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Retinoids: present role and future potential

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Summary Vitamin A and its biologically active derivatives, retinal and retinoic acid (RA), together with a large repertoire of synthetic analogues are collectively referred to as retinoids. Naturally occurring retinoids regulate the growth and differentiation of a wide variety of cell types and play a crucial role in the physiology of vision and as morphogenic agents during embryonic development. Retinoids and their analogues have been evaluated as chemoprevention agents, and also in the management of acute promyelocytic leukaemia. Retinoids exert most of their effects by binding to specific receptors and modulating gene expression. The development of new active retinoids and the identification of two distinct families of retinoid receptors has led to an increased understanding of the cellular effects of activation of these receptors. In this article we review the use of retinoids in chemoprevention strategies, discuss the cellular consequences of activated retinoid receptors, and speculate on how our increasing understanding of retinoid-induced signalling pathways may contribute to future therapeutic strategies in the management of malignant disease.

Keywords: differentiation; apoptosis; drug resistance; retinoids

RETINOIDS AND THEIR RECEPTORS

Chemoprevention

Many cancers develop as a result of exposure to carcinogens and cancer-promoting agents in a multistep process including both initiation and promotion. Attempts to delay, reverse or block cancer development by intervening in this process form the basis of cancer chemoprevention strategies. An increased susceptibility to chemical carcinogens and a higher incidence of cancer have been observed in experimental animals with vitamin A deficiency (Moon et al, 1994). This, and the observation that individuals with a lower dietary intake of vitamin A are at a higher risk of developing cancer (Hong and Itri, 1994), gave rise to the notion that physiological levels of retinoids may, in some way, protect the individual against the development of premalignant and malignant disease. Furthermore, the efficacy of pharmacological doses of retinoids as chemopreventive agents has been demonstrated in experimental models of carcinogenesis for numerous animal tumours (reviewed in Lotan, 1996).

Preclinical data

Retinoids can inhibit the transformation of cultured mouse embryo cells in vitro by either 3-methylcholanthrene (Bertram, 1983) or γ-rays. Retinoids can also inhibit the ability of malignant cells to form colonies in semisolid medium, an anchorage-independent property that is a characteristic of transformed cells (Lotan, 1995). Moreover, all-*trans* retinoic acid (ATRA) can inhibit immortalization of human epidermal keratinocytes during or after transfection with HPV16 (Creek et al, 1994). In addition, the synthetic retinoid *N*-(4-hydroxyphenyl)retinamide (4HPR) can inhibit prolactin-induced DNA synthesis and end-bud proliferation in mouse mammary gland in whole organ culture (Moon et al, 1994), and retinoids can also modulate normal rat mammary epithelial cell proliferation, morphogenesis and functional differentiation (Lee et al, 1995).

The use of retinoids to suppress tumour development has been evaluated in several animal models of carcinogenesis including models of skin, breast, oral cavity, lung, hepatic, gastrointestinal, prostatic and bladder cancers (reviewed in Lotan, 1996). Many of these studies have shown that retinoids possess antipromotion activities. However, long-term retinoid treatment was required to suppress carcinogenesis since the effects of the retinoids were reversible when stopped. Furthermore, some retinoids were found to be active in certain animal models of carcinogenesis and not in others, that is retinoids exhibit some degree of tissue selectivity. Moreover, certain retinoids may be active inhibitors of carcinogenesis in certain tissues but can act as enhancers of carcinogenesis in the same tissue in another strain of mice, or in another carcinogenesis model. For example, dietary addition of ATRA had no effect on tumour initiation in the two-stage mouse skin carcinogenesis model, but acted as an antipromoter by inhibiting progression of papilloma to carcinoma (De Luca et al, 1994). However, retinoids were either ineffective in preventing, or enhanced, papilloma formation when an alternate mouse skin carcinogenesis model was used (De Luca et al, 1994). Indeed, vitamin A deficiency was more effective than excess retinoid in inhibiting skin carcinogenesis using an alternative strain of mice (Lotan, 1996). Similarly, both suppression and enhancement by retinoids have been reported in different models of liver carcinogenesis (Moon et al, 1994). Indeed, 4HPR suppressed carcinogenesis in two strains of mice and enhanced carcinogenesis in two other strains (Moon et al, 1994). Inconsistent results were also obtained in models of lung, oesophageal and pancreatic carcinogenesis (Moon et al, 1994). Studies using rat mammary models have confirmed

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the importance of tissue distribution and metabolism of retinoids. Retinoids that accumulated in the mammary gland and the surrounding fat pad were more effective inhibitors of carcinogenesis than those that failed to concentrate in the target tissue. Again, retinoids were ineffective as inhibitors of initiation in these rat mammary models but, interestingly, administration of retinoid in early phases of carcinogenesis resulted in sustained inhibition of carcinogenesis even after stopping retinoid treatment, whereas a delay in retinoid administration until later in the carcinogenic process required continuous retinoid administration to maintain an inhibition of carcinogenesis. In contrast, pre-treatment of rats with retinoids for 2 months prior to initiation resulted in an increased incidence of carcinomas unless retinoid administration was continued through to the promotion step (reviewed in Moon et al, 1994; Lotan, 1996). Consequently, understanding the timing of administration in addition to tissue distribution and metabolism is crucial if retinoids are to be effectively used as chemopreventive agents.

Clinical data

Many clinical trials of retinoids as chemoprevention agents are in progress or have been completed. Most of these trials focus on individuals at an increased risk of developing cancer, such as patients with pre-malignant lesions or patients who have been successfully treated for an early-stage carcinoma and have a high risk of developing a second primary cancer. The use of retinoids in patients with cutaneous actinic keratoses results in a significant decrease in the incidence of squamous cell carcinomas of the skin (Moon et al, 1997), and this is also observed in renal transplant patients with this pre-malignant condition (Bavinck et al, 1995; Rook et al, 1995). Similarly, topical ATRA can lead to a clinical and histological improvement in patients with the dysplastic nervous syndrome (Edwards and Jaffe, 1990; Halpern et al, 1994). Oral pre-malignant lesions such as leukoplakia or erythroplakia are frequently extensive or multiple and consequently are not amenable to surgery. Therefore, patients with these lesions are ideal candidates for chemoprevention studies. In an original placebo-controlled randomized study, 13-*cis* retinoic acid was evaluated (Hong et al, 1986). Although there was a significant clinical response in the treatment arm (67%) compared to the placebo arm (10%), the treatment was unacceptably toxic and half of the responding patients had relapsed within 3 months of stopping the retinoid (Hong et al, 1986). Subsequently, patients received this dose for only a 3-month induction period, and were then randomized to either low-dose retinoid or β-carotene for 9 months (Lippman et al, 1993, 1995). This study demonstrated the feasibility of using low-dose 13-*cis* retinoid acid in maintaining initial responses, whilst underlining the inability of β-carotene to do so. Furthermore, topical 4HPR also shows promising activity in the management of pre-malignant oral lesions (Chiesa et al, 1993; Costa et al, 1994). Cancer of the cervix, which develops in a multistep fashion through progressive intra-epithelial neoplasia (CINI–III) is another logical candidate for a chemoprevention approach. Topical application of ATRA-induced regression in 43% of patients with moderate dysplasia (CIN II) in comparison to a spontaneous regression in 27% of patients treated with placebo, but had no effect on more severe dysplasia (Meyskens et al, 1994). Moreover, *N*-(4-ethoxycarbophenyl) retinamide decreased the incidence and increased survival in a population at high risk of oesophageal cancer (Han, 1993), but retinoids have had consistently no effect on reversal of bronchial metaplasia (Lee et al, 1994).

An unexpected observation from the adjuvant studies of 13-*cis* retinoic acid in early stage head and neck cancers was the reduction in incidence of second primary tumours, although there was no reduction in the rate of recurrence or metastases from the original primary (Hong et al, 1990; Benner et al, 1994). In contrast, etretinate was ineffective in preventing second primary tumours of the oral cavity and oropharynx (Bolla et al, 1994). Similarly, retinyl palmitate can reduce the incidence of second primary tumours in comparison to observation after resection of stage I non-small cell lung cancer (Pastorino et al, 1993). Moreover, initial studies evaluating 13-*cis* retinoic acid in the prevention of recurrent early-stage bladder cancer had to be terminated due to toxicity and a lack of positive results (Prout and Barton, 1992), but etretinate in contrast can increase the time to recurrence in patients with superficial papillary bladder cancers with a reduction in the number of annual transurethral resections (Studer et al, 1995). Based on the preclinical data, a randomized study comparing 4HPR with observation alone has been initiated in women with node-negative breast cancer, and accrual to this study has been completed (Costa et al, 1995). The end-point is the incidence of contralateral primary carcinoma, and clearly if 4HPR can reduce this risk (estimated at 0.8% per year within 10 years of primary treatment), then it would be appropriate to evaluate this agent as a chemoprevention strategy in women at high risk of developing breast cancer on the basis of family history.

However, the most prominent example of the role of retinoids as differentiating agents in current oncology practice is the remarkable activity of all-*trans* retinoic acid in patients with acute promyelocytic leukaemia (APL). Numerous phase II studies have confirmed that ATRA induces complete remission in the vast majority of patients, with rapid resolution of the characteristic, life-threatening coagulopathy (Huang et al, 1988; Castaigne et al, 1990; Chen et al, 1991; Warrell et al, 1991; Fenaux et al, 1992; Ohno et al, 1993; Frankel et al, 1994; Kanamaru et al, 1995). The duration of complete remission with ATRA alone is usually brief and post-remission chemotherapy is required to diminish the likelihood of relapse. A randomized study has confirmed that ATRA as induction or maintenance treatment improves disease-free and overall survival as compared with chemotherapy alone, and should be included in the treatment of APL (Tallman et al, 1997). This therapeutic approach has also contributed to our understanding of retinoid-induced signalling pathways and in particular the role of the retinoic acid receptors.

The retinoid receptors

Retinoids exert most of their effects by binding to specific receptors and modulating gene expression. The nuclear retinoid receptors are members of the steroid/thyroid hormone superfamily of receptors (Evans, 1988) with which they share common structural and functional properties. The diversity of retinoid-induced signalling pathways is mediated by at least six retinoid receptors which fall into two subfamilies: retinoic acid receptors (RARs), α, β and γ, and the retinoid X receptors (RXRs), α, β and γ (Chambon, 1995). In common with other members of the steroid hormone receptor superfamily, these two subfamilies of receptors contain a DNA-binding domain and a ligand-binding domain united by a short hinge region that may also serve as a nuclear

translocation signal (reviewed in Giguere, 1994). The DNA-binding domain contains two 'zinc fingers' involved in recognition of specific DNA sequences and in activation of target genes (Evans, 1988; Freedman, 1992). The RARs bind ATRA with high affinity (Giguere et al, 1987; Petkovich et al, 1987), whereas the stereoisomer 9-*cis* retinoic acid is a bifunctional ligand which can bind to and activate both RARs and RXRs (Mangelsdorf et al, 1992; Allenby et al, 1993). Despite these similarities, the RXRs belong to a subgroup of nuclear receptors distinct from the RARs (Laudet et al, 1992) suggesting that these two groups of retinoid receptors have distinct roles in retinoid signalling.

In keeping with the models by which steroid hormone receptors bind DNA as dimers, the hormone response elements of the target genes for retinoid receptors are direct repeats separated by 1 or 5 nucleotides. The RARs and RXRs bind the half-site consensus sequence PuGGTCA, as can several of the other ligand-activated nuclear receptors, and consequently enabling cross-talk among the gene networks controlled by various ligands. Both negative and positive effects on transcription can occur in the absence of ligand and these bimodal transcriptional properties of retinoid receptors are mediated, in part, by the ability of these receptors to associate with various co-activators and co-repressors such as SMRT and N-CoR (Chen and Evans, 1995; Kurokawa et al, 1995; Horwitz et al, 1996). Transcriptional regulation by receptors would therefore seem to be controlled by selective recruitment of co-activators and co-repressors in response to hormone, and in turn, control of activity of a target promoter. It may be that the role of ligand binding is to cause a conformational change in the receptor, and as a result of this a co-repressor protein is dissociated from the receptor and a co-activator binds to the receptor thereby initiating transcription (reviewed in Perlmann and Evans, 1997). However, although in vitro-binding experiments suggest that DNA-binding is not usually ligand-dependent, in vivo footprinting suggest that RAR–RXR heterodimers bind to a specific RARE in the RARβ gene promoter in a ligand-dependent manner (Dey et al, 1994; Chen et al, 1996). Nevertheless it is not certain that ligand-binding is necessary for steroid hormone receptors to bind DNA (Perlmann and Evans, 1997).

The complexity of retinoid signalling mechanisms is increased by the diversity of the dimer complexes that can occur. Both RARs and RXRs can bind response elements as homodimers, albeit at high protein concentrations (Mangelsdorf et al, 1991; Yang et al, 1991; Mader et al, 1993), although heterodimerization of RARs and RXRs enables high affinity binding of RARs to response elements (Kliewer et al, 1992; Zhang et al, 1992). A large number of different RXR–RAR heterodimer complexes can be formed by combinatorial pairing of the RAR and RXR receptor, with each heterodimer complex having cell and promoter-specific acitivity (Nagpal et al, 1992), and consequently may control distinct gene networks. Retinoid signalling can also be regulated by positive and negative feedback mechanisms. The retinoid binding proteins CRBP-I and CRABP-II, which are involved in retinoid metabolism, may also be involved in retinoid signalling autoregulation (Smith et al, 1991; Durand et al, 1992). However, knockout mice deficient in both CRAB-I and CRABP-II proteins have no adverse phenotype, so the significance of these proteins is unclear (De Bruijn et al, 1994; Gorry et al, 1994). In addition, RXRs also serve as promiscuous partners in a multitude of other hormonal response systems, including vitamin D signalling pathways (Kliewer et al, 1992). However, much of the complexity of these signalling networks has yet to be elucidated.

Cellular consequences of retinoid stimulation

At the cellular level, activation of the retinoid receptors can inhibit cell proliferation, induce differentiation and induce apoptosis in mesenchymal, neuroectodermal, haematopoietic and epithelial cells during normal development as well as in normal and transformed cells in tissue culture. However, the specific receptor which mediates these effects varies with each cell line. Furthermore, the induction of apoptosis is related to cell growth and differentiation in various ways, depending on the cell type. For example, retinoids initially induce the human myeloid leukaemia HL-60 cells to differentiate into neutrophils that subsequently undergo apoptosis (Martin et al, 1990). Using receptor-selective ligands and sub-lines with different retinoid responsiveness, it appears that ligand-induced activation of RARs alone is sufficient to induce differentiation, but activation of RXRs is essential for induction of apoptosis, although the necessary dimerization complexes are unknown (Nagy et al, 1995). In contrast, retinoids can induce differentiation and apoptosis concurrently as in F9 embryonal carcinoma cells (Atencia et al, 1994), or can induce apoptosis by a process that is independent of differentiation as in neuroblastoma cells (Piacentini et al, 1992). Apoptosis can be induced in small cell lung cancer cells by 4HPR (Kalemkerian et al, 1995), in melanoma cells by the synthetic retinoid CD437 (Schadendorf et al, 1996), and in ovarian and breast cancer cells by 4HPR (Sheikh et al, 1995; Supino et al, 1996). Moreover, retinoids can also suppress growth and squamous differentiation in head and neck squamous cell cancer lines (Lotan, 1994), and also induce apoptosis in these cell lines (Oridate et al, 1996). Furthermore, tumour regression on treatment with retinoids has been demonstrated for in vivo xenograft models of experimental tumours including lip squamous cell carcinoma (Gottardis et al, 1996*b*) and melanoma (Schadendorf et al, 1996). Anti-proliferative response appears to be mediated through the RARγ in melanoma and teratocarcinoma cell lines (Moasser et al, 1994; Schadendorf et al, 1994), although no correlation was noted with any receptor in ovarian cancer cell lines (Harant et al, 1993). Several groups have observed that differentiating agents such as butyrate and DMSO can induce p21 and terminal differentiation in a p53-independent manner, although p21 induction by differentiating agents can occur in the presence of wild-type p53 (reviewed in Liebermann et al, 1995). However, uncoupling of p21 induction from growth arrest can occur in the presence of deregulated c-*myc* (Selvakumaran et al, 1994). Retinoids are also able to induce p21 and, consequently, growth arrest and differentiation (Shao et al, 1995; Liu et al, 1996) but the mechanism of induction of apoptosis remains unclear. Putative mechanisms include a role for activation of the AP-1 complex, for which activation of the retinoid receptors is not necessary (Schadendorf et al, 1996), possible suppression of bcl-2 expression and/or induction of transforming growth factor β (TGF-β) (Roberts and Sporn, 1992), induction of insulin-like growth factor-binding protein 3 (Gucev et al, 1996) and activation of downstream effectors of p53 in a p53-independent manner (Shao et al, 1995). The receptor dimerization patterns associated with these cellular events are also unknown.

THE FUTURE USES OF RETINOIDS IN CANCER THERAPY

In addition to the natural retinoids, ATRA, 9-*cis* retinoic acid and 13-*cis* retinoic acid, several novel retinoid compounds have been synthesized including 4HPR, CD437 (Schadendorf et al, 1996),

the RAR-selective ligand ALRT1550 (Shalinksy et al, 1997), and the RXR-selective ligand LGD1069 (Gottardis et al, 1996*a*; Miller et al, 1997). The initial reason for developing these new compounds has been to identify chemoprevention agents with an acceptable toxicity profile suitable for use in chronic administration. However, these agents may well differ from the naturally occurring retinoids in their mechanism of action. Any new potential chemoprevention agent will need to be evaluated in preclinical models of carcinogenesis, prior to entering dose-finding studies, phase II chemoprevention studies and randomized placebo-controlled phase III studies in patients (a) at high risk of developing malignant tumours (e.g. breast cancer on the basis of family history), (b) with pre-malignant conditions such as in situ carcinoma of the cervix, or (c) at risk of developing a second primary tumour. However, these agents, particularly those specific for RAR- or RXR-binding, may also enable us to dissect the retinoid signalling pathway and enable us to explore these agents in advanced disease, either (a) in combination with agents acting cooperatively on other steroid hormone receptors, (b) in combination with other agents which inhibit intracellular pathways, and (c) in combination with other conventional cytotoxic agents to overcome drug resistance.

Cooperative effects with agents acting on other steroid hormone receptors

Retinoids can inhibit the growth of many human hormone-dependent breast cancer cells (Fontana, 1987; Fontana et al, 1990), although many ER-negative cell lines are resistant to the effect of retinoids (Van der Burg et al, 1993). Although RARα can be expressed in both ER-positive and ER-negative breast cancer cell lines, expression of RARα may be higher in ER-positive cell lines and also in human breast cancer samples (Roman et al, 1993). Oestradiol can induce RARα expression in human breast cancer cells (Roman et al, 1993), while transfecting ER-negative cells with RARα leads to retinoid-sensitivity in these cell lines (Sheikh et al, 1994; Rishi et al, 1996). In addition, retinoids down-regulate ER RNA and protein expression in hormone-dependent breast cancer cells (Rubin et al, 1994), as well as inhibiting ER function (Pratt et al, 1996). Taken together, this suggests that retinoids could inhibit ER function. Furthermore, retinoids and anti-oestrogens appear to target different cell cycle regulatory molecules to initiate cell cycle arrest (Wilcken et al, 1997). Indeed, retinoids and tamoxifen appear to have additive effects in the chemoprevention of breast cancer in animal models (Anzano et al, 1994). If these additive effects could be demonstrated in advanced breast cancer in clinical trials then this combination could be evaluated in the adjuvant treatment and for chemoprevention of human breast cancer. If this additive effect could be demonstrated in the clinic it would be important to determine whether it is restricted to tumours expressing RARα and ER.

The biologically active form of vitamin D, 1,25-dihydroxyvitamin D3, is another agent that can induce differentiation and inhibit cellular proliferation with induction of apoptosis. The actions of this ligand are mediated by the vitamin D receptor (VDR) which is part of the steroid hormone family of receptors, again having structural and functional similarities to the retinoid receptors. The VDR has been found in a variety of cancer cell lines including prostate cancer (Miller et al, 1992), pancreatic, breast, colon, thyroid, bladder and cervical carcinoma, osteosarcoma, melanoma and fibrosarcoma (Reichel et al, 1989). The clinical use of vitamin D3 is limited by its calcaemic effect, but a number of analogues have been synthesized that inhibit cancer cell growth but with reduced calcaemic activity such as EB1089 and KH1060 (Colston et al, 1992; Shabahang et al, 1994). Cooperative effects on growth inhibition using a combination of a retinoid with a vitamin D3 analogue have been observed in several experimental systems, including lung cancer cells (Higashimoto et al, 1996), pancreatic cancer cells (Zugmaier et al, 1996) and the HL-60 leukaemic cells (Elstner et al, 1996). However, VDR can bind to response elements either as a homodimer or as a heterodimer complex with RXR. Therefore, in addition to the cooperative effects between retinoids and vitamin D which have been observed, there is also the possibility that these agents could have antagonistic activity if their respective receptors compete for RXR partners to bind their response elements. Consequently, the cellular events which occur with combination therapy in any particular cell may be dictated by the relative abundance of VDR, RARs and RXRs, within that cell, the relative concentration of ligand (including the relative affinity of the retinoids for RARs or RXRs) and the resulting rate and pattern of the various possible heterodimer complexes. Clearly considerable effort will be required to determine the optimal concentrations and combinations of these drugs to produce optimal inhibition of cell proliferation in experimental models of any given tumour. Nevertheless, the results of these preliminary studies are promising, and may develop therapeutic strategies that will add to the treatment options available for tumours that express the relevant receptors.

Combination therapy with inhibition of intracellular signalling pathways

Interferons are a group of multifunctional cytokines with antiviral, antiproliferative and cellular-differentiating activities. Two classes of interferons – type I (interferon α/β) and type II (interferon γ) – acting on different receptors are known. Preclinical data suggest that a combination of retinoids and interferons has synergistic antiproliferative and differentiating effects in some haematological and solid tumour models (reviewed in Eisenhauer et al, 1994), although the mechanisms underlying the cross-talk between the intracellular pathways activated by the retinoids and the interferons have yet to be defined. On the basis of these preclinical observations, a combination of 13-*cis* retinoic acid and interferon α-2a has been evaluated in a number of clinical trials in human solid tumours (reviewed in Eisenhauer et al, 1994). Initial studies yielded dramatic results, with a 50% response rates in patients with previously untreated stages IB–IVA cervical cancer and a 68% response rate in patients with advanced squamous cell carcinoma of the skin. However, these high response rates have not been reproduced in other squamous cell cancers that have been evaluated (head and neck, lung, pre-treated cervix), (Rinaldi et al, 1993; Voravud et al, 1993; Arnold et al, 1994; Hallum et al, 1995) and no benefit was observed in studies of two non-squamous tumours (lung and melanoma) (Arnold et al, 1993; Dhingra et al, 1993). However, these studies did not always evaluate an optimal population of previously untreated patients, and the results of the cervical studies suggest that this is a relevant consideration.

There is evidence to suggest that interferons may modulate the retinoid-signalling pathways by inducing or increasing RAR or RXR expression, rendering cells more sensitive to the retinoid action and even restoring retinoic acid sensitivity in RA-insensitive cell lines (Marth et al, 1986; Widschwendter et al, 1995;

Fanjul et al, 1996). In addition, retinoids are able to induce and activate key components of interferon-signalling pathways including the Stat proteins (Kolla et al, 1996; Gianni et al, 1997) and interferon-regulatory factor (Matikainen et al, 1996). Further dissection of the mechanisms for cross-talk between these two signalling pathways may answer some of the questions raised by the important observations of early retinoid/interferon combination studies. Issues include the specific tumour sensitivities to this combination and the mechanism of acquired resistance in pre-treated sensitive tumour types (e.g. cervix) as well as the optimal combination and doses of the analogues to be used in this combination, and the identification of new targets for inhibiting intracellular signalling. Furthermore, combinations of retinoids and interferons, and also retinoids and vitamin D, can inhibit angiogenesisis in preclinical tumour models (Bollag et al, 1994); this represents an exciting further area for future drug development.

The *ras* family of genes encodes proteins involved in signal transduction across the cell membrane. Constitutive activation of *ras* by point mutation is one of the most common genetic aberrations in malignant disease. Oncogenic (constitutively activated) *ras* reduces the level of RARα in NIH3T3 cells, altering the responsiveness of these cells to RA (Scita et al, 1996), and reducing the level of RARα and RARγ in keratinocytes (Darwiche et al, 1996). Moreover, inhibition of protein kinase C with bryostatin in these cells can restore RAR protein levels to near normal levels (Darwiche et al, 1996). This raises the interesting possibility of synergistic anti-tumour effect by using retinoids in combination with protein kinase C inhibitors or inhibitiors of *ras*-induced signalling pathways such as farnesyltransferase inhibitors.

The use of retinoids in combination with cytotoxic chemotherapy agents

One of the most intriguing possibilities for the use of retinoids in advanced disease is to enhance the sensitivity of tumours to cytotoxic agents and to overcome drug resistance by adjusting the apoptopic set-point. Among the many mechanisms of chemotherapeutic drug resistance, a key factor is likely to be the p53 tumour suppressor gene mutations of which are associated with decreased sensitivity of Burkitt's lymphoma cells to treatment with ionizing radiation and DNA-damaging chemotherapy drugs (Fan et al, 1994). Studies have also suggested that the p53 tumour suppressor gene is required for efficient induction of cell death by chemotherapy drugs (Lowe et al, 1993, 1994). Indeed, disruption of p53-mediated apoptosis, e.g. by mutations of the p53 genes, contributes both to tumour development and acquisition of drug resistance (Lowe et al, 1994; Symonds et al, 1994; Tsang et al, 1995). However, in a subsequent study, induction of apoptosis correlated with chemosensitivity in a number of human tumour cell lines independent of p53 status or bcl-2 protein levels in vitro (Wu and El-Deiry, 1996). This is supported by the evidence that overexpression of WAFI/CIPI increased the susceptibility of p53 non-functional malignant glioma cells to cisplatin-induced apoptosis even though overexpression of WAFI/CIPI alone inhibited DNA synthesis but did not induce apoptosis (Kondo et al, 1996). Thus the relationship between p53 function and chemosensitivity probably varies according to cell type.

Although the mechanisms of retinoid-induced apoptosis remain unclear, it is worth noting that the putative mechanism with some of the novel synthetic retinoids include inhibition of AP-1 activity independent of receptor activation, and induction of G0/G1 arrest and apoptosis in a p53-independent manner by activating downstream effectors of p53 (Shao et al, 1995). Therefore, by inducing apoptosis, retinoids may be able to enhance sensitivity of tumours to cytotoxic agents and overcome cytotoxic drug resistance even though the precise mechanism of induction of apoptosis by retinoids is not known. It is encouraging that pre-treatment of ovarian cancer cell lines with ATRA potentiates the cytotoxicity of these cells to cisplatin (Caliaro et al, 1997). Synergy between these two agents was observed only in cells sensitive to ATRA, regardless of their relative sensitivity to cisplatin. Indeed, in a variant cell line resistant to cisplatin but sensitive to ATRA, the IC_{50} for cisplatin was reduced with combination therapy in the clonogenic assay. ATRA can also increase the sensitivity of a murine embryonal carcinoma cell line to cisplatin (Guchelaar et al, 1993), can potentiate the cytotoxicity of cisplatin, etoposide and bleomycin in a human ovarian teratocarcinoma (Le Ruppert et al, 1992) and is synergistic with cisplatin and 5-fluorouracil in squamous cell carcinoma cells (Sacks et al, 1995). Furthermore, enhanced antitumour efficacy of cisplatin is observed in combination with 9-*cis* retinoic acid in human oral squamous cell carcinoma xenografts in nude mice, with no change in systemic toxicity or dose tolerance of the individual agents (Shalinsky et al, 1996).

Encouraging preliminary clinical results for retinoid chemotherapy combination therapy have also been reported in one small study (20 patients) using ATRA with cisplatin and VP16 in advanced non-small cell lung cancer, with a 53% objective response rate (Thiruvengadam et al, 1996). However, the optimal retinoid agent, dose, schedule and combination for a given tumour has yet to be determined in animal models. It also remains to be determined whether this enhancement of cytotoxicity is restricted to cisplatin or also occurs with other cytotoxic agents, and whether using immunocytochemistry to determine the presence of RARs or RXRs in a given tumour specimen will predict for tumour response or enhanced cytotoxicity. Nevertheless this is a promising approach in an attempt to overcome cytotoxic drug resistance which remains a significant cause of treatment failure.

In summary, the discovery of new, synthetic retinoid analogues may enable longer term administration with less toxicity than the naturally occurring retinoids. These agents will also be useful in the laboratory to further dissect the retinoid signalling pathway, which in turn may identify new therapeutic targets. In addition to revisiting the chemoprevention approach in certain tumour groups, these new agents may be useful in advanced disease or as adjuvant therapy in combination with other steroid hormones, inhibitors of specific signal transduction pathways, or in combination with cytotoxic chemotherapy agents currently in use in the clinic. Retinoid resistance, both intrinsic and acquired, represents a further major challenge in the field of differentiation therapy.

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