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Estrogen alpha and progesterone receptor expression in the normal mammary epithelium in relation to breast cancer risk

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Estrogens play a central role in the etiology of breast cancer, and results from observational studies and randomized trials have also implicated progestins. The effects of these hormones in the mammary tissue are exerted through binding with specific receptor proteins in the cell nucleus. It has been proposed that higher estrogen receptor alpha expression in the normal breast epithelium may increase breast cancer risk. In a study in Greece, we determined estrogen alpha and progesterone receptor expression in normal mammary tissue adjacent to the pathological tissue from 267 women with breast cancer and 299 women with benign breast disease. Mouse monoclonal antibodies specific for estrogen receptor alpha and progesterone receptor were applied. The H–index, which incorporates frequency and intensity of staining of the cells, and can range from 0 to 300, was deemed positive when it exceeded 9. Among premenopausal women, there was no evidence for an association with breast cancer risk for expression of either type of receptors. Among postmenopausal women, breast cancer risk was inversely associated with expression of both estrogen alpha (odds ratio (OR) = 0.39; $p = 0.015$) and progesterone (OR = 0.40; $p = 0.008$) receptors. The hypothesis that overexpression of estrogen receptors alpha or progesterone receptors in normal breast epithelium may increase the risk of breast cancer was not supported by our data. Instead, we found evidence that overexpression of these receptors may be associated with reduced risk for breast cancer in line with the well-known association of expression of these receptors in the malignant tissue and better breast cancer prognosis.

Key words: breast cancer; estrogen; progesterone; receptors

There is a consensus that estrogens play a central role in the etiology of breast cancer, and results from both observational studies and randomized trials have also implicated progestins.[1–3] The effects of estrogens and progesterone in the mammary tissue are exerted through binding with specific receptor proteins in the cell nucleus.[4,5] Khan *et al*. have suggested that higher estrogen receptor alpha expression in the normal breast epithelium may increase breast cancer risk.[6] They undertook a study to evaluate this hypothesis by examining expression of estrogen receptors (at that time only estrogen receptors alpha were routinely evaluated) in the apparently normal mammary gland tissue adjacent to the pathological tissue in 174 women with breast cancer and 202 women with benign breast diseases (BBDs).[7] The results were interpreted as indicating that overexpression of estrogen receptors alpha in normal breast epithelium may augment estrogen sensitivity and, hence, the risk of breast cancer. In an editorial accompanying this study, the authors found the results intriguing, but also pointed out some limitations of the study and the need for additional investigations.[8] Since then, the results of 2 partially overlapping ecological studies in Asia[9,10] were interpreted as compatible with those of the analytical investigation previously undertaken by Khan *et al*.[7] No study based on individuals however, using an approach similar to that of Khan *et al*.,[7] has been reported, possibly because such an investigation presents considerable practical difficulties. We have undertaken such a study in Greece also examining progesterone receptors, given the long known positive correlation of the expression of these 2 types of receptors in malignant breast tissue.[11]

Material and methods

From March 2001 till May 2005, women who have undergone mammary biopsy in 2 major breast clinics in Athens, Greece were asked to participate in the study. Those who agreed to join the study provided informed consent for an in person interview, review of their medical records and use for research purposes of biological specimens collected in the context of their standard medical care. The study was approved by the Bioethics Committee of the University of Athens.

In breast clinic 1, women who underwent a breast biopsy during the duration of the study and women who had undergone a biopsy prior to the study initiation (but were interviewed during the indicated study period) were included. All women in breast clinic 2 underwent biopsy during the study period. Among eligible women in both clinics, we estimate that about 75% agreed to participate in the study. In several instances, women refused to allow any recording of information concerning agreement to participate in the study; thus, the refusal proportion cannot be accurately calculated.

All women completed an interviewer-administered questionnaire with information on sociodemographic and lifestyle factors as well as on gynecological and general medical history. We were able to determine receptors in normal mammary tissue in 267 women with breast cancer and 299 women with BBD. In breast clinic 1, histological samples were made available in the form of paraffin-embedded tissue (PET) blocks, whereas in breast clinic 2, samples obtained during biopsy were frozen in liquid nitrogen before being fixed in 10% neutral buffer formalin at 25°C for 24 hr and processed to PETs. In all instances, samples from surgical biopsies were examined.

The streptavidine-biotin-superoxidase method[12,13] was applied on paraffin sections. The sections that were prepared from fresh

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frozen tissue were fixed in a 10% formol solution of pH 7.4, at 25°C for 24 hr, in the automatic immunochemical BioGenex i6000 Consolidated Staining System. The primary specific mouse monoclonal antibodies were obtained from Novocastra Laboratories, Newcastle upon Tyne, UK. Clone 6F11, specific for estrogen receptor alpha, was applied in a 1:60 dilution, and clone 1A6, specific for the progesterone receptor, was applied in a 1:40 dilution.

The immunocytochemical results were scored in a semiquantitative way using the "H-score," which incorporates both the number of cells with positive staining and the intensity of staining.[12,14] Intensity of staining was evaluated on the basis of percentages of stained cells under 4 categories, denoted as 0 (no staining), 1 (weak but detectably above control), 2 (distinct) and 3 (strong). The H-score was calculated from the formula $[(a_0 \times 0) + (a_1 \times 1) + (a_2 \times 2) + (a_3 \times 3)] \times 100$, in which a_0 is the percent (expressed as a fraction of 1) of cells with intensity of staining 0, a_1 the percent of cells with intensity staining 1, a_2 the percent of cells with intensity of staining 2 and a_3 is the percent of cells with intensity of staining 3. The H-score, therefore, ranges from 0 to 300. We have considered scores from 0 to 9 (inclusive) as indicative of estrogen alpha (or progesterone) negative tissues and scores from 10 or more as indicative of estrogen alpha (or progesterone) positive tissues.

Multiple logistic regression was used to calculate odds ratios (ORs) contrasting breast cancer to BBD on the basis of expression of estrogen alpha or progesterone receptors in the corresponding normal mammary tissue. In all analyses, data were stratified by clinic and adjusted for age, age at menarche and body mass index (BMI) (continuously) as well as for menopausal status (pre- and perimenopausal women *vs*. postmenopausal), parity (parous *vs*. nulliparous) and family history of breast cancer in 1st degree relatives (yes *vs*. no). The above indicated cuttoff point of the H-score was used, although, in secondary analyses, additional cutoff points and trend tests were utilized. The p value was set at 0.05. Analyses were conducted with the SPSS 16.0 statistical package.

TABLE I – DISTRIBUTION OF WOMEN WITH BENIGN BREAST DISEASE OR BREAST CANCER BY DESCRIPTIVE CHARACTERISTICS, SOURCE OF TISSUE SAMPLES AND EXPRESSION OF ESTROGEN ALPHA AND PROGESTERONE RECEPTORS IN THE NORMAL MAMMARY TISSUE ADJACENT TO THE PATHOLOGICAL TISSUE

Diagnosis	Benign breast disease, $N = 299$	Breast cancer, $N = 267$
Age (years)		
−39	105	12
40–49	109	69
50–59	56	91
60+	29	95
Age at menarche (years)		
−11	51	40
−12	70	76
−13	77	78
−14	69	37
14+	29	33
Menopausal status		
Pre- and peri menopausal	227	99
Post menopausal	72	167
Parity		
Nulliparous	76	34
Parous	223	233
Body mass index (kg/m^2)		
−25	161	102
25–29, 99	78	85
30+	38	56
Family history of breast cancer		
Yes	28	33
No	271	234
Paraffin-embedded tissue (PET)		
Stored PETs	79	41
Newly processed PETs	220	226
Expression of receptors in the normal tissue	295[1]	267
Estrogen receptors alpha	196 (66%)	165 (62%)
Progesterone receptors	219 (74%)	165 (62%)

[1]For 4 women only estrogen receptor alpha expression was determined, whereas for another 4, only progesterone receptor expression was determined. For the remaining 291 women expression of both types of receptors was assessed.

Results

Table I shows the distribution of women with BBD or breast cancer by descriptive characteristics, source of tissue samples and expression of estrogen alpha and progesterone receptors in the normal mammary tissue adjacent to the pathological tissue. Because both clinics are involved in mammographic screening, participating women with breast cancer are somewhat younger than women with breast cancer in general. As expected, women with BBD are younger than breast cancer patients. All variables indicated in Table I have been adjusted for in the analyses, whereas the data were stratified by source of tissue samples.

Table II presents the odds for breast cancer *versus* BBD by, alternatively, expression of estrogen alpha or progesterone receptors in the normal mammary tissue adjacent to the pathological tissue, overall and by menopausal status. Overall, there were suggestive inverse associations between expression of estrogen alpha and progesterone receptors and breast cancer risk, which, however, were not statistically significant. For estrogen receptors alpha, the OR and 95% confidence interval (CI) were 0.68 and 0.45–1.03, whereas for progesterone receptors, the corresponding figures were 0.69 and 0.45–1.05. Among premenopausal women, associations were essentially null with respect to both estrogen receptors alpha and progesterone receptors. In contrast, among postmenopausal women, expression of both estrogen receptors alpha and progesterone receptors in the normal mammary tissue was strongly and statistically significantly inversely associated with breast cancer risk. The OR and 95% CIs were 0.39 and 0.18–0.84 for expression of estrogen receptors alpha and 0.40 and 0.20–0.79 for expression of progesterone receptors. Tests for interaction by menopausal status generated p values 0.085 for estrogen alpha and 0.046 for progesterone receptors. Although we opted for a cutoff point frequently used in clinical practice, we have also repeated the analyses using different cutoff points for receptor expression, as well as trend tests, using as exposure the H-score in successive categories. The results were consistent with those presented in Table II in documenting an inverse association of expression of estrogen alpha and progesterone receptors with breast cancer risk among postmenopausal women, whereas no clear pattern was evident among premenopausal women. In additional analyses, we

TABLE II – ODDS RATIO (OR)[1] AND 95% CONFIDENCE INTERVALS (CI) FOR BREAST CANCER VS. BENIGN BREAST DISEASE BY EXPRESSION OF ESTROGEN ALPHA OR PROGESTERONE RECEPTORS IN THE NORMAL MAMMARY TISSUE ADJACENT TO THE PATHOLOGICAL TISSUE, OVERALL AND BY MENOPAUSAL STATUS

Receptor expression	All women		Pre- and perimenopausal women		Postmenopausal women	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Estrogen alpha	0.68 (0.45–1,03)	0.069	0.93 (0.54–1.60)	0.788	0.39 (0.18–0.84)	0.015
Progesterone	0.69 (0.45–1.05)	0.085	1.14 (0.63–2.09)	0.662	0.40 (0.20–0.79)	0.008

[1]Stratified by type of tissue sample (stored or fresh samples) and controlling for age, age at menarche and BMI (continuously) as well as menopausal status, parity and family history of breast cancer (categorically).

have estimated the OR for breast cancer *versus* BBD contrasting women expressing both receptors in their normal mammary tissue to women expressing neither (and ignoring women discordant with respect to expression of the 2 studied receptors). Over all women, the OR was 0.64 (95% CI 0.39–1.04, $p = 0.069$), whereas among pre- and perimenopausal women it was 1.03 (95% CI 0.53–2.00, $p = 0.914$), and among postmenopausal women it was 0.31 (95% CI 0.13–0.73, $p = 0.008$). p value for interaction by menopausal status was 0.045.

Discussion

There have been 2 fairly large case–control studies examining the expression of estrogen receptors alpha in the normal mammary tissue in relation to breast cancer risk: the study by Khan *et al.*[7] and this investigation. An earlier study by Khan *et al.*[6] was based on data included in their later report.[7] The study by Khan *et al.*[7] and this investigation point to opposite directions. Khan *et al.* reported a nonsignificant positive association among premenopausal women (p for trend = 0.384) and a significant positive association among postmenopausal women (p for trend = 0.032). Specifically, in comparison to women with the lowest level of estrogen receptor expression, those with the highest level of estrogen receptor expression had an age-adjusted OR of 1.6 (95% CI 0.7–3.5) among premenopausal women and 3.1 (95% CI 1.3–7.6) among postmenopausal women. Further adjustment for breast cancer risk factors tended to accentuate the associations, but with much larger CIs.[7] We have found a nonsignificant inverse association of estrogen receptor alpha expression with breast cancer risk among premenopausal women and a significant inverse association among postmenopausal women. Moreover, among postmenopausal women, we found a significant inverse association of breast cancer risk with expression of progesterone receptors in the normal mammary epithelium. The reason for the discrepant results is not apparent, but the biologically plausible hypothesis advanced by Khan *et al.*[6] is not supported by our data. Instead our findings indicate that overexpression of estrogen alpha and progesterone receptors may be associated with reduced risk for breast cancer in line with the well-known association of expression of these receptors in the malignant tissue and better breast cancer prognosis.[15]

Strengths of this investigation are the larger sample size compared to previous investigations,[6,7] the comprehensive assessment of covariates and the consistency of the findings, irrespectively of the cutoff points used for the assessment of receptor expression. A limitation of the study is the unavoidable selection of study participants, who were volunteers from 2 clinics. Factors affecting selection, however, are unlikely to be correlated with receptor expression and, thus, generate selection bias. Another limitation is the use of BBD patients as a control group. It is imposed by ethical considerations, but it introduces complexity in the interpretation of the results of our study as well as those of any relevant study.

Investigation of an association between expression of estrogen receptors alpha and/or progesterone receptors in the normal mammary tissue with breast cancer risk could be of importance and deserves further investigation. If an association, positive or inverse, were conclusively documented from additional studies and were inferred as causal, it could provide a critical insight into the pathogenesis of breast cancer and, conceivably, a window of opportunity for possible breast cancer prevention.

Modulators of the expression of estrogen alpha and progesterone receptors in the normal mammary gland have not been identified, mostly because little research has been done on this subject. Such modulators, however, are likely to exist given the apparently large variability of receptor expression among women.

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