Aberrant Retinoid Signaling and Breast Cancer: the View From Outside the Nucleus

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Retinoids are synthetic and natural analogues of vitamin A (retinol). In 1925, Wolbach and Howe (1) reported that the epithelium of vitamin A-deficient rats develops squamous metaplasia resembling lesions that occur early during carcinogenesis. Notably, this squamous metaplasia reversed with vitamin A treatment. This notion that retinoids inhibit carcinogenesis was supported by extensive animal studies conducted in the 1970s that demonstrated retinoid chemopreventive effects on the epithelium of various tissues after exposure to chemical mutagens (2). However, the mechanisms involved were unknown. Several cytosolic or cellular proteins that bind retinoids with high affinity were identified (3), yet it was unclear what direct role, if any, these proteins had in mediating retinoid actions.

The discovery of the nuclear retinoid receptors in the 1980s revolutionized the retinoid field by providing a firm mechanistic basis for the cellular actions of retinoids (4,5). It was learned that the active derivative of dietary vitamin A was not retinol but isomers of retinoic acid that directly activate nuclear retinoid receptors. At this time, the first successful clinical trials were reported [reviewed in (6,7)] that studied the use of retinoids in treating premalignant diseases, reducing second malignancies, and treating overt malignancies. During the past decade, associations between nuclear retinoid receptor abnormalities and specific cancers suggest that the dietary vitamin A deficiency reported by Wolbach and Howe may be mimicked in specific tissues, perhaps precipitating early carcinogenic events. Findings indicate that at least some of these events are reversible with pharmacologic retinoid doses. This activity of retinoids is linked to their ability to promote differentiation and cell cycle arrest at G1 phase (8,9). In this issue of the Journal, a report by Kuppumbatti et al. (10) presents the first detailed analyses of the expression of cellular retinol-binding protein (CRBP) in normal breast tissue and breast carcinoma. Confirmation of the main finding, a loss of CRBP expression in a subset of breast cancers, will spur future work aimed at understanding the biologic impact of CRBP repression in breast cancer and the prevalence and importance of aberrant retinoid metabolism in other malignancies.

Nuclear retinoid receptors are members of the steroid receptor superfamily (4,5). Nuclear retinoid receptors function as ligand (hormone)-dependent transcription factors. Two subtypes of retinoid receptors exist, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). RARs are activated by all-trans-retinoic acid (RA), and 9-cis-retinoic acid activates both RARs and RXRs. Retinoid-mediated changes in gene expression occur by the binding of RAR/RXR heterodimers or RXR/RXR homodimers to defined retinoic acid-responsive elements within target genes. Three RARs (α, β, and γ) and three RXRs (α, β, and γ) exist and have cell-context-dependent expression patterns and interact with coregulators. Selective nuclear retinoid receptor agonists and antagonists designed to increase the therapeutic window of naturally occurring retinoids are under study (11). The genes coding for RARs themselves possess retinoic acid-responsive elements and are retinoid inducible. In addition to the nuclear receptors, several cellular proteins bind retinoids with high affinity and likely participate in the metabolism of retinoids, although their precise role is complex and not yet well understood (3,12). CRBP (or CRBPI) and CRBPII selectively bind retinol and retinol with high affinity. CRBPII is expressed exclusively in the small intestine and functions in packaging of dietary vitamin A into chylomicrons that are absorbed by the liver. CRBP is widely expressed in many tissues. Accumulating data suggest that CRBP has an important role in regulating RA availability (13).

The concentration of retinol, the main circulating retinoid, is tightly regulated by the liver. Retinol must be converted to RA in target tissues. The majority of cellular retinol is bound by CRBP and, in most tissues, retinol levels are much higher than RA levels. It is proposed that CRBP shuttles retinol between various retinoid-dependent enzymes (13). Many retinol-metabolizing enzymes have increased access to CRBP-bound retinol compared with free retinol. CRBP is proposed to facilitate formation of retinol esters for storage or conversion of retinol to RA via a retinol intermediate. CRBP may participate in the entry of retinol into the cell. Distinct proteins called cellular retinoic acid-binding proteins I and II bind RA selectively and appear to regulate the metabolism of RA (3). Because of the complexity of retinoid metabolism, the precise role of CRBP in retinoid signaling remains controversial. Recent analyses (14) of CRBP-null mice indicate a major role for CRBP in maintaining adequate retinol ester stores in the liver. When fed a vitamin A-restricted diet, these mice develop vitamin A-deficiency syndrome that includes squamous metaplasia, reminiscent of prolonged vitamin A deprivation in rats or in certain RAR-null mice (15).

Retinoids have activity in cancer therapy and prevention [reviewed in (6,7)]. These activities include the treatment of premalignant lesions and the reduction of second cancers of the head and neck, lung, and liver. As a single agent, RA induces complete remissions in acute promyelocytic leukemia. When
combined with interferon α2A, 13-cis-retinoic acid causes complete and partial remissions of squamous cell carcinomas of the skin and cervix. This regimen is active in the treatment of disseminated renal cancer. Recently, the role of retinoids in the management of high-risk neuroblastoma was reported (16). Clinical effects of retinoids in preventing or reversing carcinogenesis indicate that preneoplastic epithelium may have retinoid-signaling defects that are overcome by pharmacologic retinoid treatments. Thus, attention was focused on the nuclear retinoid receptors (17). The best example of a retinoid-signaling defect that is overcome by retinoid treatment is the t(15;17) chromosomal rearrangement that results in fusion of the genes PML and RARα in acute promyelocytic leukemia. The presence of this balanced chromosomal translocation is tightly linked to the RA response [reviewed in (18)]. Repressed RARβ expression occurs in oral leukoplasia, head and neck squamous cell carcinoma, and non-small-cell lung cancer (19 and references therein). Decreased expression of RARγ is found in retinoid-resistant human embryonal carcinoma cells (20). Loss of RARβ expression is reported in breast cancer (21). Notably, RARα overexpression in estrogen receptor (ER)-positive relative to ER-negative breast cancers is associated with the observed insensitivity of retinoids toward ER-negative breast carcinoma cells (22). A clinical trial has suggested that N-(4-hydroxyphenyl) retinamide reduces the risk of second breast cancers in premenopausal women but not in postmenopausal women (23). This finding indicates that a subset of breast tumors may respond to retinoid therapy and underscores the importance of defining these subsets at the molecular level.

The report by Kuppumbatti et al. (10) extends prior work from this laboratory (24) that demonstrated a loss of basal expression and retinoid-inducible expression of CRBP and RARs in breast cancer cell lines as compared with cultured normal breast epithelial cells and fibroblasts. This study using in situ hybridization (10) reveals that CRBP is expressed in all six normal mammaryplasty specimens examined. An additional nine normal mammaryplasty specimens were CRBP positive by northern or western blotting. Moreover, 33 of 35 normal breast tissues adjacent to breast carcinomas were positive for CRBP. In contrast, CRBP was not detected in 24% (12 of 49) of breast carcinomas. A statistically significant association between loss of expression of CRBP and breast carcinoma was observed, yet the small sample size and minority of cases having loss of CRBP expression (compared with 67% [eight of 12] of tumors that failed to express RARβ) and the limited clinical correlates available for CRBP stress the need for confirmatory studies. Loss of CRBP was found to be as frequent in early ductal carcinoma in situ, suggesting a role for CRBP in early breast carcinogenesis. CRBP expression was not associated with putative breast cancer prognostic markers, including age, tumor grade, ER status, or expression of RARβ. A prior reverse transcription–polymerase chain reaction study (25) concluded that CRBP was expressed in all breast cancers examined. Kuppumbatti et al. correctly point out that contamination from normal adjacent tissues may account for these discrepant results.

The study by Kuppumbatti et al. is notable because it identifies a tumor-associated abnormality in a protein linked to retinoid metabolism. A critical question is whether loss of CRBP is associated with altered retinoid levels within these breast tumors. Searching for similar CRBP expression abnormalities in other tumors where retinoids have known activity (especially lung and aerodigestive tract) will be informative. Perhaps this retinoid defect will be overcome by retinoid treatments. RA, because it is the biologically active retinoid that binds RARs, should bypass any CRBP defects in retinol uptake, storage, or conversion to RA. In contrast, irreversible loss of RARβ may permanently disable retinoid signaling. The mechanism for loss of CRBP and RARβ in breast carcinoma and RARβ in lung carcinoma is unclear. Loss of heterozygosity at chromosome 3p, where RARβ resides, is reported to occur at high frequency in lung cancer (26,27). Of interest, the CRBP gene is also located at chromosome 3p (28). An added complexity to consider is that the genes for CRBP and RARβ contain retinoic acid-responsive elements and are retinoid inducible (5). Thus, a loss of RARβ may mediate a secondary repression of CRBP or a defect in the availability of RA because a loss of CRBP may repress RARβ.

Preliminary evidence by Kuppumbatti et al., indicating that the breast tumors that did not express CRBP are contained in a larger subset of RARβ nonexpressors, points toward the former mechanism. Whether retinoid treatment restores CRBP expression in breast cancer may address this question.

In conclusion, the report by Kuppumbatti et al. (10) describes a novel association between the repressed expression of CRBP and breast carcinoma. This observation suggests that multiple defects at the level of retinoid receptors and at the level of retinoid metabolism may disrupt cellular vitamin A homeostasis. It will be important to learn whether retinoid treatment reverses these defects in breast cancer. The challenge will be to use this information to identify breast cancers that are sensitive or resistant to retinoid treatments.

REFERENCES


