Published in final edited form as:

Drug Discov Today Dis Mech. 2012 ; 9(1-2): e1–e3. doi:10.1016/j.ddmec.2012.12.001.

Preface

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In women, the most commonly occurring cancer is breast cancer, with more than one million cases and almost 500,000 deaths each year throughout the world [1]. In the United States, approximately 234,000 new cases and 40,000 deaths are estimated for 2012 [2]. Prolonged exposure to estrogens has long been recognized as a risk factor for breast cancer.

It is logical, then, to investigate what is the role of estrogens in relation to the development of breast cancer. Two mechanisms have been hypothesized for the role of estrogens in breast cancer: one is hormonal and the other is nonhormonal. In the hormonal mechanism, estrogens bind to the estrogen receptor (ER) to generate a variety of cellular signals that promote the proliferation of cells. This event can contribute to the development of breast cancer.

The nonhormonal mechanism is represented by the genotoxicity of estrogens. In this mechanism, estrogens act as chemical carcinogens and initiate cancer via formation of estrogen-DNA adducts that generate the critical mutations leading to cancer. Both the hormonal and nonhormonal mechanisms are operative in the development of breast cancer, but at different stages in the process of carcinogenesis. The eight research groups represented in this issue deal with various aspects of the role of estrogens in breast cancer.

Rajkumar has investigated the effects of dose, duration and route of administration on breast cancer risk associated with use of hormone replacement therapy (HT) in postmenopausal women [3]. Overall, studies to date are insufficient to support the conclusion that differences in breast cancer risk can be achieved by changing the dosage, duration or route of administration of HT. Additional detailed studies are needed to assess the effects of HT on breast cancer risk.

Antiestrogens, such as tamoxifen, faslodex and raloxifene, are among the most effective chemotherapeutic treatments for ER-positive breast cancer. Resistance to these drugs, however, is a major problem with this therapeutic approach. Investigating resistance by using single genes or signaling pathways has had limited success because antiestrogen resistance appears to arise from genomic and nongenomic mechanisms. A systems biology approach could be effective for tackling this multifaceted problem. Clarke presents an approach [4] that systematically reviews the information available from studies of the

genome, proteome and methylome to give new insights into combating antiestrogen resistance in the treatment of ER-positive breast cancer.

One factor in ER-positive breast cancer is the androgen receptor (AR), and its various roles in breast cancer have been delineated by Fuqua [5]. The AR interacts with ER- α and affects many other signaling pathways. A majority of ER-positive breast tumors express AR. The AR may also play a role in tumor metastasis. Under various circumstances, expression of the AR increases or decreases the effectiveness of breast cancer chemotherapies. It is possible that blocking AR action could be an effective way to overcome resistance to hormone-based breast cancer chemotherapeutic regimens.

The genotoxicity of estrogens depends upon the balance between estrogen activating and deactivating enzymes. A key deactivating enzyme discussed by Montano [6] is the anti-oxidative stress enzyme quinone reductase, which reduces catechol estrogen quinone metabolites to catechol estrogens. Among the selective estrogen receptor modulators (SERMs), the antiestrogen tamoxifen upregulates expression of quinone reductase. Tamoxifen carries out the upregulation through ER- β and the regulatory factor hPMC2, enabling tamoxifen to block ER-mediated cell proliferation. Furthermore, when tamoxifen induces quinone reductase, a lower level of estrogen-DNA adducts is observed, as well as decreased estrogen-induced mammary tumorigenesis in ACI rats.

The progressive steps involved in transformation of the ER-negative human breast epithelial MCF-10F cell line to malignant cells in the absence of ER- α have been investigated by Russo [7]. Because these cells do not contain ER- α , initiation of transformation derives from the genotoxicity of estrogens. This initial step produces transformed trMCF cells, which are invasive in Matrigel chambers. The invasive trMCF cells are tumorigenic, as demonstrated by their ability to induce tumors when injected into the mammary fat pad of female severely compromised immunodeficient (SCID) mice. The resulting caMCF cells are metastatic and induce lung tumors when injected into the tail of SCID mice. Dr. Russo has investigated the differential expression of genes in these four stages of cancer progression and found effects on the extracellular matrix, cell adhesion and immune response. These results demonstrate breast cancer to be a polygenic disease, initiated by estrogen-induced genetic aberrations in the absence of ER- α .

The genotoxicity of estrogens requires endogenous or exogenous estrogens to be metabolized to catechol estrogen-3,4-quinones, that react with DNA to form specific depurinating estrogen-DNA adducts. Cytochrome P450 (CYP)1B1 and catechol-*O*-methyltransferase (COMT) are two key enzymes in estrogen metabolism. CYP1B1 is the activating enzyme that metabolizes estradiol to 4-hydroxyestradiol, which can be oxidized further to the electrophilic reactive estradiol-3,4-quinone. By contrast, COMT is a key deactivating, or protective, enzyme. Polymorphisms in the COMT gene that affect the activity of the enzyme have been studied in a variety of human populations, as discussed by Yager [8], but no consistent patterns associating lower levels of COMT activity with increased susceptibility to breast cancer have been found. To bring clarity to this area of investigation, Yager suggests that COMT needs to be studied in the context of other

estrogen-metabolizing enzymes and, importantly, in conjunction with cancer risk biomarkers, such as depurinating estrogen-DNA adducts.

The role of estrogens as chemical carcinogens via reaction of the catechol estrogen-3,4-quinones with DNA is stressed in Bolton's review of the effects of widely used botanical dietary supplements to relieve menopausal symptoms [9]. This article reviews the ability of various botanical supplements to induce estrogen-deactivating enzymes and/or inhibit estrogen-activating enzymes. By modulating these estrogen-metabolizing enzymes the botanical dietary supplements could increase or decrease a woman's risk of developing breast cancer.

Cavalieri and Rogan [10] review the evidence from experiments on estrogen metabolism, formation of DNA adducts, mutagenicity, cell transformation and carcinogenicity that demonstrates the role of estrogen genotoxicity in cancer initiation. Estrogens are metabolized to catechol estrogens, which are further oxidized to catechol estrogen-3,4-quinones, the predominant carcinogenic metabolites of estrogens. These quinones react with DNA to form specific estrogen-DNA adducts that depurinate, leaving apurinic sites in the DNA. The apurinic sites can generate the critical mutations leading to initiation of breast cancer. The depurinating estrogen-DNA adducts are potential biomarkers for risk of developing breast cancer because they are detected at significantly higher levels in the urine or serum of women at high risk for breast cancer, or diagnosed with the disease, compared to women at normal risk. Prevention of breast cancer could be achieved by blocking the formation of estrogen-DNA adducts, either by inhibiting formation of catechol estrogen-3,4-quinones or their reaction with DNA. *N*-Acetylcysteine and resveratrol are two dietary supplements that can block formation of estrogen-DNA adducts, offering a potential widely applicable approach to prevention of breast cancer. When initiation is blocked, promotion, progression and development of breast cancer would be prevented.

As stated above, both hormonal and nonhormonal effects play a role in breast cancer. The receptor-mediated effects of steroid hormones govern the proliferation of cells that have already undergone oncogenic mutations and started on the path to become malignant. Hormonal effects are of particular concern to women using HT to deal with menopausal symptoms, but we do not yet have sufficient knowledge to minimize risk of breast cancer for individual women using them. The use of antiestrogens in chemotherapy to combat ER-positive breast cancer is limited by the development of resistance to the antiestrogens. A systems biology approach provides a novel, effective way to incorporate a wide variety of factors to resolve this complex problem. Crucial insights have arisen recently through research on the AR as a therapeutic target for ER-positive breast cancer. At the same time, insights into the role of ER- β in the effects of the antiestrogen tamoxifen offer yet another avenue for progress in combatting breast cancer.

While hormonal effects are a necessary aspect of research to improve chemotherapy approaches, investigation into breast cancer etiology and prevention is focused in this issue on nonhormonal mechanisms of estrogen action. Estrogens are weak chemical carcinogens, due to the formation of catechol estrogen-3,4-quinone metabolites. These are the electrophilic ultimate carcinogenic metabolites capable of reacting covalently with DNA to

yield predominantly the N3adenine and N7guanine depurinating adducts. These adducts leave apurinic sites in the DNA that can generate the specific mutations leading to the initiation of breast cancer. The initiating step of cancer can be blocked by the dietary supplements *N*-acetylcysteine and resveratrol. These agents act by inhibiting formation of catechol estrogen quinones and their reaction with DNA. Preventing the initiation of breast cancer eliminates the possibility of promotion, progression and development of cancer.

Biographies

Ercole L. Cavalieri is a Professor in the Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, Nebraska. He was awarded a Doctor of Science in chemistry from the University of Milan in 1962 and carried out postdoctoral research in chemical carcinogenesis at the University of California-Berkeley from 1968 to 1970. He has conducted research on carcinogenesis of polycyclic aromatic hydrocarbons and estrogens for his entire career. The major focus of his research has been mechanistic aspects of the chemical reactions involved. In 1994 he received the Outstanding Research and Creative Activity Award from the University of Nebraska. From 1997 to 2010 he served as the Director of the Center for Environmental Toxicology at the University of Nebraska. Dr. Cavalieri is the author of 235 peer-reviewed articles, including many reviews on the mechanisms of carcinogenesis of polycyclic aromatic hydrocarbons and estrogens.

Eleanor G. Rogan is a Professor in the Department of Environmental, Agricultural and Occupational Health, College of Public Health, University of Nebraska Medical Center, Omaha, Nebraska. Since 2007 Dr. Rogan has served as the founding chair of the department. She received an A.B. from Mount Holyoke College in 1963 and a Ph.D. in biochemistry from The Johns Hopkins University in 1968. She has conducted research in the Eppley Institute on metabolism and mechanisms of carcinogenesis of polycyclic aromatic hydrocarbons and estrogens since 1973. In 2006 she received the Linus Pauling Award for Functional Medicine from the Institute for Functional Medicine. Dr. Rogan is the author of 199 peer-reviewed articles concerning mechanisms of metabolism, DNA adduct formation and carcinogenesis of polycyclic aromatic hydrocarbons and estrogens.

References

1. Jemal A, et al. Global cancer statistics. *CA Cancer J. Clin.* 2011; 61:69–90. [PubMed: 21296855]

2. Siegel R, et al. Cancer statistics, 2012. *CA Cancer J. Clin.* 2012; 62:10–29. [PubMed: 22237781]
3. Rajkumar L. Dose, duration and mode of administration of hormones influence breast cancer risk. *Drug Discov. Today: Dis. Mech.* 2012; 9:e5–e9.
4. Bouker KB, et al. Antiestrogen resistance and the application of systems biology. *Drug Discov. Today: Dis. Mech.* 2012; 9:e11–e17. [PubMed: 23539064]
5. Iacopetta E, et al. The role of androgen receptor in breast cancer. *Drug Discov. Today: Dis. Mech.* 2012; 9:e19–e27.
6. Montano MM, et al. Targeting the genotoxic effects of estrogens. *Drug Discov. Today: Dis. Mech.* 2012; 9:e29–e33. [PubMed: 23795205]
7. Zhai Y, et al. An in vitro-in vivo model of epithelial mesenchymal transition in triple negative breast cancer. *Drug Discov. Today: Dis. Mech.* 2012; 9:e35–e40. [PubMed: 23585768]
8. Yager J. Catechol-O-methyltransferase: characteristics, polymorphisms and role in breast cancer. *Drug Discov. Today: Dis. Mech.* 2012; 9:e41–e46. [PubMed: 23734165]
9. Snelton CS, et al. Modulation of estrogen chemical carcinogenesis by botanical supplements used for postmenopausal women's health. *Drug Discov. Today: Dis. Mech.* 2012; 9:e47–e54.
10. Cavalieri EL, Rogan EG. The etiology and prevention of breast cancer. *Drug Discov. Today: Dis. Mech.* 2012; 9:e55–e69. [PubMed: 26246832]