

Retinoids in cancer therapy and chemoprevention: promise meets resistance

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Retinoids (natural and synthetic derivatives of vitamin A) signal potent differentiation and growth-suppressive effects in diverse normal, premalignant, and malignant cells. A strong rationale exists for the use of retinoids in cancer treatment and chemoprevention based on preclinical, epidemiological, and early clinical findings. Despite the success of all-*trans*-retinoic acid (RA)-based differentiation therapy in acute promyelocytic leukemia (APL), the broad promise of retinoids in the clinic has not yet been realized. In addition to the expected limited activity of any single therapeutic agent, translation of retinoid activities from the laboratory to the clinic has met with intrinsic or acquired retinoid resistance. Evidence suggests that solid tumors develop intrinsic resistance to retinoids during carcinogenesis. In contrast, relapse of APL is often associated with acquired resistance to retinoid maturation induction. This review discusses what is known about retinoid resistance mechanisms in cancer therapy and chemoprevention. Strategies to overcome this resistance will be discussed, including combination therapy with other differentiation-inducing, cytotoxic or chromatin-remodeling agents, as well as the use of receptor-selective and nonclassical retinoids. Opportunities exist in the post-genomic era to bypass resistance to classical retinoids by identifying target genes and associated pathways that directly mediate the antineoplastic effects of retinoids. In this regard, the retinoids are useful pharmacological tools to reveal important pathways targeted in cancer therapy and chemoprevention.

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Introduction

The retinoids are natural and synthetic derivatives of vitamin A that regulate a variety of important cellular functions. A strong rationale exists for the use of retinoids in cancer therapy and chemoprevention based on preclinical, epidemiological, and clinical findings, as reviewed by Hong and Itri (1994), Hong and Sporn

(1997), and Nason-Burchenal and Dmitrovsky (1999). Preclinical studies, first reported by Wolbach and Howe (1925), indicated that vitamin A-deficient rodents developed squamous metaplasia. These metaplastic changes in the lung were reminiscent of alterations found in smokers, and were reversed by vitamin A repletion. Experimental animal model studies revealed retinoid chemopreventive effects on the epithelium of tissues exposed to chemical mutagens (Moon *et al.*, 1994). Epidemiological evidence indicated an inverse relationship between cancer incidence at specific sites and serum vitamin A or β -carotene levels, as reviewed by Hong and Itri (1994).

These findings provided a basis for the use of retinoids in clinical cancer chemoprevention. Preneoplastic diseases including oral leukoplakia, cervical dysplasia, and xeroderma pigmentosum have been successfully treated with retinoids, as reviewed by Hong and Sporn (1997), Nason-Burchenal and Dmitrovsky (1999), and Sun and Lotan (2002). Retinoids have also been reported to reduce second malignancies in the liver or aerodigestive tract and in the breast (Hong and Sporn, 1997; Nason-Burchenal and Dmitrovsky, 1999; Sun and Lotan, 2002; Kitareewan *et al.*, 2003). Retinoids were also shown to be active in the treatment of certain malignancies such as acute promyelocytic leukemia (APL), juvenile chronic myelogenous leukemia, mycosis fungoides, Kaposi's sarcoma, and high-risk neuroblastoma, as reviewed by Cheer and Foster (2000), Reynolds and Lemons (2001), and Kitareewan *et al.* (2003). When combined with interferon- α 2A, 13-*cis*-retinoic acid is active in the treatment of specific epithelial malignancies that include squamous cell cancers of the skin or cervix, and advanced renal cancer (Moore *et al.*, 1994; Berg *et al.*, 2000).

These and other findings anticipated the broad activity of retinoids in cancer therapy or chemoprevention. In contrast to the reported beneficial clinical effects that are summarized in Table 1, evidence of intrinsic or acquired resistance has limited retinoid clinical activity. Recent randomized clinical trials using β -carotene or classical retinoids have not shown a benefit in reduction of primary or second lung cancers, especially in smokers (Khuri and Lippman, 2000; Lippman *et al.*, 2001). Mechanisms responsible for the biological effects of retinoids are not fully understood. For this reason, models have been established to identify anticarcinogenic pathways activated by retinoids in defined cellular

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Table 1 Clinical activity of classical and nonclassical retinoids in cancer therapy and chemoprevention (representative clinical examples are depicted)

Therapeutic application	Compound
Malignancy	
Promyelocytic leukemia	All- <i>trans</i> -retinoic acid (tretinoin)
Neuroblastoma	13- <i>cis</i> -retinoic acid (isotretinoin)
Kaposi's sarcoma	9- <i>cis</i> -retinoic acid (alitretinoin)
Cutaneous T-cell lymphoma	Bexarotene (targretin)
Premalignancy	
Oral leukoplakia	13- <i>cis</i> -retinoic acid (isotretinoin)
Xeroderma pigmentosum	13- <i>cis</i> -retinoic acid (isotretinoin)
Chemoprevention	
Second breast cancers	Fenretinide (4-HPR)
Second primary hepatocellular carcinomas	Acyclic retinoid (polyphenic acid)
Combination therapy	
Squamous cell skin cancer	13- <i>cis</i> -retinoic acid with interferon- α -2A
Cervical cancer	

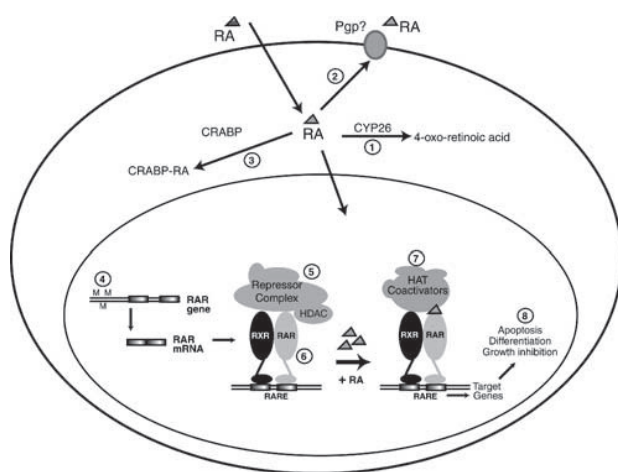


Figure 1 Potential mechanisms of RA resistance. Cellular retinoid resistance may occur through (1) increased *P*450 catabolism, (2) drug export (P-glycoprotein (Pgp) mediated?), (3) sequestration of retinoids by CRABPs or other proteins, (4) decreased expression of RARs through promoter methylation, as depicted (M), (5) persistent histone deacetylation, (6) RAR rearrangement or mutation in the RAR ligand-binding domain, (7) coactivator alteration, or (8) alterations downstream of target gene expression

systems. Critical for their rational use in the clinic is the identification of mechanisms of intrinsic or acquired resistance to retinoids (Figure 1). This knowledge should predict those most likely to benefit from retinoid therapy, and provide strategies to optimize single-agent or combination retinoid regimens to overcome resistance. This review summarizes retinoid resistance mechanisms and potential strategies to overcome or reduce the clinical impact of this resistance.

Mechanisms of retinoid actions

Retinoids are needed for normal embryonic development through postnatal development, and in adult life

for vision, growth, immune function, reproduction, and homeostasis for diverse tissues. All-*trans*-retinoic acid (RA) activates the classical nuclear retinoic acid receptors (RAR), while 9-*cis*-retinoic acid activates the RAR and nonclassical nuclear retinoid X receptors (RXRs), as reviewed by Piedrafita and Pfahl (1999) and Kitareewan *et al.* (2003). There are six genes encoding retinoid receptors: RAR α , RAR β , and RAR γ , as well as RXR α , RXR β , and RXR γ . Multiple receptor isoforms exist through the alternate usage of splice sites and promoters. The ligand-binding domains of RARs and RXRs are distinct, and can be pharmacologically targeted separately, as shown in Table 2. RARs can heterodimerize with RXRs, while RXRs heterodimerize with other nuclear receptors, including the thyroid hormone receptors (TR), vitamin D receptor (VDR), and peroxisomal proliferator activated receptor (PPAR γ), among others, as reviewed by Kitareewan *et al.* (2003).

RAR-RXR heterodimers bind to specific genomic DNA sequences designated as retinoic acid response elements (RARE), which are characterized by two half sites with the consensus sequence AGGTCA. These are generally arranged as direct repeats (DR) separated by two or five nucleotides. Other RXR heterodimers bind to similar half sites with different preferences for spacing and orientation, as reviewed by Rastinejad (2001). Direct target genes of retinoid receptors have been described, including genes involved in retinoid signaling such as RAR β , CRBP II, and CRABP II, as well as transcription factors or cofactors including Oct3/4, Hoxa1, and Hoxb4. Other candidate retinoid targets have been reviewed (McCaffery and Drager, 2000), and with the advent of microarray-based technology the complement of RA-regulated genes should be uncovered. Initial microarray data revealed novel as well as previously recognized candidate retinoid target genes, and confirmed the expected cell context differences (Tamayo *et al.*, 1999; Lian *et al.*, 2001; Dokmanovic *et al.*, 2002; Freemantle *et al.*, 2002; Houldsworth *et al.*, 2002).

Table 2 Receptor specificity of classical and nonclassical retinoids

Retinoid class	Receptor
<i>Physiological retinoids</i>	
All- <i>trans</i> -retinoic acid	RAR α , RAR β , and RAR γ
9- <i>cis</i> -retinoic acid	RAR α , RAR β , and RAR γ and RXR α , RXR β , and RXR γ
<i>Synthetic retinoids — receptor dependent</i>	
Targretin/bexarotene	RXR α , RXR β , and RXR γ
<i>Synthetic retinoids — receptor-dependent and -independent</i>	
4-HPR/fenretinide	RAR β and RAR γ
CD437/AHPN	RAR γ
TAC-101	RAR α

In the absence of ligand, RXR-RAR heterodimers are bound to DNA in complex with corepressors that actively repress transcription. Silencing mediator of RAR and TR (SMRT) and nuclear receptor corepressor (NCoR) were the first members of the repressor complex identified (Chen and Evans, 1995; Horlein *et al.*, 1995). Transcriptional repression occurs through recruitment of histone deacetylases (HDAC) that prevent opening of chromatin associated with deacetylation of nucleosomes, as reviewed by Xu *et al.* (1999), Privalsky (2001), and Urnov *et al.* (2001).

Upon ligand binding, corepressors are released and several multiprotein coactivator complexes are recruited to activate transcription. CREB-binding protein (CBP) and p300 associate with nuclear receptors in a ligand-dependent manner, and have intrinsic histone acetyltransferase (HAT) activity. The p160 family of coactivators interacts with ligand-activated receptors to enhance transcription. A novel arginine methyltransferase (CARM1) was recently found to be associated with p160 coactivators, and to methylate histone H3 and other coactivators (Xu *et al.*, 2001). Comprehensive reviews of coactivators have been previously published (Naar *et al.*, 2001; Rachez and Freedman, 2001; Rosenfeld and Glass, 2001).

The TRAP/DRIP complex was isolated by affinity purification of proteins bound to activated VDR and TR (Fondell *et al.*, 1996; Rachez *et al.*, 1999). This complex consists of at least nine proteins and, unlike the previously discussed coactivators, is devoid of HAT activity. The ligand-dependent binding of this complex is through a single subunit (DRIP205/TRAP220). Other components of the complex contain part of the 'mediator complex' that interacts directly with the basal transcriptional machinery, including RNA polymerase II. Complexes with ATP-dependent chromatin-remodeling activity, from the SWI/SNF family, associate with nuclear receptors in a ligand-dependent manner (Wallberg *et al.*, 2000). Distinct complexes have been isolated, and a recent study found that the remodeling complex designated PBAF is required for transcription through ligand binding to nuclear receptors (Lemon *et al.*, 2001).

The metabolism and distribution of retinol is tightly controlled by intracellular and extracellular binding proteins and by metabolizing enzymes, as reviewed by Blaner *et al.* (1999). Several components are directly

regulated by retinoids, acting as a feedback mechanism to affect retinoid response. The cytochrome P450-dependent retinoic acid-4-hydroxylase enzyme that affects retinoid metabolism is rapidly induced in the liver and other tissues following retinoid treatment (White *et al.*, 1997). Also induced are the cellular retinoid-binding proteins (CRABP-I and CRABP-II). CRABPs function in retinoid storage and transport from the cytosol to the nucleus, and may exert other effects.

Key aspects of the retinoid-signaling pathway are illustrated in Figure 1. Alterations associated with retinoid resistance are displayed. Despite advances in understanding how retinoids induce transcription, much less is known about downstream targets that signal retinoid biological effects. Microarray expression profiling has permitted the identification of candidate retinoid target genes and the complex network of signals that generate retinoid biological effects in different cell contexts (Tamayo *et al.*, 1999; Lian *et al.*, 2001; Dokmanovic *et al.*, 2002; Freemantle *et al.*, 2002; Houldsworth *et al.*, 2002). Of the retinoid-responsive malignancies, mechanisms responsible for RA response and resistance have been most extensively studied in APL, and these are summarized in the next section.

Retinoid response and resistance in APL

RA has dramatically changed the clinical course of APL (FAB M3) from one that was highly lethal to one that now appears highly curable, as reviewed by Nason-Burchenal and Dmitrovsky (1996), Tallman and Nabhan (2002), and Kitareewan *et al.* (2003). Prior to inclusion of RA, conventional anthracycline therapy for APL resulted in long-term remission in a subset of cases. With combined RA and chemotherapy, long-term remissions occur in almost 70% of cases, although a minority of APL cases exhibit intrinsic or acquired resistance to RA or anthracycline-based therapies. RA intervention in APL has been particularly valuable in illuminating mechanisms of retinoid resistance. Most APL cases present with the reciprocal t(15;17) translocation resulting in the fusion product PML-RAR α (Nason-Burchenal and Dmitrovsky, 1996). Nearly all cases that are PML-RAR α positive undergo complete

clinical remission with RA monotherapy through induction of granulocytic differentiation. However, many patients will relapse with disease that is often resistant to a second clinical remission with RA. This acquired resistance occurs even in those patients on continuous RA therapy.

Two mechanisms of pharmacological resistance have been implicated. One involves catabolism of RA through the P450 system, and the other involves induction of cytoplasmic-binding proteins that sequester RA. These mechanisms may cooperate to reduce plasma RA levels following treatment, as reviewed by Gallagher (2002). Genetic mechanisms of RA resistance also exist. These include intrinsic resistance, as is found in the rare t(11,17) APL cases that express PLZF-RAR α , as well as in acquired resistance that occurs with the appearance of mutations in the ligand-binding domain of the RAR α portion of PML-RAR α , as reviewed by Melnick and Licht (1999), Slack (1999), and Gallagher (2002). An improved understanding of the mechanisms of retinoid resistance in APL should provide insights into strategies to overcome this resistance in APL, and perhaps other malignancies that are currently refractory to retinoid-based therapy.

Mechanisms of retinoid response in APL

There has been an intense interest in the role of PML-RAR α in RA response in APL, as reviewed by Slack (1999) and Piazza *et al.* (2001). PML-RAR α plays an etiological role in leukemogenesis, as well as a central role in mediating RA response in APL. PML-RAR α acts in a dominant-negative manner to inhibit wild-type RAR α activity. This is due to transcriptional repression mediated by a stable association of PML-RAR α , with HDAC containing corepressor complexes that are resistant to physiologic levels of RA. This results in transcriptional repression of the program required for granulocytic maturation of APL blasts (Grignani *et al.*, 1998; He *et al.*, 1998; Lin *et al.*, 1998). In contrast, therapeutic levels of RA dissociate the corepressor complex and recruit coactivator complexes, restoring the regulation of target genes. It has been proposed that PML-RAR α inhibits normal PML function (Piazza *et al.*, 2001). PML has tumor-suppressive properties, but it does not bind DNA directly. It does possess domains consistent with a transcription factor. The precise role of PML inhibition in the pathology of APL needs to be understood, since other transcription factors (PLZF, NuMA, NPM, and STAT5b) can substitute for the PML portion of PML-RAR α in rare APL cases that express variant translocation products (Melnick and Licht, 1999; Zelent *et al.*, 2001).

Transgenic PML-RAR α expression causes abnormal myelopoiesis or leukemia, with long latencies that implicate cooperating alterations in leukemogenesis (Early *et al.*, 1996; Brown *et al.*, 1997; Grisolan *et al.*, 1997; He *et al.*, 1997). Notably, promyelocytic leukemia triggered in PML-RAR α transgenic mice responds to RA treatment. RA-resistant NB4 APL lines that no longer express PML-RAR α fail to differentiate despite

RA treatment (Dermime *et al.*, 1993). This illustrates the important role of PML-RAR α in mediating RA response. Consistent with this, PML-RAR α -transfected U937 cells have an enhanced response to RA-mediated differentiation (Testa *et al.*, 1994). PML-RAR α can sequester RXR through heterodimerization, leading to aberrant partnering of RXR with RAR and with other nuclear receptors. The PML portion of PML-RAR α can also induce the formation of homo-oligomers with enhanced corepressor-recruiting activity leading to transcriptional silencing (Lin and Evans, 2000).

Mechanisms of retinoid resistance in APL

RA therapy in APL has been associated with preclinical and clinical evidence of multifactorial mechanisms of resistance. Pharmacokinetic studies demonstrated that sustained RA treatment induced a catabolic response associated with reduced plasma RA levels (Warrell, 1993). RA metabolism occurs through the cytochrome P450 system that is induced in the liver and target tissues following RA treatment. Treatment with P450 enzyme inhibitors can inhibit the anticipated decline in peak plasma RA levels, as reviewed by Njar (2002). A novel P450 enzyme, CYP26, is an RAR target, and can mediate the conversion of RA to its major 4-hydroxy and 4-oxo RA metabolites (White *et al.*, 1997). This implicates CYP26 as a mediator of RA metabolism autoregulation. APL cases may exhibit resistance due to the induction of cytosolic binding proteins that sequester RA (Cornic *et al.*, 1994). Intermittent RA therapy may limit the emergence of pharmacological resistance (Gallagher, 2002). Since RA resistance is frequent in APL, RA has been combined with cytotoxic chemotherapy.

Decreased availability of RA in APL

Conventional APL therapy combines RA with anthracyclines, agents known to induce multidrug resistance. The role of P-glycoprotein (Pgp) in mediating RA resistance in APL has been investigated. APL cells express lower Pgp levels than retinoid unresponsive subsets of AML (Paietta *et al.*, 1994). Yet, conflicting evidence exists for the involvement of Pgp in mediating retinoid resistance in APL. Pgp expression is increased in RA-resistant HL-60 cells relative to sensitive cells, and RA response could be partially restored by verapamil treatment (Kizaki *et al.*, 1996). Ribozymes that target MDR1 also conferred increased retinoid sensitivity in RA-resistant HL60 cells (Matsushita *et al.*, 1998). Yet, evidence for differential RA uptake in RA-sensitive as compared to resistant APL cells was not found when examined in primary versus RA-relapsed cases (Takeshita *et al.*, 2000). No differences were reported in intracellular RA levels between wild-type, RA-resistant, and MDR1-transfected NB4 cells (Takeshita *et al.*, 2000).

An alternative proposed mechanism that reduced RA levels in APL cells involved induction of the cytoplasmic binding protein CRABP II, leading to RA sequestration.

The CRABP^{II} promoter contains a retinoid response element that leads to CRABP^{II} induction after RA treatment. Initial evidence in APL cases suggested that increased expression of CRABP^{II} was associated with relapsed APL (Delva *et al.*, 1993). However, a larger study did not confirm this association (Zhou *et al.*, 1998). CRABP^{II} may be a positive regulator of RA signaling through direct delivery of RA to nuclear RARs, and thereby serves as a coactivator (Budhu and Noy, 2002). The precise role of CRABP species in retinoid response and resistance still needs to be determined, since CRABP^I and CRABP^{II} knockout mice do not exhibit major defects in RA metabolism or homeostasis (Lampron *et al.*, 1995).

PML-RAR α degradation

A hallmark of RA response in APL is PML-RAR α degradation that could reverse PML-RAR α oncogenic effects (Yoshida *et al.*, 1996; Nervi *et al.*, 1998). Proteasomal inhibitors prevent PML-RAR α proteolysis, despite RA treatment, implicating a proteasomal pathway in this degradation, although caspase-mediated degradation may also play a role (Yoshida *et al.*, 1996; Nervi *et al.*, 1998). An RA-resistant NB4 cell line had altered ability to degrade wild-type PML-RAR α despite RA treatment, also indicating the involvement of the proteasome degradation pathway in retinoid response (Nason-Burchenal *et al.*, 1997). Persistent PML-RAR α expression and RA resistance are found in other RA-resistant APL cell lines, as reviewed by Gallagher (2002). One potential mechanism for this degradation involves the ubiquitin-activating enzyme E1-like protein (UBE1L), which is a direct RA target. Transfection of UBE1L results in PML-RAR α degradation, and in NB4 cells triggers apoptosis but not differentiation (Kitareewan *et al.*, 2002). Evidence that PML-RAR α is an antiapoptotic factor that promotes APL survival was found by ribozyme targeting of PML-RAR α that triggered apoptosis (Nason-Burchenal *et al.*, 1998). These findings were consistent with previous work indicating that RA promotes ubiquitination and degradation of G1 cyclins and Cdk4 during retinoid-induced differentiation or growth suppression, as reviewed by Dragnev *et al.* (2001). Interestingly, an RA-resistant NB4 line that failed to express PML-RAR α showed restored RA response upon proteasome inhibition of PML-RAR α degradation (Fanelli *et al.*, 1999). Together, these findings underscore the dual role of PML-RAR α in APL. PML-RAR α is an antiapoptotic and oncogenic factor that blocks maturation at physiologic RA levels, but PML-RAR α is required in maturation induction at pharmacologic levels of RA. The precise mechanism and biological impact of RA-mediated degradation of PML-RAR α awaits further study.

PML-RAR α mutations

RA resistance was associated with mutations of the ligand-binding domain of RAR α in an RA-resistant subclone of the HL-60 myeloid leukemia cell line

(Collins *et al.*, 1990). Subsequently, several RA-resistant NB4 APL cell lines were shown to contain mutations in the ligand binding E domain of PML-RAR α , as reviewed by Gallagher (2002). These mutations appear to cluster in two regions that are at or near residues in direct contact with RA, based on crystallographic analysis. Several of these mutations have been shown to disrupt RA binding to PML-RAR α , but retain the ability to heterodimerize with RXR and bind DNA, and thereby confer dominant-negative activity. This was shown to be associated with constitutive binding of the SMRT corepressor (Shao *et al.*, 1997). Mutations in the ligand-binding domain of PML-RAR α were also found in APL cells isolated from RA relapsed cases, as reviewed by Gallagher (2002). These mutations are variably associated with loss of RA binding and dominant-negative activity. Only a subset of RA-relapsed APL cases have PML-RAR α mutations. Future studies with larger series of RA-resistant APL cases should clarify the clinical impact of PML-RAR α mutations in APL.

Increased histone deacetylation

Complex transcriptional machinery is involved in nuclear receptor function (Naar *et al.*, 2001; Rachez and Freedman, 2001; Rosenfeld and Glass, 2001). Four classes of multiprotein complexes are involved in mediating the RAR/RXR signaling. These are the HDAC-associated corepressor, histone acetylase-associated p160 coactivator, DRIP/TRAP, and the chromatin-remodeling Swi/Snf complexes. Acetylation, methylation or phosphorylation of specific residues in histone tails caused by these complexes control the gene transcriptional activity of nuclear receptors. Alterations in corepressors and coactivators have been associated with other malignancies, but have not yet been reported in APL (Carapeti *et al.*, 1998). Dominant-negative effects of specific RAR α fusion proteins have been associated with increased affinity for corepressors (Grignani *et al.*, 1998; He *et al.*, 1998; Lin *et al.*, 1998). Thus, signaling is constitutively repressed by corepressor-associated HDAC activity at physiologic RA levels, leading to transcriptional silencing and a maturation block. Corepressor association with PML-RAR α is disrupted and differentiation induced at pharmacologic RA dosages. This is a mechanism for the oncogenic properties of PML-RAR α and for the therapeutic activity of RA in APL. The structural basis for pharmacologic RA dissociation of corepressors has not yet been elucidated. Prior reports found that the affinity of RA for RAR α and PML-RAR α were similar (Benedetti *et al.*, 1997).

Intrinsic RA resistance in PLZF-RAR α -expressing APL cases appears due to a second corepressor-binding site on PLZF, resulting in constitutive association of PLZF-RAR α with the corepressor complex, despite pharmacologic RA doses (He *et al.*, 1998; Lin *et al.*, 1998). Cellular and transgenic models expressing PLZF-RAR α have shown that combining RA with HDAC inhibitors can restore transcriptional activation to PLZF-RAR α , and subsequent maturation response in

promyelocytic leukemia (He *et al.*, 2001). RA in combination with an HDAC inhibitor induced clinical remission in RA-relapsed PML-RAR α and RA refractory PLZF-RAR α -expressing APL cases (Petti *et al.*, 2002; Zhou *et al.*, 2002). Primary blasts from leukemic cells derived from AML cases treated with HDAC inhibitors restored RA-dependent transcriptional activation and triggered terminal differentiation (Ferrara *et al.*, 2001). This indicated that HDAC inhibition combined with RA is a potential strategy for differentiation-based therapy of AML and perhaps other malignancies currently refractory to single-agent retinoid treatment.

RA resistance in APL has also been linked to a loss of induction of p21 or transglutaminase, as reviewed by Ozpolat *et al.* (2001). RA-induced differentiation in APL and other tumors is associated with repression of telomerase activity (Albanell *et al.*, 1996; Nason-Burchenal *et al.*, 1997; Pendino *et al.*, 2002). PML is localized to nuclear multiprotein complexes designated as PML oncogenic domains (PODs), which are disorganized in APL and reorganized by RA treatment of APL cells. This is disrupted in RA-resistant NB4 cells (Nason-Burchenal *et al.*, 1997). Further studies are warranted to address the impact of this disruption in RA resistance in APL.

Retinoids and solid tumors

RAR β as a tumor suppressor

To identify why preclinical retinoid activity did not readily translate into clinical responses, the retinoid-signaling pathway was studied in normal and neoplastic tissues. *In situ* hybridization was used to compare retinoid receptor expression profiles in head and neck squamous cell carcinomas, dysplastic lesions, adjacent normal tissues, and in tissues from normal volunteers (Xu *et al.*, 1994). RAR α , RAR β , and RAR γ and RXR α and RXR β mRNAs were expressed in all samples from normal volunteers. The levels of RAR α and RAR γ and RXR α and RXR β mRNAs were similar to that in most of the adjacent normal, hyperplastic, dysplastic, and malignant tissues. However, RAR β mRNA levels were detected in only 70% of dysplastic and adjacent normal tissues, and were repressed further in dysplastic and malignant epithelium. RAR β repression was also found in preneoplastic oral cavity lesions (Lotan *et al.*, 1995), non-small-cell lung cancer (Castillo *et al.*, 1997; Xu *et al.*, 1997a; Picard *et al.*, 1999), breast cancers (Widschwendter *et al.*, 1997; Xu *et al.*, 1997b), and esophageal cancer (Qiu *et al.*, 1999). Other retinoid receptors were expressed in these tissues, but only RAR β levels were significantly lower in the premalignant and tumor tissues. The correlation of RAR β repression with epithelial carcinogenesis led to the hypothesis that RAR β could act as a tumor suppressor. This view was supported by experiments where RAR β was overexpressed in cell lines. In retinoid-sensitive human lung carcinoma cells, constitutive overexpression of RAR β 2

inhibited cellular proliferation (Houle *et al.*, 1993). In a retinoid-resistant human lung cancer cell line, only overexpression of RAR β 2 sensitized these cells to retinoids. Clones stably overexpressing RAR α 1 or RAR β 1 did not exhibit growth inhibition when treated with RA (Si *et al.*, 1996; Toulouse *et al.*, 2000). These studies were extended to the *in vivo* setting, whereby RAR β antisense expression in transgenic mice increased pulmonary tumors as compared to controls (Berard *et al.*, 1996).

RAR β expression is selectively lost in premalignant oral lesions, and can be restored by RA treatment (Lotan *et al.*, 1995). The restoration of RAR β expression was associated with a clinical response, suggesting a role for RAR β both as a mediator of RA response and as a biological marker in chemoprevention trials (Lotan *et al.*, 1995). This was confirmed in renal cancers, where upregulation of RAR β correlated with response to 13-*cis*-retinoic acid and interferon- α -2a (Berg *et al.*, 1999). Thus, an agent that can induce RAR β expression should be considered for evaluation in the treatment of epithelial malignancies.

A frequent mechanism of RAR β repression was found to involve DNA methylation-induced silencing (Cote and Momparler, 1997). By sequencing the bisulfite-modified DNA of tumor cells, specific 5-methylcytosine residues, in the region of -46 to +251 bp from the transcription start site of the RAR β 2 gene, were mapped (Cote and Momparler, 1997; Cote *et al.*, 1998; Virmani *et al.*, 2000). Tumor-specific hypermethylation of the RAR β 2 promoter has been found in diverse epithelial malignancies (Hayashi *et al.*, 2001; Nakayama *et al.*, 2001; Ivanova *et al.*, 2002; Kwong *et al.*, 2002). These findings implicated DNA methylation as a marker for early carcinogenesis. PCR-based methylation assays performed in sputum samples have supported this viewpoint (Palmisano *et al.*, 2000). Use of a demethylating agent combined with a classical retinoid has been proposed to overcome resistance due to RAR β silencing, as will be discussed.

Loss of histone H3 acetylation was associated with retinoid resistance in the presence and absence of RAR β 2 hypermethylation, indicating that multiple mechanisms could repress RAR β (Suh *et al.*, 2002b). Pharmacological inhibition of chromatin acetylation is under investigation to restore retinoid sensitivity. Other mechanisms engaged in RAR β suppression could include loss of coactivators (Moghal and Neel, 1995) or aberrant nuclear receptor expression (Wu *et al.*, 1997). Repression of RXR β has been associated with poor prognosis in non-small-cell lung cancer (Brabender *et al.*, 2002), implicating the altered expression of other retinoid receptors in retinoid resistance.

Although RAR β silencing is associated with increased tumorigenicity in certain cell types, other cell contexts depend on different retinoid receptors for retinoid response. For example, in NT2/D1 human embryonal carcinoma cells, RAR γ is required for retinoid-mediated differentiation (Spinella *et al.*, 1998; Kitareewan *et al.*, 1999). The retinoid-resistant NT2/D1-R1 cell line exhibits RAR γ repression that is not overcome by RA

treatment (Moasser *et al.*, 1994). Only by introducing RAR γ into NT2/D1-R1 cells can the differentiation response be restored. In contrast, in RA-resistant HL-60 myeloid leukemia cells, where retinoid resistance can be attributed to an aberrant retinoid receptor, mutations occur in RAR α (Collins *et al.*, 1990; Robertson *et al.*, 1992; Li *et al.*, 1994; Brigati *et al.*, 1999; Mori *et al.*, 1999). Thus, specific retinoid receptors confer cell- and tissue-dependent retinoid response.

Alternate mechanisms of retinoid resistance

Repression of RAR β is viewed as a critical step in epithelial carcinogenesis. The high frequency of RAR β repression in epithelial carcinogenesis has not excluded the presence of other potentially cooperating mechanisms of retinoid resistance. The possibility that other frequent oncogenic events induce retinoid resistance has been examined. For example, aberrant expression of p53 was linked to clinical resistance to 13-*cis*-retinoic acid (Lippman *et al.*, 1995). Consistent with this finding, studies of retinoid-resistant embryonal carcinoma cells found that activation of normal p53 function was required for retinoid-mediated differentiation and cell cycle arrest (Curtin *et al.*, 2001). Deregulation of *c-myc* was associated with retinoid resistance in neuroblastoma cells (Reynolds *et al.*, 2000). Retinoid-mediated repression of *c-myc* by RA treatment was observed in some models (Miller *et al.*, 1990; Dimberg *et al.*, 2002), while retinoids induced *c-myc* in others (Guernsey and Yen, 1988).

Breast cancers are classified based on estrogen receptor (ER) status. Breast cancers that no longer depend on estrogen for proliferation are often resistant to retinoids (van der Berg *et al.*, 1993). Overcoming this resistance could increase the therapeutic activity of retinoids in breast cancer. Evidence exists for retinoid activity in suppressing breast carcinogenesis. Nearly 3000 women with stage I breast cancer were randomized to the nonclassical retinoid *N*-(4-hydroxyphenyl)retinamide (4-HPR) versus no intervention (Torrise and Decensi, 2000). The incidence of contralateral breast cancer and local recurrence were compared between groups, and overall results indicated no chemopreventive activity for 4-HPR. However, decreased incidence of contralateral breast cancer and decreased incidence of local recurrence in premenopausal women were observed (Torrise and Decensi, 2000). There was also a trend for ER-negative tumors benefiting less from 4-HPR therapy than ER-positive tumors. These responses could be due to crosstalk between the retinoid and estrogen-signaling pathways. Selective ER modifiers (SERMs), such as tamoxifen, have proven chemopreventive activity in breast cancer, as reviewed by Zujewski (2002). There is evidence that retinoids and SERMs in combination will act synergistically in the chemoprevention of breast cancer, and this is discussed below (Suh *et al.*, 2002a).

Intrinsic retinoid resistance has also been linked to levels of reactive oxygen species in some cells. Oxidative states diminished, whereas reducing conditions enhanced DNA binding of RXR/RAR heterodimers *in*

vitro (Demary *et al.*, 2001). Through gain and loss of function studies, it was determined that CRABP-II regulated retinoid response in human mammary carcinoma cells. Reduced expression of CRABP-II rendered these cells retinoid resistant (Budhu and Noy, 2002). Methylation of the CRABP-I promoter occurred in breast tumors, suggesting that this may be involved in epigenetic silencing of retinoid response (Esteller *et al.*, 2002).

Overcoming resistance

Intrinsic or acquired retinoid resistance has limited the clinical activity of retinoid-based therapy and chemoprevention. Clinical strategies to overcome this resistance include efforts to reduce toxicity, retain bioavailability, and develop effective combination regimens. Other approaches include the use of nonclassical retinoids that have retinoid receptor-independent properties, or that target RXRs that would bypass RAR β repression. One example is bexarotene, an RXR agonist (rexinoid), that can trigger effects even in cells that do not respond to a classical retinoid (Heald, 2000). Other examples of nonclassical retinoids include 4-HPR, CD437, and TAC101, that have reported ligand-dependent activity for RARs as well as apoptogenic activity that can be ligand independent, as shown in Table 2.

Since retinoids can induce their metabolism, RA metabolism-blocking agents (RAMBA) such as liarozole have been developed, as reviewed by Njar (2002). To increase the levels of endogenous retinoids, these compounds were selected to inhibit cytochrome P450-dependent retinoic acid-4-hydroxylase enzyme(s) responsible for RA metabolism. More selective inhibitors of RA metabolism are being developed, for example R116010 which increases RA toxicity in cultured human breast cancer cells and is growth suppressive in an estrogen-independent murine mammary tumor model (Van Heusden *et al.*, 2002). Another method to address RA metabolism and toxicity include liposomal RA, which was designed to maintain plasma concentrations and to overcome RA resistance in APL. Systemic toxicity can also be avoided by targeting administration to the desired tissue site. Examples of this include immunotargeting of liposomal retinoid formations or aerosolization of retinoids (Wang *et al.*, 2000; Ozpolat and Lopez-Berestein, 2002).

Therapies combining retinoids with other agents are currently being evaluated. In preclinical breast cancer chemopreventive studies, the combination of retinoids with SERMs was more effective than either agent alone (Suh *et al.*, 2002a). 4-HPR with tamoxifen has been evaluated in patients at high risk for breast cancer (Conley *et al.*, 2000), and it is anticipated that trials combining bexarotene and raloxifene as an alternative retinoid-SERM combination will be initiated. Other potential retinoid regimens include modifiers of ceramide metabolism (Maurer *et al.*, 2000), docetaxol (Nehme *et al.*, 2001), arsenic (Calleja and Warrell, 2000), and melatonin (Nowfar *et al.*, 2002).

Epigenetic mechanisms of RAR β 2 silencing include promoter methylation and decreased chromatin acetylation. Unlike DNA mutations or chromosome loss, DNA methylation and chromatin acetylation are reversible biological modifications that can be pharmacologically targeted. There is considerable interest in demethylating agents and HDAC inhibitors for cancer therapy and chemoprevention. The next section reviews the current status of these agents and their potential to reverse intrinsic retinoid resistance.

DNA methylation is now accepted as a contributing factor in human carcinogenesis. There are several mechanisms by which DNA methylation could repress gene transcription: inhibition of activating transcription factor binding, transcriptional repression by methylated DNA-specific binding proteins, and through DNA methyltransferases that have repressor-binding domains, as reviewed by Karpf and Jones (2002). 5-Aza-2'-cytidine (5-aza-CR) and 5-aza-2'-deoxycytidine (5-aza-CdR) inhibit DNA methyltransferase 1 (DNMT-1) noncompetitively after incorporation into cellular DNA. 5-Aza-CdR may inhibit the growth of cancer cells by reactivating growth-regulatory genes silenced by *de novo* methylation (Bender *et al.*, 1998). In a subset of human lung cancer and breast cancer cell lines and in breast cancer xenografts, RAR β 2 expression occurred only after demethylation and treatment with RA (Widschwendter *et al.*, 2000; Sirchia *et al.*, 2002; Suh *et al.*, 2002b). Clinically, 5-aza-CdR and 5-aza-CR have been used to treat certain leukemias and myelodysplastic syndromes (Lubbert, 2000; Christman, 2002; Silverman *et al.*, 2002). However, they have toxicity, and have not been as effective in other settings. Antisense oligonucleotides and siRNA that target DNMT-1 can hypomethylate DNA and reactivate methylation-silenced genes (Milutinovic *et al.*, 2000; Robert *et al.*, 2003). A DNMT-1-specific antisense inhibitor is being evaluated in clinical trials. It inhibits the growth of cancer cells *in vitro* and in preclinical *in vivo* models, as reviewed by Reid *et al.* (2002). Perhaps where RAR β 2-methylation silencing confers retinoid resistance, a combination regimen with a methylation inhibitor and an appropriate retinoid would restore retinoid response.

Another epigenetic chromatin modification found to affect RAR β 2 gene expression is acetylation. Repressors of transcription are frequently associated with HDAC activity. Acetylation relaxes chromatin structure, allowing transcriptional activation, and deacetylation closes chromatin structure, repressing transcription. HDAC inhibitors have restored retinoid-dependent transcriptional activation, and triggered terminal differentiation in leukemic cell blasts from AML cases (Ferrara *et al.*, 2001). AML1/ETO, the most common AML-associated fusion protein, is an HDAC-dependent repressor of retinoid signaling. The fusion of the AML transcription

factor to ETO converts it from a transcriptional activator to a repressor, as reviewed by Licht (2001). These leukemic cells expressed retinoid receptors, but only in the presence of the HDAC inhibitor was retinoid-dependent transcription activated, confirming the association between suppression of the retinoid-signaling pathway and carcinogenesis. This highlights a role for combined transcription and differentiation-based therapies. HDAC inhibitors, including phenylbutyrate and SAHA, are being examined in clinical trials as single agents and as part of combination regimens, as reviewed by Pandolfi (2001) and Johnstone (2002). At least three of the proteins that bind methylated DNA (MBD1, MBD2, and MeCP2) directly repress transcription and can recruit HDAC, as reviewed by Karpf and Jones (2002). DNA methyltransferases 1, 3a, and 3b can also recruit HDACs and/or other corepressor proteins. This demonstrates a link between methylation and acetylation, and provides a rationale for use of a combination regimen with demethylating agents and inhibitors of deacetylation.

Conclusions

Retinoids are active in cancer therapy and chemoprevention, but retinoid resistance in the clinic is frequent. Deregulation of retinoid signaling is a common event in carcinogenesis. Epigenetic silencing of RAR β or translocation of RAR α to form oncogenic fusion proteins are examples of aberrant retinoid-signaling mechanisms. The use of retinoids alone to treat cancer or to prevent the onset of second cancers may not be optimal if suppression of retinoid signaling has already occurred. Early intervention with retinoids might overcome this. Another therapeutic or chemopreventive strategy would be to reverse or inhibit retinoid resistance. This could be achieved by activating key target genes with nonclassical retinoids that bypass resistance, or by use of combination regimens that include demethylating agents or HDAC inhibitors. The challenge for the future is to realize the promise of retinoid therapy by overcoming the consequences of intrinsic or acquired resistance.

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References

Albanell J, Han W, Mellado B, Gunawardane R, Scher HI, Dmitrovsky E and Moore MA. (1996). *Cancer Res.*, **56**, 1503–1508.

Bender CM, Pao MM and Jones PA. (1998). *Cancer Res.*, **58**, 95–101.

- Benedetti L, Levin AA, Scicchitano BM, Grignani F, Allenby G, Diverio D, Lo Coco F, Avvisati G, Ruthardt M, Adamo S, Pelicci PG and Nervi C. (1997). *Blood*, **90**, 1175–1185.
- Berard J, Laboune F, Mukuna M, Masse S, Kothary R and Bradley WE. (1996). *FASEB J.*, **10**, 1091–1097.
- Berg WJ, Divgi CR, Nanus DM and Motzer RJ. (2000). *Semin. Oncol.*, **27**, 234–239.
- Berg WJ, Nanus DM, Leung A, Brown KT, Hutchinson B, Mazumdar M, Xu XC, Lotan R, Reuter VE and Motzer RJ. (1999). *Clin. Cancer Res.*, **5**, 1671–1675.
- Blaner WS, Piantadosi R, Sykes A and Vogel S. (1999). *Retinoids: Handbook of Experimental Pharmacology*. Nau H and Blaner WS (eds), Vol. 139. Springer: Berlin, pp. 117–152.
- Brabender J, Danenberg KD, Metzger R, Schneider PM, Lord RV, Groshen S, Tsao-Wei DD, Park J, Salonga D, Holscher AH and Danenberg PV. (2002). *Clin. Cancer Res.*, **8**, 438–443.
- Brigati C, Nobile L, Fugazza G, Zohouri M, Gallagher R and Cannizzaro L. (1999). *Leuk. Res.*, **23**, 105–113.
- Brown D, Kogan S, Lagasse E, Weissman I, Alcalay M, Pelicci PG, Atwater S and Bishop JM. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 2551–2556.
- Budhu AS and Noy N. (2002). *Mol. Cell. Biol.*, **22**, 2632–2641.
- Calleja EM and Warrell Jr RP. (2000). *Curr. Oncol. Rep.*, **2**, 519–523.
- Carapeti M, Aguiar RC, Goldman JM and Cross NC. (1998). *Blood*, **91**, 3127–3133.
- Castillo L, Milano G, Santini J, Demard F and Pierrefite V. (1997). *Clin. Cancer Res.*, **3**, 2137–2142.
- Cheer SM and Foster RH. (2000). *Am. J. Clin. Dermatol.*, **1**, 307–314.
- Chen JD and Evans RM. (1995). *Nature*, **377**, 454–457.
- Christman JK. (2002). *Oncogene*, **21**, 5483–5495.
- Collins SJ, Robertson KA and Mueller L. (1990). *Mol. Cell. Biol.*, **10**, 2154–2163.
- Conley B, O'Shaughnessy J, Prindiville S, Lawrence J, Chow C, Jones E, Merino MJ, Kaiser-Kupfer MI, Caruso RC, Podgor M, Goldspiel B, Venzon D, Danforth D, Wu S, Noone M, Goldstein J, Cowan KH and Zujewski J. (2000). *J. Clin. Oncol.*, **18**, 275–283.
- Cornic M, Delva L, Castaigne S, Lefebvre P, Balitrand N, Degos L and Chomienne C. (1994). *Leukemia*, **8**, 914–917.
- Cote S and Momparler RL. (1997). *Anticancer Drugs*, **8**, 56–61.
- Cote S, Sinnett D and Momparler RL. (1998). *Anticancer Drugs*, **9**, 743–750.
- Curtin JC, Dragnev KH, Sekula D, Christie AJ, Dmitrovsky E and Spinella MJ. (2001). *Oncogene*, **20**, 2559–2569.
- Delva L, Cornic M, Balitrand N, Guidez F, Miclea JM, Delmer A, Teillet F, Fenaux P, Castaigne S, Degos L and Chomienne C. (1993). *Blood*, **82**, 2175–2181.
- Demary K, Wong L, Liou JS, Faller DV and Spanjaard RA. (2001). *Endocrinology*, **142**, 2600–2605.
- Dermime S, Grignani F, Clerici M, Nervi C, Sozzi G, Talamo GP, Marchesi E, Formelli F, Parmiani G, Pelicci PG and Gambacorti-Passerini C. (1993). *Blood*, **82**, 1573–1577.
- Dimberg A, Bahram F, Karlberg I, Larsson LG, Nilsson K and Oberg F. (2002). *Blood*, **99**, 2199–2206.
- Dokmanovic M, Chang BD, Fang J and Roninson IB. (2002). *Cancer Biol. Ther.*, **1**, 24–27.
- Dragnev KH, Freemantle SJ, Spinella MJ and Dmitrovsky E. (2001). *Ann. N.Y. Acad. Sci.*, **952**, 13–22.
- Early E, Moore MA, Kakizuka A, Nason-Burchenal K, Martin P, Evans RM and Dmitrovsky E. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 7900–7904.
- Esteller M, Guo M, Moreno V, Peinado MA, Capella G, Galm O, Baylin SB and Herman JG. (2002). *Cancer Res.*, **62**, 5902–5905.
- Fanelli M, Minucci S, Gelmetti V, Nervi C, Gambacorti-Passerini C and Pelicci PG. (1999). *Blood*, **93**, 1477–1481.
- Ferrara FF, Fazi F, Bianchini A, Padula F, Gelmetti V, Minucci S, Mancini M, Pelicci PG, Lo Coco F and Nervi C. (2001). *Cancer Res.*, **61**, 2–7.
- Fondell JD, Ge H and Roeder RG. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 8329–8333.
- Freemantle SJ, Kerley JS, Olsen SL, Gross RH and Spinella MJ. (2002). *Oncogene*, **21**, 2880–2889.
- Gallagher RE. (2002). *Leukemia*, **16**, 1940–1958.
- Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, Fanelli M, Ruthardt M, Ferrara FF, Zamir I, Seiser C, Grignani F, Lazar MA, Minucci S and Pelicci PG. (1998). *Nature*, **391**, 815–818.
- Grisolano JL, Wesselschmidt RL, Pelicci PG and Ley TJ. (1997). *Blood*, **89**, 376–387.
- Guernsey DL and Yen A. (1988). *Int. J. Cancer*, **42**, 576–581.
- Hayashi K, Yokozaki H, Goodison S, Oue N, Suzuki T, Lotan R, Yasui W and Tahara E. (2001). *Differentiation*, **68**, 13–21.
- He LZ, Guidez F, Tribioli C, Peruzzi D, Ruthardt M, Zeleat A and Pandolfi PP. (1998). *Nat. Genet.*, **18**, 126–135.
- He LZ, Tolentino T, Grayson P, Zhong S, Warrell Jr RP, Rifkind RA, Marks PA, Richon VM and Pandolfi PP. (2001). *J. Clin. Invest.*, **108**, 1321–1330.
- He LZ, Tribioli C, Rivi R, Peruzzi D, Pelicci PG, Soares V, Cattoretti G and Pandolfi PP. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 5302–5307.
- Heald P. (2000). *Clin. Lymphoma Suppl.*, **1**, S45–S49.
- Hong WK and Itri LM. (1994). *The Retinoids: Biology, Chemistry and Medicine*. Sporn MB, Roberts AB and Goodman DS (eds), 2nd edn. Raven Press: New York, pp. 597–630.
- Hong WK and Sporn MB. (1997). *Science*, **278**, 1073–1077.
- Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK and Rosenfeld MG. (1995). *Nature*, **377**, 397–404.
- Houldsworth J, Heath SC, Bosl GJ, Studer L and Chaganti RS. (2002). *Cell Growth Differ.*, **13**, 257–264.
- Houle B, Rochette-Egly C and Bradley WE. (1993). *Proc. Natl. Acad. Sci. USA*, **90**, 985–989.
- Ivanova T, Petrenko A, Gritsko T, Vinokourova S, Eshilev E, Kobzeva V, Kisseljov F and Kisseljova N. (2002). *BMC Cancer*, **2**, 4.
- Johnstone RW. (2002). *Nat. Rev. Drug Discov.*, **1**, 287–299.
- Karpf AR and Jones DA. (2002). *Oncogene*, **21**, 5496–5503.
- Khuri FR and Lippman SM. (2000). *Semin. Surg. Oncol.*, **18**, 100–105.
- Kitareewan S, Pitha-Rowe I, Ma Y, Freemantle SJ and Dmitrovsky E. (2003). *The Retinoids and Cancer Prevention Mechanisms. Vol. 1: Promising Cancer Chemopreventive Agents*. Kelloff GJ, Hawk ET and Sigman CC (eds). Humana Press: New Jersey (in press).
- Kitareewan S, Pitha-Rowe I, Sekula D, Lowrey CH, Nemeth MJ, Golub TR, Freemantle SJ and Dmitrovsky E. (2002). *Proc. Natl. Acad. Sci. USA*, **99**, 3806–3811.
- Kitareewan S, Spinella MJ, Allopenna J, Reczek PR and Dmitrovsky E. (1999). *Oncogene*, **18**, 5747–5755.
- Kizaki M, Ueno H, Yamazoe Y, Shimada M, Takayama N, Muto A, Matsushita H, Nakajima H, Morikawa M, Koeffler HP and Ikeda Y. (1996). *Blood*, **87**, 725–733.
- Kwong J, Lo KW, To KF, Teo PM, Johnson PJ and Huang DP. (2002). *Clin. Cancer Res.*, **8**, 131–137.

- Lampron C, Rochette-Egly C, Gorry P, Dolle P, Mark M, Lufkin T, LeMeur M and Chambon P. (1995). *Development*, **121**, 539–548.
- Lemon B, Inouye C, King DS and Tjian R. (2001). *Nature*, **414**, 924–928.
- Li YP, Said F and Gallagher RE. (1994). *Blood*, **83**, 3298–3302.
- Lian Z, Wang L, Yamaga S, Bonds W, Beazer-Barclay Y, Kluger Y, Gerstein M, Newburger PE, Berliner N and Weissman SM. (2001). *Blood*, **98**, 513–524.
- Licht JD. (2001). *Oncogene*, **20**, 5660–5679.
- Lin RJ and Evans RM. (2000). *Mol. Cell*, **5**, 821–830.
- Lin RJ, Nagy L, Inoue S, Shao W, Miller Jr WH and Evans RM. (1998). *Nature*, **391**, 811–814.
- Lippman SM, Lee JJ, Karp DD, Vokes EE, Benner SE, Goodman GE, Khuri FR, Marks R, Winn RJ, Fry W, Graziano SL, Gandara DR, Okawara G, Woodhouse CL, Williams B, Perez C, Kim HW, Lotan R, Roth JA and Hong WK. (2001). *J. Natl. Cancer Inst.*, **93**, 605–618.
- Lippman SM, Shin DM, Lee JJ, Batsakis JG, Lotan R, Tainsky MA, Hittelman WN and Hong WK. (1995). *Cancer Res.*, **55**, 16–19.
- Lotan R, Xu XC, Lippman SM, Ro JY, Lee JS, Lee JJ and Hong WK. (1995). *N. Engl. J. Med.*, **332**, 1405–1410.
- Lubbert M. (2000). *Curr. Top. Microbiol. Immunol.*, **249**, 135–164.
- Matsushita H, Kizaki M, Kobayashi H, Ueno H, Muto A, Takayama N, Awaya N, Kinjo K, Hattori Y and Ikeda Y. (1998). *Blood*, **91**, 2452–2458.
- Maurer BJ, Melton L, Billups C, Cabot MC and Reynolds CP. (2000). *J. Natl. Cancer Inst.*, **92**, 1897–1909.
- McCaffery P and Drager UC. (2000). *Cytokine Growth Factor Rev.*, **11**, 233–249.
- Melnick A and Licht JD. (1999). *Blood*, **93**, 3167–3215.
- Milutinovic S, Knox JD and Szyf M. (2000). *J. Biol. Chem.*, **275**, 6353–6359.
- Miller Jr WH, Moy D, Li A, Grippo JF and Dmitrovsky E. (1990). *Oncogene*, **5**, 511–517.
- Moasser MM, DeBlasio A and Dmitrovsky E. (1994). *Oncogene*, **9**, 833–840.
- Moghal N and Neel BG. (1995). *Mol. Cell. Biol.*, **15**, 3945–3959.
- Moon RC, Mehta RG and Rao KVN. (1994). *The Retinoids: Biology, Chemistry, and Medicine*. Sporn MB, Roberts AB and Goodman DS (eds), 2nd edn. Raven Press: New York, pp. 573–595.
- Moore DM, Kalvakolanu DV, Lippman SM, Kavanagh JJ, Hong WK, Borden EC, Paredes-Espinoza M and Krakoff IH. (1994). *Semin. Hematol.*, **31** (Suppl. 5), 31–37.
- Mori J, Suzuki S, Hara M, Kaneko A, Yamashita K, Kumagai M, Sakuma T, Kakizawa T, Yamazaki M, Takeda T, Miyamoto T, Ichikawa K and Hashizume K. (1999). *Jpn. J. Cancer Res.*, **90**, 660–668.
- Naar AM, Lemon BD and Tjian R. (2001). *Annu. Rev. Biochem.*, **70**, 475–501.
- Nakayama T, Watanabe M, Yamanaka M, Hirokawa Y, Suzuki H, Ito H, Yatani R and Shiraishi T. (2001). *Lab. Invest.*, **81**, 1049–1057.
- Nason-Burchenal K and Dmitrovsky E. (1996). *Molecular Biology of Cancer*. Bertino JR (ed). 1st edn Academic Press: San Diego, pp. 1547–1560.
- Nason-Burchenal K and Dmitrovsky E. (1999). *Retinoids: Handbook of Experimental Pharmacology*. Nau H and Blaner WS (eds), Vol. 139. Springer: Berlin, pp. 301–322.
- Nason-Burchenal K, Maerz W, Albanell J, Allopenna J, Martin P, Moore MA and Dmitrovsky E. (1997). *Differentiation*, **61**, 321–331.
- Nason-Burchenal K, Takle G, Pace U, Flynn S, Allopenna J, Martin P, George ST, Goldberg AR and Dmitrovsky E. (1998). *Oncogene*, **17**, 1759–1768.
- Nehme A, Varadarajan P, Sellakumar G, Gerhold M, Niedner H, Zhang Q, Lin X and Christen RD. (2001). *Br. J. Cancer*, **84**, 1571–1576.
- Nervi C, Ferrara FF, Fanelli M, Rippo MR, Tomassini B, Ferrucci PF, Ruthardt M, Gelmetti V, Gambacorti-Passerini C, Diverio D, Grignani F, Pelicci PG and Testi R. (1998). *Blood*, **92**, 2244–2251.
- Njar VC. (2002). *Mini. Rev. Med. Chem.*, **2**, 261–269.
- Nowfar S, Teplitzky SR, Melancon K, Kiefer TL, Cheng Q, Dwived PD, Bischoff ED, Moro K, Anderson MB, Dai J, Lai L, Yuan L and Hill SM. (2002). *Breast Cancer Res. Treat.*, **72**, 33–43.
- Ozpolat B and Lopez-Berestein G. (2002). *Leuk. Lymphoma*, **43**, 933–941.
- Ozpolat B, Lopez-Berestein G and Mehta K. (2001). *J. Biol. Regul. Homeost. Agents*, **15**, 107–122.
- Paietta E, Andersen J, Racevskis J, Gallagher R, Bennett J, Yunis J, Cassileth P and Wiernik PH. (1994). *Leukemia*, **8**, 968–973.
- Palmisano WA, Divine KK, Saccomanno G, Gilliland FD, Baylin SB, Herman JG and Belinsky SA. (2000). *Cancer Res.*, **60**, 5954–5958.
- Pandolfi PP. (2001). *Oncogene*, **20**, 3116–3127.
- Pendino F, Sahraoui T, Lanotte M and Segal-Bendirdjian E. (2002). *Leukemia*, **16**, 826–832.
- Petti MC, Fazi F, Gentile M, Diverio D, De Fabritiis P, De Propriis MS, Fiorini R, Spiriti MA, Padula F, Pelicci PG, Nervi C and Lo Coco F. (2002). *Blood*, **100**, 1065–1067.
- Piazza F, Gurrieri C and Pandolfi PP. (2001). *Oncogene*, **20**, 7216–7222.
- Picard E, Seguin C, Monhoven N, Rochette-Egly C, Siat J, Borrelly J, Martinet Y, Martinet N and Vignaud JM. (1999). *J. Natl. Cancer Inst.*, **91**, 1059–1066.
- Piedrafitia FJ and Pfahl M. (1999). *Handb. Exp. Pharm.*, **139**, 153–184.
- Privalsky ML. (2001). *Curr. Top. Microbiol. Immunol.*, **254**, 117–136.
- Qiu H, Zhang W, El-Naggar AK, Lippman SM, Lin P, Lotan R and Xu XC. (1999). *Am. J. Pathol.*, **155**, 1519–1523.
- Rachez C and Freedman LP. (2001). *Curr. Opin. Cell Biol.*, **13**, 274–280.
- Rachez C, Lemon BD, Suldan Z, Bromleigh V, Gamble M, Naar AM, Erdjument-Bromage H, Tempst P and Freedman LP. (1999). *Nature*, **398**, 824–828.
- Rastinejad F. (2001). *Curr. Opin. Struct. Biol.*, **11**, 33–38.
- Reid GK, Besterman JM and MacLeod AR. (2002). *Curr. Opin. Mol. Ther.*, **4**, 130–137.
- Reynolds CP and Lemons RS. (2001). *Hematol. Oncol. Clin. North Am.*, **15**, 867–910.
- Reynolds CP, Wang Y, Melton LJ, Einhorn PA, Slamon DJ and Maurer BJ. (2000). *Med. Pediatr. Oncol.*, **35**, 597–602.
- Robert MF, Morin S, Beaulieu N, Gauthier F, Chute IC, Barsalou A and MacLeod AR. (2003). *Nat. Genet.*, **33**, 61–65.
- Robertson KA, Emami B and Collins SJ. (1992). *Blood*, **80**, 1885–1889.
- Rosenfeld MG and Glass CK. (2001). *J. Biol. Chem.*, **276**, 36865–36868.
- Shao W, Benedetti L, Lamph WW, Nervi C and Miller Jr WH. (1997). *Blood*, **89**, 4282–4289.

- Si SP, Lee X, Tsou HC, Buchsbaum R, Tibaduiza E and Peacocke M. (1996). *Exp. Cell. Res.*, **223**, 102–111.
- Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, Stone RM, Nelson D, Powell BL, DeCastro CM, Ellerton J, Larson RA, Schiffer CA and Holland JF. (2002). *J. Clin. Oncol.*, **20**, 2429–2440.
- Sirchia SM, Ren M, Pili R, Sironi E, Somenzi G, Ghidoni R, Toma S, Nicolo G and Sacchi N. (2002). *Cancer Res.*, **62**, 2455–2461.
- Slack JL. (1999). *Curr. Opin. Oncol.*, **11**, 9–13.
- Spinella MJ, Kitareewan S, Mellado B, Sekula D, Khoo KS and Dmitrovsky E. (1998). *Oncogene*, **16**, 3471–3480.
- Suh N, Lamph WW, Glasebrook AL, Grese TA, Palkowitz AD, Williams CR, Risingsong R, Farris MR, Heyman RA and Sporn MB. (2002a). *Clin. Cancer Res.*, **8**, 3270–3275.
- Suh YA, Lee HY, Virmani A, Wong J, Mann KK, Miller Jr WH, Gazdar A and Kurie JM. (2002b). *Cancer Res.*, **62**, 3945–3949.
- Sun SY and Lotan R. (2002). *Crit. Rev. Oncol. Hematol.*, **41**, 41–55.
- Takeshita A, Shinjo K, Naito K, Ohnishi K, Sugimoto Y, Yamakawa Y, Tanimoto M, Kitamura K, Naoe T and Ohno R. (2000). *Br. J. Haematol.*, **108**, 90–92.
- Tallman MS and Nabhan C. (2002). *Curr. Oncol. Rep.*, **4**, 381–389.
- Tamayo P, Slonim D, Mesirov J, Zhu Q, Kitareewan S, Dmitrovsky E, Lander ES and Golub TR. (1999). *Proc. Natl. Acad. Sci. USA*, **96**, 2907–2912.
- Testa U, Grignani F, Barberi T, Fagioli M, Masciulli R, Ferrucci PF, Seripa D, Camagna A, Alcalay M, Pelicci PG and Peschle C. (1994). *Cancer Res.*, **54**, 4508–4515.
- Torrisi R and Decensi A. (2000). *Curr. Oncol. Rep.*, **2**, 263–270.
- Toulouse A, Morin J, Dion PA, Houle B and Bradley WE. (2000). *Lung Cancer*, **28**, 127–137.
- Urnov FD, Wolffe AP and Guschin D. (2001). *Curr. Top. Microbiol. Immunol.*, **254**, 1–33.
- van der Burg B, van der Leede BM, Kwakkenbos-Isbrucker L, Salverda S, de Laat SW and van der Saag PT. (1993). *Mol. Cell. Endocrinol.*, **91**, 149–157.
- Van Heusden J, Van Ginckel R, Bruwiere H, Moelans P, Janssen B, Floren W, van der Leede BJ, van Dun J, Sanz G, Venet M, Dillen L, Van Hove C, Willemsens G, Janicot M and Wouters W. (2002). *Br. J. Cancer*, **86**, 605–611.
- Virmani AK, Rath A, Zochbauer-Muller S, Sacchi N, Fukuyama Y, Bryant D, Maitra A, Heda S, Fong KM, Thunnissen F, Minna JD and Gazdar AF. (2000). *J. Natl. Cancer Inst.*, **92**, 1303–1307.
- Wallberg AE, Neely KE, Hassan AH, Gustafsson JA, Workman JL and Wright AP. (2000). *Mol. Cell Biol.*, **20**, 2004–2013.
- Wang DL, Marko M, Dahl AR, Engelke KS, Placke ME, Imondi AR, Mulshine JL and De Luca LM. (2000). *Clin. Cancer Res.*, **6**, 3636–3645.
- Warrell Jr RP. (1993). *Blood*, **82**, 1949–1953.
- White JA, Beckett-Jones B, Guo YD, Dilworth FJ, Bonasoro J, Jones G and Petkovich M. (1997). *J. Biol. Chem.*, **272**, 18538–18541.
- Widschwendter M, Berger J, Daxenbichler G, Muller-Holzner E, Widschwendter A, Mayr A, Marth C and Zeimet AG. (1997). *Cancer Res.*, **57**, 4158–4161.
- Widschwendter M, Berger J, Hermann M, Muller HM, Amberger A, Zeschnigk M, Widschwendter A, Abendstein B, Zeimet AG, Daxenbichler G and Marth C. (2000). *J. Natl. Cancer Inst.*, **92**, 826–832.
- Wolbach BS and Howe PR. (1925). *J. Exp. Med.*, **42**, 753–777.
- Wu Q, Li Y, Liu R, Agadir A, Lee MO, Liu Y and Zhang X. (1997). *EMBO J.*, **16**, 1656–1669.
- Xu L, Glass CK and Rosenfeld MG. (1999). *Curr. Opin. Genet. Dev.*, **9**, 140–147.
- Xu W, Chen H, Du K, Asahara H, Tini M, Emerson BM, Montminy M and Evans RM. (2001). *Science*, **294**, 2507–2511.
- Xu XC, Ro JY, Lee JS, Shin DM, Hong WK and Lotan R. (1994). *Cancer Res.*, **54**, 3580–3587.
- Xu XC, Sozzi G, Lee JS, Lee JJ, Pastorino U, Pilotti S, Kurie JM, Hong WK and Lotan R. (1997a). *J. Natl. Cancer Inst.*, **89**, 624–629.
- Xu XC, Sneige N, Liu X, Nandagiri R, Lee JJ, Lukmanji F, Hortobagyi G, Lippman SM, Dhingra K and Lotan R. (1997b). *Cancer Res.*, **57**, 4992–4996.
- Yoshida H, Kitamura K, Tanaka K, Omura S, Miyazaki T, Hachiya T, Ohno R and Naoe T. (1996). *Cancer Res.*, **56**, 2945–2948.
- Zelent A, Guidez F, Melnick A, Waxman S and Licht JD. (2001). *Oncogene*, **20**, 7186–7203.
- Zhou DC, Hallam SJ, Lee SJ, Klein RS, Wiernik PH, Tallman MS and Gallagher RE. (1998). *Cancer Res.*, **58**, 5770–5776.
- Zhou DC, Kim SH, Ding W, Schultz C, Warrell Jr RP and Gallagher RE. (2002). *Blood*, **99**, 1356–1363.
- Zujewski J. (2002). *Environ. Mol. Mutagen.*, **39**, 264–270.