge can be put to use in the development of better strategies to control cancer development.

6. Molecular pathways involved in the mediation of tumor suppression by RAR- β_2

Although the precise molecular mechanisms responsible for RAR- β_2 -mediated antitumor activity are not fully understood, we previously found that RAR- β_2 suppressed the expression of EGFR, activating protein-1 (AP-1), and COX-2 and the phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (Erk1/2) [19]. We also found that COX-2 expression was downregulated in RAR- β_2 -transfected esophageal cancer cells and that the restoration of sensitivity to RA was mediated by suppression of COX-2 expression [18]. Further, we unexpectedly found that stable transfection with RAR- β_2 cDNA did not restore the sensitivity of COX-2-negative esophageal cancer cells to RA or inhibit tumor formation in nude mouse xenograft models. These findings indicate that the antitumor effect of RAR- β_2 may require the suppression of COX-2 expression (our unpublished data). To further substantiate this notion, we studied the expression of RAR- β_2 and COX-2 mRNA in tissue specimens and found RAR- β_2 expression to be associated with low levels of COX-2 expression in esophageal cancer tissues. We also found that after a 3-month treatment with 13-cis RA, the induction of RAR- β_2 expression in human oral leukoplakia tissues correlated with a reduction in COX-2 expression and a clinical response (our unpublished data). Collectively, our findings suggest that the antitumor activity of RAR- β_2 occurs through the suppression of COX-2 expression, although further study is needed to determine whether manipulation of COX-2 expression in these cells could antagonize this antitumor activity. We also determined that downregulation of COX-2 expression by RAR- β_2 took place through the inhibition of EGFR, with subsequent decreases in ERK1/2 phosphorylation and AP-1 expression [19]. Most importantly, we showed that induction of COX-2 expression by the carcinogen BPDE depended on both the expression and the inhibition of RAR- β in esophageal cells [19] (Fig. 2). A previous study showed that stable transfection with RAR- β_2 exhibited strong inhibition of AP-1 activity in tumor cells, even in the absence of RA. Moreover, expression of the endogenous AP-1-responsive gene collagenase I was strongly repressed in cancer cells stably transfected with RAR- β_2 (108). Another line of evidence comes from a study showing that a high-fat diet reduced the expression of PPAR- γ and RAR- β_2 mRNA, increased COX-2 and β -catenin levels, and increased the number of aberrant crypt foci in rat colon tissue. However, vitamin A was able to prevent these high-fat-diet-induced alterations of PPAR- γ and RAR- β_2 and the increases in COX-2 and β -catenin [109]—further evidence that that RAR- β_2 can suppress COX-2 expression.

A cDNA microarray analysis comparison of a parental and an RAR- β_2 -transfected lung cancer cell line revealed 27 genes expressed at different levels between the two cell lines [16]. Several of the affected genes code for proteins whose functions augment apoptosis or host immune response. Other investigators identified and characterized genes that are differentially expressed in wild-type cells and RAR- $\beta_2^{-/-}$ F9 teratocarcinoma cells by using subtractive hybridization and DNA array analysis [17]. The identified genes, which encode transcription factors, cell surface signal-transduction molecules, and metabolic enzymes, included c-myc, FOG1, GATA6, glutamate dehydrogenase, Foxq1, Hic5, Meis1a, Dab2, midkine, and the PDGF- α receptor [17]. Again, induction of RAR- β_2 expression suppressed the potential for metastasis of breast cancer cells. Another cDNA microarray study showed that RAR- β_2 induced the expression of tumor-cell antigens (CTAG1 and CTAG2), the innate immune response (e.g., RIG-I/DDX58), and a tumor suppressor gene (TYRP1) but reduced the expression of several genes involved in cell adhesion (LSAMP, PCDH11Y, and CD64), nutrient availability (FABP6, SLC38A2, PCSK4, SRPB1), and transcription/AP-1 activity (HOXB7 and JUN) [20].

We identified and cloned a retinoid receptor-induced gene, RRIG1, which is expressed differentially by RAR- β_2 -positive and -negative esophageal cancer cells, by using restriction fragment differential display-polymerase chain reaction techniques [21]. RRIG1 is expressed in a broad range of normal tissues but is lost in various types of cancer [21,110]. RRIG1 mediates the effect of RAR- β_2 in the regulation of cancer cell growth and gene expression [21]. The RRIG1 protein is expressed in the cell membrane and binds to and inhibits small GTPase RhoA activity. Whereas induction of RRIG1 expression inhibits RhoA activation and f-actin formation and consequently reduces colony formation, invasion, and proliferation of esophageal cancer cells, antisense RRIG1 increases RhoA activity and f-actin formation and thus induces the colony formation, invasion, and proliferation of these cells. These findings indicate the existence of a novel molecular pathway involving RAR- β_2 regulation of RRIG1 expression and RRIG1-RhoA interactions [21]. Further studies of RRIG1 and the pathways involved may translate into better control of esophageal cancer.

In summary, a large body of evidence has established that RAR- β_2 participates in the regulation of cell growth and the suppression of carcinogenesis, although the molecular mechanisms of action deserve further study. However, the role of RAR- β_4 in cancer development and its molecular signaling transduction pathways has remained virtually unexplored.

7. Future directions

The associations between loss of RAR- β_2 expression and tumorigenesis, and induction of RAR- β_2 expression and the suppression of cancer development, have been well established [3,4]. However, expression of the closely related isoform RAR- β_4 is increased in various types of cancer [83–86]. RAR- β_4 transgenic mice developed hyperplasia and neoplasia in various tissues [83], and induction of RAR- β_4 expression increased growth of tumor cells that do not express RAR- β_2 (our unpublished data). Therefore, future studies are needed of the molecular pathways triggered by RAR- β_2 to suppress cancer development, as are other studies of the role of RAR- β_4 in cancer development. Issues remaining to be clarified include whether RAR- β_4 -mediated oncogenic effects on normal and cancerous cells are attributable to its modulation of RAR- β_2 -mediated signal pathways, or whether RAR- β_4 acts on separate signaling pathways leading to malignant transformation and cancer development. Answers to these questions will provide crucial clues for resolving the occasionally contradictory and controversial findings from studies of retinoids for cancer prevention and treatment.

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Fig. 1.

Comparison of RAR- β_2 and RAR- β_4 cDNA (modified from ref. 90). **A.** Organization of RAR cDNA is shown at the top and RAR- β isoforms at the bottom. The location of sequences that encode regions B to F and the 3'-untranslated region (UTR) common to all RAR- β isoforms is indicated, as well as those of the 5'-UTR and A region, which is isoform-specific. Numbers correspond to nucleotide positions in the human RAR- β_2 cDNA sequence. The "A" regions of RAR- β_2 and RAR- β_4 are represented as a solid box. The region of RAR- β_2 that is spliced to give RAR- β_4 (between 266–619 bp) is indicated with a dashed line. **B.** Sequence and schematic representation of donor splice sites for RAR- β_4 and RAR- β_2 (positions 265 and 619, respectively).

Fig. 2.

A molecular pathway triggered by RAR-β₂ for the suppression of human carcinogenesis. Benzo (a)pyrene diol epoxide (BPDE) exposure is a risk factor in the development of esophageal cancer. BPDE is thought to act by suppressing RAR-β₂ expression and, in turn, upregulating EGFR expression and Erk1/2 phosphorylation, resulting in the induction of AP-1 and COX-2 expression. Further, BPDE can reduce the expression of retinoid receptor-induced gene-1 (RRIG1), perhaps through reduction of RAR-β₂. RRIG1 mediates RAR-β₂ effects on the regulation of cancer cell growth and gene expression. RRIG1 protein binds to and inhibits RhoA activity and consequently suppresses Erk1/2 phosphorylation and expression of COX-2

and cyclin D1, resulting in reductions in tumor cell colony formation, invasion, and proliferation.

Table 1

Suppression of Nuclear Retinoid Receptors in Premalignant Lesions and Tumors

Lesion	Lost receptor expression
Oral premalignant lesions	RAR- β_2
Bronchial squamous metaplasia	RAR- β_2
Head and neck cancer	RAR- β_2
Lung cancer	RAR- β_2 , RAR- γ , RXR- β
Pancreatic cancer	RAR- β_2
Esophageal cancer	RAR- β_2
Breast cancer	RAR- β_2 , RAR- α
Prostate cancer	RAR- β_2 , RXR- β