Estradiol Metabolism: An Endocrine Biomarker for Modulation of Human Mammary Carcinogenesis

Nitin T. Telang, Meena Katdare, H. Leon Bradlow, and Michael P. Osborne

Strang Cancer Research Laboratory, The Rockefeller University, New York, New York

The natural estrogen 17β-estradiol (E2) has a profound influence on proliferation and neoplastic transformation of mammary epithelium. The role of cellular metabolism of E2 in mammary carcinogenesis, however, remains to be elucidated. Explant culture and cell culture models developed from noncancerous human mammary tissue were used to examine modulation of E2 metabolism in response to treatment with prototype rodent mammary carcinogens and the ability of the naturally occurring phytochemical indole-3-carbinol (I3C) to influence E2 metabolism and regulate aberrant proliferation. In the two models, treatment with the chemical carcinogens 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene altered the metabolism of E2 as determined from the radiometric (tritium release) and gas chromatography–mass spectrometry (GC–MS) assays. This alteration in E2 metabolism was accomplished by aberrant proliferation and abrogation of apoptosis as determined by the extent of replicative DNA synthesis, S-phase fraction and Sub G0 (apoptotic) peak. Exposure of carcinogen-initiated cultures to I3C resulted in induction of C2-hydroxylation of E2 and of apoptosis and downregulation of hyperproliferation. Determination of altered cellular metabolism of E2 in response to initiators and modulators of carcinogenesis and evaluation of cell cycle related markers for proliferation and apoptosis may provide a mechanism-oriented approach to validate E2 metabolism as an endocrine biomarker for induction and prevention of human mammary carcinogenesis. — Environ Health Perspect 105(Suppl 3):559–564 (1997)

Key words: estrogen metabolites, mammary carcinogenesis, chemoprevention, in vitro models

Introduction

Breast cancer is one of the prevalent causes of death in women in the United States. The American Cancer Society has estimated a 31% incidence of breast cancer (184,300 new breast cancer cases) and about 17% mortality (44,300 cancer related deaths) in 1996 (1). These estimates emphasize a need to identify markers for risk, early detection, and effective prevention.

Laboratory investigations on animal models have provided compelling but circumstantial evidence that human mammary carcinogenesis may be a multifactorial and multistep process involving early occurring molecular, biochemical, and cellular events that represent preneoplastic transformation and late-occurring epigenetic events that represent promotion and progression of the preneoplastic phenotype to tumorigenic phenotype with metastatic potential (2,3). Identification and validation of biomarkers for preneoplastic transformation, therefore, may provide important leads not only for identifying markers of risk for developing breast cancer but also for evaluating effective primary or secondary prevention (4–9).

In the estrogen-responsive mammary tissue, the natural estrogen 17β-estradiol (E2), in concert with other steroid and polypeptide hormones that have mammotrophic or lactogenic effects, supports epithelial cell proliferation and neoplastic transformation (6–8). The mitogenic stimulus mediated via E2 may predispose nontransformed cells for initiation of carcinogenesis, while in preinitiated cells, this stimulus may promote the expression of the transformed cell phenotype (2,3,5–8,10–12).

The cellular biotransformation of E2 represents a complex enzymatic process by which metabolically competent cells convert the mitogenic estrogen E2 to its less active metabolites. Cytochrome P450 (cyp450)-dependent steroid hydroxylases are critical for E2 metabolism, while estrogen receptor, a nuclear transcription factor, is indispensable for transcriptional activation, expression of early response genes c-fos, c-jun, c-myc, and resultant E2-mediated positive regulation of growth (12–16).

Our studies on murine mammary explant cultures and on immortalized non-tumorigenic mammary epithelial cell cultures have demonstrated that treatment with chemical carcinogens and transfection with oncogenes results in altered cellular metabolism of E2 and aberrant hyperproliferation in vitro prior to tumorigenicity in vivo (17–19). In these studies alteration in E2 metabolism was detected by specific and significant increase in C16α-hydroxylation, with a concomitant decrease in C2-hydroxylation pathways, while aberrant hyperproliferation was quantified by the relative extent of cell proliferation in anchorage-dependent and anchorage-independent conditions of growth. Altered cellular metabolism of E2 and aberrant hyperproliferation, therefore, represent biochemical and cellular surrogate end point biomarkers for mammary carcinogenesis (4,9,17–21). The clinical relevance of these biochemical and cellular perturbations, however, depends on extrapolation and therefore is largely equivocal. Clinical investigations on breast cancer patients, disease-free subjects,
and subjects at risk have demonstrated a correlation between elevated C16α-hydroxylation of E2, presence of atypical hyperplasia or proliferative breast disease and increased risk for developing breast cancer (2,5,6–8,13,14,20,21). Thus, altered E2 metabolism may represent a biochemical or endocrine marker for breast cancer development. A systematic investigation of this aspect using appropriate human tissue-derived models should provide important leads that will help researchers evaluate the clinical relevance of specific molecular, biochemical, endocrine, and cellular biomarkers for human mammary carcinogenesis and its prevention (4,9,22).

The present report provides an overview of the experiments designed on explant and cell culture models developed from noncancerous human mammary tissue to establish potential clinical relevance of E2 metabolism as a biochemical or an endocrine biomarker for effective chemoprevention of human mammary carcinogenesis.

**Experimental Systems and Biomarker Assays**

**Human Mammary Explant Culture and Cell Culture Models.** The mammary explant culture system and mammary epithelial cell culture system provide useful in vitro models to examine the responsiveness of noncancerous mammary tissue to agents that affect cell proliferation, cytodifferentiation, and neoplastic transformation at the molecular, biochemical, and cellular levels (2,4,17–19). The tissue culture technology and biomarker assays established for the murine models have been optimized for human mammary tissue (4,22,23).

The explant cultures were prepared from human mammary terminal duct lobular unit (TDLU) obtained from surgical samples. The TDLU are the endocrine responsive and proliferatively active intact organoids that represent target tissue for carcinogenesis (2,4,8). These organoids were maintained in a chemically defined, serum-free Waymouth’s MB 752/1 medium (GIBCO/BRL, Grand Island, NY) supplemented with 5 μg/ml insulin, 1 ng/ml E2, 2 mM L-glutamine, and antibiotics. The medium was routinely changed every 48 hr and the cells were subcultured by a 1:4 split when approximately 70% confluent.

**Chemical Carcinogens and Chemopreventive Agent.** The stock solutions (1000x) of the chemical carcinogens 7,12-dimethylbenz(a)anthracene (DMBA) and benz[a]pyrene (B[α]P) were made up in dimethyl sulfoxide (DMSO).

The stock solution of the naturally occurring phytochemical indole-3-carbolin (I3C), to be used as the chemopreventive test compound, was made up in 100% ethanol. These stock solutions were appropriately diluted with the culture medium to obtain the effective nontoxic concentrations. The selection of chemical carcinogens and of the naturally occurring phytochemical was based on their documented tumorigenic or tumor modulating effects on the rodent models (3,10,26–28).

**Cellular Metabolism of 17β-estradiol.** The metabolism of E2 by TDLU and 184-B5 cultures was determined by the radiometric assay that measures the tritium exchange from specifically labeled E2 to form [3H]H2O (4,17,19) and by the gas chromatography–mass spectrometry (GC–MS) assay that involves product isolation and identification of the metabolites (29).

For the radiometric assay, cultures were incubated with 8.0 × 10−10 M [C2-3H]E2 or [C16α-3H]E2 for 48 hr at 37°C. Aliquots of 500 μl of the incubation medium were diluted to 3.0 ml with distilled water and lyophilized to separate [3H]H2O from the residual radioactive E2. The relative extent of [3H]H2O formed provided an indirect measure of 2-hydroxyestrone (2-OHE1) or 16α-hydroxyestrone (16α-OHE1) formed via the C2-hydroxylation and C16α-hydroxylation pathways of E2 metabolism, respectively. Based on the stoichiometric conversion and the specific activity of labeled E2, the amounts of the metabolites formed were calculated. The data were expressed as pmol metabolite/48 hr/mg tissue.

For the GC–MS assay, cultures were incubated for 48 hr at 37°C with 10−8 M nonradioactive E2. Fifteen milliliters of incubation medium was added with deuterated E2 as an internal standard and processed for solid phase extraction. The extracted sample was derivatized in the presence of 50 μl dry pyridine and 10 μl bis(trimethylsilyl)trifluoroacetamide (BSTFA). The derivatized samples were analyzed under selected ion mode and mass ion, and GC elution times of the metabolites were determined (29). The data were expressed as relative abundance of E2 metabolites per 107 cells.

**Cell Cycle Analysis and Cellular Apoptosis.** The effect of initiators and modulator of carcinogenesis on alteration in cell cycle progression and on the relative extent of cellular apoptosis was examined on 184-B5 cells using the fluorescence-assisted cell sorting (FACS) flow cytometric assay. For this assay, trypsinized cell suspensions fixed in 2% formaldehyde and subsequently in ice-cold 70% ethanol were stained with propidium iodide according to the published procedure (30). The extent of proliferation was expressed as percent synthesis (S)-phase fraction, while the extent of apoptosis was expressed as the intensity of Sub G0 (apoptotic) peak obtained from FACS analysis.

**Results**

**Effect of Chemical Carcinogens on Terminal Duct Lobular Units.** The experiment presented in Table 1 was performed on explant cultures of human mammary TDLU to examine the effects of well known rodent carcinogens DMBA and B[α]P on the cellular metabolism of E2. The relative extent of E2 metabolism via C2-hydroxylation and C16α-hydroxylation pathways was determined using the radiometric assay. The results obtained from

**Table 1.** Effect of chemical carcinogens on the metabolism of 17β-estradiol (E2) in explant cultures of human mammary terminal duct lobular units.

| Treatment | 2-OHE1 (pmol/mg tissue) | 16α-OHE1 (pmol/mg tissue) | C2/16α ratio |
|-----------|-------------------------|---------------------------|--------------|
| DMSO      | 2.32 ± 0.24             | 0.48 ± 0.08               | 4.83 ± 0.30  |
| DMBA      | 1.36 ± 0.24             | 2.24 ± 0.72               | 0.61 ± 0.20  |
| B[α]P     | 1.12 ± 0.08             | 3.12 ± 0.24               | 0.36 ± 0.10  |

Explain cultures incubated with 0.1% DMSO, 39 μM DMBA, or 39 μM B[α]P for 24 hr and with 10−8 M [C2-3H]E2 or [C16α-3H]E2 for the subsequent 48 hr. The culture medium was processed for the radiometric assay. Calculated from the stoichiometric convertibility and the specific activity of [4H]-E2. Values are mean ± SD; n = 12.

- c,d,e,p = 0.005; f,g,h,p = 0.001.
this experiment clearly demonstrate that both the carcinogens DMBA and B[a]P inhibit 2-OHE\(_1\) formation and increase 16\(\alpha\)-OHE\(_1\) formation. This alteration in metabolism leads to an 87.4% and a 92.5% decrease by DMBA and B[a]P, respectively, in C2/C16\(\alpha\)-hydroxylation ratio relative to that observed in the solvent-treated control cultures. The preliminary experiments on the TDLU explant culture system were designed to examine whether the solvents DMSO or ethanol used to solubilize DMBA, B[a]P, and 13C, respectively, influence E\(_2\) metabolism. The extent of C2/C16\(\alpha\)-hydroxylation ratios obtained from untreated cultures (master controls) and those treated with 0.1% DMSO or 0.1% ethanol (solvent controls) was 5.7 \pm 1.1, 4.9 \pm 0.2, and 5.1 \pm 0.1, respectively. The lack of substantial difference in the ratios from the three groups indicates that the two solvents at 0.1% concentrations did not influence the constitutive level of E\(_2\) metabolism.

In the explant cultures of TDLU the epithelial component is surrounded by intralobular and extraductular stroma. It is therefore not possible to demonstrate whether the epithelial component is directly responsible for E\(_2\) metabolism. In addition, the radiometric assay measures the reaction kinetics of C2- and C16\(\alpha\)-hydroxylation pathways and therefore represents an indirect assay for the formation of 2-OHE\(_1\) or 16\(\alpha\)-OHE\(_1\). In an attempt to eliminate the above-mentioned limitations, experiments were conducted on human mammary epithelial 184-B5 cells, and E\(_2\) metabolism was evaluated by the GC–MS assay that measures the specific metabolites formed.

**Effect of Chemical Carcinogens on 184-B5 Cells.** The data presented in Table 2 demonstrate that treatment of 184-B5 cells with DMBA and with B[a]P results in a decreased abundance of 2-OHE\(_1\) relative to that observed in the solvent-treated control cultures. Furthermore, B[a]P was more effective in upregulating 16\(\alpha\)-OHE\(_1\) formation than was DMBA. Because of the observed distinct effects of DMBA and B[a]P on the metabolic pathways, the carcinogen-mediated alteration in the 184-B5 system also resulted in an 87.6% and a 91% inhibition in the C2/C16\(\alpha\)-hydroxylation ratio, respectively, relative to that observed in the solvent-treated controls. The effect of DMBA and B[a]P on cellular apoptosis in confluent cultures of 184-B5 cells is presented in Figures 1A–C. The differential effects of DMBA and B[a]P were also evident on the cell cycle progression. Treatment of 184-B5 cells with DMBA exhibited 63.3 \pm 0.5% S-phase fraction and 5.8 \pm 1.3% apoptosis. In contrast, treatment with B[a]P resulted in 34.5 \pm 0.3% S-phase fraction and 1.6 \pm 0.9% apoptosis, in comparison with 26.1 \pm 3.6% S-phase fraction and 15.1 \pm 3.1% apoptosis that was observed in 0.1% DMSO-treated solvent controls. It is possible that the two carcinogens enhance aberrant hyperproliferation and inhibit cellular apoptosis via distinct mechanisms. Additional experiments focused on the specific cell cycle regulatory gene expression may elucidate the possible mechanisms responsible for effects of DMBA and B[a]P on 184-B5 cells.

**Effect of 13C on Chemical Carcinogenesis in Terminal Duct Lobular Units.** Having demonstrated that treatment of TDLU explant cultures or 184-B5 cell cultures with the chemical carcinogen B[a]P results in decreased

### Table 2. Effect of chemical carcinogens on the metabolism of 17\(\beta\)-estradiol (E\(_2\)) in human mammary epithelial 184-B5 cells.

| Treatment | 2-OHE\(_1\) (relative abundance) | 16\(\alpha\)-OHE\(_1\) (relative abundance) | C2/C16\(\alpha\) ratio |
|-----------|-----------------------------------|----------------------------------------|----------------------|
| DMBA      | 342 \pm 73\(^*\)                 | 53 \pm 13\(^\text{+}\)                 | 6.45 \pm 0.20        |
| B[a]P     | 52 \pm 3\(^\text{+}\)            | 65 \pm 5\(^\text{+}\)                 | 0.80 \pm 0.20        |
| DMBA + 13C| 45 \pm 5\(^\text{+}\)            | 70 \pm 6\(^\text{+}\)                 | 0.58 \pm 0.10        |

*Cell cultures incubated with 0.1% DMSO, 39 \(\mu\)M DMBA or 39 \(\mu\)M B[a]P for 24 hr and with 10\(^{-4}\)M E\(_2\) for the subsequent 48 hr. The culture medium was processed for the product isolation and identification by the GC–MS assay. \(^*\)Values are mean \pm SD; \(n = 4\); \(^*\)\(p < 0.0001\); \(^\text{+}\)\(p < 0.05\); \(^\text{\#p} = 0.01\).
Table 3. Effect of I3C on chemical carcinogen-treated human mammary explant cultures.

| Treatment | Replicative DNA synthesis | C2/C16α-hydroxylation ratio |
|-----------|---------------------------|-----------------------------|
| DMSO      | 3.0 ± 0.4 a                | 3.6 ± 0.9 b                 |
| B[a]P     | 8.3 ± 0.3 a                | 5.6 ± 0.6 a                 |
| B[a]P + I3C | 3.6 ± 0.9 b               | 6.5 ± 1.6 a                 |

Explant cultures incubated with 0.1% DMSO or 39 μM B[a]P for 24 hr and were maintained with or without 50 μM I3C for the subsequent 10 days. *Pulse labeled with 5μCi/mL of [3H]-thymidine between days 9 and 10 of culture. Radioactivity was determined as trichloroacetic acid precipitable counts. **Determined by the radiometric assay as in Table 1. Mean ± SD, n = 6 per treatment group. *Mean ± SD, n = 18 per treatment group. *p < 0.001.

Table 4. Effect of I3C on chemical carcinogen-treated human mammary epithelial 184-B5 cells.

| Treatment | S-Phase fraction | Apoptosis | E2 Metabolism in (C2/C16α ratio) |
|-----------|------------------|-----------|----------------------------------|
| DMSO      | 29.8 ± 0.9 b     | 14.9 ± 1.2 b | 3.7 ± 0.5/0.6                   |
| B[a]P     | 34.5 ± 0.3 b     | 1.6 ± 0.9 b  | 0.6 ± 0.1*                     |
| B[a]P + I3C | 17.0 ± 1.7 b   | 5.1 ± 0.5 b  | 2.6 ± 0.3 b                    |

*Cell cultures were incubated with 0.1% DMSO or 39 μM B[a]P for 24 hr and were maintained with or without 50 μM I3C for the subsequent 48 hr. **Determined from propidium iodide stained cell suspensions using FACS. Mean ± SD, n = 6/treatment group. *Mean ± SD, n = 6/treatment group. *p < 0.001. *p = 0.005. *p = 0.001.

C2/C16α-hydroxylation ratio, it was important to provide evidence for validation of E2 metabolism as an endocrine marker for preventive efficacy of agents known to inhibit rodent mammary carcinogenesis. In the experiment presented in Table 3, the naturally occurring plant product I3C represented the chemopreventive test compound, while replicative DNA synthesis and E2 metabolism provided two independent markers for quantification. Treatment of TDLU explant cultures with 50 μM I3C resulted in a 3H-thymidine uptake of 3.6 ± 0.9 cpm × 10^6/mg DNA and a C2/C16α-hydroxylation ratio of 5.0 ± 0.6. These data were comparable to those observed in the DMSO-treated or ethanol-treated solvent controls (data not shown). Exposure of TDLU explants to B[a]P resulted in increased DNA synthesis activity and in decreased C2/C16α-hydroxylation ratio. The B[a]P-initiated explant cultures, upon treatment with I3C, exhibited a 42.8% decrease in replicative DNA synthesis and a 10-fold increase in C2/C16α-hydroxylation ratio. These results indicate that I3C by itself does not influence the constitutive status of the biomarkers; however, this agent is able to reverse the hyperproliferative effects of B[a]P, in part, by upregulating the C2/C16α-hydroxylation ratio.

**Effect of I3C on Chemical Carcinogenesis in 184-B5 Cells.** The experiment presented in Table 4 was conducted on the 184-B5 cells to examine the effect of I3C on B[a]P-induced cellular and biochemical alterations. Treatment of 184-B5 cells with B[a]P resulted in increased S-phase fraction, inhibited Sub G0 (apoptotic) peak and decreased C2/C16α-hydroxylation ratio. In the 184-B5 cell culture system, treatment with 50 μM I3C exhibited 29.7 ± 0.7% S-phase fraction, 15.5 ± 0.6% apoptosis, and a C2/C16α-hydroxylation ratio of 4.1 ± 0.3. These data were comparable to those observed in untreated master controls and in 0.1% ethanol-treated solvent controls (data not shown). Thus, I3C, at the dose level tested, does not appear to influence the constitutive status of the biochemical and cellular quantitative end points.

The effect of DMBA appears to be distinct from that of B[a]P in the present experimental system. It will therefore be of considerable interest to examine whether I3C in DMBA-initiated cells downregulates the status of S-phase specific gene expression.

**Discussion.** The experiments in this study were conducted on in vitro models for human mammary carcinogenesis to examine the role of E2 metabolism in chemical carcinogen-induced initiation of tumorigenic transformation and to validate E2 metabolism as a surrogate end point biomarker for efficacy of chemopreventive agents. Human mammary TDLU explant cultures and 184-B5 cell cultures represent the experimental systems derived from noncancerous human mammary tissue. Biochemical determination of E2
metabolism by radiometric and GC-MS assays, replicative DNA synthesis by [3H]thymidine uptake assay, and cell cycle analysis by FACS flow cytometry assay represented the quantitative parameters.

The observed increase in replicative DNA synthesis or S-phase fraction in response to the chemical carcinogens may be due to a combination of replicative DNA synthesis and DNA repair synthesis. Mammary tissue has been reported to effectively metabolize the procarcinogens and generate DNA-damaging oxidative metabolites that may induce DNA repair synthesis (2,18,31-35).

In the multistep process of mammary carcinogenesis, the natural estrogen E2 functions as a potent tumor promoter, acting prevalently during the late-occurring, postinitial (promontional) stage of tumorigenesis (3,6,7,10). The possible mechanisms responsible for the positive growth regulation by E2 include mitogenic signal transduction via upregulation of early response genes c-jun, c-fos, and c-myc; induction of DNA synthesis in quiescent cells; and activation of nuclear transcriptional factors estrogen receptor and nuclear factor (12,15,16,36). In contrast, the biological activity of various oxidative metabolites of E2 is pleiotropic depending upon the experimental systems (37-41), and therefore the role of E2 metabolites in mammary carcinogenesis remains equivocal.

The experiments on TDLU explant culture system clearly demonstrated that prototypic rodent carcinogens DMBA and B[a]P decrease the C2/C16α-hydroxylation ratio of E2 metabolism. This observation is consistent with our previous studies that demonstrated a similar perturbation in in vitro models of rodent mammary carcinogenesis in response to DMBA or c-myc (17,18,20,21,31,32). In addition, clinical investigations on patients with breast cancer, as well as laboratory investigations on noncancerous breast tissue, or on human mammary carcinoma-derived cell lines, have provided convincing evidence for a role of E2 metabolism in breast cancer development (4,13,14,20,22,23,40-42). Taken together, these observations suggest that altered metabolism of E2 may represent an endocrine biomarker for carcinogenic insult to the mammary tissue.

The presence of interlobular and interductular stroma in the TDLU explant culture system represents the nontarget component for mammary carcinogenesis. This cellular heterogeneity therefore compromises the specificity of E2 metabolism as a biomarker assay. In addition, the radiometric assay measuring the reaction kinetics of the two pathways provides an indirect measurement for the formation of E2 metabolites. The two technical limitations were eliminated by experiments that utilized the mammary epithelial 184-B5 cells to determine E2 metabolites by the GC-MS assay. The experiments on the 184-B5 cell culture system demonstrated that while both DMBA and B[a]P decrease 2-OH-E2 formation, upregulation of 160-OHE1 is observed only in response to B[a]P. Decrease in C2/C16α-hydroxylation ratio by the two carcinogens in the two experimental systems is comparable, and the human mammary tissue is more susceptible to B[a]P than it is to DMBA. The observed preferential susceptibility of human tissue to the polycyclic aromatic hydrocarbons is consistent with that reported previously (33-35). The results obtained from the TDLU explant culture and 184-B5 cell culture systems, taken together, suggest that the target epithelial component responds directly to the carcinogenic insult.

The naturally occurring plant product 13C represents a major phytochemical in such cruciferous vegetables as cabbage, broccoli, and brussels sprouts. Our previous in vitro and in vivo studies on rodent models of mammary carcinogenesis have shown that exposure to 13C protects the target tissue from chemical carcinogen-induced transformation or from mammary tumor virus-induced mammary tumorigenesis (27,31). Furthermore, 13C has been reported to induce C2-hydroxylation of E2 in human mammary carcinoma cells as well as in human subjects (42-45). We therefore sought to examine whether 13C is also an effective inhibitor of carcinogenesis in human mammary TDLU explant culture and cell culture models. In the experiments utilizing the TDLU explant culture system, treatment of cultures with B[a]P resulted in induction of aberrant proliferation as evidenced by increased replicative DNA synthesis, and in altered E2 metabolism, as seen by inhibition of C2/C16α-hydroxylation ratio. B[a]P-initiated cultures in the presence of 13C exhibited inhibition of aberrant proliferation and enhancement of C2/C16α-hydroxylation ratio. In the experiments on 184-B5 cell culture system, exposure to B[a]P exhibited an increase in S-phase fraction, and a decrease in cellular apoptosis and in C2/C16α-hydroxylation ratio. The B[a]P-initiated cultures in the presence of 13C exhibited downregulation of cell proliferative activity and upregulation of apoptosis and of C2/C16α-hydroxylation ratio. The possible mechanisms responsible for the protective effect of 13C against B[a]P-induced human mammary carcinogenesis, however, remain to be identified. In this context, it is noteworthy that 13C functions as an inducer of cyp4501A1-dependent C2-hydroxylation of estradiol (42) and 2-OHE1 has been reported to antagonize the genotoxic and transforming effects of DMBA (31). These observations, taken together, raise the possibility that increased production of antiproliferative E2 metabolite 2-OHE1 by 13C in carcinogen-initiated human mammary tissue may negatively regulate aberrant hyperproliferation, in part by induction of cellular apoptosis.

In conclusion, the present study on human mammary tissue-derived in vitro models for carcinogenesis has provided evidence that E2 metabolism and cell cycle-related markers for proliferation and apoptosis may represent valuable surrogate end point biomarkers to evaluate efficacy of chemopreventive agents for human mammary carcinogenesis.

REFERENCES

1. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics. CA Cancer J Clin 65:5–27 (1996).
2. Russo J, Calaf G, Sohi N, Tahir Q, Zhang PL, Alvarado ME, Estrado S, Russo IH. Critical steps in breast carcinogenesis. Ann NY Acad Sci 698:1–20 (1995).
3. Welsh CW. Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. Cancer Res 45:3415–3443 (1985).
4. Telang NT, Bradlow HL, Osborne MP. Molecular and endocrine biomarkers in noninvolved breast: relevance to cancer chemoprevention. J Cell Biochem 16G:161–169 (1992).
5. Henderson BE, Ross RK, Pike MC. Toward the primary prevention of breast cancer. Science 254:1131–1138 (1991).

6. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogens, normal breast cell proliferation and breast cancer risk. Epidemiol Rev 15:17–35 (1993).

7. Bernstein L, Ross RK. Endogenous hormones and breast cancer risk. Epidemiol Rev 15:48–65 (1993).

8. Howell AE, Anderson E, Laidlaw I. Cyclical activity and 'aging' of the human breast: clues to assessment of risk and strategies for prevention. In: Endocrine Therapy of Breast Cancer (Howell A, ed). New York:Springer-Verlag, 1994:27–46.

9. Kelloff GJ, Boone CW, Steele VE, Crowell JA, Luber B, Doody LA, Greenwald P. Development of breast cancer chemopreventive drugs. J Cell Biochem 17G:2–13 (1993).

10. Welsch CW. Rodent models to examine in vivo hormonal regulation of mammary gland tumorigenesis. In: Cellular and Molecular Biology of Mammary Cancer (Medina D, Kidwell W, Hepner G, eds). New York:Plenum Press 1987:163–179.

11. Nandi S, Guzman RC, Yang J. Hormones and mammalian carcinogenesis in mice, rats and humans: a unifying hypothesis. Proc Natl Acad Sci USA 92:3650–3657 (1995).

12. Sekeris CE. Hormonal steroids act as tumor promoters by modulating oncogene expression. J Cancer Res Clin Oncol 117:96–101 (1991).

13. Martucci CJ, Fishman J. P450 enzymes of estrogen metabolism. Pharmacol Rev 57:237–257 (1993).

14. Adams JB. Enzymatic regulation of estradiol 17b-concentrations in human breast cancer cells. Breast Cancer Res Treat 20:145–154 (1992).

15. Weisz A, Bresciani F. Estrogen regulation of protooncogenes coding for nuclear proteins. Crit Rev Oncol 4:361–388 (1989).

16. Dubik D, Shiu RPC. Mechanism of estrogen activation of c-myc oncogene expression. Oncogene 7:1587–1594 (1992).

17. Telang NT, Kurilah R, Wong GYC, Bradlow HL, Osborne MP. Prenoplastic transformation in mouse mammary tissue: identification and validation of intermediate biomarkers for chemoprevention. Anticancer Res 11:1021–1028 (1991).

18. Garg A, Suto A, Osborne MP, Gupta RC, Telang NT. Expression of biomarkers of transformation in 7,12-dimethylbenz(a)anthracene-treated mammary epithelial cells. Int J Oncol 3:105–109 (1993).

19. Telang NT, Narayan R, Bradlow HL, Osborne MP. Coordinated expression of intermediate biomarkers for tumorigenic transformation in Ras-transfected mouse mammary epithelial cells. Breast Cancer Res Treat 18:155–163 (1991).

20. Fishman J, Osborne MP, Telang NT. The role of estrogen in mammary carcinogenesis. Ann NY Acad Sci 768:91–100 (1995).

21. Telang NT. Oncogenes, estradiol biotransformation and mammary carcinogenesis. Ann NY Acad Sci 784:277–287 (1996).

22. Osborne MP, Bradlow HL, Wong GYC, Telang NT. Upregulation of estradiol C160-hydroxylation in human breast tissue: a potential biomarker of breast cancer risk. J Natl Cancer Inst 85:1917–1920 (1993).

23. Telang NT, Axelrod DM, Wong GYC, Bradlow HL, Osborne MP. Biotransformation of estradiol by explant cultures of human mammary tissue. Steroids 56:37–43 (1991).

24. Stamper MR, Bartley JC. Induction of transformation and continuous cell lines from normal human mammary epithelial cells after exposure to benzo[a]pyrene. Proc Natl Acad Sci USA 82:2394–2398 (1985).

25. Zhai YF, Beitermiller H, Wang BC, Gould MN, Oakley C, Esselman WJ, Welsch CW. Increased expression of specific protein tyrosine phosphatases in human breast epithelial cells neoplastically transformed by the neu oncogene. Cancer Res 53:2272–2278 (1993).

26. McCormick DB, Burns FJ, Albert RE. Inhibition of benzo[a]pyrene-induced mammary carcinogenesis by retinyl acetate. J Natl Cancer Inst 66:559–564 (1981).

27. Bradlow HL, Michnovicz JJ, Telang NT, Osborne MP. Effect of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumorigenesis in mice. Carcinogenesis 12:1571–1574 (1991).

28. Kojima T, Tanaka T, Mori H. Chemoprevention of spontaneous endometrial cancer in female Dntruy rats by dietary indole-3-carbinol. Cancer Res 54:1446–1449 (1994).

29. Sepkovic DW, Bradlow HL, Michnovicz J, Murrezani S, Levy I, Osborne MP. Catechol estrogen production in rat microsomes after treatment with indole-3-carbinol, ascorbigen, or β-naphthaflavone: a comparison of stable isotope dilution, GC–MS and radiometric methods. Steroids 59:318–323 (1994).

30. Goldberg Y, Nasisif H, Pittas A, Tsai L, Dynlacht BD, Rigas B, Schiff SJ. The antiproliferative effect of sulindac and sulindac sulfide on HT-29 colon cancer cells: alterations in tumor suppressor and cell cycle regulatory proteins. Oncogene 12:893–901 (1996).

31. Suto A, Bradlow HL, Wong GYC, Osborne MP, Telang NT. Experimental down-regulation of intermediate biomarkers of carcinogenesis in mouse mammary epithelial cells. Breast Cancer Res Treat 27:193–202 (1993).

32. Telang NT, Suto A, Wong GYC, Osborne MP, Bradlow, HL. Induction by estrogen metabolite 16α-hydroxysterone of genotoxic damage and proliferative alteration in mouse mammary epithelial cells. J Natl Cancer Inst 84:634–638 (1992).

33. Moore CJ, Tricomi WA, Gould MN. Interspecies comparison of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene binding to nuclear macromolecules in human and rat mammary epithelial cells. Cancer Res 46:4946–4952 (1986).

34. Moore CJ, Eldridge SR, Tricomi WR, Gould MN. Quantitation of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene binding to nuclear macromolecules in human and rat mammary epithelial cells. Cancer Res 47:2609–2613 (1987).

35. Stampfer MR, Bartholomew JC, Smith HS, Bartley JC. Metabolism of benzo[a]pyrene by human mammary epithelial cells: toxicity and DNA adduct formation. Proc Natl Acad Sci USA 78:6251–6255 (1981).

36. Mauvais-Jarvis P, Kuttten F, Gompel A. Estradiol/progestosterone interaction in normal and pathologic breast cells. Ann NY Acad Sci 464:152–167 (1986).

37. Zhu BT, Bui QD, Weisz H, Liehr JG. Conversion of estrone to 2-and 4-hydroxysterone by hamster kidney and liver microsomes: implication for the mechanisms of estrogen-induced carcinogenesis. Endocrinology 135:1772–1779 (1994).

38. Li JJ, Li SA. Estrogen carcinogenesis in hamster tissues: a critical review. Endocr Rev 11:524–531 (1990).

39. Lottering ML, Haag M, Seegers JC. Effects of 17β-estradiol metabolites on cell cycle events in MCF-7 cells. Cancer Res 52:5926–5932 (1992).

40. Schneider J, Huh MM, Bradlow HL, Fishman J. Antiestrogen action of 2-hydroxysterone on MCF-7 human breast cancer cells. J Biol Chem 259:4840–4845 (1984).

41. Vandewalle B, Lefebre J. Opposite effects of estrogen and catechol estrogen on hormone-sensitive breast cancer cell growth and differentiation. Mol Cell Endocrinol 61:239–246 (1989).

42. Tiwari RK, Li G, Bradlow HL, Telang NT, Osborne MP. Selective responsiveness of human breast cancer cells to indole-3-carbinol, a chemopreventive agent. J Natl Cancer Inst 86:126–131 (1994).

43. Michnovicz JJ, Bradlow HL. Induction of estradiol metabolism by dietary indole-3-carbinol in humans. J Natl Cancer Inst 50:947–950 (1990).

44. Bradlow HL, Sepkovic DW, Telang NT, Osborne MP. Indole-3-carbinol: a novel approach for breast cancer prevention. Ann NY Acad Sci 768:180–200 (1995).

45. Davis D, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H. Medical hypothesis: xenosterogens as preventable causes of breast cancer. Environ Health Perspect 101:372–377 (1993).