Roles of Retinoids and Their Nuclear Receptors in the Development and Prevention of Upper Aerodigestive Tract Cancers

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Vitamin A analogs (retinoids) suppress oral and lung carcinogenesis in animal models and prevent the development of second primary tumors in head, neck, and lung cancer patients. These effects result from changes in the expression of genes that regulate cell growth and differentiation. Retinoic acid receptors (RARs; \(\alpha\), \(\beta\), and \(\gamma\)) and retinoid X receptors (RXRs; \(\alpha\), \(\beta\), and \(\gamma\)) are retinoid-activated transcription factors, which mediate effects of retinoids on gene expression. Therefore, alterations in receptor expression or function could interfere with the retinoid signaling pathway and thereby enhance cancer development. We found that the expression of RAR\(\beta\) was suppressed in more than 50% of oral and lung premalignant lesions in individuals without cancer and in dysplastic lesions adjacent to cancer in patients with oral and lung carcinomas. The expression of the other receptors was not different among normal, dysplastic, and malignant oral tissues. However, the expression of RXR\(\gamma\) and RAR\(\beta\) was somewhat decreased in lung cancers. These results show that RAR\(\beta\) expression is lost at early stages of carcinogenesis in the aerodigestive tract and support the hypothesis that the loss of RAR\(\beta\) expression may facilitate the development of some of these cancers. — Environ Health Perspect 105(Suppl 4):985–988 (1997)

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Need for Chemoprevention for Aerodigestive Tract Cancers

Lung cancer in the United States leads other cancers in both incidence (17% in men, 12% in women) and mortality (34% in men, 22% in women). Head and neck (HN) cancers account for about 3.2% of new cancer cases and 2.2% of cancer deaths (1). Unfortunately, the overall survival rate among HN cancer patients has remained unchanged for the last 3 decades (approximately 45%) despite adjuvant chemotherapy (2). The situation with lung cancer is more grim because of the severe morbidity and mortality and the discouraging 15% overall 5-year survival rate, which has not been improved despite advances in treatment modalities (3,4). Smoking tobacco is a major risk factor for developing HN and lung cancer (2–4) and even ex-smokers continue to be at a higher risk for more than 10 years (5). The exposure to carcinogenic substances and promoters in tobacco smoke leads to genetic changes over large areas of the oral cavity and the airway epithelium, resulting in a “field cancerization” with potential multifocal unsynchronized premalignant and primary malignant lesions (6–7). This may explain the high recurrence rate and the development of second primary tumors following resection of early stage (I or II) HN and lung cancers (2,8). Thus novel approaches to control cancers of the aerodigestive tract should include treatment of surrounding “condemned” airway epithelium. Because these cancers develop over a prolonged period of exposure to carcinogens and promoters and because of the multistep nature of carcinogenesis, there is an opportunity to intervene in the process using chemical agents for prevention, termed chemoprevention (9). Indeed, chemoprevention with retinoids (vitamin A analogs) is now being examined for its potential to reduce the incidence of and mortality from HN and lung cancers (8,10–13).

Retinoids in Head, Neck, and Lung Cancer Prevention

Epidemiological studies have shown that vitamin A deficiency is associated with increased incidence of lung cancer (10). Vitamin A deficiency also induces squamous metaplasia in the mucosa of the upper aerodigestive tract (14) that is similar to premalignant changes in the mucosa in heavy smokers (15). Vitamin A supplementation reversed squamous metaplasia in the trachea from vitamin A-deficient animals in vivo (16) and various retinoids exhibited a similar activity in vitro (9). Furthermore, retinoids inhibited carcinogenesis in experimental animal models (17) including a model for oral cancer (18), suppressed premalignant oral lesions (e.g., leukoplakia) (8,11,13), and also prevented the development of second primary tumors in HN cancer patients (8,12) and in lung cancer patients (19). The association between vitamin A status and cancer development in the upper airways and the ability of retinoids to suppress premalignant lesions suggest that physiological levels of retinoids are required for a natural antitumorogenesis surveillance mechanism and that retinoid-dependent signaling pathways play a role in suppression of carcinogenesis. This hypothesis implies that abnormalities in retinoid levels or in their signal transduction pathway could enhance carcinogenesis and that pharmacological levels of retinoids might activate the signaling mechanisms even in normal cells.

Mechanisms of Retinoid Action

We have shown that retinoids suppress the proliferation of head and neck squamous cell carcinoma (HNSCC) cells in monolayer cultures, inhibit the formation of squamous cell carcinoma colonies in semisolid agarose or Matrigel (Collaborative Biomedical Products, Bedford, MA) and decrease the growth of HNSCC multicellular spheroids.
(20–27). In addition, retinoids suppress the expression of squamous differentiation markers (e.g., involucrin, type I transglutaminase, keratin K1, cholesterol sulfate, and others) and inhibit cornified envelope formation in HNSCCs (22,24,26,28).

It is thought that the anticanonogenesis and antitumor effects of retinoids are the result of retinoid-induced changes in cell growth and differentiation resulting from changes in the expression of specific genes such as oncogenes, growth factors, and growth factor receptors (29). Retinoids exert most of their effects on gene expression by activating a signal transduction pathway in which nuclear retinoid receptors play a pivotal role (30–34). These receptors are members of the steroid hormone receptor superfamily. Two types of receptors have been identified: retinoic acid (RA) receptors (RARs) and retinoid X receptors (RXRs). Each type of receptor includes three subtypes—α, β, and γ—with distinct amino- and carboxy-terminal domains. The RARs bind all-trans-RA (ATRA) and 9-cis-RA (9cRA), a natural RA isomer, whereas the RXRs bind only 9cRA (30–34). RARs form heterodimers with RXRs, bind to specific DNA sequences—RA response elements (RAREs)—characterized by direct repeats of (A/G)GGTCA separated by two or five nucleotides, and act as ligand-dependent transcriptional regulators for RA-responsive genes (30,32). Because each subtype exhibits distinct patterns of expression during embryonal development and different distributions in adult tissues, each is thought to regulate the expression of a distinct set of genes as well as a common set of genes (30–34). We found that normal buccal mucosa specimens (35) and HNSCC cell lines express RARα, RARβ, RARγ, RXRa, and RXRβ (26) and that the RXRβ levels were lower in more squamous differentiated cells (26).

Several isoforms of RXRβ mRNA that are transcribed from two different promoters have been identified and RXRβ2 was found to be the major isoform in RA-treated cells (36). This receptor is expressed constitutively in a limited variety of cells; however, its expression is increased in response to RA in many cell types (37). This ligand-dependent induction is due to the presence of an RARE (RXRβ/RARβ2, GGTCTAACCAGGTCTCA) that binds RXR–RAR heterodimers preferentially in the RXRβ gene promoter and mediates ligand-dependent transcriptional activation of the RXRβ2 gene (30–34,38).

Aberrations in Retinoid Receptor Expression

Nuclear retinoid receptors are the proximate mediators of many of retinoids' effects on gene expression. Therefore, it is plausible to assume that changes in their expression and function may cause aberrations in the response of cells to retinoids and thereby alter the regulation of cell growth, differentiation, and expression of the transformed phenotype. This assumption makes investigations of the expression patterns of retinoid receptors in normal, premalignant, and malignant tissues very timely in that they may provide important clues to the roles of these receptors in cancer development and the response of these tissues to retinoid treatment.

In Vivo

Some indications that abnormalities in receptor expression exist have come from studies of lung cancer cell lines. It was found that whereas most human lung carcinomas cell lines express RARα, RARγ, and RXRα constitutively, the expression of RXRβ is suppressed in many of the cell lines (39–42). Because the normal counterparts of these cancer cells in the lung tracheobronchial epithelium and in the nonkeratinizing oral cavity mucosa express RXRβ mRNA, it was concluded that the selective suppression of the expression of RXRβ may be related to the process of malignant transformation in epithelial cells (39–42). Support for this contention was provided by the report that lung carcinoma cells expressing a transfected RXRβ exhibited decreased tumorigenicity in nude mice (43). The abnormalities in RXRβ expression suggested that this receptor may be involved in the pathogenesis of lung cancer (39,40). Similarly, RXRβ expression decreased in several human HNSCC cell lines (44).

The mechanism underlying the lack of expression of the RXRβ gene is not known. There appear to be no homozygous deletions or rearrangements of the gene as indicated by southern blotting of DNA from HNSCC cell lines that do not express the RXRβ mRNA (44), and studies with HeLa cells suggest that the loss of RXRβ expression was not caused by mutations in the RARE or other proximal regulatory elements of the RXRβ promoter (45). Another possibility is aberrant transcription of the RXRβ gene as reported for lung cancer cells by others (46) and for HNSCC cells by our group (38). These studies have demonstrated that reporter gene constructs containing 5′-flanking sequences of the native RXRβ gene promoter fused upstream of the luciferase coding region could not be activated effectively in some HNSCC or lung cancer cells by either ATRA or 9cRA treatment.

In Vivo

The RXRβ gene is located on chromosome 3p24 close to a region that is often deleted in HN and lung cancer. However, rearrangements or deletions of the RXRβ gene were not detected in several surgical specimens of lung cancer (39). Thus the molecular mechanism underlying the relationship between aberrant expression of RXRβ and the development of lung cancer is still unknown. We studied specimens from human HN and lung premalignant and malignant tissues by a nonradioactive in situ hybridization method that we adapted for the analysis of receptor expression in formalin-fixed, paraffin-embedded surgical specimens (47). To determine whether the expression of the receptors’ mRNAs is related to the development of HNSCC, we used digoxigenin-labeled antisense riboprobes of RARα, RARβ, RXRα, and RXRβ for in situ hybridization to histological sections of specimens from seven normal volunteers and 31 HNSCC patients. All 31 tissue specimens from HNSCC patients contained carcinomas; 16 also contained dysplastic lesions, 22 also contained hyperplastic lesions, 17 also contained adjacent normal tissue, and 6 contained all four types of tissue. All specimens from normal volunteers expressed the five receptors. Similar levels of RARY, RXRα, and RXRβ mRNAs were detected in most of the adjacent normal, hyperplastic, dysplastic, and malignant tissues. RARα mRNA was detected in 94% of adjacent normal and hyperplastic tissues and in 87% of dysplasias and 77% of HNSCCs. In contrast, RXRβ mRNA was detected in about 70% of adjacent normal and hyperplastic lesions, and its expression decreased further to 56% of dysplastic lesions and 35% of HNSCCs. The difference in RXRβ level in carcinoma and adjacent normal tissues was significant (48). Similar studies with specimens from normal lung (n = 17) and non-small cell lung cancers (NSCLC) (35 adenocarcinoma- and 37 squamous cell carcinomas) revealed that 89% of normal specimens expressed RXRβ. In contrast, only about 50% of dysplastic tissues and NSCLC expressed this receptor’s mRNA (49). These results indicate that the decreased expression of RXRβ occurs in
dysplastic lesions and may be associated with HN and lung cancer development.

To determine whether the decrease in RARβ can be detected in premalignant lesions from patients without cancer, we examined the expression of nuclear retinoid receptors in specimens from normal oral mucosa (n = 7) and in specimens from oral premalignant lesions before (n = 52) and after (n = 39) a 3-month treatment with 13-cis-retinoic acid (13cRA). All normal specimens expressed RARβ. In contrast, RARβ mRNA was detected in only 21 of 52 oral premalignant lesions. The suppression of RARβ expression in the premalignant lesions was significant (p = 0.003). Thirty-five of the 39 specimens examined after treatment expressed RARβ. The increase in expression was significant (p < 0.00001). All normal and premalignant specimens expressed similar levels of RARα and RARγ and RXRs-α, -β, and -γ. Eighteen of 22 cases (82%) in which RARβ level increased responded clinically to 13cRA (35). These results show that a selective loss of RARβ mRNA expression occurs at early stages of oral carcinogenesis and demonstrate that RARβ can be upregulated by 13cRA in humans. Furthermore, the association between the increase in RARβ expression and clinical response suggests that RARβ has a role in mediating retinoid response. RARβ expression may depend on the cellular level of retinoids because this receptor's gene contains an RARE in the 5' region and is inducible by retinoids (37). This conclusion is supported by the finding that RARβ expression is selectively reduced in several organs during vitamin A deficiency and enhanced by retinoic acid, as demonstrated in studies with rats (50, 51). Although it is unlikely that people in the United States are vitamin A deficient due to severe malnutrition or other causes, one cannot exclude the possibility that some premalignant tissues are vitamin A deficient. Indeed, we have demonstrated recently that monoclonal antibody (MAb) against ATRA bound to 100% of normal oral tissue (n = 7) compared to only 20% of 43 premalignant oral lesions. Similarly, 7 of 7 normal specimens contained RARβ mRNA compared to only 14 of 43 premalignant oral lesions. Twenty-four specimens were available before and after a 3-month treatment with 13cRA in vivo. Anti-RA-MAb binding to these specimens increased from 10 of 24 before to 22 of 24 after treatment and the expression of RARβ mRNA increased from 7 of 24 before to 21 of 24 after treatment, respectively. There was a strong agreement between the binding of anti-RA-MAbs and the expression of RARβ (32). Thus, we propose that the binding anti-RA-MAbs reflects the level of retinoids in the tissues and that this level is related strongly to RARβ expression.

**Conclusions**

The results serve as the basis for the following hypotheses. First, RARβ plays a role in suppression of carcinogenesis; thus the downregulation of RARβ enables premalignant cells to escape the anticarcinogenic activity of physiological levels of retinoids. Second, at pharmacological doses, some retinoids can overcome the resistance of certain premalignant and malignant cells to the effects of retinoids by mechanisms that may include an increase in RARβ level. Third, RARβ may be a useful intermediate marker in HN carcinogenesis prevention studies because its expression is decreased in early stages of carcinogenesis and its expression is induced in vivo during treatment with 13cRA, and this increase in vivo is associated with response to the preventive agent.

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