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Hormone replacement therapy dependent changes in breast cancer-related gene expression in breast tissue of healthy postmenopausal women☆,☆☆

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ABSTRACT

Risk assessment of future breast cancer risk through exposure to sex steroids currently relies on clinical scorings such as mammographic density. Knowledge about the gene expression patterns in existing breast cancer tumors may be used to identify risk factors in the breast tissue of women still free of cancer. The differential effects of estradiol, estradiol together with gestagens, or tibolone on breast cancer-related gene expression in normal breast tissue samples taken from postmenopausal women may be used to identify gene expression profiles associated with a higher breast cancer risk. Breast tissue samples were taken from 33 healthy postmenopausal women both before and after a six month treatment with either 2 mg micronized estradiol [E2], 2 mg micronized estradiol and 1 mg norethisterone acetate [E2 + NETA], 2.5 mg tibolone [T] or [no HRT]. Except for [E2], which was only given to women after hysterectomy, the allocation to each of the three groups was randomized. The expression of 102 mRNAs and 46 microRNAs putatively involved in breast cancer was prospectively determined in the biopsies of 6 women receiving [no HRT], 5 women receiving [E2], 5 women receiving [E2 + NETA], and 6 receiving [T]. Using epithelial and endothelial markers genes, non-representative biopsies from 11 women were eliminated. Treatment of postmenopausal women with [E2 + NETA] resulted in the highest number of differentially ($p < 0.05$) regulated genes (16.2%) compared to baseline, followed by [E2] (10.1%) and [T] (4.7%). Among genes that were significantly down-regulated by [E2 + NETA] ranked estrogen-receptor-1 (*ESR1*, $p = 0.019$) and androgen receptor (*AR*, $p = 0.019$), whereas *CYP1B1*, a gene encoding an estrogen-metabolizing enzyme, was significantly up-regulated ($p = 0.016$). Mammary cells triggered by [E2 + NETA] and [E2] adjust for steroidogenic up-regulation through down-regulation of the estrogen-receptor pathway. In this prospective study, prolonged administration of [E2 + NETA] and to a lesser extent of [E2] but not [T] were associated in otherwise healthy breast tissue with a change in the

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expression of genes putatively involved in breast cancer. Our data suggest that normal mammary cells triggered by [E2 + NETA] adjust for steroidogenic up-regulation through down-regulation of the estrogen-receptor pathway. This feasibility study provides the basis for whole genome analyses to identify novel markers involved in increased breast cancer risk.

1. Introduction

Breast cancer risk assessment is an important issue in clinical medicine and various methods such as scoring systems based on epidemiological characteristics (Gail et al., 1989), sex steroid levels in the serum (Key et al., 2002; Missmer et al., 2004; Zeleniuch-Jacquotte et al., 2004) and mammographic density (Byrne et al., 1995) have been proposed for the early prediction of a higher breast cancer risk in individual postmenopausal women. All these parameters point to the crucial role of sex steroids in creating the environment in the breast which ultimately leads to the manifestation of overt breast cancer disease. The present study was based on the hypothesis that the changes caused by hormonal therapeutics on the expression levels of genes in the mammary tissue of postmenopausal women can be used to identify additional markers for the prediction of incident breast cancer.

Various hormonal treatments have been shown to modify the breast tissue differently and to alter the risk of breast cancer. With mammographic density known to be increased during prolonged administration of hormone replacement therapy (HRT, Greendalem et al., 2003) and to be reduced under tamoxifen (Cuzick et al., 2004), both mammographic density and circulating sex steroid levels have been described as independent markers of an increased risk of breast cancer by HRT (Tamimi et al., 2007). Combined treatments with estrogens and progestagens and to a lesser extent with estrogen-only preparations enhance mammographic density, whereas tibolone [T] does not affect mammographic density (Lundström et al., 2002; Valdivia et al., 2004). Continued treatment with both estrogens and progestagens increases the incidence of invasive breast cancer in postmenopausal women (Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Schairer et al., 2000; Writing Group for the Women's Health Initiative Investigators, 2002). In contrast, the risk of breast cancer was not significantly influenced in hysterectomized women receiving conjugated estrogens alone (The Women's Health Initiative Steering Committee, 2004) as for elderly women with chronic low circulating estrogen levels at risk of osteoporosis receiving [T] (Cummings et al., 2008), although the recurrence rate was increased by [T] in women previously diagnosed with breast cancer (Kenemans et al., 2009). In case–control studies involving early postmenopausal women, the results with respect to the effect of [T] on breast cancer risk are conflicting (Beral et al., 2003; Banks et al., 2006; Opatrny et al., 2008).

The aim of this prospective study was to explore the effects of three distinctive hormonal preparations, each with a different effect on mammographic density and on circulating sex hormone levels, on the expression of a list of genes putatively known to be involved in breast cancer, such as proliferation and apoptosis markers, breast cancer subtype specific stromal and stem cell markers as well as markers for steroid receptors, steroidogenic and steroid-metabolizing enzymes (Supplementary Table 1, on line only). This list of genes was extracted from large data bases collected from well characterized breast cancer samples which were used so far to establish prognostic and predictive patterns in already existing breast cancer cases. To evaluate the expression of these genes in relation to increased breast cancer risk of healthy women due to the use of HRT core needle biopsies were taken from the breasts of healthy postmenopausal women before and after six months of hormonal treatment.

2. Subjects and methods

This research project was conceived as a prospective study aiming at identifying differences in the expression profile of genes in core needle biopsies taken from the breasts of postmenopausal women. Except for [E2], which was following established guidelines only given to women after hysterectomy, the allocation to each of the three other treatments, estradiol and norethisterone acetate [E2 + NETA], tibolone [T] or [no HRT], was randomized using sealed envelopes. The compliance of the volunteers was controlled by measuring the plasma levels of FSH and SHBG both at the onset and at the end of the intake of the respective hormonal preparations. The serum samples were stored frozen at −70 °C and the assay of both FSH and SHBG concentrations was carried out in one single run.

As gene expression profiles detected in the core needle biopsy of a breast tumor have been shown to be representative of the entire tumor (Zanetti-Dällenbach et al., 2006), we made use of such biopsies taken from the upper outer quadrant of the left breast of healthy postmenopausal women before and after 6 months of each treatment modality.

The inclusion criteria were as follows: healthy postmenopausal women at least three months after natural menopause as identified by secondary amenorrhea in the presence of FSH levels above 30 IU/L and estradiol levels below 40 pmol/L; normal BMI below 32 kg/m^2, and having normal prolactin levels. All participants were to be Caucasians. The exclusion criteria were intake of HRT, dihydroepiandrosteron or phytoestrogens at least four weeks before recruitment, intake of cardiac medication, any excessive abuse of drugs or alcohol, known existing pathology of the breast, claustrophobia, the presence of metallic implants, such as pacemakers, and an endometrial thickness above 5 mm.