REVIEW ARTICLE

Estrogen Metabolism and Breast Cancer Risk – A Review

Okobia M.N.[1,2] *and Bunker C.H.*[2]

ABSTRACT

The standard paradigm providing a general mechanistic explanation for the association of cumulative, excessive estrogen exposure and breast cancer risk is that estrogen and perhaps progesterone affect the rate of cell division; and thus manifest their effect on the risk of breast cancer by causing proliferation of breast epithelial cells. Proliferating cells are susceptible to genetic errors during DNA replication which, if uncorrected, can ultimately lead to a malignant phenotype. This standard paradigm has recently been expanded to encompass emerging research data supporting a complementary genotoxic pathway mediated by the generation and redox cycling of reactive oxygen species through the metabolic effects of estrogen metabolites such 4- and 16a-hydroxy catechols. This paradigm shift is necessitated by evidence of estrogen-induced carcinogenesis in several animal and human models following exposure to these estrogen metabolites. This review examines some of the available evidence relating these estrogen metabolites to animal and human breast carcinogenesis. (*Afr J Reprod Health* 2006; 10[1]:13-25)

RÉSUMÉ

Le métabolisme oestrogénique et le risque du cancer de sein—Compté rendu. Un paradigme standard qui donne une explication mécaniste par l'association de l'exposition cumulative et excessive de l'oestrogène et le risque du cancer du sein est que l'oestrogène et peut-être la progestérone affecte le rythme de la division de la cellule et manifeste ainsi leur effet sur le risque du cancer du sein en provoquant la prolifération des cellules épithéliale du sein. Les cellules qui se prolifèrent sont succeptibles aux erreurs génétiques pendant la réplication de l'ADN. Si cela n'est pas corrigé, il risque d'aboutir éventuellement à un phénotype maligne. Ce paradigme standard a connu récemment une expansion qui permet d'encompasser les données de recherche naissante appuyant une voie génotoxique complimentaire dont la génération et le recyclage redox des espèces de l'oxygène réactive servaient d'intermédiaire à travers les effets métaboliques des métabolites de l'oestrogène tels 4– et les catéchols 16a – hydroxyl. Ce déplacement du paradigme a été causé par l'évidence de la carinogenèse provoquée par l'oestrogène dans beaucoup de modèles humain et animal suite à une exposition à ces métabolites de l'oestrogène aux carcinogènes du sein de l'animal et des êtres humains. (*Rev Afr Santé Reprod* 2006; 10[1]:13-25)

KEY WORDS: *Estrogens, catecholestrogens, methoxyestrogens, breast cancer*

[1]*Department of Surgery, College of Medical Sciences, University of Benin, Benin City, Nigeria and* [2]*Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261, U.S.A.*

Correspondence: *Dr. M.N. Okobia, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Room A521, Crabtree Hall, 130 Desoto Street, Pittsburgh, PA 15261, U.S.A. E-mail: michaelokobia@Yahoo.com*

Introduction

A large volume of literature has accumulated in the past half century documenting the possible role of endogenous and exogenous estrogen exposure in breast cancer susceptibility in both animals and humans. This has been made possible as a result of better appreciation of the biological activities of the diverse estrogen metabolites and the identification of candidate genes encoding the enzymes involved in the aromatisation of C-19 androgens to C-18 estrogens, phase I hydroxylation of estrogens to catechol estrogens, and the phase II metabolic reactions involving O-methylation, and conjugation of catechol estrogens to methoxyestrogens. It has been demonstrated that contrary to previous beliefs, in-situ estrogen synthesis in the breast by aromatisation in breast stromal cells is a major source of estrogens in the breast of humans.[1] There is evidence for a complex interaction between aromatase and cytokines such as TNF-a, Interleukin-6 (IL-6), and PGE_2 in in-situ estrogen synthesis in the human breast. There has also been a better understanding of the biological activities of various estrogen metabolites. The catechol estrogens including 4-, and 16a-hydroxyestradiol have been shown to bind more tightly and activate the classical estrogen receptor, stimulating higher cell proliferation rate compared to estradiol while 2-catechol estrogen has much less binding affinity to the estrogen receptor.[2,3] 4-Hydroxyestradiol has been shown to undergo metabolic redox cyling leading to the generation of superoxide radicals and chemically-reactive semiquinone/quinone intermediates. All these metabolites have been demonstrated to damage DNA and other cellular macromolecules, induce cell transformation and initiate tumorigenesis.[4] In contrast, 2-catechol estrogens lack carcinogenic activity and in fact, 2-methoxyestradiol has been shown to be a very potent inhibitor of tumour cell proliferation and angiogenesis.[5,6]

Molecular epidemiology studies relating the above actions of estrogens and its metabolites in animal models to human breast carcinogenesis have yielded conflicting results. For example, studies on the aromatase (CYP19) gene have reported positive association between the heterozygous tetranucleotide simple tandem repeat polymorphism (STRP) and breast cancer risk in some Caucasian populations but not in others.[7,8] Also, some CYP1A1 polymorphic variants such as the exon 7 polymorphism are associated with increased risk of breast cancer in African-American women but not in Caucasians.[9] In addition, the catecho-O-methyltransferase (COMT) gene polymorphisms have been shown to confer increased breast cancer risk more in Asian populations compared with most Caucasian populations studied to date despite the fact that the low-activity allele of the COMT gene has a much higher prevalence in Caucasian populations compared with Asian populations.[10-14]

This review examines the literature on the role of estrogen and its diverse metabolites on carcinogenesis in animal models and the emerging evidence demonstrating involvement of these estrogenic agents in human breast carcinogenesis.

Sources of endogenous estrogen

The most important estrogens in humans are 17b-estradiol (E_2), estrone (E_1) and estriol (E_3). They are all steroids consisting of 18 carbon atoms and characterised by an aromatic A ring. E_2, the most potent and important estrogen in non-pregnant women, is predominantly produced in the ovary by the granulosa cells of the active follicle from androgens delivered by the theca interna. During pregnancy, E_3 produced from androgenic precursors provided by the fetus and the mother, represents the major estrogen.[15] E_1, the third of the major endogenous estrogens, exists in metabolic equilibrium with E_2 due to the action of 17b-hydroxysteroid dehydrogenase.

In the classical pathway, the estrogen synthesis starts from cholesterol provided by lipoproteins. The sources of E_2 production in women are important to consider, since over-production may

result from altered regulation at any site. Estrogen can be made in several tissues. Aromatase, the enzyme catalysing the rate-limiting step in estrogen biosynthesis, is widely present throughout the body. The premenopausal ovary, which contains the highest level of aromatase, except for the placenta, is the major source of E_2 during the premenopausal years. Peripheral adipose tissue also contains aromatase and is a major source of this enzyme, since the mass of adipose tissue (particularly in obese women) is substantial. Breast tissue itself contains aromatase, both in its fatty components and in its epithelial cells, and can synthesise estrogen in-situ.

Importance of in-situ aromatase activity in breast cancer susceptibility

Emerging evidence suggests that estrogen produced in-situ, as opposed to E_2 made in other tissues and delivered to the breast via an endocrine mechanism, plays a major biologic role in breast physiology. Several lines of evidence support this concept:

1) demonstration of the aromatase enzyme and its messenger RNA in breast tissue by immunohistochemical and molecular biologic techniques,
2) studies in nude mice showing that the amount of estrogen made locally causes biologic effects, and
3) clinical studies of aromatase inhibitors in patients provide proof of the importance of in-situ production of estrogen in breast tissue.

Immunohistochemical studies have provided evidence for significant in-situ aromatase activity in breast cancer cells. Resulting data demonstrated high levels of aromatase staining in individual cells, supporting the concept that aromatase might act in an autocrine or paracrine fashion in breast tissue[16].

Further support for the importance of aromatase in breast tissue itself derives from studies in a nude mouse model developed by Yue *et. al*[17]. Using this model, these investigators examined the relative importance of uptake from plasma versus local E_2 synthesis in the breast tissue. This model involves the use of MCF-7 breast cancer cells transfected stably with aromatase (A+) that were implanted on one side of castrated nude mice. On the other side, sham-transfected MCF-7 cells (A-) were implanted. Administration of the aromatase substrate androstenedione caused no growth stimulation of aromatase-negative cells. This important control demonstrated that no aromatase activity is present in non-breast tissue in the mouse. Aromatase-positive cells implanted on the other side of the same animals were stimulated to grow by androstenedione, providing evidence of the biologic effect of aromatase present locally in the breast. This series of experiments in mice supports the hypothesis that an important determinant of tissue E_2 level is local production in the breast. If this hypothesis is correct, the level of E_2 produced in breast tissue may be the most important determinant of E_2-induced carcinogenesis. This conclusion is supported by the direct measurement of aromatase activity with elegant isotopic techniques in human breast tumours by Reed *et. al*[18]. These investigators demonstrated that 83%±9% (Standard deviation) of tumour estrogen levels resulted from in-situ aromatase in four of six tumors. If the local synthesis hypothesis is correct, measurement of concentration of E_2 in breast tissue itself would be the most precise predictor of later development of breast cancer.

Additional evidence for the role of in-situ aromatase synthesis in the breast is provided by the use of aromatase inhibitors in breast cancer therapy. Several studies including the European Organization for Research and Treatment of Cancer (EORTC) trial,[19] the ATAC (Arimidex, tamoxifen, alone or in combination) adjuvant breast cancer trial,[20] the Intergroup Exemestane Study (IES),[21] and the MA-17 trials[22] have demonstrated the benefits of selective aromatase inhibitors in the treatment of breast cancer both in the adjuvant setting and patients with metastatic disease.

Taken together, these data suggest that certain factors present in breast tissue can influence local production of estrogen and that these may be the prime determinants of tissue estrogen concentrations. If these concepts are correct, elevated plasma levels of estrogen would be associated with high tissue concentrations in some, but not in all, patients and breast cancer risk might only be increased in those with high tissue levels. Given emerging evidence suggesting that aromatase functional activity may vary in different individuals as a result of polymorphic variants in the aromatase gene, one can speculate that lifetime breast tissue estrogen exposure and susceptibility to breast cancer might differ from one individual to another.

Multiple pathways of NADPH-dependent estrogen hydroxylation

Endogenous estrogens (estradiol and estrone) can be hydroxylated at multiple positions by NADPH-dependent cytochrome P450 enzymes. Several extrahepatic target tissues or cultured cells from target tissues express estrogen-hydroxylating enzyme activities.[23] Although the functional role of several estrogen metabolites detected in experimental systems using liver and different target tissue microsomes are not fully known, attention has focused on the 2-, 4-, and 16a-hydroxylation pathways because of the known properties of metabolites in these pathways in animals and humans.

2-Hydroxylation

2-Hydroxylation of estradiol or estrone to a catechol is a major metabolic pathway in the liver whereas 4-hydroxylation to a different catechol represents a quantitatively minor pathway (usually <15% of 2-hydroxylation) in this organ.[24] In humans, cytochrome P450 1A2 and the 3A family are major enzymes for hepatic estrogen 2-hydroxylation.[25] It is of considerable interest that there are large interindividual differences in the 2-hydroxylation of estradiol or estrone by human liver samples[25] reflected by person-to-person differences in estrogen action in different individuals.

NADPH-dependent 2-hydroxylation of estradiol and/estrone has been observed with microsomes prepared from various extrahepatic tissues including the breast.[26] Available data indicates that 2-hydroxylation of estradiol by MCF-7 human breast cancer cells treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is catalysed predominantly by cytochrome P450 1A1/1A2.[27] Since cytochrome P450 3A4 (which has high estradiol 2-hydroxylase activity) is present in several extrahepatic tissues[28], it is believed that this cytochrome P450 isoform may contribute substantially to estradiol 2-hydroxylation in these tissues.

Important consequences of locally-formed 2-hydroxyestradiol and 2-hydroxyestrone

There is data suggesting that locally formed 2-hydroxyestrogens (2-hydroxyestradiol and 2-hydroxyestrone) may play important role in carcinogenesis in various organs including the breast. 1) Both catechol estrogens can bind the classical estrogen receptor, but with a markedly reduced binding affinity[2], and these metabolites possess much weaker hormonal potency as compared with the parent hormone, estradiol.[29] 2-Hydroxyestrone was reported to partially antagonize the growth stimulatory effect of estradiol in cultured human MCF-7 breast cancer cells.[30] 2) 2-Hydroxyestradiol and 2-hydroxyestrone (like 4-hydroxyestradiol) can undergo metabolic redox cycling[31,32] to generate free radicals such as superoxide and the chemically-reactive estrogen semiquinone/quinone intermediates[4] which may damage DNA and other cellular constituents.[33-35] Despite their potential for undergoing metabolic redox cycling and generating free radicals, 2-hydroxyestradiol and 2-hydroxyestrone (but not 4-hydroxyestradiol) have little or no tumorigenic activity towards the male Syrian hamster kidney.[36]

It is now well documented that lean women or women on low fat diets have a lower risk of breast cancer than obese women or women consuming high fat diets. This finding is explained by the fact that in lean women or those on low fat diets, metabolism of estrogens via the 2-hydroxylation pathway predominates with subsequent formation of 2-methoxyestrogens.[37] Consequently, this causes downregulation of cytokine and PGE_2 receptors in breast adipose stromal cells and reduces in-situ estrogen synthesis via peripheral aromatisation. In contrast, in obese women or subjects on high fat diets that are associated with reduced synthesis of 2-methoxyestrogens, any cytokines or PGE_2 within the breast could result in increased production of estrogens and subsequent risk of tumor development. There is also evidence suggesting that pesticides, which have been implicated in breast cancer, can also decrease estrogen 2-hydroxylation but increase 16a -hydroxylation.[38]

4-Hydroxylation

Although 2-hydroxylation of estradiol and estrone is the dominant pathway for catechol estrogen formation in liver microsomes, small amounts of 4-hydroxylated estradiol and estrone are also formed.[24-26] In human liver microsomes, the cytochrome P450 3A family is believed to play a major role in the 4-hydroxylation of estradiol.[25]

In contrast to the above observations indicating that 4-hydroxylation of estrogens is a minor pathway for catechol estrogen formation in liver, recent studies showed that 4-hydroxylation of estradiol is a dominant pathway for catechol estrogen formation in several extrahepatic target tissues including the breast.[39]

Actions of 4-hydroxyestradiol

Selective expression of estradiol 4-hydroxylase activity in target cells does not inactivate the parent estrogen but may be a mechanism for maintaining strong hormonal activity in these cells or for exerting other unknown biological effects that are not shared with estradiol. Some suggested important functions of 4-hydroxyestradiol related to estrogen carcinogenesis include:

1) 4-Hydroxyestradiol is similar to estradiol in its ability to bind and activate the classical estrogen receptor.[40] Interestingly, the interaction of this estrogen metabolite with the estrogen receptor appears to occur with a reduced dissociation rate compared with estradiol[40] suggesting that the association of 4-hydroxyestradiol with the estrogen receptor may last longer than that for its parent hormone, estradiol.
2) 4-Hydroxyestradiol undergoes metabolic redox cycling[31,32] to generate free radicals such as superoxide and the chemically-reactive estrogen semiquinone/quinone intermediates. These metabolic intermediates may damage DNA and other cellular constituents,[33-35] induce cell transformation and initiate tumorigenesis.[41-43]
3) 4-Hydroxyestradiol is a strong carcinogen towards the hamster kidney (~100% tumor incidence) under conditions where 2-hydroxyestradiol is not carcinogenic.[36]

The strong carcinogenicity of 4-hydroxyestradiol may be due to its potential genotoxicity (redox cycling plus reactive semiquinone intermediate) as mentioned above and its potent growth stimulatory effect as recently demonstrated with cultured kidney proximal tubule cells.[44,45]

16a -Hydroxylation

In male and female rats, the constitutively expressed hepatic cytochrome $P450_{C\text{-}M/F}$ (thought to be a member of the 2D family[46]) has a high catalytic activity for the 2- and 16a-hydroxylation of estradiol and weak activity for 6a-, 6b-, and 15a-hydroxylation of estradiol and for the conversion of estradiol to estrone.[47] In humans, a recent study showed that cytochrome CYP3A4

has strong catalytic activity for estrone 16a-hydroxylation.[69]

16a-Hydroxylated estrogen metabolites are known to possess some unique properties including; 1)16a-hydroxyestrone and 16a-hydroxyestradiol, like 4-hydroxyestradiol, retain potent hormonal activity by activating the classical estrogen receptor[49], 2) a covalent reaction of 16a-hydroxyestrone with the estrogen receptor has been reported[50], and there is a preliminary study suggesting that 16a-hydroxyestrone may activate the classical estrogen receptor-mediated oncogene expression and growth stimulation for a prolonged period[51]. Mechanistically, a Schiff base is formed from 16a-hydroxyestrone by reacting with amino groups in proteins[52]. In principle, 16a-hydroxyestrone may also react covalently with other amino-containing macromolecules including DNA.

The observations of Fishman and colleagues[53] are in keeping with the carcinogenic potential of 16a-hydroxyestradiol. These investigators found that whole-body 16a-hydroxylation of estradiol was ~50% greater in post-menopausal patients with breast cancer than in healthy control subjects and enhanced 16a-hydroxylation was also detected in healthy women at high risk for breast cancer (from cancer-prone families).[54] In addition, they noted that estrogen 16a-hydroxylation was higher in the terminal duct lobular units in cancerous or non-cancerous tissues from women with breast cancer compared with breast tissue (reduction mammoplasties) from women without cancer.[55]

O-Methylation and Methoxyestrogens

The O-methylation of catechol estrogens is catalysed by catechol-O-methyltransferase (COMT), an enzyme that also catalyzes the O-methylation of physiologically important catecholamines and many other catechols.[56] Catechol-O-methyltransferase activity is present in large amounts in liver and kidney, and it also exists in significant amounts in red blood cells, uterine endometrium, the mammary gland and many other tissues.[57] In all tissues examined thus far, catechol-O-methyltransferase activity is found almost exclusively in the cytosol, but some activity is also found in the membrane-bound form.[58] Because of the rapid enzymatic O-methylation of catechol estrogens, 2-methoxyestrone was previously shown to be one of the most abundant estrogen metabolites in human plasma and urine.[59] In pregnant women, the mean plasma concentration of unconjugated 2-methoxyestrone is ~400 pg/ml.[60] Interestingly, 2-methoxyestrone and 2-methoxyestradiol have higher binding affinities for sex hormone binding globulin than estradiol and 2-hydroxyestradiol,[61] and the high binding affinities of these two methoxyestrogens may contribute to their high plasma levels.

The monomethylated estrogen metabolites have little or no estrogen receptor binding affinities (<1%) when compared to estradiol,[59] and they lack estrogenic effects on the uterus.[62] Previous studies on the chemical reactivity and potential genotoxicity of catechol estrogens[33-35,43] have led to the suggestion that enzymatic O-methylation was primarily a detoxication pathway for these catechol intermediates. However, there are several studies indicating that 2-methoxyestradiol exerts unique biological effects that are not associated with estradiol, 2-hydroxyestradiol or other methoxy derivatives of estradiol.[63] For instance, treatment of rats with 2-methoxyestradiol decreases cholesterol and triglyceride levels in the blood and this effect is not associated with activation of the classical estrogen receptor.[64] In addition, 2-methoxyestradiol inhibits the growth of certain human breast cancer cell lines in vitro and in vivo, and it is a potential inhibitor of estrogen-dependent carcinogenesis. 2-Methoxyestradiol inhibits the proliferation of several cancer cell lines in vitro,[63] and human breast cancer cell lines (estrogen receptor positive or negative) were particularly sensitive to the cytotoxic effect of 2-methoxyestradiol.[65] Additional studies indicated that 2-

methoxyestradiol disrupted microtubule function and was a potent inhibitor of angiogenesis.[63] Administration of 2-methoxyestradiol inhibited the growth of transplanted meth-A sarcoma and B16 melanoma in C3H mice,[63] and oral administration of 2-methoxyestradiol also inhibited the growth of a human breast carcinoma cell line (estrogen receptor negative) in immunodeficient mice.[66] It is noteworthy that 2-methoxyestradiol is among the most potent endogenous inhibitors of angiogenesis known, and its antiangiogenic effect as tested in vitro is highly specific and is not shared by several closely related structural analogs.[63] The effects of 2-methoxyestradiol to disrupt microtubule function, to inhibit angiogenesis and to inhibit the proliferation of breast cancer cells in vitro and in vivo suggest that factors enhancing the 2-hydroxylation of estradiol and the subsequent formation of 2-methoxyestradiol may inhibit estrogen-induced breast cancer.

Molecular epidemiology evidence

In the past decade, effort has been made to relate animal laboratory data and evidence from human cell culture systems to human molecular epidemiology literature. The discovery of the existence of polymorphic variants in genes encoding enzymes involved in various metabolic pathways has been accentuated by the Human Genome Project. There is now evidence of the existence of polymorphic variants in genes encoding various enzymes involved in estrogen metabolism. Preliminary efforts are currently underway to characterise the functional role of these polymorphisms and it is hoped that a better picture will emerge in the next few decades. A role for estrogen 2-hydroxylase activity in human breast cancer risk has been documented in seven molecular epidemiological studies in different populations. Of these, three studies including those of Taioli *et. al*,[9] Ambrosone and colleagues,[67] and Huang *et. al*.[68] reported significant association between various CYP1A1 polymorphims and increased risk of breast cancer in African Americans, Caucasians and Chinese populations while Miyoshi[69] found an inverse association between the MspI and exon 7 polymorphism and breast cancer risk in Japanese women. Other investigators including Rebbeck *et. al*.[70] and Ishibe *et. al*.[71] found no association between various CYP1A1 variants and breast cancer risk.

Since Cytochrome P450 1B1 is responsible for the 4-hydroxylation of estradiol particularly in extrahepatic target tissues including the breast, investigators have been searching for evidence of a relationship between polymorphic variants of CYP1B1 gene and breast cancer risk. Some of these studies have reported significant association between some of the polymorphisms and breast cancer risk while others have reported contrary findings. While there appear to be no overall association between CYP1B1 polymorphisms and breast cancer risk in Caucasian populations, Bailey *et. al*.[72] noted that Caucasian patients with the Val/Val genotype of the M1 polymorphism had a significantly higher percentage of breast cancer that were positive for estrogen or progesterone receptors. Wanatabe *et. al*.[73] reported increased risk of breast cancer among Japanese women carrying the Ala-Ser polymorphism at codon 119 in the substrate recognition site of the CYP1B1 gene while Zheng *et. al*.[74] found that the codon 432 Leu/Leu genotype of the CYP1B1 gene was associated with elevated risk of breast cancer among Chinese women in Shanghai.

As noted in the preceding paragraphs, 16a-hydroxylation is a major complimentary pathway for both hepatic and extrahepatic hydroxylation of estrogens and there is laboratory data suggesting a relationship between increased 16a-hydroxylation and estrogen-induced carcino-genesis in animals. There is evidence to support an association between the relative amount of C-2 and C-16 metabolites and human breast cancer risk.[58] Women with breast can-

cer[75] and women at increased risk of breast cancer[76] have higher levels of C-16 metabolites compared with those without the disease. Among a subgroup of women in a population-based case control study in Shanghai, China, Zheng *et .al.*[77] reported a positive association between urinary 6-beta-OH:cortisol ratio and breast cancer risk and the association was stronger in postmenopausal women in whom estrone is the major form of estrogen. Two recent genotyping studies of CYP3A4 and breast cancer risk have been published; one a case control study among Caucasian women in Australia[78] and the second recruiting a multi-ethnic population in the U.S.[79] In the Australian study, CYP3A4*1B polymorphism was not associated with breast cancer (odds ratio [OR] = 0.86, 95% confidence interval [CI], 0.54-1.33).[78] In a group of US girls (n = 137; 39 African American, 57 Hispanic, and 41 Caucasian) early-onset menarche, a breast cancer risk factor, was associated with the inheritance of the CYP3A4*1B allele (odds trend = 3.21, 95% CI, 1.62-6.89).[79]

The suggestion that women harboring the low-activity allele of the COMT gene might experience higher lifetime exposure to catechol estrogens created the impetus for association studies between COMT polymorphism and breast cancer risk. Studies among Caucasians have reported inconsistent findings. While Lavigne *et. al.*[10] found an increased risk of breast cancer among postmenopausal women carrying the COMT met/met (low-activity) genotype, Thompson *et .al.*[12] noted a reversal of effects with menopausal status with premenopausal women harboring at least one low-activity allele showing significantly increased risk of breast cancer and an inverse association between low-activity alleles and postmenopausal breast cancer. Studies in Asian populations appear to report more consistent associations between the low-activity genotype and breast cancer risk. Among Korean women, Yim *et .al.*[14] showed that subjects with at least one COMT low-activity allele had an almost two-fold increased risk of breast cancer compared with the homozygous high-activity COMT individuals (OR=1.7; 95% CI=1.04-2.78). Huang *et .al.*[13] also reported increased risk of breast cancer among Taiwanese women harboring the low-activity COMT genotype compared with those homozygous for the high-activity COMT genotype.

Conclusion

Effort has been made in the preceding paragraphs to highlight recent knowledge in our understanding of the pivotal role of estrogen and its metabolites in breast carcinogenesis. Central to the new paradigm of estrogen-mediated carcinogenesis is the role of metabolites including 2-, 4- and 16a -hydroxyestradiol. While 4-hydroxy estradiol retain potent estrogenic activity via activation of estrogen receptor-a, its ability to generate apurinic sites in DNA through unstable catechol-adduct formation and undergo metabolic redox cycling leading to the generation of reactive oxygen species capable of damaging DNA and other cellular macro-molecules[33-35] marks it out as a potent carcinogen in the breast. In addition, 16a -hydroxyestradiol, retains potent estrogenic action through binding tightly to the estrogen receptor and forms a Schiff base with the amino groups of proteins and other cellular macromolecules,[49-52] a process culminating in aberrant DNA synthesis and anchorage-independent growth of tumor cells.[57] On the other hand, 2-methoxyestradiol, a 2-hydroxy derivatives of estradiol is considered the body's natural anticancer metabolite. By altering microtubule stability, inducing apoptosis and inhibiting angiogenesis in tumor cells and downregulating cytokine and PGE, induced in-situ aromatase synthesis in the breast,[80] 2-methoxyestradiol has distinguished itself as the new focus for the new generation anti-cancer chemotherapy for both hormone dependent and hormone-independent breast cancer. In fact, 2-methoxyestradiol is undergoing a phase I trial as

an angiogenesis inhibitor and search is ongoing for synthetic derivatives of this metabolite for anti-cancer chemotherapy.[81]

Overall, our understanding of the functional role of estrogen metabolites in breast carcinogenesis has improved in the past two decades. It is interesting that a metabolite of estradiol (currently classified a carcinogen by the International Agency for Research on Cancer [IARC]) is now the focus of new generation cancer chemotherapy. In addition, the preferential 2-hydroxylation of estrogens in lean women and women on low fat diet further justifies current efforts at combating obesity as a worthy fight in the battle against breast cancer. Much still needs to be done in the identification and functional characterisation of various polymorphic variants in the genes encoding enzymes involved in estrogen metabolism. In addition, carefully designed molecular epidemiology studies employing haplotype-based polygenic models are required to tease out the contributions of these variants to differential lifetime exposure to estrogens and their metabolites and consequently breast cancer risk. Understanding how differences in estrogen metabolism influences exposure to various genotoxic agents has the potential to considerably improve our ability to characterise individual risk of breast cancer and enhance our ability to design individual and population-specific control and preventive measures. This is particularly important in developing countries where obesity is likely to assume an epidemic with increasing westernisation of diet and lifestyle.

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