Journal of Cellular Biochemistry 102:886–898 (2007)

# Retinoids in Biological Control and Cancer

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**Abstract** More than 80 years ago, Wolbach and Howe provided the first evidence suggesting a link between alterations within human cells that lead to malignancies and vitamin A deficiencies (Wolbach and Howe [1925] Nutr. Rev. 36: 16–19). Since that time, epidemiological, preclinical and clinical studies have established a causative relationship between vitamin A deficiency and cancer. Laboratory research has provided insight into the intracellular targets, various signaling cascades and physiological effects of the biologically-active natural and synthetic derivatives of vitamin A, known as retinoids. Collectively, this body of research supports the concept of retinoids as chemopreventive and chemotherapeutic agents that can prevent epithelial cell tumorigenesis by directing the cells to either differentiate, growth arrest, or undergo apoptosis, thus preventing or reversing neoplasia. Continued refinement of the retinoid signaling pathway is essential to establishing their use as effective therapeutics for tumor subtypes whose oncogenic intracellular signaling pathways can be blocked or reversed by treatment with retinoids. J. Cell. Biochem. 102: 886–898, 2007.


**Key words:** retinoids; retinoic acid; ovarian cancer; cancer; apoptosis; differentiation; growth arrest; RAR/RXR

## NATURAL AND SYNTHETIC RETNOIDS

Retinoids are a group of over 4000 different natural and synthetic derivatives of vitamin A. Vitamin A and its retinoid derivatives are essential to the body's important processes such as vision, immune function, reproduction, maintenance of epithelial tissue, and differentiation [De Luca, 1991]. Consequently, vitamin A deficiency presents many significant health consequences such as night blindness, loss of vision, retardation, shortening and thickening of bones, atrophy of the testes, fetal reabsorption, and immunodeficiency leading to increased morbidity and mortality [Shils, 2006]. Vitamin A itself cannot be naturally synthesized and therefore must be ingested as a dietary pro-vitamin such as β-carotene or as preformed retinol or its esters. β-carotene is the major dietary source of vitamin A and is abundant in green-leaf vegetables, carrots, sweet potatoes and egg yolks. Each molecule of β-carotene is cleaved to form retinal by the enzyme 15–15′ β-carotene oxygenase, located in the intestinal epithelium. Retinal, active only in vision, is either irreversibly oxidized to all-trans retinoic acid by retinal dehydrogenases or reduced to retinol, the major transport form of vitamin A, by retinaldehyde reductases. Another major dietary source of vitamin A is retinyl esters, which are found in milk, red meat products, and liver. Retinyl esters, the major storage form of vitamin A, can be hydrolyzed into retinol in the small intestine. Retinol is then absorbed by the intestinal mucosa cells through passive diffusion and complexed with cellular retinol-binding protein II (CRBP II). Regardless of the dietary source of vitamin A, retinol is esterified by lecithin-retinol-acyl transferase in the intestinal mucosa cells. Chylomicrons deliver retinyl-ester to the liver and to a lesser extent to tissues of the bone marrow, spleen, adipose tissue, and the kidneys. When the body is in need of vitamin A, retinyl esters in the liver are hydrolyzed to retinol and transported to needy tissues bound to retinol binding protein

Grant sponsor: NIH; Grant number: CA64945.

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Received 6 July 2007; Accepted 10 July 2007

DOI 10.1002/jcb.21530

(RBP) [Harant et al., 1993; Chao et al., 1997; Blomhoff and Blomhoff, 2006; Soprano et al., 2006].

Synthetic retinoids are compounds that are systematically manufactured to have similar structure and function as natural retinoids. The impetus for their design is to exploit the functional activity of naturally occurring retinoids while minimizing the toxicity at therapeutic doses. The large number of synthetic retinoids is a reflection of the many attempts made to obtain synergism among the structural moieties for optimal function. Synthetic retinoids can also act as agonist or antagonist in either pan- or selective-retinoid receptor binding. The restriction of the retinoid conformation has been shown to reduce the binding to a subset of the receptors and hence provide a more focused physiological effect [Willhite and Dawson, 1990]. For example, 6-naphthalenecarboxylic acid (CD437) and Fenretinide N-[4-hydroxyphenylretinamide (4-HPR)] are clinically important conformationally restricted synthetic retinoids that selectively bind RAR-$\gamma$ [Fanjul et al., 1996]. While naturally occurring retinoids induce differentiation and growth arrest, synthetic retinoids, such as fenretinide or 4HPR, and AHPN or CD437 inhibit cell growth through induction of apoptosis [Sheikh et al., 1995; Fanjul et al., 1996; Wu et al., 1998a].

## RETINOID MECHANISM OF ACTION

Retinoid activity is mediated by cellular retinol or cellular retinoic acid binding proteins (CRBP and CRABP), which sequester the retinoid and facilitate transport to different enzymes involved in its metabolism [Takase et al., 1986]. Once in the nucleus, the retinoid signal is transduced by two families of nuclear receptors, the retinoic acid receptor (RAR) family and the retinoid X receptor (RXR) family. RARs and RXRs includes three isotypes (designated $\alpha$, $\beta$, and $\gamma$), which are encoded by three different genes. Each isotype consists of a number of isoforms that are generated by the mechanisms of alternate promoter usage or by alternative splicing of transcripts [Giguere et al., 1987; Kastner et al., 1995]. These receptors, which function as ligand-activated transcription factors, exist as RAR/RXR heterodimers and to a lesser extent as RXR/RXR homodimers [Tsai et al., 1998]. In the presence of their natural ligands, 9-cis-RA in the case of RXR and 9-cis-RA and ATRA in the case RAR, the ligand-receptor complexes act as inducible transcription factors by binding to retinoic acid response elements (RAREs) found in the promoter regions of target genes [Schrader et al., 1993] (Fig. 1). Two types of RAREs on retinoic acid regulated genes bind the RAR/RXR heterodimer, the DR-2 type and the more common DR-5 type [Predki et al., 1994] DR-2 and DR5 are composed of two hexameric canonical 5′PuG(G/T)TCA3′ repeat sequences spaced 2 and 5 bps apart, respectively [Kato et al., 1995]. In the absence of ligands, co-repressors such as nuclear receptor corepressor (NCoR) or silencing mediator for retinoid and thyroid hormone receptors (SMRT) associate with the RAR/RXR heterodimer and recruit histone deacetylase (HDAC)-containing complexes that consequently lead to the compaction of chromatin. When an RAR ligand is available, it destabilizes the CoR-binding interface and induces transconformation that allows the interaction with coactivators (CoAs). These CoAs can activate the RAR/RXR dimers by recruiting histone acetyltransferases (HATs) which lead to chromatin decondensation over the target gene promoter region [Chen and Evans, 1995; Lavinsky et al., 1998]. It is now well established that at least one of the mechanisms by which retinoids carry out their functions is by activating or repressing specific genes via the action of the RAR/RXR nuclear receptors. However, it is becoming even more clear that retinoids also act via post transcriptional mechanisms which alter the stability of cell cycle regulatory proteins such as Rb2/p130 and p27 (see below).

## RETINOIDS AS DIFFERENTIATING AGENTS

The role of vitamin A during embryonic development was first recognized in the 1930s when maternal vitamin A deficiency was found to be associated with a number of defects [Mason, 1935; Hale, 1937]. Later, it was demonstrated that an excess of vitamin A caused a number of congenital abnormalities [Cohlan, 1935]. Following these initial observations, a large number of studies have investigated the role of vitamin A and more specifically RA, RARs and RXRs in differentiation in both the adult and the embryo. In vitro studies, using pluripotent embyronal carcinoma (EC) and embryonic stem (ES) cells as a model system provide evidence of RA-induced cellular

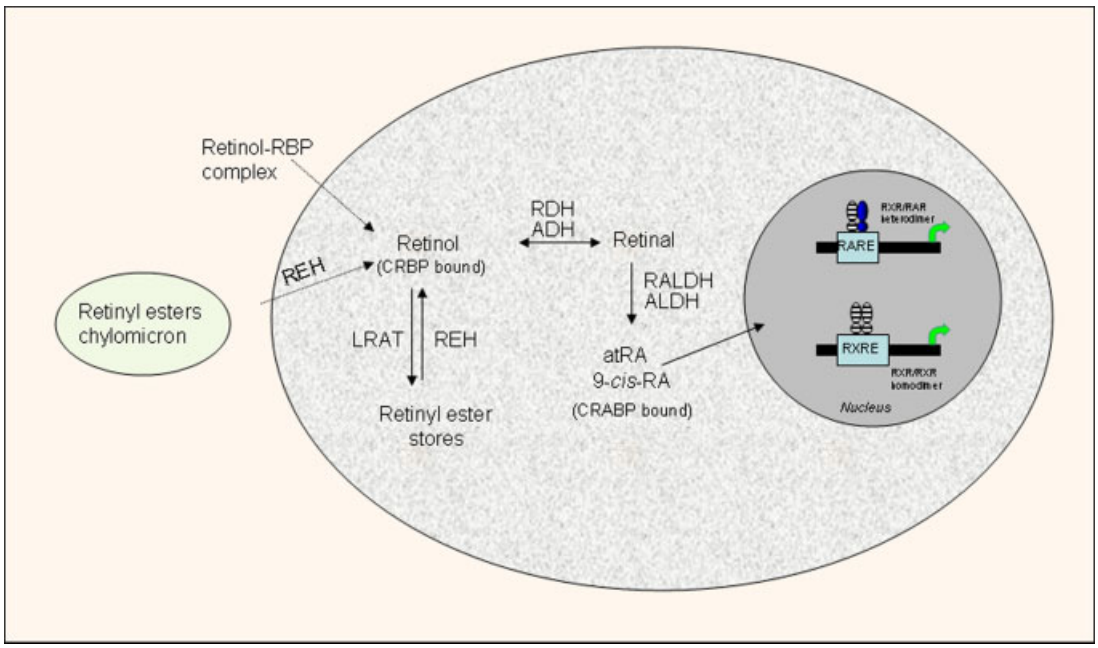


**Fig. 1.** Metabolism of vitamin A in target cells to biologically active retinoic acid. Once in the nucleus the retinoid signal is transduced by means of gene expression by two families of nuclear receptors, the retinoic acid receptor (RAR) and the retinoid X receptor (RXR). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

differentiation of EC and ES cells into such cell types as, endodermal, mesodermal, neuronal, myocardial, and fibroblast cells. Furthermore, other studies indicate that RA is a critical regulator of early embryonic development as well as adult neurogenesis [Jacobs et al., 2006] (Fig. 2). Still, more studies provide evidence that the molecular mechanism of RA-induced differentiation occurs by regulating a cascade of gene expression events (Table I). The genes whose expression is altered by RA during differentiation include those which function as transcription factors, RA metabolism and transport proteins, protooncogenes, apoptosis-related proteins and growth factors among others. The temporal pattern of their expression occurs in phases regardless of the pathway. There are subsets of genes whose expression changes very quickly, within 16 h upon RA treatment. These genes have RAREs in their promoters and hence respond directly to RA binding to the RAR/RXR heterodimer. A larger group of genes exhibit altered expression patterns 1 or more days after RA treatment and hence are indirectly regulated by RA treatment and more restricted to a particular differentiation pathway. There can also be a third phase which involves the expression of genes representative of the differentiated phenotype.

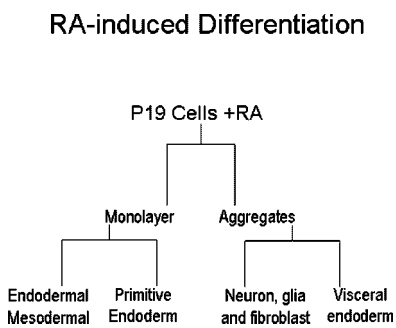


**Fig. 2.** One example of an RA-dependent differentiation pathway as exhibited by P19 cells.

The genes whose expression pattern changes early after RA treatment include RARα and RARβ whose increased expression is mediated by RAREs located in the promoter of each gene [Shen et al., 1991]. Studies have shown that RARβ is necessary for the RA-dependant increase in p27 transcription, translation and protein half-life [Li et al., 2004] and is also required for the sustained expression of other RA-responsive genes [Zhuang et al., 2003]. Hoxa-1 and Hoxb-1 genes, which also have RAREs located in their 3′ enhancer regions, also increases rapidly upon RA treatment in EC

**TABLE I. Molecular Mechanism of RA-Induced Cellular Differentiation Occurs by Regulating a Cascade of Gene Expression Events**

| Phase | RA effect | RA response | Event |
|---|---|---|---|
| Retinoids-induced differentiation regulated by a cascade of gene expression events | | | |
| I | Direct | Immediate 0–16 h | Genes with RAREs in promoters (transcription regulators): Ex. RARα, RARβ, Hoxa-1, Hoxb-1, Cyp26A1 |
| II | Indirect | 1–3 days | Secondary responses mediating differentiation: Ex. Pbx, Sox6, Rex-1, Wnt-1 |
| III | Indirect | 3+ days | Genes expressed in the differentiated phenotype: Ex. Troma, neurofilament, laminin |

The temporal pattern of their expression occurs in phases regardless of the pathway.

and ES cells [Langston and Gudas, 1992; Langston et al., 1997]. Hoxa-1 was shown to regulate the expression of genes that affect cell morphology during differentiation to neuroectoderm and mesoderm and represses differentiation to endoderm [Martinez-Ceballos et al., 2005]. However, it is not known whether Hoxa-1 effects are direct or indirect. Pre-B-cell leukemia transcription factors (Pbx) family of proteins function as cofactors for the transcriptional regulation of Hox proteins. PBX2 is expressed early and PBX1 and PBX3 proteins are expressed later. They all function to promote RA-induced endodermal and neuronal differentiation [Knoepfler and Kamps, 1997; Qin et al., 2004]. Cyp26 is an RA-inducible P450 cytochrome that regulates the catabolism of RA producing hydroxylated products. Overexpression of cyp26a1, in the presence of low concentrations of RA, promotes rapid neuronal differentiation [Pozzi et al., 2006]. Sox6 was shown to promote the aggregation and neural differentiation of p19 cells upon RA treatment. Inhibition of Sox6 resulted in the inhibition of neuronal differentiation and the induction of apoptosis [Hamada-Kanazawa et al., 2004]. Furthermore Sox6 overexpression leads to an increase in Wnt-1 protein expression. Wnt-1 was shown to be important, although not solely sufficient for RA-induced neuronal differentiation.

## RETINOIDS AS CHEMOPREVENTION AGENTS

Epidemiological and animal studies have long suggested an inverse correlation between cancer development and dietary consumption of vitamin A or beta-carotene. As early as 1925, Wolbach and Howe [1925] reported similar epithelia keratinization and augmented growth in tissues from vitamin A deficient rats to that observed in neoplastic tissues obtained from a vitamin A deficient human. In 1981, Kark published the results of a 16-year study investigating the serum retinol levels of 174 individuals of whom half developed cancer. They determined that the 85 patients who eventually developed cancer had significantly lower mean serum retinol levels than their age, race and sex matched cancer-free counterparts [Kark et al., 1981].

Preclinical models demonstrating the efficacy of both natural and synthetic retinoids as chemopreventive and chemotherapeutic agents in the treatment of cancer was provided in 1974, when Lasnitzki [1976] reported that the suppression of experimentally induced precancerous hyperplasia, parakeratosis, and metaplasia of the mouse prostate epithelial cells with concomitant administration of either retinol, retinoic acid, or a synthetic analog with the MCA carcinogen. Additionally, clinical evidence of the chemopreventive effects of retinoids have been reported in breast cancer [Veronesi and Decensi, 2001], renal-cell carcinoma [Miller et al., 2000] oral premalignant lesions [Han et al., 1990; Lippman et al., 1993; Klaassen and Braakhuis, 2002], bronchial epithelium of chronic smokers [Misset et al., 1986; Pastorino et al., 1993], skin premalignant lesions [Greenberg et al., 1990; Tangrea et al., 1992; Moon et al., 1997], cervical neoplasia [Meyskens et al., 1994; Braud et al., 2002], and several other precancer conditions [De Palo et al., 1995].

## RETINOIDS AS CHEMOTHERAPEUTIC AGENTS

### Retinoids and APL

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML). Patients with APL have an accumulation of undifferentiated hematopoietic blast cells and suffer from severe hemorrhagic events caused by a coagulation disorder. In 1977, it was observed that acute promyelocytic leukemia

patients presented a consistent t(15:17) chromosomal translocation [Rowley et al., 1977]. Later, the translocation was determined to fuse the PML growth suppressor gene, located on chromosome 15, to the RARα gene, located on chromosome 17q21 [Park and Fairweather, 1996]. Furthermore, the PML-RARα fusion protein was shown to form homodimers (or oligomers) of itself with greater affinity than heterodimers with RXR, while still retaining the ability to bind RA as a ligand and bind to RAREs of target genes [Benedetti et al., 1997]. As a consequence, the homodimerized PML-RARα has a dominant negative effect on RAR/RXR heterodimer function by (1) reducing the RA available to bind to RAR/RXR heterdimers, (2) interfering with the binding of RAR/RXR heterodimers to the RAREs of target genes and (3) actively repressing transcription of target genes by binding corepressors with enhanced affinity. The increased binding of the corepressor mediates increased HDAC recruitment, chromatin condensation and ultimately increased repression of transcriptional activity [Soprano et al., 2004]. However, pharmacological doses of ATRA were shown to completely dissociate the corepressor and associated chromatin remodeling complex from the PML-RARα homodimer, allowing the binding of coactivators and transcriptional activation [Lin et al., 2000; Soprano et al., 2004]. Early studies suggested that treatment of APL patients with 13-cis-RA could induce APL cell differentiation [Flynn et al., 1983]. However it was the clinical trails of 1987 that provided evidence of retinoids as potential chemotherapeutic agents for the treatment of APL. Huang reported complete remission of 24 APL patients treated with all-trans retinoic acid. The patients additionally suffered significantly less toxicities and side effects with ATRA treatment than with the previous treatment protocol. More impressive is that of the 24 patients that obtained complete remission, 8 were previously nonresponsive or had developed resistance to first line therapies [Huang et al., 1987, 1988; Sanz, 2006]. The success of treating APL patients with ATRA highlighted the fact that cell differentiation therapy is a potent and practical method for the treatment of human cancer.

### Retinoids and Breast Cancer

Breast cancer is the most frequently diagnosed cancer among American women, however mortality has decreased significantly as a result of improved treatment and early diagnosis. Low intake of β-carotene has been shown to increase the risk of breast cancer [Hislop et al., 1990; Moon, 1994; Rohan et al., 1998]. Several studies have established an inhibitory role of retinoids in breast cancer in experimental animal models [Moon and Mehta, 1990; Moon et al., 1992; Veronesi and Costa, 1992; Costa, 1993; Moon, 1994]. For example, Moon et al. [1976] reported a 52% reduction in the incidence of mammary cancer in rats treated with retinyl acetate. However, of all of the retinoids evaluated for efficacy against chemically-induced mammary cancer, retinyl acetate and fenretinide (4-HPR) appear to be the most efficacious. However, in contrast to retinyl acetate which accumulates in the liver, causing significant hepatotoxicity [Moon et al., 1979], 4-HPR accumulates in the mammary gland in a dose-related manner [Hultin et al., 1986; Costa et al., 1995].

In vitro studies indicate that retinoids inhibit the growth of estrogen receptor (ER)-positive but not ER-negative human breast cancer cells [Fontana et al., 1987; Koga and Sutherland, 1991]. These ER-negative cells were demonstrated to express significantly lower RAR-β levels compared to their ER-positive matched cells [Roman et al., 1992; van der Burg et al., 1993; Sheikh et al., 1993b]. In addition, ER-negative cells transfected with RAR-β exhibited retinoid-induced growth inhibition [Sheikh et al., 1994; van der Leede et al., 1995]. Furthermore, protein expression analysis of human breast cancer cell lines revealed either a consistent loss or down-regulation of RAR-β mRNA expression. Exogenous overexpression of RAR-β in these breast cancer cell lines led to RA-induced growth arrest and apoptosis [Seewaldt et al., 1995; Liu et al., 1996]. The growth of ER-positive RA breast cancer cells were shown to be inhibited by treatment with 9-cis-RA by blocking entry into S phase [Zhao et al., 1995]. Since 9-cis RA serves as a ligand for both RAR and RXR, it was further revealed that the mechanism responsible for the retinoid sensitivity of breast cancer cells does not involve transcriptional modulation of the RXRs but instead involves signaling through RAR [Zhao et al., 1995].

The downstream signaling events that lead to retinoid-induced breast cancer cell growth arrest is thought to occur at least in part through AP-1 antagonism [Fanjul et al., 1994].

A class of retinoids, which include SR11328 and SR11302, selectively inhibits AP-1 activity but does not activate transcription, has been shown to effectively inhibit the proliferation of several breast cancer cell lines [Fanjul et al., 1994]. However, anti-AP-1 activity may not be the only mechanism involved in growth inhibition. p53, an important tumor suppression gene, has been found upregulated in MCF-7 breast cancer cells by 9-cis RA [James et al., 1995]. Furthermore, a novel synthetic retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) could directly induce p21WAF1/CIP1 and cause apoptosis of breast cancer MCF-7 cells and MDA-MB-231 cells independent of p53 [Shao et al., 1995]. Also, one study showed that RA-induced growth arrest in MDA231-RAR-β transduced cells was associated with c-myc mRNA down-regulation [Seewaldt et al., 1995]. In addition to the effects on the expression of these cell growth associated genes, retinoids also block growth stimulation by IGF-I. For example, RA inhibition of cell proliferation may occur through induction of IGF binding proteins (IGFBP-1,2,3), which could bind IGF-I and inhibit the IGF-I-mediated mitogenic signal transduction pathway [Fontana et al., 1991; Adamo et al., 1992; Sheikh et al., 1993a; Chen et al., 1994]. In clinical trials, 4-HPR was found to decrease plasma IGF-I levels in early breast cancer patients [Torrisi et al., 1993]. All of these facts indicate that retinoids can serve as potential chemotherapeutic agents in the treatment of breast cancer. There is evidence in breast cancer models that IRS-1 is the main adaptor molecule activated by IGF-1R and plays a role in cell proliferation. IRS-2 on the other hand is thought to regulate cell motility and plays a role in metastasis [Dearth et al., 2007; Gibson et al., 2007]. A number of studies have demonstrated that retinoids inhibit breast cancer cell growth through interference in the IGF-1R signaling pathway. Inhibition of IGF-I stimulated, increased production of IGF binding protein 3 and recently, downregulation of Akt pathway due to degradation of IRS-1 [del Rincon et al., 2003, 2004] are some of the mechanisms by which retinoids inhibit growth of breast cancer cells.

In clinical studies, synergism has been reported when retinoids have been combined with other growth inhibitory agents, such as estrogen antagonists [Fontana et al., 1987; Koga and Sutherland, 1991], interferon α and β [Fanjul et al., 1996; Marth et al., 1986, 1987, 1993; Moore et al., 1994], and vitamin D derivatives [James et al., 1995; Saunders et al., 1995].

## Retinoids and Ovarian Cancer

Ovarian cancer is the sixth most frequent cancer in women worldwide [Parkin et al., 2005]. It is the second most commonly diagnosed gynecologic cancer in women worldwide, the deadliest gynecologic malignancy, and the fourth leading cause of cancer-related deaths in women in the USA [McGuire et al., 2002a,b; Jemal et al., 2005]. Nulliparity, never having given birth, is one of the few recognized risk factors for ovarian cancer, with nulliparous women having a two- to threefold increased risk when compared to their parous counterparts.

Attempts to arrest ovarian cancer cell growth have included a variety of chemotherapies, including alkylating and platinum compounds, the most common of which is a regimen of Paclitaxel plus Cisplatin. Unfortunately, patients ultimately become resistant to this therapy and only 20–30% of patients live to 5 years [Zhang et al., 2000]. Retinoids represent a group of molecules that show promise as a chemotherapeutic agent. ATRA has been shown to inhibit ovarian tumor cell growth through multiple pathways: (1) repression of AP-1 activity [Soprano and Soprano, 2003], (2) induction of suppressor growth factors such as TGF-beta, (3) alteration of the G1-specific cell regulatory gene expression [Zhang et al., 2001].

The mechanism of RA-induced growth arrest of ovarian tumor cells has been well studied in our laboratory. ATRA has been shown to arrest the growth of ovarian carcinoma cells in G0/G1 and to elicit the elevation of Rb2/p130, PP2A, and p27 proteins levels [Wu et al., 1997; Vuocolo et al., 2003, 2004; Zhang et al., 2001; Soprano et al., 2006] (Fig. 3). The critical role of both retinoid nuclear receptor (RAR) and retinoid-X-receptors (RXR-α) in mediating RA inhibitory functions has been demonstrated previously using anti-sense and dominant-negative approaches to reduce the expression and function of retinoid receptors [Wu et al., 1998b]. Likewise, overexpression of RARs and RXR-α nuclear receptors in the RA-resistant ovarian tumor cell line, SKOV3, was shown to partially restore growth arrest upon RA treatment [Wu et al., 1998b]. However, failure to revert completely to the wildtype phenotype suggests that the

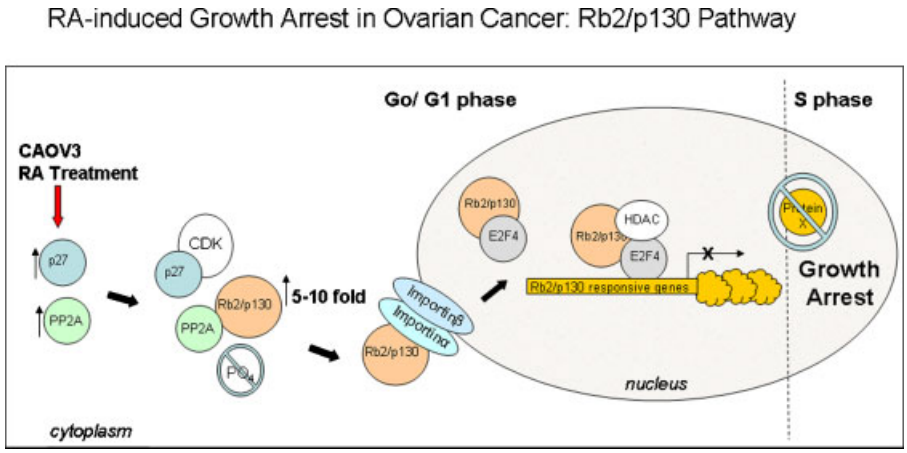


**Fig. 3.** Retinoic acid-induced growth arrest of ovarian carcinoma cells in G0/G1 requires the function of such cell cycle proteins as p27, PP2A, Rb2/p130, E2F family of transcription factors and CDKs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mechanism of RA-resistance in SKOV3 cells is not simply low RA nuclear receptor levels.

Further in vitro studies indicate that ATRA-induced growth arrest requires the function of such cell cycle proteins as Rb2/p130, p27, PP2A, cyclins, Cdks and the E2F family of transcription factors. Rb2/p130 is a member of the highly homologous retinoblastoma (Rb) family of tumor suppressors [Paggi et al., 1996; Mayol and Grana, 1997]. It is currently known that this family of proteins functions by binding and sequestering members of the E2F family of transcription factors that, when active, promote the expression of genes required for cell cycle progression [Moberg et al., 1996; Mayol and Grana, 1997; Ferreira et al., 1998]. The activity and stability of the Rb2/p130 tumor suppressor is regulated through phosphorylation, which is modulated by p27, PP2A, GSK3, cyclin A/CDK2, cyclin D/CDK4 and cyclin D/CDK6 complexes. The hypophosphorylated form has been shown to selectively bind to E2Fs thus mediating growth arrest and the most hyperposphorylated form has been shown to be targeted by ubiquitin for degradation [Ludlow et al., 1993; Mayol and Grana, 1997, 1998; Smith et al., 1998; Hansen et al., 2001; Tedesco et al., 2002; Vuocolo et al., 2003, 2004; Litovchick et al., 2004]. Following ATRA treatment, PP2A—a serine/threonine phosphatase, binds at the nuclear localization signals in the carboxy terminus of Rb2/p130 and catalyzes its dephosphorylation and increase of the half-life of Rb2/p130 in the cell [Purev et al., 2006]. These NLS sites have also been shown to be important for the binding to importin alpha and the targeting of Rb2/p130 to the nucleus [Purev et al., 2006]. Thus in the ATRA-treated ovarian carcinoma cells, PP2A binds to the Rb2/p130 and dephosphorylates the NLS of Rb2/p130 leading to the interaction of importin α with Rb2/p130. Importin β then binds to the importin α-Rb2/p130 complex, leading to the translocation of the Rb2/p130 to the nucleus where it acts to arrest ovarian cancer cells in G1 and suppress proliferation.

### Retinoids and Apoptosis

Apoptosis, also known as programmed cell death, is important for maintaining normal tissue and cell physiology in multicellular organisms. Apoptosis is essential for the development and maintenance of cellular homeostasis. It can be induced from stimuli originating from outside the cell via the extrinsic pathway or by stimuli originating from inside the cell via the intrinsic pathway. For the extrinsic pathway, apoptosis is initiated by cell membrane molecules, such as Fas and TNFR (tumor necrosis factor receptor). For the intrinsic pathway, apoptosis is initiated by an internal signal within cells, usually the mitochondria. Dysfunction of the apoptosis pathway can lead to the development of tumors. Many cancer therapeutic agents, including synthetic retinoids such as 4-HPR and CD437 exert their effect by inducing apoptosis (Fig. 4).

CD437 has been demonstrated to inhibit the growth of both ATRA-sensitive (CA-OV3) and ATRA-resistant (SK-OV3) ovarian tumor cell lines as well as to induce apoptosis [Wu et al.,

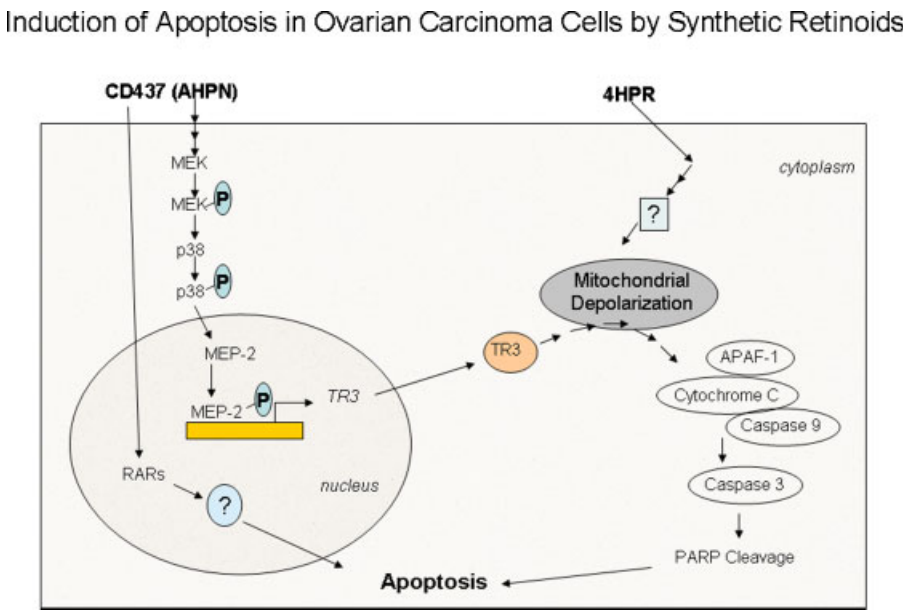


**Fig. 4.** A model of CD437- and 4HPR-induced apoptosis in ovarian carcinoma cells. CD437 induces apoptosis via RAR-dependent or MAP kinase-dependant pathway. Both CD437 and 4HPR requires the depolarization of the mitochondria and the release of apoptotic proteins such as cytochrome *c* and caspases. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

1998b; Holmes et al., 2000]. Since it is well established that RARs mediate retinoid signaling that ultimately leads to growth arrest, it is logical to ask whether the nuclear receptors also mediate the retinoid signaling pathway that results in apoptosis. To address this, RAR protein levels and function was modulated by overexpressing either wild type RARγ or a pan dominant negative mutant of all RAR subtypes called RAR-β (R269Q) [Tairis et al., 1995], or with treatment of an RAR-γ antagonist, MM11253. Inhibition of RAR function reduced but did not eliminate induction of apoptosis in both CA-OV3 and SK-OV3 cells by CD437. Furthermore, overexpression of wild type RARγ increased apoptosis after treatment with CD437. These data suggest that CD437 induces apoptosis via both RAR dependent and RAR independent pathways. In contrast, 4-HPR induces apoptosis via only an RAR independent pathway [Holmes et al., 2000].

Caspase activity is a hallmark of the induction of apoptosis. In response to CD437 and 4-HPR treatment caspase-3 activity is induced in both the CA-OV-3 and the SK-OV-3 ovarian carcinoma cell lines. Using caspase-3 inhibitors, caspase-3 activation was shown to be essential for the induction of apoptosis by both CD437 and 4-HPR [Holmes et al., 2002, 2003]. In contrast, caspase-8 activity was not necessary for the induction of apoptosis by either CD437 or 4-HPR because no appreciable caspase-8 activity was detected and caspase-8 inhibitors did not block induction of apoptosis by these retinoids. However, caspase-9, which functions as an activator of caspase-3, was shown to be induced following both CD437 and 4-HPR treatment and was required for activation of caspase-3 and induction of apoptosis. Mitochondrial membrane depolarization was also shown to be necessary for the activation of apoptosis by CD437 and 4-HPR. Using two chemical reagents, Bongkreikic and Betulinic acid, which inhibit the depolarization of mitochondrial membranes and induces the depolarization of mitochondrial membranes respectively through the activation of caspase-9 and caspase-3, apoptosis was abrogated and reinstated [Holmes et al., 2002, 2003]. In response to the depolarization of the mitochondrial membrane, pro-apoptotic proteins such as cytochrome *c*, procaspase-9, and APAF-1 are released. These proteins can then associate and cleave procaspase-9 to the active caspase-9. Active caspase-9 then cleaves procaspase-3 to active caspase-3. Thus the order of events in the late stages of apoptosis induction by CD437 and 4-HPR are similar: mitochondrial depolarization, caspase-9 activation, subsequent activation of capase-3. The induction of apoptosis by CD437 and 4-HPR

however, utilizes separate early molecular pathways that converge at the depolarization of the mitochondrial membrane.

## SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

The mechanisms by which ATRA causes growth inhibition of ovarian carcinoma cells are complex. Our lab has identified several distinct mechanisms to date, which bring to light the multifunctional activities of ATRA. We have identified changes in expression of proteins such as Rb2/p130, p27, and PP2A. These proteins are upregulated in response to ATRA treatment and cause a response via their normal activities, which are to slow cell growth through several post-transcriptional mechanisms summarized earlier. Our studies are the first to show that ATRA treatment lends to the binding of PP2A to the Rb2/p130 protein, which renders Rb2/p130 non-susceptible to ubiquitination dependent proteosomal degradation. Moreover, interaction of PP2A with Rb2/p130 C-terminus also plays an important role in translocating Rb2/p130 to the nucleus by mediating interaction of the Rb2/p130 with the nuclear transport proteins importin $\alpha$ and importin $\beta$. Once in the nucleus, Rb2/p130 acts to inhibit the transcription of E2F-regulated genes required for G1 progression and entry into S phase.

Understanding the molecular mechanism of ATRA-mediated growth arrest and/or apoptosis will allow us to design more effective therapy for a variety of human cancers including ovarian cancer. For example, based on our findings, assays for Rb2/p130 and p27 protein level, Rb2/p130 phosphorylation status, mutations in Rb2/p130 NLS sequences, PP2A activity and presence of Rb2/p130 in the nucleus could provide invaluable prognostic tools to evaluate the efficacy of ATRA treatment for late stage ovarian cancer. Likewise, modulation of these proteins through gene therapy could be used to increase sensitivity of drug resistant ovarian tumor cells to retinoid treatment.

Little is known about the mechanism of retinoid resistance. Preliminary studies from our lab suggest that one mechanism that contributes to ATRA resistance involves the sequestration of the Rb2/p130 tumor suppressor protein in sub-nuclear bodies or as aggregates. When the levels of these proteins or their interacting proteins are modified in vitro, ATRA-sensitivity is restored and growth arrest occurs. Since innate and acquired resistance to ATRA and other retinoids limits their usefulness as chemotherapeutic agents, high throughput screening can (1) assist in determining the suitability of the patient for ATRA treatment based on the protein profile of the tumor and (2) modulation of those proteins that confer retinoid resistance can potentially make a tumor sensitive to retinoid treatment. Therefore continued refinement of the retinoid signaling pathway and understanding the mechanisms that govern retinoid resistance will be essential to future employment of retinoids as effective anti-cancer therapeutics.

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