RESEARCH ARTICLE

Preferential Induction of CYP1A1 over CYP1B1 in Human Breast Cancer MCF-7 Cells after Exposure to Berberine

Chun-Jie Wen[1], Lan-Xiang Wu[1], Li-Juan Fu[1], Dong-Ya Shen[1], Xue Zhang[1], Yi-Wen Zhang[2], Jing Yu[2], Hong-Hao Zhou[1]*

Abstract

Estrogens are considered the major breast cancer risk factor, and the carcinogenic potential of estrogens might be attributed to DNA modification caused by derivatives formed during metabolism. 17β-estradiol (E_2), the main steroidal estrogen present in women, is metabolized via two major pathways: formation of 2-hydroxyestradiol (2-OH E_2) and 4-hydroxyestradiol (4-OH E_2) through the action of cytochrome P450 (CYP) 1A1 and 1B1, respectively. Previous reports suggested that 2-OH E_2 has putative protective effects, while 4-OH E_2 is genotoxic and has potent carcinogenic activity. Thus, the ratio of 2-OH E_2/4-OH E_2 is a critical determinant of the toxicity of E_2 in mammary cells. In the present study, we investigated the effects of berberine on the expression profile of the estrogen metabolizing enzymes CYP1A1 and CYP1B1 in breast cancer MCF-7 cells. Berberine treatment produced significant induction of both forms at the level of mRNA expression, but with increased doses produced 16~ to 52~fold greater induction of CYP1A1 mRNA over CYP1B1 mRNA. Furthermore, berberine dramatically increased CYP1A1 protein levels but did not influence CYP1B1 protein levels in MCF-7 cells. In conclusion, we present the first report to show that berberine may provide protection against breast cancer by altering the ratio of CYP1A1/CYP1B1, could redirect E_2 metabolism in a more protective pathway in breast cancer MCF-7 cells.

Keywords: Berberine - CYP1A1 - CYP1B1 - estrogen metabolism - MCF-7 breast cancer cells

Asian Pac J Cancer Prev, **15 (1)**, 495-499

Introduction

Breast cancer is one of the most common malignancies and the leading cause of mortality in females worldwide (Jemal et al., 2011). Estrogens have been recognized as the prime risk factor for the development of breast cancer (Feigelson et al., 1996; Cavalieri et al., 1997). In 2001 the US government added steroidal estrogens to the list of known human carcinogens (Yager et al., 2006; Clemons et al., 2007). Excessive estrogen exposure can initiate cancer development and promote carcinogenesis by the reaction of their oxidation products with DNA (Cavalieri et al., 1997).

17β-estradiol (E_2), the main estrogen in the breast, can be metabolically transformed to 2-OH E_2 and 4-OH E_2 (Parl et al., 2009). The catechols 2-OH E_2 and 4-OH E_2 can be oxidized to quinones (Cavalieri et al., 1997). Quinones derived from 2-OH E_2 (E_2-2,3-Q) do not seem to generate mutations (Cavalieri et al., 2000), moreover, 2-OH E_2 have putative protective effects via its 2-methoxy form (Zhu et al., 1998). In contrast, quinones derived from 4-OH E_2 (E_2-3,4-Q) is likely to have genotoxic and carcinogenic effects, which can react with DNA via its quinine to form predominantly depurinating and stable adducts (Cavalieri et al., 1997). Thus, this 4-OH E_2 appears to be one of the most genotoxic metabolites of E_2 in the breast epithelium (Liehr et al., 1996). It is critical to note that the ratio 2-OH E_2/4-OH E_2 could be a critical parameter of the carcinogenicity of E_2.

In breast epithelium, the production of estrogen catechols depends on the activity of enzymes, especially CYP1A1 and CYP1B1 (Badawi et al., 2001; Parl et al., 2009). CYP1A1 displays hydroxylase activity at the C2 position to form 2-OH E_2 metabolite, whereas CYP1B1 displays its primarily activity on the C4 position to produce 4-OH E_2 metabolite (Spink et al., 1992; Hayes et al., 1996). As in the case of the catechol ratio, the relative expression of these genes and the CYP1A1/CYP1B1 ratio could be critical in terms of carcinogenesis.

An understanding of the pathways in which natural compounds alter estrogen metabolism is a key step in the development of new breast cancer prevention strategies (Sarah et al., 2008). Dietary or pharmaceutical agents that can reduce the production of genotoxic estrogen metabolites may be effective in preventing estrogen-induced breast carcinogenesis. For example, a compound that increases the formation of 2-OH E_2 catalyzed by CYP1A1 would reduce the level of 4-OH E_2 catalyzed

*[1]Institute of Life Sciences, Chongqing Medical University, Chongqing, [2]Institute of Clinical Pharmacology, Central South University, Changsha, China *For correspondence: hhzhou2003@163.com*

by CYP1B1 and thereby lower the potential for DNA damage.

Many herbs have been reported to be chemopreventive ingredients that have a potential for inhibition or induction of cytochrome P450 enzymes. Berberine, an isoquinoline alkaloid, isolated from many kinds of medical herbs such as Coptis chinensis and berberis aristata, exerts a modulating role on the metabolism of drugs and xenobiotics by acting as inducers or inhibitors of CYPs. In human liver microsomes, berberine inhibits CYP2D6 and CYP3A4 activities, in mice, berberine inhibits CYP3A11 and CYP3A25 expression while induces CYP1A2 expression (Guo et al., 2011a; Guo et al., 2011b). In renal-transplant recipients, berberine was reported to markedly elevate the blood concentration of cyclosporine A, a drug metabolized by CYP3A4 (Wu et al., 2005). Prior to our work, little has been reported about the effects of berberine on estrogen-metabolizing enzymes CYP1A1 and CYP1B1 in human breast cells. Thus, in the current study we have investigated the effects of the berberine on the RNA and protein expression of the estrogen-metabolizing enzymes CYP1A1 and CYP1B1 using the human breast cancer cell line MCF-7.

Materials and Methods

Materials

Berberine (>95% of purity; Sigma, St. Louis, MO) was dissolved in dimethylsulfoxide (DMSO; Sigma). The final concentration of the vehicle was always less than 0.1%.

Cell culture

Human breast cancer cells MCF-7 were purchased from the American Type Culture Collection (ATCC; Manassas, VA). The cells were grown in RPMI 1640 medium (Hyclone, South Logan, UT) with 10% fetal calf serum (FBS; HyClone), 100U/ml penicillin and 100μg/ml streptomycin. Cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Cells were sub-cultured twice weekly. Berberine was used at 5, 10, 20 and 40μM dose. Cells were cultured for either 24, 48 and 72 h. All treatments were done in quadruplicate.

Real-time PCR

Total RNA from the treated and control cells were isolated by Trizol (Invitrogen Valenica, CA, USA) according to the manufacturer's instructions. Sample (2μg) of RNA was reverse-transcribed using the RevertAid First Strand cDNA Synthesis Kit (Fermantas, St. Leon-Rot, Germany) to prepare first strand cDNA. qPCR amplification was carried out using SYBR-green detection of PCR products in real time with an ABI-7500 Sequence Detection System (Applied Biosystems, Foster City, CA). The PCR program was initiated by 30s at 95°C before 40 thermal cycles, each of 5s at 95°C, 30s at 55°C, and 34s at 72°C. Data were analyzed according to the $2^{-\triangle\triangle Ct}$ method (Livak et al., 2001). β-actin gene was used as an endogenous control to normalize the expression of the samples. The following primers were used for forward and reverse: CYP1A1 (5' TCC TGG AGA CCT TCC GAC AC 3' and 5' GGG TTG ACC CAT AGC TTC TG 3'), CYP1B1 (5' CTA GGC AAA GGT CCC AGT TC 3' and 5' CAG CAC CGA CAG GAG TAG CA 3'), β-actin (5' TTG CCG ACA GGA TGC AGA AGG A 3' and 5' AGG TGG ACA GCG AGG CCA GGA T 3').

Western Blot

The adherent cells were lysed in RIPA lysis buffer supplemented with proteinase inhibitors phenyl methyl sulfonyl fluoride (PMSF), and incubated on ice for 30 min. Cell lysate was centrifuged at 14,000 rpm for 15 min and the supernatant was recovered. The protein concentration of the lysates was determined by BCA method. Twenty micrograms total protein was resolved in 10% SDS-polyacrylamide gels and transferred to PVDF membranes (Immobilon P, Millipore, Bedford, MA) under standard conditions. After blocking with 5% skimmed milk, the membranes were probed with a 1:100 dilution primary antibody for CYP1A1 antibody H-70 (Santa Cruz, sc-20772) and CYP1B1 antibody H-105 (Santa Cruz, sc-32882) and a 1:5000 dilution primary β-actin antibody (sigma, A5441) for overnight at 4 °C. Membranes were washed and then incubated with a 1:10000 dilution of DyLight 800-labeled second antibodies (KPL, Maryland, USA) for 1 h. The amount of immunoreactive production in each lane was determined using Odyssey Two-Color Infrared Imaging System (LI-COR Bioscience, St. Lincoln. NE, USA). Blots were reprobed with antibodyβ-actin protein to normalize the results.

Statistical analysis

The data were expressed as mean ± standard deviation. The results were analyzed using the one-way analysis of variance (ANOVA) test. The statistical significance between the experimental groups and their respective controls was assessed by Tukey's post hoc test by using GraphPad Prism 4 software package (San Diego, CA, USA). A p value <0.05 was considered statistically significant.

Results

The effect of berberine on the expression of CYP1A1 in MCF-7 cells

Treatment of MCF-7 cells with increased doses of

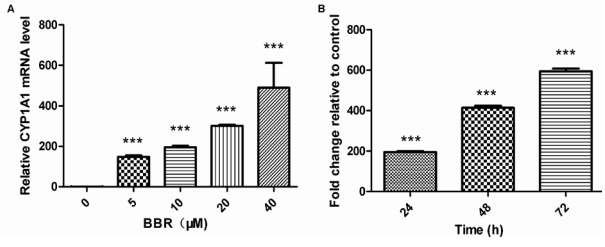

Figure 1. Regulation of CYP1A1 mRNA Expression by Berberine. (A) MCF-7 cells were treated with increased concentrations of berberine (0, 5, 10, 20 and 40 μM) for 48 h. (B) MCF-7 cells were treated with 20 μM concentration of berberine for 24-72h. Total RNA was isolated using TRIzol reagent, and CYP1A1 mRNA was quantified by RT-PCR. Duplicate reactions were performed for each experiment, and the values are presented as mean ± SD. ***P<0.001compared with the control group. BBR, berberine

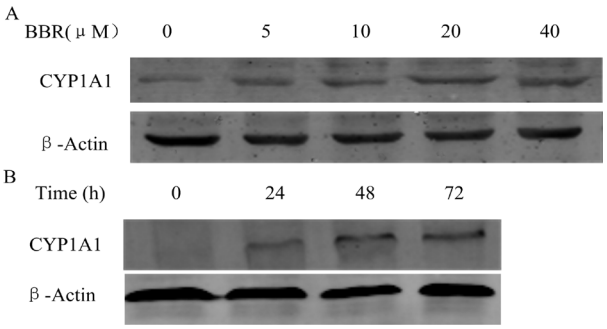

Figure 2. Regulation of CYP1A1 Protein Expression by Berberine. (A) MCF-7 cells were treated with increased concentrations of berberine (0, 5, 10, 20 and 40 μM) for 48h. (B) MCF-7 cells were treated with 20μM concentration of berberine for 24-72h. Protein was isolated and subjected to Western blot analysis. PVDF membranes were probed using antibodies to CYP1A1 and β-actin. Equal amounts of protein were loaded into each lane

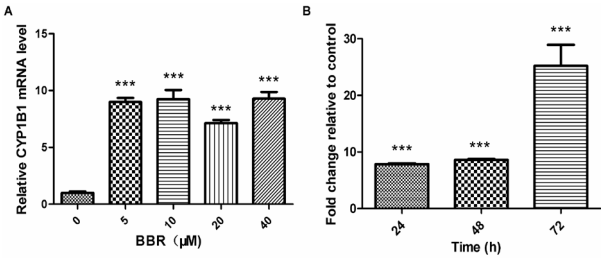

Figure 3. Regulation of CYP1B1 mRNA Expression by Berberine. (A) MCF-7 cells were treated with increased concentrations of berberine (0, 5, 10, 20 and 40μM) for 48h. (B) MCF-7 cells were treated with 20μM concentration of berberine for 24-72h. Total RNA was isolated using TRIzol reagent, and CYP1A1 mRNA was quantified by RT-PCR. Duplicate reactions were performed for each experiment, and the values are presented as mean ± SD. ***P<0.001compared with the control group. BBR, berberine

berberine for 48 h resulted in a dose-dependent increase in CYP1A1 mRNA levels that was significantly different from control groups (Figure 1A). 40μM berberine caused ~500 fold increase in the CYP1A1 mRNA expression. A 20μM concentration of berberine induced a time-dependent increase in CYP1A1 mRNA expression (Figure 1B). Maximal induction of CYP1A1 by 20μM berberine occurred after 72 h treatment and was equal to a 600~fold compared to control groups.

To further examine whether the induction of CYP1A1 mRNA in MCF-7 cells in response to berberine treatment is translated into protein levels, we performed western blot analysis. As shown in Figure 2, The protein expression of CYP1A1 was dramatic increased following 48h exposures to increased doses of berberine. And also MCF-7 cells displayed a dramatic increase in CYP1A1 protein levels following 24, 48 and 72h exposures to 20μM doses of berberine. An increase in CYP1A1 protein in correlation with the observed increased in CYP1A1 mRNA levels in MCF-7 cells.

The effect of berberine on the expression of CYP1B1 in MCF-7 cells

After 48h treatment with increased doses berberine, CYP1B1 mRNA expression was markedly increased

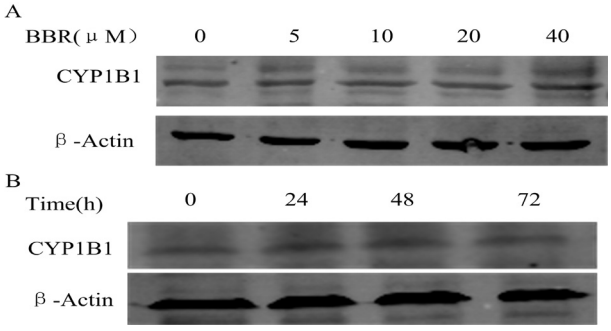

Figure 4. Regulation of CYP1B1 Protein Expression by Berberine. (A) MCF-7 cells were treated with increased concentrations of berberine (0, 5, 10, 20 and 40 μM) for 48 h. (B) MCF-7 cells were treated with 20μM concentration of berberine for 24-72h. Protein was isolated and subjected to Western blot analysis. PVDF membranes were probed using antibodies to CYP1A1 and β-actin. Equal amounts of protein were loaded into each lane

Table 1. Ratio of CYP1A1 to CYP1B1 mRNA Expression in Increased Doses of Berberine-treated MCF-7 Cells at the 48 h

Dose (μM)	CYP1A1 fold change	CYP1B1 fold change	Ratio (CYP1A1/CYP1B1)
5	147.61	9	16.4
10	195.38	9.24	21.1
20	301.89	7.14	42.3
40	489.78	9.3	52.7

Table 2. Ratio of CYP1A1 to CYP1B1 mRNA Expression in 20 μM Berberine-treated MCF-7 Cells Ranging from 24 to 72 h Time Point

Time (h)	CYP1A1 fold change	CYP1B1 fold change	Ratio (CYP1A1/CYP1B1)
24	194.92	7.86	24.8
48	414.35	8.6	48.2
72	594.97	25.24	23.6

compared to control cells (Figure 3A). The maximal induction of CYP1B1 expression produced by 40μM berberine was equal to a 10~fold increase over control cells after 48 h treatment. Significant elevations in CYP1B1 mRNA expression were observed at the 24, 48 and 72h after exposure to 20 μM berberine. The maximal increase in CYP1B1 expression after exposure to 20μM berberine was observed at the 72 h and was equal to a 25~fold increase over DMSO-treated cells.

Next, we investigated whether the changes at the RNA levels were translated into protein. CYP1B1 protein levels, however, in our study were not changed. The difference between mRNA levels and the corresponding protein levels may indicate that mRNA molecules do not reach the translational machinery, probably because the translation mechanism is saturated in these conditions.

Berberine treatment elevates the expression of CYP1A1 to significantly higher levels than CYP1B1 in MCF-7 cells

To compare the relationship between CYP1A1 and CYP1B1 mRNA expression in response to berberine exposure in MCF-7 cells, the ratios of CYP1A1 to

CYP1B1 fold changes were calculated for each dose and time point. The ratios greater than one indicate that the expression of CYP1A1 was induced to a higher level than the expression of CYP1B1. As shown in Table 1, the treatment of MCF-7 cells with berberine in all doses resulted in the ratio of CYP1A1 to CYP1B1 greater than one. At all time points tested, 20μM doses of berberine produce a ratio of CYP1A1 to CYP1B1 greater than one (Table 2). The highest ratios of CYP1A1 to CYP1B1 caused by 40μM berberine at 48h and were equal to 52.7. According to the western blot analysis, berberine strikingly increases CYP1A1 but not CYP1B1 protein levels, indicating berberine preferential induction of CYP1A1 over CYP1B1 in MCF-7 cells.

Discussion

The prolonged exposure of estrogens is well known to play a role in breast cancer etiology (Feigelson et al., 1996; Cavalieri et al., 1997). Accumulative evidences suggest that estrogen-induced carcinogenesis depends on estrogen metabolism. Hydroxylation, which is the first step in the metabolism of estrogens, is initiated by CYP enzymes, especially CYP1A1 and CYP1B1 (Parl et al., 2009). CYP1A1 generates primarily 2-OH E_2, which is less toxic and has been considered as protective (Spink et al., 1992; Schumacher et al., 1999). However, CYP1B1 displays its primary activity on the C4 position to produce 4-OH E_2 (Hayes et al., 1996). Additionally, 4-OH E_2 generates free radicals with corresponding semiquinone and quinone forms, which cause depurinating adducts and readily lead to mutation events (Cavalieri et al., 1997; Parl et al., 2009). In various studies, the ratio of 2-OH E_2/4-OH E_2 has been shown to be a cancer marker. The relative activities of CYP1A1 and CYP1B1 are critical determinants of this ratio. Evidence from previous studies suggests that exposure to exogenous compounds alter the pathways of estrogen metabolism by modulating CYP1A1 and CYP1B1 in vivo and in vitro. The current study was conducted to assess the effects of berberine on the expression of estrogen-metabolizing enzymes CYP1A1 and CYP1B1.

Berberine, one of the major alkaloids, has been shown to possess anti-cancer activity including inhibition of protein synthesis, cell cycle progression and apoptosis in breast cancer cells. In the present study, we set out to explain the alterative mechanisms by which berberine may exert its anti-cancer effects. We found that berberine treatment produced greater inductions of the expression of CYP1A1 gene over CYP1B1 gene. Results from this study suggests that berberine, may shift E_2 metabolism to the production of 2-OH E_2 and reduce the level of the 4-OH E_2, thereby reducing the opportunity for carcinogenicity of this reactive estrogen metabolite.

The results of the present study showed also that berberine strongly induced CYP1B1 mRNA expression; however, the protein levels were unchanged in all cases. The lack of correlation between the CYP1B1 transcripts and protein levels was reported also in the previous studies (Sissung et al., 2006; Sarah et al., 2008; Szaefer et al., 2012). The difference between mRNA expression and the corresponding protein levels may indicate that the translation mechanism is saturated in the conditions of enhanced transcription. For example, quercetin treatment dramatically increased CYP1B1 mRNA levels but only slightly increased protein levels (Sarah et al., 2008). The transcripts of CYP1B1 were increased 11~fold after exposure to indole-3-carbinol, however, the protein levels were not changed (Szaefer et al., 2012). It is probably because that some posttranscriptional modifications such as proteolytic degradation can modulate CYP1B1 protein levels (Bandiera et al., 2005).

In the present study, we have reported that berberine differentially affects the expression of CYP1A1 and CYP1B1; this could be one possible mechanism by which the berberine exerts protective effects against estrogen-related breast carcinogenesis. Further studies are required to analyze the ratio of 2-OH E_2/4-OH E_2 following exposure to berberine by using high performance liquid chromatography (HPLC) analysis in vivo and in vitro, and the mechanisms of regulation of these genes by berberine also need to be further elucidated.

Acknowledgements

This research was supported by the National Science Foundation Research Grant of China (No.84402511), Natural Science Foundation of Chongqing (No. cstc2011jjA10004), Postdoctoral Science Foundation of China (No.2011M500669) and Special Postdoctoral Research Foundation of Chongqing (No.Yu xm201102007).

References

Badawi AF, Cavalieri EL, Rogan EG, (2001) Role of Human Cytochrome P450 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16α-Hydroxylation of 17β-Estradiol. Metabolism, **50**, 1001-3.

Bandiera S, Weidlich S, Harth V, et al (2005) Proteasomal degradation of human cytochrome P450 1B1 (CYP1B1): effect of the Asn453Ser polymorphism on the post-translational regulation of CYP1B1 expression. *Mol Pharmacol*, **67**, 435-43.

Cavalieri E, Frenkel K, Liehr JG, et al (2000) Estrogens as endogenous genotoxic agents-DNA adducts and mutations. *J Natl Cancer Inst Monogr*, **27**, 75-93.

Cavalieri EL, Stack DE, Devanesan PD, et al (1997) Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc Natl Acad Sci USA*, **94**, 10937-42.

Clemons M, Goss P, (2001) Estrogen and the risk of breast cancer. *N Engl J Med*, **344**, 276-85.

Feigelson HS, Henderson BE, (1996) Estrogens and breast cancer. *Carcinogenesis*, **17**, 2279-84.

Guo Y, Li F, Ma XC, et al (2011a) CYP2D plays a major role in berberine metabolism in liver of mice and humans. *Xenobiotica*, **41**, 996-1005.

Guo Y, Pope C, Cheng X, et al (2011b) Dose-response of berberine on hepatic cytochromes P450 mRNA expression and activities in mice. *J Ethnopharmacol*, **138**, 111-8.

Hayes CL, Spink DC, Spink BC, et al (1996) 17b-Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. *Proc Natl Acad Sci USA*, **93**, 9776-81.

Jemal A, Bray F, Center MM, et al (2011) Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Liehr JG, Ricci MJ, (1996) 4-Hydroxylation of estrogens as marker of human mammary tumors. *Proc Natl Acad Sci USA*, **93**, 3294-6.

Livak KJ, Schmittgen TD, (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(–Delta Delta C(T)) Method. *Methods*, **25**, 402-8.

Parl FF, Dawling S, Roodi N, et al (2009) Estrogen Metabolism and Breast Cancer A Risk Model. *Ann N Y Acad Sci*, **1155**, 68-75.

Sarah M, Chhabra MJ, Bhat HK, (2008) Preferential induction of cytochrome P450 1A1 over cytochrome P450 1B1 in human breast epithelial cells following exposure to quercetin. *J Steroid Biochem Mol Biol*, **110**, 157-62.

Schumacher G, Kataoka M, Roth JA, et al (1999) Potent antitumor activity of 2-methoxyestradiol in human pancreatic cancer cell lines. *Clin Cancer Res*, **5**, 493-9.

Sissung TM, Price DK, Sparreboom A, et al (2006) Pharmacogenetics and regulation of human cytochrome P450 1B1: implications in hormone-mediated tumor metabolism and a novel target for therapeutic intervention. *Mol Cancer Res*, **4**, 135-50.

Spink DC, Eugster HP, Lincoln DW, et al (1992) 17b-Estradiol hydroxylation catalyzed by human cytochrome P450 1A1: a comparison of the activities induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in MCF-7 cells with those from heterologous expression of the cDNA. *Arch Biochem Biophys*, **293**, 342-8.

Szaefer H, Licznerska B, Krajka-Kuźniak V, et al (2012) Modulation of CYP1A1, CYP1A2 and CYP1B1 Expressio by Cabbage Juices and Indoles in Human Breast Cell Lines. *Nutr Cancer*, **64**, 879-88.

Wu X, Li Q, Xin H, et al (2005) Effects of berberine on the blood concentration of cyclosporin A in renal transplanted recipients: clinical and pharmacokinetic study,. *Eur J Pharmacol*, **61**, 567-72.

Yager JD, Davidson NE, (2006) Estrogen carcinogenesis in breast cancer. *N Engl J Med*, **354**, 270-82.

Zhu BT, Conney AH, (1998) Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? *Cancer Res*, **58**, 2269-77.