RESEARCH ARTICLE

CYP3A4 Expression in Breast Cancer and its Association with Risk Factors in Mexican Women

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Abstract

Background: In Mexico, breast cancer (BCa) is the leading type of cancer in women. Cytochrome P450 (CYP450) is a superfamily of major oxidative enzymes that metabolize carcinogens and many antineoplastic drugs. In addition, these enzymes have influence on tumor development and tumor response to therapy. In this report, we analyzed the protein expression in patients with BCa and in healthy women. Links with some clinic-pathological characteristic were also assessed. Materials and Methods: Immunohistochemical analyses were conducted on 48 sets of human breast tumors and normal breast tissues enrolled in Hospital Militar de Especialidades de la Mujer y Neonatologia and Hospital Central Militar, respectively, during the time period from 2010 to 2011. Informed consent was obtained from all participants. Statistical analysis was performed using χ^2 or Fisher exact tests to estimate associations and the Mann Whitney U test for comparison of group means. Results: We found a significant CYP3A4 overexpression in BCa stroma and gland regions in comparison with healthy tissue. A significant association between protein expression with smoking, alcoholism and hormonal contraceptives use was also observed. Additionally, we observed estrogen receptor (ER) and progesterone receptor (PR) positive association in BCa. Conclusions: We suggest that CYP3A4 expression promotes BCa development and can be used in the prediction of tumor response to different treatments. One therapeutic approach may thus be to block CYP3A4 function.

Keywords: Breast cancer - CYP3A4 - protein expression - gene environment interactions - Mexico

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Introduction

In 2011, World Health Organization (WHO) estimated that the leading cause of worldwide death during 2008 was cancer with 7.6 million cases mainly lung, stomach, liver, colon and breast cancer (BCa) (World Health Organization, 2012). In Mexico, BCa is the most common malignancy in women, followed by cervical cancer. Currently, BCa etiology has been attributed to factors such as advanced age, reproductive, personal and family history, estrogen metabolism, geographic area and diet (Lopez-Carrillo, 2003). These observations suggest that environmental factors, diet, physical inactivity and obesity, as well as the increase in the consumption of alcohol and saturated fat and sugar diet are involved in BCa progression (Blacam et al., 2012; Canchola et al., 2012; Nelson et al., 2012; Xing et al., 2014).

Cytochromes P450 (CYP450) is a superfamily of constitutive and inducible enzymes that metabolize a large amount of environmental chemicals and endogenous substances such as environmental toxins, therapeutic drugs and steroid hormones. This group of enzymes is involved in several biological processes, including carcinogenesis, drug metabolism and cell signaling (Mc Fadyen et al., 2004). Therefore, CYP450 may be a valuable therapeutic target, since they may influence the response of tumors to chemotherapeutic agents (Mc Fadyen et al., 2004; Bruno and Njar, 2007; Michael and Doherty, 2007).

CYP3A4 is involved in the metabolism of therapeutic agents, environmental and endogenous compounds. CYP3A4 is expressed in the prostate, breast, stomach, colon and small intestine and liver (Huang et al., 1996; Lown et al., 1997; Guengerich, 1999). CYP3A4 plays an important role in the testosterone (2β, 6β, o 15β-hydroxytestosterone) and estrogen (4-y 16α-hydroxylation) oxidation (Shou et al., 1997; Niwa et al., 1998). In recent years have been described several genetic polymorphisms that affect the expression of this gene (Chu and Fyles, 2007). CYP3A4 protein expression has been observed in cancer tissues (Takahara et al., 2011; Weiss and Haefeli, 2011) due the induction of different kind of substances, resulting in an increase in transcription and expression of CYP3A4

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(Basseville et al., 2011).

The evidence suggests that overexpression of CYP3A4 could be associated with BCa. Huang et al. (1996) studied CYP's gene expression levels of mRNA in BCa; CYP3A4 mRNA was more expressed in normal breast tissues (70%) than tumoral tissues (18%) (Huang et al., 1996). Zheng et al. (2001) evaluated the association between urinary cortisol levels and the risk of BCa in a subgroup of women in Shanghai, China. They found a strong association between BCa risk and urinary levels of 6β-hydroxycortisol. It also showed that increasing in the risk of dose-response manner. This association appears to be higher in older women (Zheng et al., 2001). Currently, there are no data in Mexico about the CYP3A4 expression and its possible association with the development of BCa. The aim of this study was to evaluate, protein expression of CYP3A4 in Mexican women with BCa and in normal mammary gland.

Materials and Methods

Biological samples

All samples of human mammary carcinomas were obtained from 48 BCa patients with radical mastectomy surgery diagnosed in the Department of Pathology, Hospital Militar de Especialidades de la Mujer y Neonatologia, Secretaria de la Defensa Nacional (SEDENA) in Mexico City. 48 normal tissues were obtained for reduction of mammary gland donated by the Department of Plastic Surgery, Hospital Central Militar (SEDENA).

The histological classification of the carcinomas, as well as the evaluation of non-tumor breast lesions, were made according to standard diagnostic procedures and confirm by three pathologists. The sample collection was conducted from October 2010 to November 2011 and was considered inclusion, exclusion and elimination criteria. Patients were asked to read and sign an Informed Consent in agreement with requirements of the Ethical approval provided by the Bioethics and Research Committees of the Hospital Militar de Especialidades de la Mujer y Neonatologia in Mexico with registration number SI-378. Collection information of demographic status, tumor characteristics, as well as medical history and lifestyle behavior in patients was used. The human experimentation guidelines of these committees were followed.

Immunohistochemistry study

Inmunohistochemical analyzes were performed on slide prepared with histological tissues previously fixed with 10% formalin and embedded in paraffin. The cuts included on slides were deparaffinezed and heated to unmask antigenic sites; the endogenous peroxidase activity was blocked with 0.03% H_2O_2 in absolute methanol. Tissue sections were incubated overnight at 4°C with a 1:100 dilution of polyclonal antibody against CYP3A4 (Santa Cruz Biotechnology, CA, U.S.A.), respectively in TRIS solution.

Primary antibody was removed and washed twice repeated with TRIS solution; the sections were incubated with a 1:500 dilution of rabbit or goat polyclonal antibody as secondary antibody (Santa Cruz Biotechnology, CA, U.S.A.) and were washed twice repeated with TRIS solution. Bound antibodies were detected with the Avidin-Biotin complex (ABC-kit Vectatastain) and diaminobenzidine as the substrate. After washing repeatedly with TRIS solution, sections were counterstained with hematoxylin. All sections were incubated under the same conditions with the same concentration of antibody in the same run and therefore, immunostaining was comparable. All specimens were examined by Axiovert 200M inverted confocal microscope (Carl Zeiss, Jena, Germany). For automated morphometric analysis the percentage of positive cells (brown color) was determined with a computerized image analyzer KS-3003.0 (Carl Zeiss, Jena, Germany). This equipment automatically detects positive cells per field by determining their percentage. Five random fields were examined at a magnification of 40x (total area 1,584,000 m^2). The results were expressed as a percentage.

Statistical analysis

χ^2 test or Fisher exact test was used to estimate the association between individual clinicpathological factors and risk of BCa. Mann Whitney U test was used for compare means between groups. Statistical analysis was performed using SPSS v19 for Windows XP (SPSS UK, Ltd, Woking, UK). $p<0.05$ was regarded as significant.

Results

Available clinical and histological data on all patients are summarized in Table 1. Tissue samples were collected

Table 1. Characteristics of Patients with BCa

Factors	N	Mean	Deviation	Variance
Age (years)	48	58.3	10.90	119.500
Height (meters)	45	1.5	0.06	0.004
Weight (Kilograms)	46	67.7	13.40	179.500
BMI (Kilograms/meters^2)	45	29.2	5.90	34.900
Menarche (years)	48	12.5	1.40	2

*BMI (Body Mass Index)

Table 2. Descriptive Analysis of Patients Diagnosed with BCa

Region	Frequency	Percentage
Center	32	66.7
North	9	18.8
South	6	12.5
Total	48	100

Table 3. Estrogen Receptor Association with Other Receptors, Markers and Sociodemographic Variables in Samples from Patients with BCa

Variables	Positive (N=21)	Negative (N=27)	p	OR (IC95%)
PR	11 (52.4%)	4 (14.8%)	0.007*	6.3 (1.6-4.7)
Ki67	6 (28.6%)	15 (55.6%)	0.05*	0.3 (0.0-1.0)
ERBB2	8 (40.0%)	13 (50.0%)	0.35*	0.6 (0.2-2.1)
Smoking	2 (9.5%)	8 (29.6%)	0.08*	0.2 (0.0-1.3)
Alcoholism	1 (4.8%)	7 (25.9%)	0.05*	0.1 (0.0-1.2)
Metastases	12 (57.1%)	8 (30.8%)	0.06*	3.0 (0.9-9.9)
Weight	71.9 (9.3%)	64.7 (15.0%)	0.06**	

*Chi Square was applied or, where appropriate the Fisher's Exact test in all contrasts, Estrogen receptor (ER), Progesterone receptor (PR); **Mann-Whitney U test was applied

Table 4. Association of CYP3A4 Expression with Some Clinicopathological Factors

Factor	p value	Test Used
Smoking	0.018	Chi Square
Alcoholism	0.008	Chi Square
Hormonal Contraceptives	0.042	Chi Square
Age	0.096	Chi Square

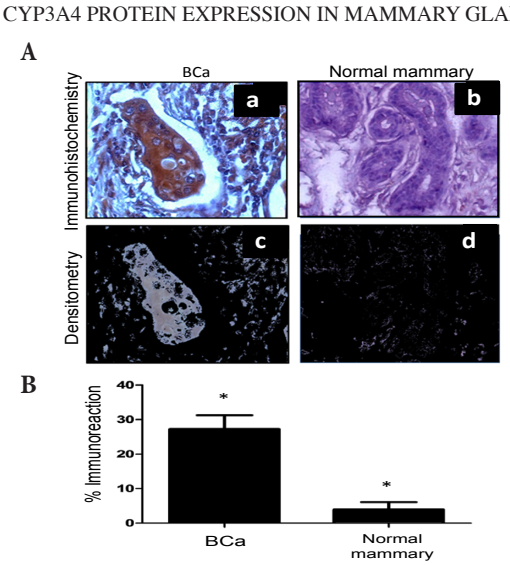

Figure 1. CYP 3A4 Expression in Gland of BCa and in Healthy Groups. A) in (a) BCa tissue immunohistochemistry; in (b) densitometric analysis in cancer tissue expression; in (c) healthy breast tissue immunohistochemistry; in (d) densitometric analysis of the healthy breast expression. In both groups was determined immunoreactivity percentage per field (40x). B) we showed a significant increase in immunoreactivity of CYP3A4 in BCa group ($p<0.0001$)

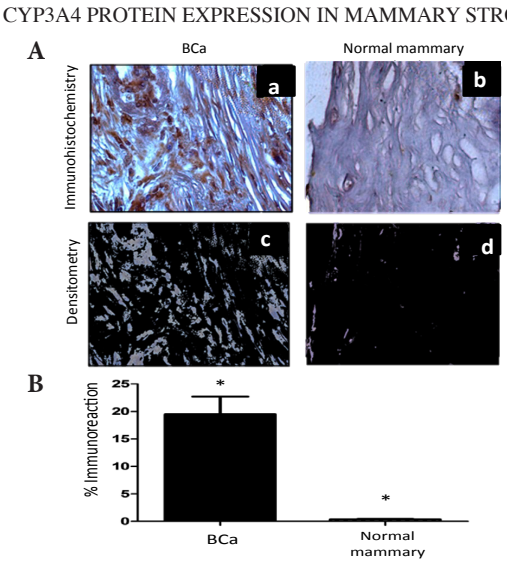

Figure 2. CYP 3A4 Expression in Stroma of BCa and in Healthy Groups. A) in (a) BCa tissue immunohistochemistry; in (b) densitometric analysis in cancer tissue expression; in (c) healthy breast tissue immunohistochemistry; in (d) densitometric analysis of the healthy breast expression. In both groups was determined immunoreactivity percentage per field (40x). B) we showed a significant increase in immunoreactivity of CYP3A4 in BCa group ($p<0.0001$)

from Mexican females who ranged from 31 to 81 years of age and the average age at diagnosis of BCa patients was 56.9±11.6 years. Patients had non physical actitivity (98%), tobacco (20.8%) and alcohol (16.8%) consumption. Only 14.6% had biomass exposure, 60.4 % did not have familiary history of BCa and 75% has overweight/obesity. Most of the patients were coming from the center of Mexico (Table 2). Table 3 shows estrogen receptor (ER) associations with other molecular markers and demographic variables studied in BCa patients. Finally we found that CYP3A4 protein expression was associated with some clinical pathological features such as hormonal contraceptives use, tobacco and alcohol consumption ($p<0.05$), see Table 4. We found no association with other factors such as drug biomass exposure, nonphysical activity, and family history of cancer, body mass index, nulliparity, chemotherapy, menopause and metastases. Figure 1 and 2 shows CYP3A4 protein expression. This cytochrome was overexpressed significantly in BCa stroma and gland regions ($p<0.05$).

Discussion

This study has defined the protein expression of CYP3A4 in Mexican women with breast cancer and associated expression of this cytochrome with some sociodemographic factors namely smoking, alcoholism, hormonal contraceptives and age. Additionally, we evaluated estrogen receptor (ER) association with histopathological markers such as progesterone receptor (PR), Ki67 and HER2 and with sociodemographic factors smoking, alcoholism, metastases, and body weight.

The results obtained in the characteristics of patients are agree with those reported in studies in Mexican women, in which, the age range of risk for developing BCa is between 40 to 69 years, the median age of menarche is 12 years and the median age of menopause has been reported between 47 and 48.2 years (Garrido-Latorre et al., 1996; Torres-Mejia et al., 2005; Mendez-Estrada et al., 2006). The BCa is more common in the north and center of Mexico (Sistema Nacional de Informacion en Salud, 2012).

We observed that the majority of patients had a sedentary life and overweight/obesity. The lifestyle behavior such as smoking, use of oral contraceptives or thyrotropin-releasing hormone, obesity or overweight and hyperglycemia are associated with the risk of BCa development as reported in other studies (Llatrakis et al., 2011; Nelson et al., 2012). In other study, we demonstrated that BCa risk was associated with chronic diseases, family history of cancer and menopausal status (Cardenas-Rodriguez et al., 2012).

In our study, ER expression was found associated only with PR status. As indicated in other studies, positive expressions of ER and PR in breast cancer correlate with better survival and response to estrogen antagonists such as tamoxifen, regardless of tumor size, stage and age (Jacquemier et al., 1998; Shapiro and Recht 2001). ER and PR association has also been reported in male breast carcinoma (Wang-Rodriguez et al., 2002). In this work showed that PR-positive associated with ER-positive

tumors appeared to have a better survival but did not offer any differences in disease-free survival. In our results no association existed between ER expression with Ki67, HER-2, smoking, alcoholism, metastases and body weight.

The human CYP3A forms have been extensively studied from several perspectives. They are collectively the most abundant P450s. have the largest number of drug substrates, and illustrate many of the before mentioned issues of expression, polymorphism, and clinical impact (Guengerich, 1999; Hassan and Yusoff, 2011). In relation with BCa, the level of CYP3A4 immunohistochemistry expression was reported to be associated with response rate to docetaxel treatment (Miyoshi et al., 2005).

In this study, we observed CY3A4 overexpression in BCa in comparison with healthy gland. Recently, CYP3A4 expression has been identified in BCa growth using specific RNAi gene silencing in cell lines MCF-7, T47D and MDA-MB-231 (Mitra et al., 2011). These authors showed that CYP3A4 synthesizes epoxyeicosatrienoic acids that promote BCa growth. In other work, CYP3A4 gene expression was observed, although in low levels, in BCa. The authors suggest that CYP3A4 expression is predictive value for any estimation of response to anticancer drugs metabolically inactivated by this cytochrome (Vaclavikova et al., 2007). In other study found positive immunohistochemical CYP3A4/A5 staining in 25% of inspected mammary tumors (Haas et al., 2006).

Moreover, we showed an association between CYP3A4 expression with alcohol consumption, tobacco consumption and oral contraceptives use. These results suggest that a change in the CYP3A4 gene expression, or polymorphisms, in BCa alters the function of this CYP in the alcohol, tobacco and oral contraceptives metabolism (Ragin et al., 2010; Bandala et al., 2012).

In summary, we suggest that the expression of CYP3A4 promotes BCa development and can used clinically in predicting tumor response to treatment against BCa. Moreover, one potential therapeutic approach may be to block CYP3A4 function.

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References

Bandala C, Floriano-Sanchez E, Cardenas-Rodriguez N, Lopez-Cruz J, Lara-Padilla E (2012). RNA Expression of cytochrome P450 in Mexican women with breast cancer. *Asian Pac J Cancer Prev*, **13**, 2647-53.

Basseville A, Preisser L, de CarneTrecesson S, et al (2011). Irinotecan induces steroid and xenobiotic receptor (SXR) signaling to detoxification pathway in colon cancer cells. *Mol Cancer*, **10**, 80-92.

Blacam C, Ogunleye AA, Momoh AO, et al (2012). High body mass index and smoking predict morbidity in breast cancer surgery: a multivariate analysis of 26,988 patients from the national surgical quality improvement program database. *Ann Surg*, **255**, 551-5.

Bruno RD, Njar VC (2007). Targeting cytochrome P450 enzymes: a new approach in anti-cancer drug development. *Bioorg Med Chem*, **15**, 5047-60.

Canchola AJ, Anton-Culver H, Bernstein L, et al (2012). Body size and the risk of postmenopausal breast cancer subtypes in the California Teachers Study cohort. *Cancer Causes Control*, [Epub ahead of print].

Cardenas-Rodriguez N, Lara-Padilla E, Bandala C, et al (2012). CYP2W1, CYP4F11 and CYP8A1 polymorphisms and interaction of CYP2W1 genotypes with risk factors in Mexican women with breast cancer. *Asian Pac J Cancer Prev*, **13**, 837-46.

Chu W, Fyles A (2007). Association between CYP3A4 genotype and risk of endometrial cancer following tamoxifen use. *Carcinogenesis*, **28**, 2139-42.

Garrido-Latorre EC, Lazcano-Ponce L, Lopez-Carrillo M, et al (1996). Age of natural menopause among women in Mexico city. *Int J Gynaecol Obstet*, **53**, 159-66.

Guengerich FP (1999). Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol*, **39**, 1-17.

Haas S, Pierl C, Harth V, et al (2006). Expression of xenobiotic and steroid hormone metabolizing enzymes in human breast carcinomas. *Int J Cancer*, **119**, 1785-91.

Hassan BA, Yusoff ZB (2011). Genetic polymorphisms in the three Malaysian races effect granisetron clinical antiemetic actions in breast cancer patients receiving chemotherapy. *Asian Pac J Cancer Prev*, **12**, 185-91.

Huang Z, Fasco MJ, Figge HL, et al (1996). Expression of cytochromes P450 in human breast tissue and tumors. *Drug Metab Dispos*, **24**, 899-05.

Jacquemier JD, Penault-Llorca FM, Bertucci F, et al (1998). Angiogenesis as a prognostic marker in breast carcinoma with conventional adjuvant chemotherapy: a multiparametric and immunohistochemical analysis. *J Pathol*, **184,** 130-5.

Kim SY, Suzuki N, Santosh Laxmi YR, et al (2003). Alpha-hydroxylation of tamoxifen and toremifene by human and rat cytochrome P450 3A subfamily enzymes. *Chem Res Toxicol*, **16**, 1138-44.

Knaul FM, Lopez Carrillo L, Lazcano Ponce E, et al (2009). Cancer de mama: un reto para la sociedad y los sistemas de salud. *Salud Publica Mex*, **51**, 135-7.

LIatrakis G, Iavazzo C, Zervoudis S, et al (2011). The role of oral contraception use in the occurrence of breast cancer. A retrospective study of 405 patients. *Clin Exp Obstet Gynecol*, **38**, 225-7.

Lopez-Carrillo L, Bravo-Alvarado J, Poblano-Verastegui O, et al (1997). Reproductive determinants of breast cancer in Mexican women. *Ann N Y Acad Sci*, **837**, 537-50.

Lopez-Carrillo L (2003). El cancer mamario: epidemiologia y prevencion. In: Tamayo RP. El cancer en Mexico. Mexico, D.F.: El Colegio Nacional, 443-56.

Lown KS, Bailey DG, Fontana RJ, et al (1997). Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest*, **99**, 2545-53.

Mc Fadyen MCE, Melvin WT, Murray GI (2004). Cytochrome P450 enzymes: novel options for cancer therapeutics. *Mol Cancer Ther*, **3**, 363-71.

Mendez -Estrada RO, Valencia M, Melendez Torres JM (2006). Menarche age in teenagers of the Northwest of Mexico. *Arch Latinoam Nutr*, **5**, 1-3.

Michael M, Doherty MM (2007). Drug metabolism by tumours: its nature, relevance and therapeutic implications. *Expert Opin Drug*, **3**, 786-03.

Mitra R, Gou Z, Milani M, et al (2011). CYP3A4 mediates growth of estrogen receptor-positive breast cancer cells in part by inducig nuclear translocation of phospho-Stat3 through biosynthesis of (+)-14, 15-epoxyeicosatrienoin acid (EET). *J Biol Chem*, **286**, 17543-59.

Miyoshi Y, Taguchi T, Kim SJ, et al (2005). Prediction of response to docetaxel by immunohistochemical analysis of CYP3A4 expression in human breast cancers. *Breast Cancer*, **12**, 11-5.

Nelson HD, Zakher B, Cantor A, et al (2012). Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis. *Ann Intern Med*, **15**, 635-48.

Niwa T, Yabusaki Y, Honma K, et al (1998). Contribution of human hepatic cytochrome P450 isoforms to regioselective hydroxylation of steroid hormones. *Xenobiotica*, **28**, 539-47.

Ragin CC, Langevin S, Rubin S, et al (2010). Review of studies on metabolic genes and cancer in populations of African descent. *Genet Med*, **12**, 12-8.

Shapiro C, Recht A (2001). Side effects of adjuvant treatment of breast cancer. *N Engl J Med*, **344**, 1997-2008.

Shou M, Korzekwa KR, Brooks EN, et al (1997). Role of human hepatic cytochrome P450 1A2 and 3A4 in the metabolic activation of estrone. Carcinogenesis, **18**, 207-14.

Sistema Nacional de Informacion en Salud (2012). www.sinais.salud.gob.mx/basesdedatos/estandar.html.

Takahara T, Nitta H, Hasegawa Y, et al (2011). Using sorafenib for recurrent hepatocellular carcinoma after liver transplantation- interactions between calcineurin inhibitor. *Transplant Proc*, **4**, 2800-5.

Torres-Mejia G, Cupul-Uicab LA, Allen B, et al (2005). Comparative study of correlates of early age at menarche among Mexican and Egyptian adolescents. *Am J Hum Biol,* **17**, 54-8.

Vaclavikova R, Hubackova M, Stribrna-Sarmanova J, et al (2007). RNA expression of cytochrome P450 in breast cancer patients. *Anticancer Res*, **27**, 4443-50.

Wang-Rodriguez J, Cross K, Gallagher S, et al (2002). Male breast carcinoma: correlation of ER, PR, Ki-67, Her2-Neu, and p53 with treatment and survival, a study of 65 cases. *Mod Pathol*, **15**, 853-61.

Weiss J, Haefeli WE (2011). Interaction potential of the endothelin-A receptor antagonist atrasentan with drug transporters and drug-metabolising enzymes assessed *in vitro*. *Cancer Chemother Pharmacol*, **68**, 1093-8.

World Health Organization (2012). www.who.int/mediacentre/factsheets/fs297/es/index.html.

Xing MY, Xu SZ, Shen P (2014). Effect of low-fat diet on breast cancer survival:a meta-analysis. *Asian Pac J Cancer Prev*, **15**, 1141-4.

Zheng W, Jin F, Dunning LA, et al (2001). Epidemiological study of urinary 6beta-hydroxycortisol to cortisol ratios and breast cancer risk. *Cancer Epidemiol Biomarkers Prev,* **10**, 237-42.

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