Retinoids in chemoprevention and differentiation therapy

Laura A. Hansen, Caroline C. Sigman1, Fausto Andreola, Sharon A. Ross2, Gary J. Kelloff3 and Luigi M. De Luca3

Laboratory of Cellular Carcinogenesis and Tumor Promotion, Division of Basic Sciences and 1Laboratory of Chemoprevention, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255 and 2FDA, CFSAN, OSN, 200 C Street SW, Washington, DC 20204, USA

Retinoids are essential for the maintenance of epithelial differentiation. As such, they play a fundamental role in chemoprevention of epithelial carcinogenesis and in differentiation therapy. Physiological retinoic acid is obtained through two oxidation steps from dietary retinol, i.e. retinol $\rightarrow$ retinal $\rightarrow$ retinoic acid. The latter retinal $\rightarrow$ retinoic acid step is irreversible and eventually marks disposal of this essential nutrient, through cytochrome P450-dependent oxidative steps. Mutant mice deficient in aryl hydrocarbon receptor (AHR) accumulate retinyl palmitate, retinol and retinoic acid. This suggests a direct connection between the AHR and retinoid homeostasis. Retinoids control gene expression through the nuclear retinoic acid receptors (RARs) $\alpha$, $\beta$ and $\gamma$ and 9-cis-retinoic acid receptors $\alpha$, $\beta$ and $\gamma$, which bind with high affinity the natural ligands all-trans-retinoic acid and 9-cis-retinoic acid, respectively. Retinoids are effective chemopreventive agents against skin, head and neck, breast, liver and other forms of cancer. Differentiation therapy of acute promyelocytic leukemia (APL) is based on the ability of retinoic acid to induce differentiation of leukemic promyelocytes. Patients with relapsed, retinoid-resistant APL are now being treated with arsenic oxide, which results in apoptosis of the leukemic cells. Interestingly, induction of differentiation in promyelocytes and consequent remission of APL following retinoid therapy depends on expression of a chimeric PML–RAR$\alpha$ fusion protein resulting from a t(15;17) chromosomal translocation. This protein functions as a dominant negative against the function of both PML and RARs and its overexpression is able to recreate the phenotypes of the disease in transgenic mice. The development of new, more effective and less toxic retinoids, alone or in combination with other drugs, may provide additional avenues for cancer chemoprevention and differentiation therapy.

Introduction

Retinol (vitamin A) is obtained in the diet as retinyl esters, mostly in animal products such as liver, eggs, milk, etc., and/or as a carotenoid precursor in plant products, particularly from green leafy vegetables. Retinoic acid (RA) is a natural product derived by oxidation of retinol (Figure 1), the parent compound for all natural retinoids. Though RA is the immediate ligand for the nuclear receptors, it is not sufficient to maintain vision and reproduction, which require retinol or its retinal derivative, neither of which may be derived by reduction of RA, because oxidation of retinal to RA is an irreversible reaction (Figure 1). RA is, however, necessary for the maintenance of epithelial differentiation. Its concentration in the blood is two orders of magnitude lower than that of retinol ($\sim 10^{-8}$ versus $10^{-6}$ M). The RA target tissue concentration is not known, but it is reputed to be in the nanomolar range, i.e. within the range required for appropriate binding to its receptors. Increasing dietary ingestion of retinol and its esters does not result in a proportional increase in RA concentration, but rather leads to accumulation of retinyl esters in liver tissue. It should, therefore, be emphasized that direct administration of RA may be required for effective chemoprevention or differentiation therapy.

Retinoid-binding proteins

Retinoids travel in the blood and within different cellular compartments as non-covalent complexes with various proteins. The prototype retinoid-binding protein is the blood retinol carrier, 21 kDa retinol-binding protein (RBP) (Figure 1). The work of Goodman, Blaner and collaborators has defined the biosynthesis of RBP in liver cells and its association with trans-thyrein (55 kDa) (1), which minimizes RBP loss by glomerular filtration. Blomhoff and collaborators have illustrated the complexity of intra- and extrahepatic transport of RBP and defined the multiplicity of compartments involved in RBP exchange (2,3). Different views are presently held to explain retinol delivery from RBP to tissue target cells. Two camps presently favor either non-receptor-mediated passive diffusion (4) or RBP-receptor-mediated retinol delivery (5).

In addition to plasma RBP, cellular retinoid-binding proteins have been characterized. The pioneering work of Ong et al. (6) has shown that retinol and RA specifically interact with cellular retinoid-binding (CRBP and CRBP II) and retinoic acid-binding (CRABP I and II) proteins. These proteins appear to mediate biological activity and/or the availability of their ligands, even though their precise function remains to be elucidated. Several pathological conditions alter expression and concentration of these proteins.

The retinoids work through gene activation, as ligands of the nuclear receptors retinoic acid receptor (RAR) and 9-cis-
retinoic acid receptor (RXR). These receptors will be discussed later in this review.

**Retinoid metabolism and the aryl hydrocarbon receptor (AHR)**

A schematic representation of the metabolic pathway of retinoids is shown in Figure 1. Excellent reviews of retinoid metabolism have been published (7–9). Here we give particular attention to the pivotal role that the AHR plays in controlling the degradation of RA via cytochromes P450 (CYP). AHR−/− mice were generated and reported to show some phenotypic abnormalities, mainly liver fibrosis (10,11). In order to determine the biochemical basis of these lesions, AHR wild-type and null mouse livers were tested for their RA, retinol and retinyl palmitate content. All of these retinoids accumulated 2- to 3-fold in the livers of AHR−/− compared with +/+ mice (12). This accumulation probably resulted from a marked reduction in the ability of AHR−/− liver cells to oxidize RA. Experiments with microsomes from wild-type and AHR−/− livers clearly showed a marked reduction in RA oxidation in the AHR−/− mice. The involvement of CYPRAI (13,14), a novel P450 active in RA 4-hydroxylation, was tested, but the AHR−/− genotype did not show any effect on its expression, at least at the mRNA level.

Moreover, increased RA levels could cause a down-regulation of RA-synthesizing enzymes. In this regard, our data showed that livers from AHR null mice express AHD2 mRNA to a lesser extent (15–20%) than controls; interestingly, a direct involvement of AHR in AHD expression has been excluded because AHD2 transcripts were not up-regulated upon 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment in either AHR−/− or +/+ strains (12). In view of these results, AHR status becomes fundamental in liver retinoid homeostasis (Figure 1). The observed large increase in retinyl esters and RA could also lead to activation of downstream target genes involved in liver fibrosis found in AHR null mice, such as type II transglutaminase and transforming growth factor β, whose expression is controlled by RA (15,16) and both of which are overexpressed in AHR−/− liver.

**Retinoic acid and maintenance of normal differentiation**

Pioneering efforts (17) have defined the essential function of vitamin A in maintenance of normal adult epithelial differentiation. Deficiency of dietary vitamin A was shown to cause alterations in epithelial differentiation, particularly in mucus secretory epithelia of the simple and pseudo-stratified phenotypes. Invariably, squamous neoplastic foci form in the epithelia of vitamin A-deficient animals (17–21). These foci, initially comprising a few cells, eventually expand to replace normal epithelia. Thus, vitamin A deficiency induces, at the same time, proliferation of squamous cells and cytostasis and eventual shedding of mucous cells (20,21). It also increases the number of benzo[a]pyrene-derived DNA adducts, at least in the hamster respiratory epithelium (22). Administration of RA replaces squamous metaplastic cells by mucus-secreting cells and, thus, re-establishes normal epithelial function. These phenotypic alterations result from profound changes in gene expression, including keratin genes (for a recent comprehensive review see ref. 20). Since squamous metaplasia is also invariably observed as a result of carcinogen exposure in mucous epithelia and excess retinoids either reverse or prevent this lesion (23), the concept that retinoids may be general chemopreventive agents against epithelial cancer has received much attention (24).

**Chemoprevention**

Chemoprevention research emphasizes the concept that retinoids, when available optimally or even at supra-physiological
levels, inhibit the development of epithelial carcinogenesis, i.e. their action is exerted during the carcinogenesis process (24). Comprehensive reviews of preclinical and clinical work have been published (25–30) and cover most organ sites in detail, including lung, cervix, intestine, prostate and bladder. This review highlights chemoprevention by retinoids in animal models of skin and breast carcinogenesis.

**Retinoids as chemopreventive agents of epidermal carcinogenesis**

Retinoids have been used extensively as chemopreventive agents for skin carcinogenesis (31). The two-stage system, which utilizes one application of the carcinogen 7,12-dimethylbenzanthracene (DMBA) and multiple applications of a tumor promoting agent, e.g. 12-O-tetradecanoylphorbol-13-acetate (TPA) (32), has been most studied (31).

Carcinogenesis develops as a stepwise process with the formation initially of benign tumors (papillomas) and later of malignant tumors (carcinomas). However, this process can be subdivided into additional stages of premalignant progression, malignant conversion and malignant progression. When mezerein is used as the promoting agent, the papillomas formed are at a higher risk of malignant conversion (31–33) compared with papillomas obtained through the DMBA/TPA system. These mezerein-promoted papillomas are also inhibited by dietary as well as topical RA, in contrast to the low risk papillomas obtained with the DMBA/TPA protocol, which do not show significant inhibition by dietary RA (34), though they are inhibited by topical RA (35). These studies are among the few in which semi-purified diets have been used, i.e. without other retinoids or carotenoids which complicate interpretation of the results.

The inhibitory action of RA on skin carcinogenesis appears to be exerted at the level of RARs. In situ hybridization analysis has shown that mouse epidermal neoplastic cells lose the ability to express RAR transcripts during tumor promotion and malignant progression (31,36). In particular, RARα transcripts are expressed in epidermis, papillomas and differentiated carcinomas, but are not expressed in undifferentiated carcinomas and in the most malignant spindle cell carcinomas (36). RARγ transcripts are abundant in epidermis and papillomas, but are absent in both differentiated and undifferentiated carcinomas and in the more malignant spindle cell carcinomas (36). RARβ transcripts are absent from the epidermis (37). In contrast to the reduction in RARα and γ during malignant progression, RXR transcript expression remains at a high level in proliferating cells in normal epidermis and tumor tissue (36).

In vitro studies of mouse epidermal keratinocyte transformation by the oncogene Ha-ras demonstrated a similar RAR transcript reduction with concomitant increased proliferation. RARγ gene expression in ras-transformed mouse keratinocytes caused RA-induced inhibition of cell growth (38). Exposure of mouse skin to tumor promoters in vivo also produced a reduction in receptor transcript expression (38).

We suggest that the mechanism underlying the chemopreventive effects of supra-physiological RA is enhanced expression of RARs, which counteracts the down-regulatory effects of tumor promoters (38). This mechanism has also been proposed to explain the action of retinoids in chemoprevention of head and neck cancer. The work of Lotan (39,40) and Khuri et al. (41) supports the involvement of RARβ as the mediator of RA action in chemoprevention of head and neck carcinogenesis.

**Retinoids as chemopreventive agents of breast carcinogenesis**

Excellent reviews of this subject have been published (25,26,29,30,42,43) and only relatively new advances in chemoprevention of breast cancer in animal models will be covered here.

The potential for hepatic toxicity of large doses of naturally occurring retinoids has inspired the search for synthetic analogs with greater chemopreventive potential and less toxicity. N-(4-hydroxyphenyl)retinamide (4-HPR) was shown to be an effective retinoid for inhibition of breast carcinogenesis in rats by N-nitroso-N-methylurea (NMU) (44). 4-HPR did not accumulate in the liver and therefore caused little hepatic toxicity in animals (44). Furthermore, 4-HPR accumulates in the mammary gland (45) and is metabolized by mammary epithelial cells in both rodents (46) and humans (47). Efficacy of 4-HPR is limited to pre-menopausal women with stage I breast cancer to prevent contralateral new primaries (48–51).

For a more extensive discussion of the importance of the timing of retinoid treatment in animal studies and additional experimental design issues with relevance to the clinical setting see Moon et al. (24), Moon and Constantinou (43) and Moon (52).

The synthetic RXR-selective ligand LDD1069 (Targretin) binds tightly to the three RXRs (α, β and γ), but has essentially no affinity for the RARs and does not appear to transactivate RA-responsive genes (53,54). After it was shown that 9-cis-RA (an agonist for all six retinoid receptors or for both retinoid and rexinoid receptors) prevented mammary carcinogenesis (55), Targretin was also tested in rats and found to prevent mammary carcinogenesis, notably without the classic pattern of retinoid toxicity (e.g. mucocutaneous toxicity, headaches and hypertriglyceridemia) seen with other retinoids (53). Additionally, the maximum inhibition in tumor incidence and multiplicity with Targretin was similar to that achieved with tamoxifen (53). Although Targretin was not shown to alter estrogen, progesterone or prolactin levels in this model, it inhibited both estrogen- and tamoxifen-induced growth stimulation of the uterus (53). The possibility that Targretin, in combination therapy with tamoxifen, may prevent the unwanted side-effects of tamoxifen agonism in the uterus should be further explored. The mechanism of action for inhibition of NMU-induced tumors by Targretin is unknown. Gottardis et al. (53) hypothesized that Targretin may suppress the formation of breast tumors by several possible mechanisms, which may involve one of the following: (i) directly activating RXR-specific pathways; (ii) enhancing activity of the endogenous retinoids that activate the RARs; (iii) weakly activating RAR signaling pathways. In additional experiments, these same investigators examined the ability of Targretin to induce regression in established tumors (56). At the highest dose studied, Targretin induced complete regression in 72% of NMU-induced rat primary tumors. In contrast, tamoxifen, at the highest dose studied, completely inhibited growth in 33% of tumors. Previously, RAR-selective ligands have failed to show effectiveness in regressing established tumors in this model. Additionally, using a moderately effective dose of Targretin (10 mg/kg) in combination with a low dose of tamoxifen (150 µg/kg), 26% of tumors regressed completely (56). This was a greater than additive efficacy compared with either tamoxifen (5.6%) or Targretin (10.5%) alone at similar doses. This finding suggests that the mechanisms of action of Targretin and tamoxifen in tumor regression do not overlap, which may be beneficial in the clinical setting.
Combination chemoprevention strategies in breast cancer chemoprevention

Chemoprevention by a single agent may be limited by both toxicity and lack of potency. Therefore, combination chemoprevention strategies are currently being explored to reduce toxicity and enhance chemopreventive activity. The combination of a differentiation/anti-proliferation agent with a hormone antagonist may be beneficial and relevant in chemoprevention of breast cancer. Estrogen is a promoting factor in mammary carcinogenesis. Therefore, antagonism of the action of estrogen receptor α in breast tissue has been an important tool in preventing mammary carcinogenesis. Recent emphasis in breast cancer prevention has concerned the use of agents that function as antagonists at sites where estrogen may promote carcinogenesis (i.e. breast) but maintain the beneficial agonist effects of estrogen at other sites (i.e. bone, brain and the cardiovascular system). Both retinoid treatment and modification of hormonal status have been shown to inhibit tumors in NMU-induced rat mammary cancer models (43,52). The combination of ovariotomy and retinyl acetate resulted in synergistic inhibition of tumor incidence and multiplicity (57). Similarly, combined ovariotomy plus 4-HPR was significantly more active in suppressing mammary cancer induction than was either treatment alone (58). In these studies 4-HPR was a more effective inhibitor of carcinogenesis in ovarioctomized rats than in intact animals. This study indicated that 4-HPR is highly effective in inhibiting ovarian hormone-independent cancers and suggested that retinoid inhibition of mammary carcinogenesis may not involve an influence on ovarian hormone action. In these models, preneoplastic and/or neoplastic cell populations may display differential sensitivity to retinoids and hormones (52).

Investigations have been performed to determine whether the effectiveness of retinoids can be enhanced by anti-estrogens and vice versa. The combinations 4-HPR and tamoxifen (59), 9-cis-RA and tamoxifen (55), 9-cis-RA and raloxifene (60) and Targetretin and tamoxifen, as discussed above (56), have been investigated in experimental mammary carcinogenesis. Following surgical excision of the first mammary carcinoma, the effect of treatment with 4-HPR and tamoxifen significantly enhanced terminal survival and reduced non-recurrent mammary cancer incidence and multiplicity in NMU-treated rats compared with either treatment alone (59). These results suggested that combination treatment with 4-HPR and tamoxifen was superior to that of either agent alone in blocking progression of neoplastic lesions at both early and later stages of the process. The combination of 9-cis-RA with low levels of tamoxifen was effective in mammary tumor inhibition; addition of 9-cis-RA to a tamoxifen regimen doubled the number of animals that were tumor-free at autopsy and significantly diminished tumor number and tumor burden (55). The authors suggested that clinical evaluation of the combination of 9-cis-RA and tamoxifen, either for chemoprevention or for adjuvant therapy, should be considered. The combination of 9-cis-RA and raloxifene, a synthetic estrogen antagonist which does not promote growth of the uterine epithelium and provides desirable estrogenic activity in bone, was found to prevent more mammary tumors in NMU-induced rats than either agent alone (60). Furthermore, this combination regimen was not found to be toxic.

In most instances, retinoid and hormone combination chemoprevention strategies resulted in greater inhibition of mammary carcinogenesis than treatment with either agent alone. These encouraging experimental results have provided an impetus to design and implement combination chemoprevention clinical trials using retinoids with other agents. One example is the combination chemoprevention trial of fenretinide and tamoxifen mentioned by Hong and Sporn in 1997 (61). Additionally, given the results in complete tumor regression experimental studies (56), future experimental and clinical studies may provide information on the effectiveness of RXR-selective ligands (e.g. Targretin) in tandem with tamoxifen or raloxifene.

Differentiation therapy of acute promyelocytic leukemia (APL)

Retinoic acid treatment of APL

Treatment which induces the differentiation of cancer cells, thus preventing further proliferation, is known as differentiation cancer therapy. The single successful example of differentiation therapy is the use of all-trans retinoic acid (ATRA), which causes frequent remission of APL by inducing the differentiation of promyelocytes. APL is a fairly rare disease, making up only 5–15% of all leukemias, and has a fairly young median age of onset, in the mid-forties (62). Before the use of ATRA therapy, up to 50% of APL patients died early on during chemotherapy (63–66). Current therapy of APL includes a central role for ATRA treatment, which results in a dramatically improved prognosis for patients, leading to complete remission in up to 95% of patients (67) and long-term survival of around 75% (68). These results are significant not only because of the real improvement in cancer survival, but also because of effective targeting of the therapy to the molecular lesion.

Molecular alterations in APL

Virtually all cases of APL are associated with chromosomal translocations involving RARα. More than 99% have a characteristic t(15;17)(q22;q11–21) chromosomal translocation which produces a fusion protein between RARα and a protein called promyelocytic leukemia protein (PML) (68). Induction of maturation of promyelocytes and consequent remission of APL by RA depends on the presence of the t(15;17) chromosomal translocation and resultant PML–RARα fusion protein (69). PML, which was originally identified as a result of this fusion, is a putative transcription factor. Several other chromosomal translocations occur less often in APL, resulting in fusion of RARα with the nuclear transcription factor PLZF on chromosome 11 (64), the nuclear mitotic apparatus protein NuMa on chromosome 11 (70) and nucleophosmin on chromosome 5 (71). The resulting fusion proteins similarly include the DNA-binding, co-repressor, co-activator and ligand-binding regions of the RARα, but differ in their sensitivity to ATRA. Leukemic cells expressing a PLZF–RARα fusion protein, in particular, do not differentiate in response to ATRA treatment (72).

PML–RARα expression induces APL in transgenic animals

Further evidence for the importance of PML–RAR in the induction of APL is provided by transgenic models. Creation of transgenic mice and chickens with expression of the PML–RARα fusion protein targeted to myeloid cells reproduces APL leukemogenesis after a long latency, demonstrating that PML–RARα is responsible for the initiation of leukemogenesis in humans (73–75). In at least one example of myeloid-driven expression of PML–RARα in transgenic mice, a block in the...
differentiation of neutrophils occurred which could progress to APL (73). Similarly to the human disease, RA treatment induces differentiation of the leukemic cells and remission of the disease in these transgenics (73).

**Functions of the RARα**

The RAR is important in the growth and differentiation of many cell lineages, including suppression of growth and induction of differentiation in myeloid cells. The RARα belongs to a family of genes, which includes RARβ and RARγ, each of which can generate several isoforms by alternative splicing or use of alternative promoters (76). RARs are nuclear steroid hormone receptors which act as ligand-inducible transcription factors. Binding of retinoid ligands induces heterodimerization of RARs with RXRs, a related family of transcription factors consisting of RXRα, β and γ and their isoforms. RAR–RXR heterodimers act as transcription factors by binding to retinoic acid response elements (RAREs). RARs can also indirectly repress transcription of genes not containing a RARE by binding of RARs to CREB-binding protein (CBP) following ligand activation. This results in decreased availability of CBP for activation of AP-1-associated transcription (77).

**Functions of PML**

The PML protein product is a member of the RBCC family of proteins and consists of a ring finger, two B boxes, a coiled-coil region and a nuclear localization sequence. PML is localized to both the nucleoplasm and discrete nuclear structures called nuclear bodies (NB) or PML oncogenic domains. Localization of PML to NBs appears to be modulated by its binding to SUMO1/PIC-1, the small ubiquitin-like modifier (78,79). Normal cells have 10–20 NBs which are part of the nuclear matrix and may be important in RNA transcription or chromatin function. NBs may also be important in cellular antiviral defense since Herpes simplex virus type 1, adenovirus and human cytomegalovirus proteins target PML NBs and disrupt their structure (80–83). Additionally, the antiviral interferon induces PML expression.

Transfection of PML into many different cell types suppresses growth (84,85) and induces apoptosis through both a caspase-dependent and a novel caspase-independent pathway (86,87). Recent experiments suggest that the effect of PML on growth may be modulated by its activity as a transcription factor. PML is associated with the AP-1 transcriptional complex and cooperates with Fos to stimulate AP-1-mediated transcription (88). Research with PML null mice further contributes to the evidence of the importance of PML in carcinogenesis. PML null mice exhibit increased sensitivity to chemical carcinogenesis in skin and salivary gland in addition to alterations in myeloid cell numbers (87,89). PML null mice also demonstrate an interaction between PML and retinoid function since retinoid-activated transcription and retinoid-induced myeloid growth suppression and differentiation are blocked in PML null cells (87,89).

**PML–RARα functions as a dominant negative towards PML and RARα signaling**

PML–RARα is believed to exhibit both dominant negative effects on PML and RARα signaling and gain of function effects as a chimeric transcription factor. In support of this hypothesis, PML–RARα can form homodimers as well as heterodimers with both PML and RXRs. PML–RARα actually causes a dominant negative transcriptional repression of RAR–RXR heterodimers (90). Additionally, PML–RARα binds RA with the same affinity as RARα and activates transcription through RAREs. In myeloid cells, PML–RARα suppresses basal level transcription by recruitment of histone deacetylase (91,92) but results in hyperinduction of expression of reporter genes from RAREs in the presence of RA (93). Also consistent with the potential for a dominant negative effect of the PML–RARα fusion protein on RARα signaling, expression of PML–RARα in leukemic cells is much higher than RARα levels (94). PML–RARα also causes redistribution of PML and RARα, with respect to subcellular localization, effects which are reversed by RA treatment. Also in support of the evidence of a dominant negative effect of PML–RARα on PML signaling are results showing that transfection of a fusion protein lacking portions of PML does not result in transformation of cells in culture. In addition, targeting of PML–RARα for cleavage with a hammerhead ribozyme results in induction of apoptosis rather than differentiation in PML–RARα-expressing NB4 APL cells, suggesting that PML–RARα may suppress PML-mediated apoptosis in leukemic cells (95). Disruption of PML NB localization by PML–RARα is reversed following RA treatment but does not seem to be responsible for differentiation of APL cells. Degradation of PML–RARα following RA treatment is due to PML–RARα-dependent caspase activation and cleavage of the PML portion of the fusion protein, but is not required for RA-induced differentiation (92). Relocation of PML to the NB, an apparent consequence of degradation of PML–RARα, is also not required for RA-induced differentiation of APL cells. Thus, RA-induced differentiation is probably mediated by release of PML–RARα from transcriptional repression (92).

**Current therapy of APL**

RA treatment causes differentiation of APL blasts and complete remission in >90% of patients with APL (67). In fact, there are no reported cases of APL with the PML–RARα translocation which are not initially responsive to ATRA treatment. Unfortunately, complete remission is short lived in most patients and only 20% of patients achieve a second complete remission following ATRA alone (96–98). The acquired resistance to ATRA treatment may be caused by increased metabolism and clearance of RA, increased cellular RA-binding protein (CRABP) levels resulting in increased removal of PML–RARα or selection of non-PML–RARα leukemic clones.

Current therapy of APL involves an initial treatment with ATRA and chemotherapy to achieve complete remission in as many as 95% of patients and disease-free survival rates of at least 60% (68). Several cycles of post-remission chemotherapy are also employed which results in molecular remission (absence of RT–PCR-detectable PML–RARα expression) in up to 95% of patients. Some form of maintenance therapy also appears to be beneficial. Using this treatment, ~20–30% of patients reportedly relapse and are treated with aggressive therapy including ATRA, chemotherapy and bone marrow transplantation (68,99).

**New drugs for the treatment of APL**

Arsenic has also been proven effective in the treatment of APL, probably through a mechanism separate from that of RA. A majority of patients with relapsed ATRA-resistant and chemotherapy-resistant APL had complete remission following arsenic treatment (up to 0.2 mg/kg body wt/day) (100,101). This response was associated with apoptosis rather than differ-
entiation of leukemic cells (100). In vitro, PML–RARα relocates PML from NBs into microspeckles, an effect which is reversed by arsenic. Arsenic treatment of cells in culture results in enhanced conjugation of PML to SUMO1 (78) and PML and PML–RARα recruitment to NBs, which is followed by degradation of both of these proteins (102). Thus, arsenic seems to specifically target the PML portion of the fusion protein (99,102), whereas RA results in differentiation of APL cells, degradation of PML–RARα by arsenic causes apoptosis (99). These differences may be the result of transcriptional activation by RA, in contrast to the effect of arsenic on NB proteins (102). However, research with PML null cells does not support the conclusion that arsenic solely targets PML and PML–RARα for its effects. As4O6 and an organic arsenical also suppress growth in PML null cells and APL cells which no longer express PML–RARα (87,103). Thus, the effects of arsenic on APL cells may be mediated by multiple pathways.

Warrell et al. (104) report clinical remission of APL following addition of the histone deacetylase inhibitor sodium phenylbutyrate to the ATRA treatment regimen in a single patient with ATRA-resistant APL, suggesting that inhibition of histone deacetylases may restore sensitivity to ATRA.

Conclusions

The relatively recent and remarkable surge of interest in retinoid biology followed the discovery that these agents function as regulators of gene transcription, through binding to nuclear receptors that belong to the steroid/thyroid hormone receptor superfamily (105,106). A nearly direct consequence of these findings is that, in addition to public health professionals (107), researchers in dermatology, oncology, cell biology, molecular biology, reproduction, embryology, etc. (20,108) are interested in vitamin A and its derivatives, the retinoids.

At least three leads from current clinical results are expected to impact on clinical development of retinoids in the next few years: (i) retinoid receptor-selective agents; (ii) cross-talk among members of the steroid superfamily; (iii) strategies for attaining sufficient tissue levels of retinoids.

We have emphasized the importance of the retinoid receptors as key molecules in differentiation and the prevention of epithelial carcinogenesis. In the mouse skin carcinogenesis model, malignant progression is associated with down-regulation of RARα and expression at the mRNA level (36,38). We propose that pharmacological RA exerts its chemopreventive actions through up-regulation of RARα expression. Tumor promoters, on the other hand, have been shown to directly down-regulate these receptors (38,109). Though RARβ does not appear to play a role in epidermal carcinogenesis, since it is not normally expressed in this tissue, this receptor has been shown to play a pivotal role in head and neck cancer and in lung cancer, where it is normally down-regulated, but is up-regulated by RA in cultured tumor cells (41,110) as well as in cervical intra-epithelial neoplasia, where it is strongly underexpressed (111). Topical retinoid administration to oral lesions, presenting with low endogenous RA and RARβ transcript levels, also results in strong up-regulation of RARβ (112).

Because of the known cytotoxicity of retinoids and the possibility of unwanted side-effects we have highlighted the fact that synthetic RXR-selective agents, such as Targretin, maintain efficacy with fewer side-effects than natural retinoids (53,55). These observations suggest that it is possible to design tissue-specific chemoprevention strategies that take advantage of receptor specificities without obvious toxicity.

Steroid hormones, retinoids, vitamin D, thyroid hormone and their receptors and their downstream signal transduction components are a very powerful family of molecules that control transcriptional events and differentiation. The synergistic chemopreventive activity of retinoids with anti-estrogens in preclinical studies in the breast are of great interest and may result, at least partially, from effects mediated by both the retinoid and steroid hormone receptor pathways of gene transcription. Similarly, the activity of fenretinide (which does not appear to bind to retinoid receptors) (113) in preventing progression of estrogen receptor positivity of retinoids in target tissues is justified because of their high affinity for multiple binding sites during absorption and their self-induced metabolism en route to target sites. Several potential strategies have been suggested to address these issues. One is the use of RA metabolism-blocking agents (114) and a second is local application (e.g. aerosol formulations for the lung) of the retinoid (115).

The concept that RA directly controls gene transcription has attracted much attention and interest from medical scientists and researchers interested in altering the behavior of cancer cells through induction of differentiation. As a consequence, a specific form of leukemia, APL, has been shown to be highly responsive to oral RA at pharmacological doses. The leukemic cells differentiate in vivo and in vitro into granulocytes and, in the process, lose the PML–RARα transcripts typical of this disease to follow the natural pathway to cell aging. Differentiation therapy may well be a more effective and less toxic avenue for cancer management and eventual eradication of the malignant clone.

The chromosomal translocation t(15;17) directly alters a retinoid receptor gene and creates a new transcript made up of a fusion between the PML and the RARα genes. We should keep in mind that we still do not understand why APL is so sensitive to RA and, in fact, this sensitivity represents a paradox, since it is difficult to understand how RA sensitivity can be associated with a mutated RARα. It is also unclear which other retinoid receptors are the molecular targets of RA in APL.

Though RA is clearly an excellent differentiation agent in APL, it may not eradicate the malignant clone, eventually causing relapse in some patients. This may in part be due to increased metabolism of RA in patients subject to pharmacological doses of the retinoid, so that lower concentrations of the retinoid may be found in the blood after prolonged treatment, and in part to resistance of some malignant clones to RA. Combined chemotherapy and differentiation therapy may provide additional protection. Moreover, treatment with agents that inhibit RA metabolism, such as lizarozol or similar compounds, may also be effective. These agents have been shown to induce differentiation of Dunning prostate carcinoma cells (114).

Progress in the treatment of APL has been surprisingly rapid following the discovery of the effectiveness of RA in inducing differentiation of APL blasts and the presence of the t(15;17) chromosomal translocation in APL. Many patients are now
References


