Effect of retinol on radiation- and estrogen-induced neoplastic transformation of human breast epithelial cells

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Abstract. Clinical, epidemiological and experimental findings have provided evidence supporting a role of free radicals in the etiology of cancer. Free radical production is enhanced in many disease states, by carcinogen exposure, and under conditions of stress contributing widely to cancer development in humans. We have established an experimental breast cancer model to examine the effects of all-trans-retinol (retinol/vitamin A) on the production of free radicals in human breast epithelial cells induced by high linear energy transfer (LET)-irradiation in the presence of 17ß estradiol. The following cell lines were used in these studies: the MCF-10F cell line, a spontaneously immortalized human breast epithelial cell line. Alpha 5 derived from MCF-10F cells irradiated with two separated doses of 60 cGy α particles in the presence of estrogens (60E/60E). Tumor 2, from a tumor formed in nude mice after injection with the cell line Alpha 5. Tumor 3, from secondary tumor formed from injecting Tumor 2 cells into nude mice. Each of the cell types examined had significantly elevated H2O2 production levels compared to MCF-10F control cells (p<0.001). Retinol (1 µl/ml) significantly (p<0.05) decreased H2O2 production in all cell types examined. Retinol significantly decreased (p<0.05) invasive capabilities of cells across matrigel coated invasion chambers and significantly reduced (p<0.05) PCNA, Fra-1, mutant p53 and increased Rb protein expression levels in comparison to non-retinol-treated ones when assayed using immunofluorescent staining coupled with confocal microscopy. The reduced H2O2 production, decrease in cell invasive capabilities and alterations in protein expression levels suggest that retinol can be used as a chemo-preventive agent in human breast cancer.

Introduction

Breast cancer is the most common cancer in women. It is a very complex disease in which a series of multiple abnormalities occur at both the cellular and molecular level (1). There have been a number of investigations in animal models as well as epidemiological studies in humans demonstrating the chemopreventive effects of selected vitamins (2-5). However, the relationship of vitamins to cancer is very complex. These authors have studied the various effects of vitamins on cell proliferation and as an anti-tumor chemoprotective agent, among others. The free radical-scavenging vitamins, such as all-trans-retinol (retinol), selenium, vitamin C have been shown to protect against cancer development in animal models (5), and may be chemopreventive in humans (3-5).

Retinoic acid, a natural metabolite of circulating vitamin A (retinol) and an irreversible oxidation product of retinol, is essential in maintaining the normal pathway of differentiation in epithelial tissue. Retinoic acid and a number of its analogs, both natural and synthetic (retinoids), have been shown to be effective in the prevention of a variety of cancers in experimental animals, and in reversing preneoplastic lesions in humans (6).

Free radical production is ubiquitous in all respirating organisms, and is enhanced in many disease states, by carcinogen exposure, and under conditions of stress, contributing widely to cancer development. Clinical, epidemiological and experimental findings have provided evidence of a role for free radicals in the etiology of cancer (2-4). Vitamins A (retinol, retinoids), ß-carotene (provitamin A), E (α-tocopherol) and C (ascorbic acid) are used in experimental, clinical and epidemiological studies for cancer chemoprevention. It has been shown that the increase in the rate of malignant transformation by free radical-generating compounds may be related to the free radical-mediated enhancement of genetic instability; thus, ultimately increasing the progression rate of non-malignant lesions (7).

The proliferating cell nuclear antigen (PCNA) is a non-histone intranuclear protein that serves as a cofactor for polymerase delta during the DNA synthesis stage of the cell cycle (8). It is synthesized in early G1 and S phases of the cell cycle and is associated with DNA synthesis (8-11). The detection of PCNA protein expression is a convenient measure of proliferation. PCNA coupled with immunocytochemistry has
been used as an alternative to traditional methods to evaluate proliferative rate of tumors (12,13).

Members of the c-fos gene family, including c-fos, and Fra-1 encode nuclear phosphoproteins are rapidly and transiently induced by a variety of xenobiotic agents by and function as transcriptional regulators for many genes (14-16). Evidence that multiple fos family genes cooperate to induce S phase progression was provided by antibody microinjection studies which showed that the inhibition of c-fos or Fra-1 function alone only partially blocked cell cycle re-entry, while inhibiting all these genes together effectively abolished cell cycle progression (17). These results raised the possibility that several c-fos family members together play a critical role in growth factor-stimulated cell cycle reentry. Experiments determined that disruption of two c-fos family members, c-fos and B fos, uncovered the role for the fos family in cell proliferation and facilitated the identification of the cell cycle targets of fos proteins (14).

The gene p53 is a tumor suppressor gene and is involved in the regulation of the cell cycle. When p53 gene is inactivated, it leads to uncontrolled cell proliferation (18-20). In response to DNA damage, p53 acts as a cell cycle checkpoint and induces a cellular response that aims to restore genomic integrity. Thus, when it is mutated, uncontrolled proliferation occurs (18). Patterns of expression of the p53 tumor suppressor in human breast tissues and tumors in situ and in vitro have been previously studied (21). Changes in expression of gene p53, and also its mutations, cause variations of cellular protein p53 concentration. The finding that gene mutations and changes in the expression form the basis of cancer processes, has prompted molecular epidemiologists to use biomarkers for detecting damaged genes or proteins synthesized under their control in easily available cellular material or systemic liquids (22). Mutations in the suppressor gene p53 are thought to be essential for cancer development. This gene is one of the most important regulators of transcription, cellular cycle, DNA repair and apoptosis detected. Observations that mutations in gene p53 appear under conditions of occupational and environmental exposures to chemical and physical carcinogens, such as vinyl chloride, radon, or aflatoxin B1, have proved to be of enormous importance for the occupational and environmental health.

The genetic predisposition to retinoblastoma is transmitted by mutant alleles of the retinoblastoma susceptibility gene (Rb) (23,24). Mutations in Rb encode a phosphorylated protein that accumulates in the nuclei of most vertebrate cells (24). Rb is differentially phosphorylated during the cell cycle, implicating a possible role in cell cycle regulation (25).

Identification of genes involved in the prevention of breast cancer and the mechanisms by which genes participate in radiation- and estrogen-induced carcinogenesis are of critical importance. In vitro models are vital to the understanding of the biological sequence of events that drive a normal cell to cancer and how such progression can be prevented. Similarly vitamins can reverse or alter such pathways. A multi-stage in vitro experimental breast cancer model was used in these studies (26) in which MCF-10F a spontaneously immortalized human breast epithelial cell line was irradiated with high-LET α particles (150 keV/μm). The MCF-10F cells were derived from the breast tissue of a 36-year-old female (27). It has the morphological characteristics of normal breast epithelial cells that include dome formation in confluent cultures, three dimensional growth in collagen gel, dependence upon hormones and growth factors for growth in vitro, lack of anchorage independence or invasive capabilities and tumorigenicity in nude mice (28,29). MCF-10F cells were irradiated with either a single 60 cGy or a double dose of 60 cGy/60 cGy α particles in the presence of estrogens. Irradiated cells showed gradual phenotypic changes including altered morphology, an increase in cell proliferation relative to control, anchorage-independent growth and invasive capabilities but no tumorigenicity in nude mice (26). However, MCF-10F cells irradiated with two doses of α particles in the presence of estrogens (60 cGy + E/60 cGy + E) showed tumorigenicity both in the SCID and nude mice.

Studies regarding the role of vitamin A in cancer cell biology are of critical importance. It has been shown that vitamins are strong regulators of cancer cell differentiation and transformation (30). Interactions of vitamin A with oncocenes, tumor suppressor genes and genes involved in cell cycle regulations play an important role in cancer cell biology. In this study, we investigated the effect of retinol on invasive capabilities and protein expression of cell cycle regulators, oncocenes and tumor suppressor genes as well as free radicals in a radiation and estrogen in vitro breast cancer model.

Materials and methods

Cell culture. Normal and transformed cell lines from an experimental in vitro breast cancer model obtained by treatment with radiation in the presence of estrogens were used in these studies (26). The following cell lines were used from the radiation- and estrogen-induced cancer model (Table I): control MCF-10F cell line (passage 98), and MCF-10F constantly treated with 17β estradiol (10^-8 M) (Sigma-
Alldrich, Chemical Co., St. Louis, MO), named MCF-10F+E (passage 62). The MCF-10F cell line was treated with double doses of 60 cGy α particles and subsequently treated with E, (60 cGy + E/60 cGy + E and named Alpha 5). Tumor 2, a cell line derived from a tumor formed in the nude mice after injection with the cell line Alpha 5. Tumor 3 is a secondary tumor line derived by injection of Tumor 2 cells into nude mice. Table I summarizes the phenotypic characteristics of this model. The immortalized MCF-10F cell line retains all the characteristics of normal epithelium in vitro including anchorage dependence, non-invasiveness and non-tumorigenicity in nude mice (26,28,29,31). The Alpha 5 cell line (passage 79), produced tumors in 3 out of 6 nude mice injected (26). Tumor 2 (passage 44) and Tumor 3 (passage 14) (29), 1 of the 3 primary tumor cell lines derived from Tumor 2, were also anchorage-independent. Phenotypic characteristics of these cell lines and their genetic alterations including differentially expressed genes were previously described (26,32-34). All the cell lines were tested from time to time to ensure they were free from mycoplasm contamination.

Preparation of culture media. All cell lines used in this present study were cultured with Dulbecco's modified Eagle's media (DMEM/F12, 1:1), medium supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml amphotericin B, 10 µg/ml insulin (all from Life Technologies, Grand Island, NY), 5% equine serum (Biofluids Inc., Rockville, MD), 0.5% µg/ml hydrocortisone (Sigma-Alldrich, Chemical Co., St. Louis, MO) and 0.02 µg/ml epidermal growth factor (Collaborative Research, Bedford, MA) (32). In cultures where the effects of estrogen were to be assessed, 17β estradiol (E) (Sigma-Alldrich Chemical Co.) was added to the culture medium at 10-8 M, a dose which gave the maximum growth stimulatory effect as described previously (26). To determine the effect of retinol in MCF-10F cell lines on cell invasion, H2O2 production and protein expression, control and transformed cells were treated with 1 µg/ml of all-trans-retinol for 24 h (Sigma-Alldrich, Chemical Co.).

Invasion assay. The cell invasion assay was performed as described previously (12,28,31,35) using modified Boyden's chambers (Transwell; Costar, Cambridge, MA) constructed with multi-well cell cultures and cell culture inserts. Cells were plated onto Matrigel-coated 96-well tissue culture plates as described (37). Briefly, 100 µl Amplex Red reagent solution (144.4 mM NaCl, 5.7 mM Na2PO4, 4.86 mM KCl, 0.54 mM CaCl2, 1.22 mM MgSO4, 75 µM Amplex Red reagent pH 7.35) containing 0.5 U/ml HRP (Molecular Probes). Cells were plated onto Matrigel-coated 96-well tissue culture until 75% confluency was achieved. Cells were then treated with either control cell media or media supplemented with 1, 3, 7 or 10 µM concentrations of all-trans-retinol and incubated them at 37°C for 4 h. Cells were rinsed in phosphate-buffered saline (pH 7.4) and hydrogen peroxide production was measured per protocol using the Amplex Red Hydrogen Peroxide Assay (Molecular Probes, Eugene, CA). We measured the fluorescence intensity of each well using a microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) with an excitation wavelength in the range of 530-560 nm. The concentration of H2O2 based on a standard curve. One µM of retinol was the best stimulatory dose for H2O2 production when the control MCF-10F cells was tested. Therefore, that dose was used in the present study.

Determination of protein expression. Determination of protein expression was analyzed as described previously (26,32). Exponentially growing cells were plated on a glass chamber slide (Nunc, Inc., Napperville, IL) at a density of 5x103 cells per 1 ml of medium and allowed to grow for 2-3 days, until reaching 70% confluence. Cells were washed twice with PBS and fixed with 3.7% paraformaldehyde in PBS (pH 7.4) at room temperature. Cells were incubated with the corresponding primary antibodies: anti-PCNA (mouse PC10; sc56), anti-Fra-1 (rabbit R20, sc605), anti-mutant p53 (Pab240, sc90) and anti-Rb (rabbit C15, sc50) (all from Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at a dilution of 1:500, overnight at 4°C. After washing with PBS, dishes were incubated with FITC-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) at a 1:1000 dilution, for 60 min at room temperature (37°C). After several washes (5 min each) with PBS, the slides were mounted with Vectorshield mounting medium (Vector Laboratories, Burlingame, CA), and then viewed on a Zeiss Axiowert 100 TV microscope (Carl Zeiss, Thornwood, NY) using a x40 1.3 NA objective lens equipped with a laser scanning confocal attachment (LSM 410, Carl Zeiss). Fluorescent images were collected using an argon/
krypton mixed gas laser. Composite images were generated using Adobe Photoshop and printed on a Kodak DS 8650 printer (Rochester, NY). A semi-quantitative estimation based on the relative staining intensity of the PCNA, Fra-1, mutant p53 and Rb protein expression by control and tumor cell lines was determined by a computer program, which gives the area and the intensity of the staining. The numbers of immunoreactive cells (30 cells/field) were counted in 15 randomly selected microscope fields (x400) per sample. All numerical data were calculated as means and error standards. Comparison between treated groups and controls were made by Student’s t-test. A p-value of ≤0.05 between groups was considered to be significant.

Results

An established in vitro breast cancer model was used in these studies (26). The spontaneously immortalized MCF-10F breast epithelial cell line (27) was transformed with high-LET α particles (double doses of 60 cGy 150 keV/µm α particles) in the presence of 17β estradiol. Transformed cells showed gradual phenotypic changes including altered morphology, increased cell proliferation, anchorage-independent growth, invasive capabilities and tumorigenicity in nude mice. Table I lists the phenotypic alterations in MCF-10F cells and various transformed derivatives. The MCF-10F and the estrogen-treated cell line were negative for these changes. The Alpha 5,
Tumor 2 and Tumor 3 cell lines were positive for anchorage independence, invasiveness and tumorigenicity.

Retinol affected the invasive capabilities of the MCF-10F-, MCF-10F+E-, Alpha 5-, Tumor 2- and Tumor 3-retinol-treated cell lines (Fig. 1). Each of the cell types examined had significantly (p<0.001) elevated invasive capabilities compared with MCF-10F control cells. However, retinol treatment reduced such capacity in all cell types, including the MCF-10F control (p<0.05) (Fig. 1). The Alpha 5 cell line had ~5 times the invasive capabilities compared to the control MCF-10F and retinol treatment reduced such effect by ~25%.

The enzymatic determination of hydrogen peroxide was done by the Amplex Red technique, by using a probe for the enzymatic determination of H$_2$O$_2$. Here we report the effect of retinol on the production of H$_2$O$_2$ in the MCF-10F-, MCF-10F+E-, Alpha 5-, Tumor 2- and Tumor 3-retinol-treated cell lines (Fig. 2). Each of the cell types examined had significantly
(p<0.001) elevated H₂O₂ production levels compared to the MCF-10F control cells. Retinol treatment reduced H₂O₂ production in all cell types, including the MCF-10F control cells (p<0.05).

To determine whether the malignant transformation process observed in this model was altered by retinol treatment, markers of cancer progression such as PCNA, Fra-1, mutant p53 and Rb protein expression were analyzed by immunofluorescence method coupled with confocal microscopy. Fig. 3A shows the quantification of the immunofluorescent image of the PCNA protein expression in MCF-10F and other transformed and tumorigenic cell lines. All 3 tumor cell lines examined, the Alpha 5, Tumor 2 and Tumor 3 had significantly (p<0.001) higher PCNA protein expression than the control MCF-10F cell line. However, there was no significant difference in PCNA expression between these cancer cell lines. Retinol treatment reduced the effect of PCNA protein expression in Alpha 5, Tumor 2 and Tumor 3 cell lines (p<0.05) in comparison to the same untreated cell lines. There was even a decreased level of PCNA protein expression in retinol-treated
MCF-10F cells. PCNA was localized mainly in the nuclei and treatment with retinol significantly reduced the immunostaining intensity of Alpha 5 and Tumor 3 (Fig. 3B).

Quantification of the immunofluorescent images of Fra-1 protein expression in MCF-10F, the transformed Alpha 5 and the tumorigenic cell lines are shown in Fig. 4A. The Alpha 5, Tumor 2 and Tumor 3 cell lines had significantly higher Fra-1 protein expression than the control MCF-10F cell line (p<0.001). However, there was no significant difference in staining intensity between these tumor cell lines. Retinol reduced Fra-1 expression in Alpha 5, Tumor 2, and Tumor 3 cell lines (p<0.05). Similarly, MCF-10F cells treated only with estrogen without radiation (MCF-10F+E) had a higher expression of Fra-1 than the parental control. Furthermore, retinol treatment reduced the Fra-1 expression by ~30% (Fig. 6). Similar to PCNA, the intracellular distribution of Fra-1 in the various cell lines examined was localized mainly in the nuclei. Fig. 4B shows a representative immunostaining of Fra-1 in Alpha 5 and Tumor 2 cells where a reduced staining pattern was clearly demonstrated among retinol-treated cells.

Fig. 5A shows the quantification of mutant p53 protein expression in MCF-10F, and the various transformed and
tumorigenic cell lines. All these tumorigenic cell lines Alpha 5, Tumor 2 and Tumor 3 cell lines had significantly (p<0.001) higher mutant p53 protein expression than the control MCF-10F cell line. However, there was no significant difference in protein expression between these various tumor cell lines. Mutant p53 protein expression was reduced in Alpha 5, Tumor 2 and Tumor 3 cell lines (p<0.05) by retinol treatment. The distribution and expression of mutant p53 was mainly localized in the nuclei of cells of MCF-10F and Alpha 5 and treatment with retinol reduced the staining pattern as shown in Fig. 5B and in Tumor 2 and Tumor 3 as shown in Fig. 5C.

Fig. 6A shows the quantification of the immunofluorescent images of the Rb protein expression in MCF-10F and retinol-treated cell lines. It was evident that Alpha 5, Tumor 2, and Tumor 3 had significantly (p<0.001) lower Rb protein expression than the control MCF-10F cell line. The transformed cell lines showed no significant difference among them. Retinol significantly (p<0.05) reduced the effect of Rb protein expression in Alpha 5, Tumor 2, and Tumor 3 cell lines. Fig. 6B shows representative images of immunofluorescent Rb protein expression in Alpha 5 cells with or without retinol treatment. The distribution and expression of Rb can be observed in the nuclei of Alpha 5.

Discussion

Cancer chemoprevention is a new challenging issue in the management of cancer. The free radical-scavenging vitamins, such as all-trans-retinol, have been shown to protect against cancer development in animal models, and may be chemopreventive in humans. Many other vitamins such as vitamin C, E, and micronutrient such as selenium may also be chemopreventive (3-5). The identification of genes involved in the prevention of breast cancer and the mechanisms by which genes participate in radiation- and estrogen-induced carcinogenesis are of critical importance. In vitro models are vital in understanding the events that drive a normal cell to cancer. Identification of factors involved in breast carcinogenesis has been facilitated by studies using breast cancer cell lines representative of different tumor phenotypes.

In this study, an established radiation and estrogen-induced breast cancer model was used (26). The tumorigenic Alpha 5, Tumor 2, and Tumor 3 cell lines responded to retinol treatment by showing reduced invasive and chemotactic properties.

Active oxygen species and other free radicals have long been known to be mutagenic. Furthermore, there is evidence that oxyradicals can modulate phenotypic and genotypic changes that ultimately lead to neoplasia (38). In the present study, retinol treatment was found to decrease free radical production suggesting that oxyradicals play a role in carcinogenesis. The enzymatic determination of hydrogen peroxide can be accomplished with high sensitivity and specificity by using N-acetyl-3, 7-dihydroxyphenoxazine (Amplex Red reagent), that is a highly sensitive and chemically stable fluorogenic probe for the enzymatic determination of H$_2$O$_2$. 

Figure 5. Representative images of immunofluorescence staining of mutant p53 protein expression of T2 and T3-treated cell lines (C).
Many chemical carcinogens have been shown to act through free radical metabolites or processes (2,7).

Our studies showed a down regulation of PCNA in the tumorigenic cell lines, as detected by immunofluorescent staining and quantified by confocal microscopy. Since PCNA is required for DNA replication, it is used as a general marker of cell proliferation. Previous study has shown that PCNA is over-expressed in MCF7, a malignant human breast epithelial cell, in response to organophosphorous pesticide treatment (39). The finding is consistent with other reports in which the PCNA is also used as an index of cell proliferation for breast cells (12). PCNA immunocytochemistry has been used to evaluate the proliferative rate of tumors (12,13). In the present study confocal microscopic imaging is a more sensitive test than the traditional fluorescent microscopes used. The expression of PCNA and products of suppressor gene p53 have been used as markers of primary invasive ductal breast carcinoma (40,41).

The anticarcinogenic and antiproliferative effects of retinol have been extensively studied in vitro. Retinoids have been reported to inhibit the growth of several breast cancer cell lines in culture and to reduce breast tumor growth in animal
models (6). Susceptibilities of breast carcinoma cell lines to growth inhibitory effects of different retinoic derivatives have been examined (42). Of the various retinoids tested, retinoic acid revealed maximum levels of activity in inhibiting cell proliferation and thymidine incorporation. The degree of inhibition of cell proliferation by the various retinoids paralleled their capacity to inhibit thymidine incorporation, suggesting suppression of DNA synthesis as a primary cause of restriction of cell growth by these compounds.

On the other hand, differentiation may play an important role in this inhibitory process. Retinoids can induce cell cycle arrest, differentiation and cell death in many cell lines (30). These compounds can act cooperatively in some of their functions and may be of potential use either individually or in combination in the treatment of breast cancer. The effects of 1 all-trans retinoic acid and several analogues have been evaluated on malignant breast cancer cell lines MCF-7, T-47D and MDA-MB-231. Such compounds caused a decrease in anchorage-independent colony formation in MCF-7 and T-47D cells in a dose-dependent manner. It induced an accumulation of MCF-7 cells in the G1 phase of the cell cycle and an associated increase in p21/WAF1/CIP1, p27KIP1 and dephosphorylation of Rb. Such data demonstrated that retinoids and induced a more differentiated phenotype in breast cancer cells. Furthermore, 2 non-epithelial human cell lines were tested for sensitivity to retinoids, and showed diminished responses compared with MCF-7 cells (42). This suggests a correlation between the ability of retinoids to exert control over cell proliferation and differentiation for a given cell type. Three other human cell lines originally isolated from metastatic infiltrating adenocarcinomas of the breast and one cell line isolated from human milk were treated with various concentrations of retinol and retinoic acid. Growth inhibition curves showed that all 3 malignant cell lines were sensitive to treatment with both retinoids. The non-tumorigenic cell line, HBL-100, derived from human milk was, not affected by treatment.

It has already been shown that treatment with retinol has an inhibitory effect on the growth of malignant cells in vitro (43). The tumorigenic CaMa-15 cell line responded to both retinol and retinoic acid under both anchorage-dependent and anchorage-independent growth conditions by as much as 50%. Fraker et al (44) studied the effects of retinol on the human breast carcinoma cell line, MDA-MB-231, in monolayer cultures and demonstrated a retinol-induced growth inhibition that was reversible as well as time- and dose-dependent.

This report is the first demonstration that retinol can decrease Fra-1 protein expression. An increase in Fra-1 expression both at protein and mRNA level among irradiated MCF-10F cell lines has already been shown (33). Those cells receiving a double dose of α particles had significantly higher Fra-1 expression levels than cells irradiated once. The transformed and tumorigenic cell lines were compared with the control cell line to identify differentially expressed genes during tumorigenic progression (33). Among 190 genes analyzed only 7 were found to be differentially expressed at the tumorigenic stage of progression. Among the late stage-associated genes, 7 genes were altered exclusively in the tumorigenic cell lines and Tumor 2. Such results confirmed the mRNA expression patterns seen in the cDNA array. Among these 6 genes, Fra-1 was up-regulated and Rb down-regulated in the transformed and tumorigenic cell lines compared with the control MCF-10F cell line (33). Rb is a tumor suppressor gene that has been shown to play an important role in breast cancer. An increase in Rb protein expression was observed suggesting a role for retinoic acid at the genetic level.

Our previous studies have shown that over-expression of oncoproteins, such as mutant p53, can potentiate progression of breast cancer by radiation and hormones (32). In the present study, we further demonstrate that retinol reduces this expression in malignant cell lines such as Alpha 5, Tumor 2 and Tumor 3.

Cancer may seem complex and intractable, but its complexity provides multiple opportunities for preventive interventions. Our present cancer model (26), using ionizing radiation and estrogen influenced breast cancer initiation and our present study with retinol show that progression can be prevented. Together, these findings strongly suggest that regulation of PCNA, Fra-1, p53, and Rb, controlled by retinol as a possible mechanism to suppress transformation of epithelial cells. Thus, retinol can inhibit the malignant progression induced by ionizing radiation and estrogen. It also suggests that the molecular targets modulated by retinol may be highly useful indicators in chemoprevention studies. However, further experiments should be required to assess the mechanism of action of retinol as an anticancer agent. Our present findings also suggest that a search for suitable retinoids as chemopreventive agents against human breast cancer is possible.

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