Aberrant Expression of Retinoid Receptors and Lung Carcinogenesis

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Lung cancer continues to be the leading cause of cancer deaths in the United States for both women and men (1). The alarmingly low 5-year relative survival rate (<14%) for all patients, regardless of stage at diagnosis, is a grim indication of the shortcomings of the current therapeutic approaches. Therefore, new strategies for preventing and treating lung cancer are urgently needed. One promising approach is chemoprevention with natural and synthetic vitamin A analogues known as retinoids (2,3). The rationale for using retinoids for lung cancer prevention is based on their ability to regulate the growth and differentiation of aerodigestive tract epithelial cells (2) and to prevent lung carcinogenesis in animal models (4). Furthermore, clinical trials have demonstrated the efficacy of retinoids in suppressing oral high-risk noncancerous lesions and reducing second primary cancers in patients with prior head and neck or lung cancer (5).

Retinoids exert most of their effects on cell growth and differentiation by regulating gene expression. This regulation is mediated by nuclear retinoid receptors that are members of the steroid receptor gene superfamily. Retinoid receptors function as ligand-dependent transcription-enhancing factors (6). Two subtypes of retinoid receptors have been identified—retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Each of these includes three isotypes designated α, β, and γ. RAR genes have been localized to chromosomes 17q21, 3p24, and 12q13, for isotypes α, β, and γ, respectively; in contrast, RXR genes have been localized to chromosomes 9q34.3, 6p21.3, and 1q22–23, for isotypes α, β, and γ, respectively (6). Each RAR and RXR subtype can be expressed in several isoforms, which differ in their amino terminal domain (6). RXRs can form heterodimers with RXRs and recognize retinoic acid response elements that consist of direct repeats (DR) PuG(G/T)TCA(X)PuG(G/T)TCA with one or five intervening nucleotides (X) or closely degenerate motifs (6). It is interesting that RAR genes themselves contain a DR5 retinoic acid response element in their promoter regions; consequently, their expression can be regulated by all-trans-retinoic acid. RARB, appears to be tightly regulated by all-trans-retinoic acid (7). RXRs can also form homodimers and activate DR1 retinoid X response element or form heterodimers with other members of the steroid receptor family, thus providing opportunities for cross-talk among different signaling pathways (6). It is thought that each receptor isotype and isoform regulates a distinct subset of retinoid-responsive genes because deletion of individual receptors by homologous recombination resulted in loss of induction by all-trans-retinoic acid of different genes (6). Genetic studies in mice by knockout of individual or multiple receptors have demonstrated both specific and redundant functions of individual RARs (8). Transcriptional regulation by retinoid receptors requires cofactors of two types: corepressors and coactivators. The complexes formed by nuclear retinoid receptors and response element are recognized by corepressors. Ligand binding can alter the conformation of the receptors such that the corepressors dissociate and the coactivators associate with the nuclear retinoid receptors and activate the transcriptional machinery (6,9).

The complex retinoid-signaling pathway may be modulated by multiple mechanisms, including changes in the uptake and metabolism of physiologic retinoids by target cells and changes in the expression or function of specific nuclear receptors, corepressors, or coactivators. Because vitamin A deficiency has been associated with enhanced lung carcinogenesis (2,5), it is plausible to suggest that defects in retinoid signaling in a vitamin A-sufficient individual may mimic vitamin A deficiency at the cell level and facilitate malignant transformation and progression.

In this issue of the Journal, Picard et al. (10) present the first detailed analysis of the expression of RARs α, β, and γ and RXRs α and β at the protein level in normal lung tissue and in non-small-cell lung cancer (NSCLC). The main findings of this investigation are as follows: (a) All of the five receptors were detected by immunohistochemistry in control tissues, which included lung cells from patients without lung cancer and normal-appearing lung tissue either distal or adjacent to the lung tumors; (b) stromal cells exhibited a receptor expression pattern similar to that of the lung epithelial cells; and (c) the expression of several types of receptor was altered in NSCLC cells compared with control cells. Several patterns of change in receptor expression were noted: Increased expression in carcinoma relative to control cell nuclei was observed for RARα and RXRα in 26% and 85% of the cases, respectively; in contrast, the rest of the cases expressed comparable levels of these receptors in carcinoma and in controls. The level of RXRβ increased moderately in 82% and decreased in 18% of the cases in carcinomas compared with controls. RARγ was detected in all of the tumor specimens, but its levels were decreased moderately in some of the carcinomas relative to control cells. Other investigators (11) have observed increased levels of RARα that were associated with higher proliferation in breast cancer relative to normal breast epithelial cells. Increased messenger RNA (mRNA) of RXR was found in ductal intraepithelial neoplasia relative to normal ductal/lobular cells (12). These authors suggested that the increased expression may be involved causally in breast carcinogenesis. It is not known whether the increase in RARα and RXRβ proteins in lung cancer is associated with cancer development.
The changes in RARβ protein levels in the study reported by Picard et al. (10) were the most statistically significant ones and included a complete absence of staining in 6% and a marked decrease of staining in 57% of the carcinoma cells relative to control cells, with only 37% of the carcinomas expressing RARβ protein at levels comparable to those in controls. In addition, Picard et al. (10) analyzed receptor expression at the mRNA level in situ hybridization using 35S-labeled antisense probes and by reverse transcription–polymerase chain reaction (RT–PCR) and found that, in general, there was a concordance between the findings at the mRNA level and the protein level. For example, in 7% of the cases the carcinoma cells showed no detectable RARβ mRNA, in 54% of the cases the tumors expressed lower levels of RARβ mRNA than control cells, and in 39% of the cases the tumors expressed amounts of mRNA similar to those of the controls. The RT–PCR data showed that RARβ levels decreased by 50% or more in 59% of the cases, despite the presence of positive stromal cells within the tumor samples from which the RNA was extracted for analysis. The changes in RARβ level showed no association with histologic type of the NSCLC (adenocarcinoma or squamous cell carcinoma), with disease stage, or with smoking status.

Picard et al. (10) also analyzed loss of heterozygosity (LOH) at chromosome 3p24 because RARβ is located at this site and LOH at 3p has been reported to occur with a high frequency in lung cancers. They found that 41% of the informative cases showed LOH and that the majority of carcinomas (75%–86%) with LOH also showed a decrease in RARβ mRNA and protein.

RARβ mRNA levels were suppressed in many NSCLC cell lines, and it has been suggested that retinoid resistance may be due to the suppression of RARβ expression (13–17). Indeed, expression of a transfected RARβ gene in NSCLC cells resulted in enhanced growth inhibition in vitro (16). It was also suggested that the loss of RARβ expression may be associated with lung cancer development (13). This idea was further supported by the finding that transfection of the RARβ gene into NSCLC cells suppressed their tumorigenicity in nude mice (18) and by the finding that transgenic mice expressing antisense RARβ2 RNA develop lung cancer (19).

To determine whether retinoid receptors are modulated in vivo, our group adapted a nonradioactive in situ method to detect mRNA for the different RARs and RXRs in formalin-fixed, paraffin-embedded tissues of cancers of the head and neck and of the lung by hybridization with digoxigenin-labeled antisense riboprobes (20,21). We found that all six receptors were expressed in normal oral tissues; however, RARβ receptors was not altered substantially in head and neck, respectively, whereas the expression of the other expressing RARβ decrease from 73% to 56% to 35% in the proportion of subjects that the loss of RARβ in many cancer specimens, Picard et al. (10) found loss in only 7% of the cases and a decreased level of RARβ mRNA or protein in nearly 60% of the cases. Their analyses appear to be more sensitive because they had used radioactive riboprobes and exposed them for 3 weeks.

The reason that we have chosen to use mRNA analysis originally is that the antibodies that we had examined, including the polyclonal anti-RARβ antibodies RPβ(F) (24) used in the study by Picard et al. (10), recognized several protein bands besides RARβ by western blotting of some head and neck cancer or lung cancer cells (Lotan R: unpublished observations). Likewise, Rochaix et al. (25), who had used apparently the same RPβ(F) antibodies, found them to bind nonspecifically to several proteins besides the RARβ in extracts of thyroid tissue and, therefore, considered them unsuitable for immunohistochemical application (25). It is interesting that the latter group was successful in applying an immunohistochemical technique to detect RARβ by using highly diluted monoclonal antibodies in conjunction with signal amplification. They found that the level of the RARβ protein was decreased in papillary thyroid carcinoma relative to normal thyroid follicular cells (25). Other investigators (26) had encountered unexpected cytoplasmic localization of antibodies against RARx in ovarian tumor specimens that had been fixed in formalin and embedded in paraffin, and these investigators limited their studies to frozen sections to minimize potential artifacts. Nonetheless, it appears that Picard et al. (10) had a better batch of anti-RARβ antibodies or that the type of tissue they used did not contain cross-reactive proteins because their western blotting analysis revealed that the antibodies used in this study, including the polyclonal anti-RARβ RPβ(F) (24), detected in lung tissue extracts a single protein band corresponding in size to the appropriate receptor; no non-specific bands were detected. Because RARβ is being evaluated as an intermediate biomarker in chemoprevention trials and immunohistochemistry is easier to perform than in situ hybridization, the former may be the method of choice once it becomes reproducible in different laboratories.

In conclusion, the novelty of the report by Picard et al. (10) is in the demonstration of receptor modulation in lung cancer at the protein level and the identification of LOH at 3p24 as a possible mechanism for the decrease in RARβ levels. In general, these findings support a considerable body of data obtained previously by analysis of lung cancer cell lines (13–17) and tissue specimens (20,21,25) for receptor mRNA levels using northern blotting and in situ hybridization implicating RARβ in lung carcinogenesis.

REFERENCES


