Retinoids in Head and Neck Chemoprevention (44179)

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Abstract. **Over the last few years, we have witnessed tremendous progress in both basic and clinical research on retinoids. Preclinical studies have indicated the potential of retinoids in cancer prevention and therapy, but the actual successful application of retinoids in clinical chemoprevention trials has been the recent and exciting development in the field of retinoid research. Our understanding of the role of retinoids in normal developmental processes and the differentiation of normal and malignant cells, and the fundamental discovery of the nuclear retinoid receptors that act as transcription modulating factors regulating specific gene expression have been major advances in the field of basic retinoid research. Chemoprevention is the newest research approach in our efforts to control upper–aerodigestive tract cancers, which have one of the lowest cure rates among epithelial malignancies, and in which the occurrence of second primary tumors further burdens the dismal prognosis of patients. The efficacy of retinoids in the reversal of oral premalignant lesions and the prevention of second primary tumors has generated tremendous enthusiasm among retinoid researchers, particularly those in the field of chemoprevention. Current explorations of combinations of retinoids with biologic response modifiers such as α-interferon, as well as new receptor-selective retinoids, hold promise for the future.**

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Clinical research in head and neck cancer has been important in establishing the role of retinoids in oncology. Despite important advances in prevention, detection, and treatment, cancer of the head and neck is still a significant cause of morbidity and mortality, accounting yearly for 12,000 deaths in the United States and 500,000 new cancer cases worldwide (1–3). Chemoprevention efforts have resulted from an understanding of the multistep nature of head and neck epithelial carcinogenesis (4) and from the concept of field cancerization (5), which describes the diffuse and multifocal epithelial injury caused by exposure to carcinogens such as tobacco smoke, and which leads to the development of multiple premalignant foci and risk for multiple primary and second primary tumors (6). Epidemiologic and laboratory investigations have supported a role for retinoids in the prevention of head and neck carcinogenesis. The knowledge that tobacco and alcohol exposure are significant risk factors has aided in the identification of individuals at increased risk, who may be candidates for retinoid chemoprevention trials. Current research efforts are focused on determining the effects of these carcinogenic exposures at the genotypic and phenotypic level, in order to develop a biological definition of increased cancer risk in terms of biomarkers that could be used as intermediate end points for chemoprevention trials (7).

Retinoids in Head and Neck Carcinogenesis—Retinoid Biology

While the interest of developmental biologists has long been focused on the biology of retinoids, it has been much more recently that investigators concerned with the prevention and treatment of malignancy have focused on this class of agents. The biologic consequences of retinoid action on normal, premalignant, and malignant cells are now better understood, and this understanding has both guided and been the result of translational chemoprevention studies in head and neck cancer. The goal of chemoprevention is to interrupt the carcinogenic process through the use of agents able to prevent initiation or reverse the progression of malignant transformation. To achieve this goal, the agent must enhance the physiologic processes that protect the organism against the growth of such abnormal cells. There are several indications of the potential of retinoids as such agents. They

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are involved in maintenance of differentiation. Further, deficiency in vitamin A has been shown to lead to metaplasia and hyperkeratinization of epithelial tissues (8), and renders animals more susceptible to chemical carcinogenesis that has been shown to be inhibited or reversed with exogenously administered retinoids (9). Numerous *in vivo* and *in vitro* model systems have been used to examine the role of retinoids as chemopreventive agents. Animal studies by many investigators have clearly established that many squamous-cell epithelial cancers evolve through a multistep carcinogenic process that is influenced by vitamin A (10). The Syrian golden hamster cheek-pouch model of carcinogenesis is probably the animal system most comparable with the development of premalignancy and malignancy in human oral cancer. The model has been extensively used by Shklar *et al.*, in chemoprevention studies that showed efficacy of isotretinoin (13-*cis* retinoic acid, or 13cRA) in decreasing tumor formation (11). The antiproliferative activity of retinoids has been demonstrated in a broad range of transformed cell types (12), along with the differentiation-inducing activity in *in vitro* cultures of cells from established cell lines (13). A major physiological function of vitamin A is to prevent abnormal squamous differentiation of epithelial cells in nonkeratinizing tissues (14). Studies of epithelial tissues and carcinomas have shown that retinoids modulate the morphology and growth of epithelial cells and tissues (15), alter keratin gene expression (16), and suppress the expression of squamous-cell differentiation markers. In fact, in cultured keratinocytes and squamous-cell carcinomas retinoids inhibit squamous differentiation through restoration of the cellular responses to normal growth controlling mechanisms, such as suppression of oncogene expression (17), restoring their normal nonkeratinizing phenotype. Further, it was demonstrated that they exert modulatory effects on dysplastic and neoplastic cell growth differentiation and apoptosis (18, 19).

The actions of retinoids are mediated by retinoid receptors, members of the steroid/thyroid/vitamin D superfamily of intracellular receptors (20). Two classes of retinoid receptors are currently recognized, RARs and RXRs. Each class has three subtypes, α, β, and γ. It now appears that retinoic acid regulates cell growth and differentiation at the level of gene expression by means of these receptors. The different nuclear receptors have different binding affinities and tissue distributions, are encoded by distinct genes, and control common as well as unique to each receptor target genes, suggesting a distinct role for each receptor in transduction of retinoid responses (21, 22). After ligand transactivation, they modulate gene expression by binding as homo- or heterodimers to specific DNA sequences known as response elements on the promoter region of genes (including growth factors, cell regulatory kinases, oncogenes, and tumor suppressor genes). Despite our extensive knowledge of nuclear retinoid receptors, there is strong evidence that additional retinoid pathways may exist, involving receptors not yet discovered. In leukemia, growth and differentiation are mediated through the RARs and apoptosis modulation by activation of the RXRs (23), while in solid tumors the modulation of these processes through the activation of these receptors needs to be further clarified. Retinoids may also inhibit cellular proliferation by inhibiting the transcription factor AP-1 without activating the RARs (24, 25).

The loss of RAR-β expression in oral premalignant and malignant squamous-cell tissues has been suggested to be associated with the development of head and neck squamous-cell carcinomas (HNSCCs) (26–28). Our studies have demonstrated that the expression of RAR-β is suppressed in early stages of head and neck carcinogenesis, in oral premalignant lesions (OPLs) in patients without cancer, and in dysplastic tissue adjacent to head and neck carcinomas (26). RAR-β mRNA was detected in 70% of normal and hyperplastic lesions adjacent to cancer, in 56% of dysplastic lesions, and in 35% of carcinomas. Its potential role in head and neck carcinogenesis was further demonstrated by the fact that its expression was upregulated *in vivo* during a chemopreventive intervention with pharmacologic doses of 13-cRA; this upregulation was associated with clinical response. In the latter study, RAR-β was detected in only 21 (40%) of the 52 oral leukoplakia specimens, but it was expressed in all of the normal specimens, and treatment with 13-cRA restored RAR-β expression to 90% of the lesions. Levels of RAR-β mRNA increased in tissue specimens from 18 of the 22 subjects whose lesions responded to 13cRA, and in 8 of 17 subjects with nonresponding lesions (29). These findings suggested also that the retinoid-signaling pathways may be abrogated by changes in expression of specific nuclear retinoid receptors, the study of which could provide important information on their role in cancer development and possible exploitation of specific defects through the use of gene transfer techniques.

The relationship between p53 alteration, one of the most common genetic changes in the pathway to head and neck cancer development, and retinoid responsiveness was also examined by our group in the OPL chemoprevention setting. Accumulation of p53 was found to increase in direct association with histologic grade. In addition, a significant correlation between the level of p53 accumulation and retinoid resistance was seen (30); p53 proteins levels were significantly higher in retinoid-resistant lesions than in retinoid-responsive lesions. Furthermore, a lack of modulation of accumulated p53 protein levels by retinoid intervention was observed, suggesting that different types of intervention might be useful in this setting.

Biology of Head and Neck Carcinogenesis and Chemoprevention

The development of clinical head and neck chemoprevention has been aided by increased understanding of the multistep nature of carcinogenesis, which implies that invasive cancer is the ultimate product of a sequence of critical events, many of which could theoretically be prevented.

Furthermore, field cancerization, which describes the diffuse abnormal epithelium observed among patients with squamous-cell carcinomas of the head and neck, has pointed out that the occurrence of genetic or phenotypic changes in one particular area of the field translates into increased risk for cancer development in the field as a whole, and can serve to identify individuals at risk and to gauge the efficacy of the chemopreventive intervention. Multistep carcinogenesis has only been observed histologically in the past but is currently under study at the molecular level. Molecular studies have revealed specific genetic alterations contributing to head and neck cancer development, but the timing of these changes is still unclear. In short-term cultures, direct visualization of interphase chromosomes, and *in situ* hybridization, critical early genetic events were detected in the upper aerodigestive tract in the form of changes in copy number of chromosomes, which increased with the development from normal epithelium to tumor, suggesting diffuse epithelial insult and genomic instability (31, 32). A high degree of genomic instability in OPLs is associated with a high frequency of subsequent onset of aerodigestive tract tumors (31), suggesting that generalized genetic instability might be a useful marker of the tumorigenic process. As previously mentioned, alterations in the p53 gene are among the most frequently observed in head and neck cancers and premalignant lesions. p53 is frequently inactivated in head and neck carcinomas, and slightly less frequently in dysplasia (33). Immunohistochemical studies showed abnormal expression of p53 in 25% of cases in normal epithelium adjacent to tumors and in 45% of cases of dysplasia, with an accompanying spatial reorganization of abnormal p53 expression from the basal into the parabasal and superficial epithelial layers in hyperplasia and dysplasia (34). The finding that a higher level of polysomy correlates with p53 positivity supports the hypothesis that loss of p53 function might lead to the outgrowth of clones of cells that are resistant to damage-induced apoptosis and that continue to accumulate genomic damage. This provides a possible explanation for our recent finding that p53 abnormalities in an initial head and neck tumor predict for increased incidence of second primary tumors as well as recurrences of the primary tumor (35).

Allelotyping of head and neck cancers has identified regions of loss of heterozygosity at 3p, 5q, 9p, 11q, 13q, and 17p, which involve in most of the cases known, or candidate tumor suppressor genes. In particular, chromosome 9p and 3p losses have been frequently observed in oral premalignant lesions and found to correlate with subsequent development of invasive cancer (36, 37). Among the genes implicated in early carcinogenic steps of head and neck cancer is *p16/MTS1/CDKN2* on chromosome 9p21–22, which encodes a cell-cycle regulator protein, p16, which controls cell proliferation by binding to cyclin dependent kinase 4 (cdk-4), inhibiting the interaction of the cyclin D-cdk4 complex with the retinoblastoma protein (pRb) (38). Evidence implicating this tumor suppressor gene in the initiation or early progression of head and neck cancer comes from studies that revealed frequent allelic losses at the 9p21–22 locus in both head and neck cancers and premalignant lesions (36, 37) and direct studies of its expression in oral premalignant lesions (39). Cyclin D1(CCND1) on chromosome 11q13 is a protooncogene that is amplified and/or overexpressed in 30%–50% of head and neck cancers (40). It encodes a protein, cyclin D1, which promotes progression through a cell-cycle checkpoint in advanced G_1 by binding and activating cdk-4 and cdk-6 kinases and phosphorylating pRb, a process that is negatively regulated by the cdk-4/6 inhibitor p16. Early cyclin D1 protein overexpression accompanied by gene amplification has been observed in the multistep carcinogenesis process (41). Accumulation of genetic alterations during multistep tumorigenesis causes sequential phenotypic abnormalities, which may reflect the extent of the carcinogenic progression. Among the most frequently studied are PCNA expression (as a marker of proliferative dysregulation) and amplification and/or overexpression of the epidermal growth factor receptor (EGFR), found to occur early in the tumorigenesis process and to be increasingly apparent as premalignant lesions progress histologically toward cancer (42, 43). Telomerase activity has also been detected in the majority of head and neck cancers studied by our group and, interestingly, in all of the premalignant lesions studied (44), suggesting that telomere stabilization may play a role in tumorigenesis. Telomerase activity might also serve as a marker for cancer risk assessment.

In practical terms, it is conceivable that a panel of specific markers, including chromosomal deletions (9p, 3p, 17p), p53 mutations, cyclin D1 gene amplification and protein overexpression, EGFR expression, proliferation markers, the status of nuclear retinoid acid receptor expression, and other markers not yet identified could be used in the selection of candidates for future chemoprevention trials. In moving from the results of animal and epidemiologic studies to clinical chemoprevention studies, the balance between efficacy and toxicity becomes vitally important. The necessity of treating patients who would not develop cancer if left untreated emphasizes the need of chemopreventive regimens that are safe and well-tolerated. One must also take into account that animal experiments suggest the need for long-term intervention in order for the chemopreventive effect to persist. Thus, the choice of population in which to use chemoprevention is critical and could be aided by the use of biomarkers as risk factors. Changes in marker modulation could be used as intermediate surrogate end points for trials that would be of shorter duration and lower cost.

Oral Premalignancy

Great interest and enthusiasm has been generated in retinoid chemoprevention by the initial positive clinical studies in oral leukoplakia, which represents the most reliable clinical model to directly test the short-term efficacy of a chemopreventive agent. Although a wide range of retinoids have now been synthesized and could be considered

for clinical trials, drug availability has been an important aspect in completed trials. Vitamin A, 13cRA, tretinoin, and fenretinamide (4-HPR) have been the most widely studied of these agents in clinical trials. Nevertheless, it is unquestionable that with the availability of new agents with improved specificity and therapeutic index, the choice of retinoids for head and neck chemoprevention will change in an effort to improve efficacy and minimize toxicity. Chemoprevention studies in the head and neck have often focused on oral leukoplakia, a premalignant lesion that is clearly associated with exposure to carcinogens and that has malignant potential. During the follow-up of 257 leukoplakia patients by Silverman *et al.*, 17.5% developed invasive squamous-cell carcinoma of the oral cavity at a mean of 7.2 years, while 36% of the individuals harboring dysplasia in their lesions developed oral cancers (45). In addition to their clear-cut malignant potential, these lesions are easily accessible and measurable. The pioneering clinical trials conducted at the M. D. Anderson Cancer Center are of fundamental importance mainly because of the strict methodology, which included randomized, double-blinded settings and detailed pathologic assessment of pre- and postintervention specimens. The first study demonstrated that treatment with 13cRA (1–2 mg/kg daily for 3 months) resulted in major regression of leukoplakia in 67% of patients (versus 10% for placebo) (46). The two issues that emerged from this trial were the high relapse rate occurring soon after treatment cessation and the substantial, dose-related toxic effects. The second study was designed to overcome these two problems. It included induction treatment with high-dose 13cRA (1.5 mg/kg/day) for a 3-month period followed by randomization to 9 months of maintenance treatment with either low-dose 13cRA (0.5 mg/kg/day) or β-carotene (30 mg/day). This study showed that low-dose isotretinoin was significantly more effective than β-carotene in maintaining clinical/histologic remission (relapse rate of 6% vs 58%), thus demonstrating that low-dose isotretinoin was an effective and well-tolerated maintenance therapy for oral premalignancy (47). This study established that induction with 13cRA followed by low-dose maintenance is effective, has acceptable toxicity, and is useful in preventing the recurrence associated with treatment cessation. The necessity of the high-dose induction, the possible integration of this approach in standard management of oral premalignancy, and the long-term reduction of cancer incidence are issues to be resolved. With a median follow-up time of 66 months, the recent update of the study revealed similar rates of malignant transformation (23% in the 13cRA arm versus 27% in the β-carotene arm). These findings further underscore the necessity for long-term intervention to suppress premalignant lesions (48). In addition, the long-term follow-up confirmed the value of oral leukoplakia as an intermediate marker toward the progression of cancer, since clinical reversal of leukoplakia correlated well with a subsequent reduced development of invasive cancer compared with retinoid-resistant lesions. The findings of this study were the basis of our current long-term randomized trial in oral premalignancy comparing low-dose 13cRA (0.5 mg/kg/day for 1 year followed by 0.25 mg/kg/day for Years 2 and 3) with retinyl palmitate for a total of 3 years. Both the maintenance study and the current long-term trial in oral premalignant lesions incorporate several laboratory correlates that may serve as validated biomarkers in the future.

Confirmation of retinoid activity in oral premalignancy has come from several other randomized studies. A trial in Italy randomized patients with prior leukoplakia that had been surgically resected by laser to treatment with 4-HPR (200 mg/day for 1 year) or placebo with the aim of preventing the development of new leukoplakia lesions, recurrence of excised leukoplakia, and development of frank oral cancer (49, 50). The preliminary results showed a significant reduction in the incidence of new leukoplakia lesions (49), confirmed by recently updated reports. During the year of intervention, 14 patients had recurrences (9 in the control group, and 5 in the 4-HPR group), and 10 showed development of new leukoplakia lesions (9 in the control, and 1 in the 4-HPR group). The overall risk of recurrence and new lesions in the patients who completed the intervention was 6% in the 4-HPR group and 30% in the control group ($P = 0.009$). The toxicity was minimal, prompting further study of this promising agent. Two other randomized trials support the efficacy of retinoids in this setting. Stich *et al.* (51) conducted a placebo-controlled 6-month study of natural vitamin A, which found that vitamin A has activity in causing remission (56% complete response rate) and reducing the progression rate of oral leukoplakia lesions in Asian betel nut chewers. A 4-month placebo-controlled study by Han *et al.* (52) also demonstrated significant activity of 4-HPR in the reversal of oral premalignant lesions.

Advanced premalignant lesions of the oral cavity and larynx (mild to severe dysplasia or carcinoma *in situ*) are the target of an ongoing pilot chemoprevention study being conducted by Hong *et al.* at M. D. Anderson. These lesions have a distinctly unfavorable prognosis, since they are almost inevitably destined to progress into invasive cancer, and since single-agent retinoids are very unlikely to cause reversal. The trial is designed to assess the efficacy of 13cRA in combination with α-tocopherol as a modulator of retinoid toxicity and also α-interferon. There is evidence for the efficacy of combined retinoids and interferons from *in vitro* and in clinical trials (53–55). Further support for this combination is provided by the fact that these agents are individually active against human papillomavirus (HPV)–transformed cells *in vitro* and against HPV-induced diseases such as recurrent respiratory papillomatosis (56), suggesting possible efficacy of the combination for advanced premalignant lesions of the upper aerodigestive tract. Preliminary results are very encouraging.

Secondary Prevention

In patients cured of an initial head and neck cancer, the lifetime incidence of second primary tumors is in excess of

20% (with a constant rate of 4%–6% per year) (6). Second primary tumors (SPT) are a major cause of death following surgical cure of head and neck cancer, and the leading cause of death in early-stage disease, supplanting even recurrence (57–59). Patients cured of an initial head and neck cancer are therefore likely candidates for chemoprevention, and compared with individuals who have never developed cancer they are more highly motivated to accept the burden of a chemopreventive intervention as an extension of long-term follow-up for their index tumor. A pioneering study in secondary prevention conducted by Hong *et al.* was carried out in 103 patients with a prior history of squamous-cell carcinoma of the head and neck, cured with surgery and/or radiotherapy. The patients were randomized to receive either 13cRA (50–100 mg/m^2/day) or placebo for 12 months (60). After a median follow-up of 32 months, the first analysis showed that retinoid treatment significantly reduced the incidence of second primary tumors: SPT developed in only 2 patients (4%) in the retinoid arm versus 12 patients (24%) in the placebo arm ($P = 0.005$). An updated analysis with 54-month follow-up confirmed a reduction in SPT in the retinoid arm (14% vs 31% in the placebo arm), but the difference was less impressive ($P = 0.04$) (61). Nevertheless, the chemopreventive effect of the retinoid on tobacco-related SPTs (aerodigestive tract) persisted at the same level of significance (7% vs 33% placebo, $P = 0.008$), and the time to SPT development was significantly longer in the retinoid arm. It is noteworthy that most SPTs occurred in the head and neck, esophagus, or lung (i.e., the carcinogen-exposed field). Treatment-related toxicity was significant, and 33% of the patients in the retinoid arm could not complete the 12-month intervention. No survival prolongation was achieved in the retinoid arm, reflecting the early detection and effective surgical salvage of patients who developed SPT (which occurred more often in the placebo group), the lack of impact on recurrence, and the high drop-out rate in the retinoid arm due to toxicity. A concurrent study conducted by a French cooperative group enrolled 323 patients with squamous cancer of the oral cavity and oropharynx (T1-2N0-1) that had been treated with curative intent. The patients were randomized to receive etretinate, a synthetic retinoid (50 mg/day for 1 month, then 25 mg/day for 24 months) or placebo (62). This study did confirm the high incidence of SPT, as well as the location of the majority of SPTs within the carcinogen-exposed field of the aerodigestive tract. SPT developed in 24% of the placebo group and 38% of the etretinate group at 41 months of follow-up, and in 80% of cases, they were located in the head and neck, lungs, or esophagus. A summary of the above-mentioned trials is shown in Table I.

In an attempt to confirm the positive results of the first M. D. Anderson study with 13cRA, two large-scale trials are underway. One is being conducted by the Radiation Therapy Oncology Group (RTOG) in cooperation with the Community Clinical Oncology Program (CCOP–M. D. Anderson). This trial differs from the earlier trial in that a lower dose of 13cRA is used, only patients with early-stage (T1,2N0) disease are included, and the period of intervention is 3 years plus 4 years of follow-up. The other study, EUROSCAN (63), is ongoing in Europe on patients treated for T1-3,N0-1 cancer of the larynx, T1-2,N0-1 cancer of the oral cavity, and pT1-2,N0-1 and T3N0 cancer of the lung. The study was designed around the favorable findings of Pastorino *et al.* (64), in which retinyl palmitate treatment for 12 months was compared with observation of patients after resection of stage I non–small-cell lung cancer. A statistically significant difference in time to development of SPT within the carcinogen-exposed field ($P = 0.045$) was observed in favor of the retinoid-treated group. The goal of the EUROSCAN study is the prevention of SPT in patients treated for upper–aerodigestive tract cancer. Four treatment arms were planned in a 2 × 2 factorial design to increase efficiency and power: (ii) retinyl palmitate and *N*-acetylcysteine (NAC); (ii) retinyl palmitate; (iii) NAC; (iv) no treatment. Randomization takes place after surgery or completion of radiotherapy, and the intervention lasts 2 years. Pastorino *et al.* (65) recently reported some of the preliminary results for the first 2244 randomized patients. At 16 months of median follow-up, there were 292 cases of primary tumor recurrence and 67 cases of SPT. Another chemoprevention study using 13cRA has been initiated through the Northern California Oncology Group (NCOG) in conjunction with the Eastern Cooperative Oncology Group (ECOG). This study involves patients with stage I or II head and neck cancer treated with 13cRA (0.15 mg/kg/day) or placebo for 2 years. The chemopreventive interven-

Table I. Chemoprevention Trials in Head and Neck Using Retinoids as Preventive Agents

Study	Design	Patients (*n*)	Agent (dose)	Outcome
Oral premalignancy				
Hong *et al.* (1986)(46)	Induction	44	Isotretinoin (2 mg/kg/day)	Positive
Stich *et al.* (1988)(51)	Induction	70	Vitamin A (200,000 IU/week)	Positive
Han *et al.* (1990)(52)	Induction	61	Retinamide (40 mg/day)	Positive
Lippman *et al.* (1993)(47)	Maintenance	70	Isotretinoin (0.5 mg/kg/day)	Positive
Costa *et al.* (1994)(49)	Maintenance	153	Fenretinide (200 mg/day)	Positive
Prior cancer				
Hong *et al.* (1990)(60)	Adjuvant	103	Isotretinoin (50–100 mg/m^2/day)	Positive
Bolla *et al.* (1994)(62)	Adjuvant	316	Etretinate (25–50 mg/day)	Negative

tion is started as adjuvant primary therapy shortly after the completion of surgery or radiotherapy. Finally, a large randomized trial in Australia is studying the efficacy of low-dose versus high-dose 13cRA versus placebo in this setting.

New Retinoids—Combinations

The best-studied retinoid agent to date is 13cRA, with established clinical activity against oral leukoplakia and in the prevention of SPT in head and neck cancer patients (46, 60). Two other classes of retinoids now used in clinical trials are the retinamides (*N*-[4-hydroxyphenyl]retinamide [4-HPR] or fenretinide) and the retinoidal benzoic acid derivatives (TTNPB). The fact that 13cRA has produced adverse effects associated with hypervitaminosis A (46, 60), at least in the high doses used in prior trials, has limited its use, particularly considering the need for long-term intervention in the chemoprevention setting. The search for new retinoids with fewer toxic effects and improved selectivity for retinoid receptors that could possibly improve their therapeutic index is ongoing, with several new agents currently in preclinical or clinical studies. Novel synthetic retinoids with retinoid receptor subtype sensitivities may regulate distinct gene pathways and thus elicit more specific physiological effects than less selective retinoid compounds. For example, the naturally occurring ligand of the RXRs, 9-*cis* retinoic acid (9cRA), is a stereoisomer of all-*trans* retinoic acid that binds and transactivates both RARs and RXRs (66). Phase I trials of 9cRA were recently completed and revealed a favorable toxicity profile (67, 68). The agent was shown to enhance the antitumor efficacy of cisplatin in a head and neck squamous-cell carcinoma tumor model (69). New retinoids that selectively activate RAR subtypes have been synthesized and examined in preclinical anticancer models. A bicyclic retinoid derivative with RXR agonist specificity, LGD 1069 is currently undergoing study in phase I and II clinical trials. It inhibits the growth of tumor cell lines of both hematopoietic and squamous epithelial origin and has the ability to induce apoptosis. Its selectivity for RXRs *in vitro* suggests unique biological activity *in vivo*, therapeutic specificity, and possibly reduced toxicity. Another novel retinoid is ALRT1550, which selectively activates RARs and has been shown to have potent antitumor activity against human oral squamous carcinoma xenografts of the UMSCC-22B cell line in nude mice (70). This activity was mediated through growth inhibition and not through suppression of differentiation. In this model, treatment with ALRT1550 did not cause upregulation of RAR-β, suggesting that RAR-β did not mediate the antiproliferative effects.

Conclusions

The knowledge that cancer is the overall result of disturbances in the normal processes of cellular growth and differentiation that are homeostatically regulated by retinoids continues to guide the research and clinical applications of retinoid chemoprevention. The significant findings of completed retinoid chemoprevention trials in head and neck cancer are the basis for future research efforts in the area of chemoprevention. Ongoing trials that incorporate an in-depth analysis of the role of genetic and phenotypic changes in the process of tumorigenesis may lead to further insight into the mechanisms of restoration of premalignant cells to patterns of normal growth and differentiation. It is hoped that these trials will strengthen our understanding of the role of retinoids as regulators of the carcinogenesis process, and lead to their incorporation in the standard management of head and neck cancer.

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