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Minireview

C2- and C4-position 17β-estradiol metabolites and their relation to breast cancer

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ABSTRACT: C2- and C4-position 17β-estradiol metabolites play an important role in breast carcinogenesis. 2-Hydroxyestradiol and 4-hydroxyestradiol are implicated in tumorigenesis via two pathways. These pathways entail increased cell proliferation and the formation of reactive oxygen species that trigger an increase in the likelihood of deoxyribonucleic acid mutations.
2-Methoxyestradiol, a 17β-estradiol metabolite, however, causes induction of apoptosis in transformed and tumor cells; thus exhibiting an antiproliferative effect on tumor growth. The 4-hydroxyestradiol:2-methoxyestradiol and 2-hydroxyestradiol:2-methoxyestradiol ratios therefore ought to be taken into account as possible indicators of carcinogenesis.

Breast cancer is one of the most common cancers affecting women (Bishop, 1999). Approximately 10% of women living in western countries will develop breast cancer during their lifetime (Bishop, 1999; Schultz and Weber, 1999). Between 5 - 10% of all breast cancer is hereditary (Bishop, 1999; Schultz and Weber, 1999). Failure of eradicating breast cancer is mainly attributed to the fact that no single etiological agent has been identified, uncertainty exists about the time of initiation and the precise molecular mechanism responsible for initiation and progression of cancer remains to be elucidated (Bishop, 1999).

The susceptibility of the breast to carcinogenesis can be related to the developmental stage of the breast when exposed to mutagenic agents. Immature breasts are especially susceptible to carcinogenesis since they contain undifferentiated structures referred to as terminal ductal lobular units. Terminal ductal lobular units have been identified as the origin of ductal carcinomas (Chodosh *et al.*, 1999). The process of terminal ductal lobular unit differentiation has a permanent protective effect against breast cancer development (Bishop, 1999).

Estrogens are regarded as key role players in advancing cell proliferation of mutually normal and neoplastic breast epithelium (Cavalieri *et al.*, 2000; Russo and Russo, 2006; Parl *et al.*, 2009). The more the breast epithelium is exposed to estrogen, the higher the chance of tumor formation. The level of estrogen that breast epithelium is exposed to over time can be viewed as a risk factor (Duncan *et al.*, 1998; Bishop, 1999; Schultz and Weber, 1999; Chodosh *et al.*, 1999; Hofmann and Schlag, 2000; Russo *et al.*, 2000; Pathak *et al.*, 2000). Established estrogen-related risk factors are early me-

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narche, late menopause, hormone replacement therapy, postmenopausal obesity and alcohol consumption. Protective factors for breast cancer are exercise, young age at first full term pregnancy, prolonged lactation and number of pregnancies (Bishop, 1999). The meaning of estrogens in breast cancer has long been implied and lately being verified by epidemiological studies (Russo and Russo, 2006).

Estradiol, the most abundant estrogen in premenopausal woman, can mediate carcinogenesis via distinct pathways (Bishop, 1999; Schultz and Weber, 1999; Hofmann and Schlag, 2000; Russo *et al.*, 2000; Pathak *et al.*, 2000). These pathways are not mutually exclusive, but can rather be perceived as complimenting each other. The first pathway involves the receptor-mediated stimulation of cell proliferation where an opportunity is created for the accumulation of random errors in deoxyribonucleic acid replication. Mutations may occur more easily giving rise to the malignant phenotype (Russo *et al.*, 2000; Henderson and Feigelson, 2000; Cavalieri *et al.*, 2000; Jefcoate *et al.*, 2000). The second pathway involves the formation of quinones and reactive oxygen species that can lead to deoxyribonucleic acid damage and mutations (Russo *et al.*, 2000; Cavalieri *et al.*, 2000; Jefcoate *et al.*, 2000). Thirdly, another mechanism entails the induction of aneuploidy (Russo and Russo, 2006).

Initially it was thought that estradiol itself was responsible for tumorigenesis (Bishop, 1999). In women younger than 45 years of age an increased incidence of breast cancer was noted because of earlier onset of birth control usage. Estradiol levels were also found to be higher in malignant tissue than in normal tissue. It has since been shown that catechol metabolites of estradiol and other estrogens may lead to carcinogenesis. Carcinogens increase the amount of catechol estrogens that are formed (Bishop, 1999). Catechol estrogens are unstable and rapidly *O*-methylated or conjugated. It was believed that *O*-methylation of these catechol estrogens was part of the breakdown and inactivation of active hormones, rendering 'biologically inactive' metabolites. It has since been shown that the *O*-methylated metabolite on the C2 position of estradiol has apoptosis-inductive activities in transformed and malignant cells (D'Amato *et al.*, 1994; Mabjeesh *et al.*, 2003).

Aromatization of androgens to estrogens is catalyzed by aromatase (estrogen synthetase) (Zhu and Conney, 1998a; Bishop, 1999). The conversion of androgens to estrogens is the rate-limiting step in the production of endogenous estrogens. Aromatase activity is found in the ovary, placenta and several non-endocrine tissues such as brain, adipose tissue, liver, fibroblasts and mammary glandular cells (Zhu and Conney, 1998a). The conversion of androstenedione to estrone shows a positive correlation with obesity and age (Zhu and Conney, 1998a). The latter suggests that adipose tissue is a major site for estrogen biosynthesis in post-menopausal women and elderly men. Aromatase activity was found to be higher in adipose tissue surrounding mammary carcinoma cells than in surrounding normal breast tissue (Zhu and Conney, 1998a). This implies that aromatization of androgens is an important source of estrogen for mammary tumors (Adams, 1998; Zhu and Conney, 1998a).

Hydroxylation of estradiol is catalyzed by members of the cytochrome P450 (CYP450) family. These enzymes are nicotinamide adenine dinucleotide phosphate-dependent mono-oxygenases and are situated in the membranes of microsomes present in target organs (Zhu and Conney, 1998a, b). The isozyme specific for hydroxylation on the C4-position is cytochrome P450 1B1. Estradiol has been found to induce tumors in those target organs where this enzyme is predominant. Isozymes specific for the 2-hydroxylation of estrogens are CYP450 1A1/1A2 (Bishop, 1999; Parl *et al.*, 2009). In the breast, the key estrogen namely 17-β-estradiol acts a substrate for the phase I enzymes cytochrome P50 1A1 and 1B1, as well as a ligand for the estrogen receptor (Parl *et al.*, 2009). Since 17-β-estradiol exerts a dual role of acting both as substrate and ligand, it has thus been linked to the development of breast cancer by concurrently causing DNA damage via its oxidation products (2-hydroxy- and 4-hydroxy-catechol estrogens) and stimulation of cell proliferation and gene expression via the estrogen receptor (Parl *et al.*, 2009). Another CYP450 isozyme, CYP450 3A, catalyzes the formation of both 2-hydroxyestradiol and 4-hydroxyestradiol (Liehr and Ricci, 1996).

2-Hydroxyestradiol has little or no carcinogenic activity compared to 4-hydroxyestradiol and more proliferating action than estradiol itself (Zhu and Conney, 1998a; Bishop, 1999). The concentration of unconjugated 2-hydroxyestradiol metabolites is low in blood and in several other tissues. This is probably because of the instability of 2-hydroxyestradiol, its rapid *O*-methylation by catechol *O*-methyltransferase and subsequent urinary excretion. The intracellular concentration of catechol estrogens is higher than that of blood and the most common estradiol metabolite in blood is 2-methoxyestradiol (Bishop, 1999). The lack of carcinogenic activity of 2-hydroxyestradiol may be ascribed to the fact that *O*-methylation of 2-hydroxyestradiol is faster than that of

4-hydroxyestradiol and that it has a more rapid clearance (Zhu and Conney, 1998a, b). Some studies showed that inducing 2-hydroxylation of estradiol might decrease spontaneous tumorigenesis in estrogen-sensitive tissues (Zhu and Conney, 1998a; Cavalieri *et al.*, 2000). 4-Hydroxyestradiol appears to be the most abundant estrogen in human breast cancer specimen extracts (Lottering *et al.*, 1992; Zhu and Conney, 1998a; Bishop, 1999). It has also been demonstrated that the differential formation of 2- and 4- hydroxyestradiol correlates with an organ's resistance or susceptibility to estrogen-induced carcinogenesis (Adams, 1998). While 4-hydroxyestradiol shows increased expression in benign or malignant neoplastic tissue compared to normal tissue, 2-hydroxyestradiol expression remains fairly constant between tissues (Zhu and Conney, 1998b). It is thus the relationship between 2-hydroxyestradiol and 4-hydroxyestradiol that should be considered as a marker for breast cancer risk. Research has shown that the ratios of 4-hydroxyestradiol/2-hydroxyestradiol formation in breast adenocarcinoma and fibro-adenoma were found to be higher when compared to normal (Lemon *et al.*, 1992). An even more significant ratio to consider as a biomarker for breast cancer risk is the 4-hydroxyestradiol/2-methoxyestradiol ratio.

2-Hydroxyestradiol, 2-hydroxy estrone and 4-hydroxyestradiol generate free radicals when they undergo metabolic redox cycling that may cause damage to deoxyribonucleic acid and other cell structures, thus initiating tumorigenesis (Zhu and Conney, 1998a; Santen *et al.*, 2009).

Normal cellular processes continuously form and degrade oxidants. Cells have an extensive anti-oxidant defense system, but with the uncalled formation of reactive oxygen species in extensive amounts, the negative effects of these reactive oxygen species become apparent (Cavalieri *et al.*, 2000; Parl *et al.*, 2009). The major type of damage caused by reactive oxygen species is the oxidation of genetic material (Cavalieri *et al.*, 2000). 2-Hydroxyestradiol and 4-hydroxyestradiol can be oxidized to semiquinones and, in the presence of molecular oxygen, further oxidized to quinones (Cavalieri *et al.*, 2000). Oxido-reduction between catechol estrogens, their semiquinones and their quinines produces reactive oxygen species like the super oxide anion radicals that can damage deoxyribonucleic acid (Cavalieri *et al.*, 2000; Jefcoate *et al.*, 2000; Santen *et al.*, 2009).

Super oxide anion radicals readily form hydrogen peroxide. This can either happen spontaneously or enzymatically via superoxide dismutase. In the presence of Fe^{2+} and Cu^{2+} ions, hydrogen peroxide is converted to powerful of oxidants, namely hydroxyl radicals (Cavalieri *et al.*, 2000). Hydrogen peroxide can readily cross cell membranes and cause damage to deoxyribonucleic acid of neighboring cells (Cavalieri *et al.*, 2000). Quinones and semiquinones are capable of redox cycling as long as molecular oxygen is available. A small amount of estradiol may thus cause substantial reactive oxygen species production and subsequent cellular damage (Cavalieri *et al.*, 2000). Quinone reductase acts to counter reactive oxygen species formation by reducing quinones to catechols using nicotinamide adenine dinucleotide phosphate as cofactor (Cavalieri *et al.*, 2000).

O-methylation of the catechol estrogens is catalyzed by catechol *O*-methyltransferase, which is the same enzyme responsible for the methylation of catecholamines (Zhu and Conney, 1998a, b). Catechol *O*-methyltransferase is situated almost exclusively in the cytosol of target tissues (Zhu and Conney, 1998a). *O*-Methylation of catechol estrogens inactivates their estrogenic potential and their ability to be further oxidized to their quinones and semiquinones. Because of the rapid *O*-methylation of 2-hydroxyestradiol, 2-methoxyestradiol was found to be the most abundant estrogen in human plasma and urine (Lottering *et al.*, 1992; Bishop, 1999; Clemons and Goss, 2001). 2-Methoxyestradiol has little or no estrogen receptor binding affinity compared to estradiol and exerts unique biological effects that cannot be ascribed to estradiol, 2-hydroxyestradiol 4-hydroxyestradiol or any other *O*-methylated form of estradiol. 2-Methoxyestradiol targets actively dividing cells by operating as a microtubule disruptor thereby disturbing cellular events associated with cell proliferation (Zhu and Conney, 1998a, b). Human clinical trials have demonstrated that 2-methoxyestradiol (PANZEM™) was orally active and well tolerated in patients suffering from breast cancer, prostate cancer and multiple myeloma (LaVallee *et al.*, 2008).

Although it was demonstrated that 17-β-estradiol is capable of stimulating the formation of tumors in severe combined immunodeficiency mice (Russo and Russo, 2006), it is apparent that a specific equilibrium exists in the metabolic pathways controlling metabolites of estradiol to maintain cell number homeostasis. In the event of disturbance of this equilibrium, carcinogenesis may occur. The 2-hydroxyestradiol:2-methoxyestradiol and 4-hydroxyestradiol:2-methoxyestradiol ratios are therefore important variables to consider as markers for tumorigenesis. In addition and when all the research studies reviewed are taken into account, it also emerges that

some of the influences of estrogen on breast cancer occur independently of estrogen receptors. The latter thus implies that prevention of estradiol metabolites formation itself might also decrease the risk of breast cancer (Santen *et al.*, 2009).

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