Expression of estrogen receptors in non-malignant mammary tissue modifies the association between insulin-like growth factor 1 and breast cancer risk

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Background: Several studies have reported that the insulin-like growth factor 1 (IGF-1) is positively associated with estrogen receptor-positive [ER(+) ] breast cancer risk, whereas there is little or no association with respect to ER(−) breast cancer. All comparisons of ER(+) breast cancer cases, however, have been made versus healthy controls, for whom there is no information about the ER expression in their mammary gland.

Patients and methods: In the context of a case–control investigation conducted in Athens, Greece, we studied 102 women with incident ERα (+) breast cancer and compared their IGF-1 blood levels with those of 178 ERα (+) and 83 ERα (−) women with benign breast disease (BBD) who underwent biopsies in the context of their standard medical care. Data were analysed using multiple logistic regression and controlling for potential confounding variables.

Results: ERα (+) breast cancer patients had higher IGF-1 levels compared with women with BBD [odds ratio (OR) 1.36, 95% confidence interval (CI): 0.95–1.94, per 1 standard deviation (SD) increase in IGF-1 levels]. When ERα status of women with BBD was taken into account, the difference in IGF-1 levels between ERα (+) breast cancer patients and women with BBD was clearly driven by the comparison with ERα (+) BBD women (OR = 1.95, 95% CI: 1.31–2.89 per 1 SD increase in IGF-1 levels), whereas there was essentially no association with IGF-1 levels when ERα (+) breast cancer patients were compared with ERα (−) BBD women. These contrasts were particularly evident among post/peri-menopausal women.

Conclusion(s): We found evidence in support of an interaction of IGF-1 with the expression of ERα in the non-malignant mammary tissue in the context of breast cancer pathogenesis. This is in line with previous evidence suggesting that IGF-1 increases the risk of ER(+) breast cancer.

Key words: benign breast disease, BBD, breast cancer, epidemiology, IGF-1

introduction

There is experimental evidence in support of biological interaction of estrogen receptors and IGF-1 in breast cancer causation [1, 2]. Importantly, several prospective studies, generally analysed through the nested case–control approach [3], as well as a recent case–control study nested in the European Prospective Investigation into Cancer (EPIC) [4], have reported that the insulin-like growth factor 1 (IGF-1) is positively associated with estrogen receptor-positive [ER(+) ] breast cancer risk, whereas there is little or no association with respect to estrogen receptor-negative [ER(−) ] breast cancer. Although the epidemiological evidence is supportive of a role of the expression of estrogen receptors in the IGF-1-dependent growth of mammary cancer cells, comparisons of ER(+) breast cancer cases are made versus healthy controls, for whom there is no information about the expression of estrogen receptors in their mammary gland. To overcome the ethical constraints that prohibit the evaluation of estrogen receptor expression in the mammary tissue of healthy women, we have used as control group, women with benign breast disease (BBD) who underwent biopsies in the context of

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their standard medical care. We compared IGF-1 levels among women with estrogen receptor α (ERα) positive breast cancer and women with ERα(+) and ERα(−) BBD and we report here results in support of a carcinogenic role of IGF-1 in the presence of estrogen receptors.

**materials and methods**

**subjects**

In the context of a case–control study conducted in Athens (2001–2008), we recruited women who had undergone mammary biopsy with a diagnosis of BBD or breast cancer in two major breast clinics [5]. In breast clinic I, women who underwent a breast biopsy during the duration of the study, as well as women who had undergone a biopsy with a diagnosis of BBD up to 4 years before the study initiation (but were interviewed and provided a blood sample during the study period) were eligible for the present study. In breast clinic II, all women underwent biopsy during the study period and were eligible for the present study. In the present investigation, we included only incident breast cancer cases from both centres, for whom treatment had not been initiated. For BBD, all available cases from both centres were studied, irrespective of the time of biopsy, as medication is generally not prescribed for BBD and in all BBD studies in the literature, prevalent cases have been used [6]. The study was approved by the Bioethics Committee of the University of Athens and only women who agreed to participate and provided informed consent were included in the study.

All women completed an extensive interviewer-administered questionnaire with information on sociodemographic and lifestyle factors, as well as on gynaecological history and general medical history. In breast clinic I, histological samples were available in the form of paraffin-embedded tissue (PET) blocks, whereas in breast clinic II, samples obtained during biopsy were frozen in liquid nitrogen before being stored in −80°C.

**hormone measurements**

Blood samples for hormone measurements were collected, at the time of the in-person interview, into sterile tubes, were centrifuged, and then aliquoted and stored for hormonal assays at −20°C at the University of Athens Medical School. Hormonal analyses were blindly done in the ‘Biomedicine’ laboratories (ISO 15189). IGF-1 and IGF binding protein 3 (IGFBP-3) were measured by chemical luminescence immunoassay kits (Immulite 2500 kit, Diagnostic Systems, Llanberis, Gwynedd, UK). Details on the hormone determinations have been previously reported [6].

**hormone receptor analyses**

We determined estrogen α receptors in malignant tissue from biopsies of women diagnosed with breast cancer and in the tissue adjacent to the lesion from the biopsies of women with BBD. In breast clinic I, histological samples were made available in the form of PET blocks, whereas in breast clinic II, samples obtained during biopsy were frozen in liquid nitrogen before being fixed in 10% neutral buffer formalin at 25°C for 24 h and processed to PETs. Details on the receptor information have been previously reported [5]. In brief, the streptavidine–biotin–superoxidase method [7, 8] was applied on paraffin sections. The sections that were prepared from fresh-frozen tissue were fixed in a 10% formal solution of pH 7.4, at 25°C for 24 h, in the automatic immunohistochemical BioGenex i6000 Consolidated Staining System. The primary specific mouse monoclonal antibodies were obtained from Novocastra Laboratories Ltd, Newcastle upon Tyne, UK. The clone 6F11, specific for estrogen receptor α, was applied in a 1:60 dilution. We scored the immunocytochemical results in a semiquantitative way using the ‘H-score’, that incorporates both the number of cells with positive staining for hormone receptor and the intensity of staining [7, 9]. The H-score, therefore, ranges from 0 to 300. We considered scores from 0 to 9 (inclusive) as indicative of ER-α negative tissues and scores from 10 or more as indicative of ER-α-positive tissues. The frequency of ERα expression was not statistically significantly different when the two sources of samples (PETs versus fresh-frozen tissue) were compared (P = 0.253).

In the present investigation, we included 117 women with breast cancer and 261 women with BBD, for whom we had determinations of both ERα expression in the tissue from their biopsies and hormone levels. Only 15 of the breast cancer patients were ERα(−); hence, comparisons were restricted to the 102 of them who were ERα(+).

**statistical analysis**

Analyses were conducted using SPSS (IBM Statistical Package for Social Sciences v. 19.0, Chicago, IL). We used multiple logistic regression to investigate the association between IGF-1 levels and the risk of ERα(+) breast cancer compared with women with BBD, overall as well as, separately, ERα (+) BBD and ERα(−) BBD. Analyses were carried out both overall controlling for menopausal status and separately among pre- and peri/post-menopausal women. In the models, we controlled for age (continuously), phase of menstrual cycle (1 = blood sample collected in days 2–11 and 0 = else) among pre-menopausal women, and external hormonal use (oral contraceptives among pre-menopausal women or hormone replacement treatment (HRT) among peri/post-menopausal women). Models adjusted also for IGFBP-3 (continuously). To test the robustness of our results, we also applied regression models that further controlled for age at menarche (continuously), parity (parous versus nulliparous), body mass index (BMI, continuously), smoking habits (current smokers versus other), educational level (ordered, with 1 = up to 6 years of schooling, 2 = 7–12 years and 3 = more than 12 years) and lactation (women who had breast-fed versus those who had not). Due to power limitations, the extensive models were not applied in the stratified analysis by menopausal status. Finally, we tested for heterogeneity between the effect estimates for ERα (+) breast cancer per SD increase in IGF-1 levels, between women with ERα(+) and those with ERα(−) BBD, using the Q test for heterogeneity.

**results**

Table 1 presents age, menstrual history and hormone use data for ERα(+) breast cancer patients (n = 102) and women with BBD overall and by ERα status [ERα(+) = 178 and ERα(−) = 83]. As expected, women with incident breast cancer are generally older than those diagnosed with BBD (mean age 57 versus 44 years, respectively). Among women with BBD, those who are ERα(+) in comparison to those who are ERα(−) are generally older (mean age 45 versus 42 years, respectively) and somewhat heavier (mean BMI 27.0 versus 25.7 kg/m², respectively). These data are not age-adjusted and serve descriptive purposes only.

Table 2 shows the median, 25th and 75th percentiles of IGF-1 and IGFBP-3 by menopausal status among ERα(+) breast cancer patients and women with BBD (overall and by ERα status). Among pre-menopausal women, those with breast cancer appear to have higher levels of IGF-1 and IGFBP-3, whereas no such differences are evident among peri/post-menopausal women. In women with BBD, irrespective of menopausal status, IGF-1 levels appear to be higher among those who are ERα(−) than among those who are ERα(+) or no differences are apparent with respect to IGFBP-3. As data in Table 2 take into account menopausal status, but no other potential confounders, they serve descriptive purposes only.
Table 1. Characteristics\(^a\) of breast cancer patients and women with benign breast disease (BBD) by estrogen receptor \(\alpha\) (ER\(\alpha\)) status\(^b\)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Cancer ER(\alpha)(+) (n = 102)</th>
<th>All BBD (n = 261)</th>
<th>BBD ER(\alpha)(+) (n = 178)</th>
<th>BBD ER(\alpha)(-) (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;49)</td>
<td>27 (26.5)</td>
<td>181 (69.3)</td>
<td>117 (65.7)</td>
<td>64 (77.1)</td>
</tr>
<tr>
<td>(\geq50)</td>
<td>75 (73.5)</td>
<td>80 (30.7)</td>
<td>61 (34.3)</td>
<td>19 (22.9)</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>12.8 (1.4)</td>
<td>12.9 (1.5)</td>
<td>12.8 (1.5)</td>
<td>13.1 (1.3)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>28 (27.5)</td>
<td>171 (65.5)</td>
<td>108 (60.7)</td>
<td>63 (75.9)</td>
</tr>
<tr>
<td>Post- and peri-menopausal</td>
<td>74 (72.5)</td>
<td>90 (34.5)</td>
<td>70 (39.9)</td>
<td>20 (24.1)</td>
</tr>
<tr>
<td>Age at menopause (years, among peri/post-menopausal)</td>
<td>49.4 (5.6)</td>
<td>48.7 (6.1)</td>
<td>48.4 (6.7)</td>
<td>49.7 (3.1)</td>
</tr>
</tbody>
</table>

| Body mass index (kg/m\(^2\)) | 26.1 (5.6) | 26.8 (6.1) | 26.6 (6.2) | 26.8 (6.3) |
| Hormone use (yes versus no)\(^c\) | 7 (6.9) | 41 (15.7) | 27 (15.2) | 14 (16.9) |

\(^a\)For quantitative: mean (standard deviation); for qualitative: number (%).
\(^b\)Malignant mammary tissue for breast cancer cases.
\(^c\)Oral contraceptives use for pre-menopausal and hormone replacement therapy for peri/post-menopausal women.

Table 2. Median and quartiles (25th–75th percentile) of insulin-like growth factor 1 (IGF-1) and its binding protein 3 (IGFBP-3) by menopausal status and estrogen receptor \(\alpha\) (ER\(\alpha\)) expression status among breast cancer patients and women with benign breast disease (BBD)

<table>
<thead>
<tr>
<th></th>
<th>Breast cancer ER(\alpha)(+)</th>
<th>All BBD</th>
<th>BBD ER(\alpha)(+)</th>
<th>BBD ER(\alpha)(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-menopausal women</td>
<td>(n = 28)</td>
<td>(n = 171)</td>
<td>(n = 108)</td>
<td>(n = 63)</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>182.5 (138.8–212.0)</td>
<td>169.0 (139.0–190.0)</td>
<td>162.0 (135.5–218.8)</td>
<td>175.0 (147.0–219.0)</td>
</tr>
<tr>
<td>IGFBP-3 ((\mu)g/ml)</td>
<td>4.4 (3.8–4.8)</td>
<td>3.9 (3.5–4.4)</td>
<td>4.0 (3.5–4.4)</td>
<td>3.9 (3.5–4.5)</td>
</tr>
<tr>
<td>Post- and peri-menopausal women</td>
<td>(n = 74)</td>
<td>(n = 90)</td>
<td>(n = 70)</td>
<td>(n = 20)</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>125.0 (95.8–160.0)</td>
<td>131.5 (92.8–158.8)</td>
<td>129.5 (92.8–153.3)</td>
<td>148.0 (96.8–188.0)</td>
</tr>
<tr>
<td>IGFBP-3 ((\mu)g/ml)</td>
<td>3.7 (2.9–4.3)</td>
<td>3.8 (3.3–4.6)</td>
<td>3.8 (3.3–4.6)</td>
<td>3.9 (3.6–4.6)</td>
</tr>
</tbody>
</table>

Table 3 presents multiple logistic regression-derived odds ratios (ORs) and 95% confidence intervals (CIs) for ER\(\alpha\)(+) breast cancer versus BBD, overall and by ER\(\alpha\) status per standard deviation increase in IGF-1 levels, after adjustment for several potential confounders. There is evidence, which however does not reach statistical significance, that ER\(\alpha\)(+) breast cancer patients have higher IGF-1 levels compared with women with BBD (OR = 1.36, 95% CI: 0.95–1.94). When ER\(\alpha\) status of women with BBD is taken into account, the difference in IGF-1 levels between ER\(\alpha\)(+) breast cancer patients and women with BBD is clearly driven by the comparison with BBD women who are ER\(\alpha\)(+) (OR = 1.95, 95% CI: 1.31–2.89), whereas there is essentially no association with IGF-1 levels when ER\(\alpha\)(+) breast cancer patients are compared with ER\(\alpha\)(-) BBD women. These contrasts are particularly evident among post/peri-menopausal women.

To test the robustness of our results, we also ran models controlling for additional potential confounders, as indicated in the Statistical analysis section; due to power limitations, these models were not applied in the stratified analysis by menopausal status, but for the overall sample. For the comparison between ER\(\alpha\)(+) breast cancer and all BBD, the OR was 1.52 (95% CI: 1.04–2.23, \(P = 0.031\)), whereas for the comparison between ER\(\alpha\)(-) breast cancer and ER\(\alpha\)(+) BBD, the OR was 2.22 (95% CI: 1.45–3.42, \(P < 0.001\)). Finally, when we tested for heterogeneity of the effect estimates for ER\(\alpha\)(+) breast cancer per SD increase in IGF-1 levels, between women with ER\(\alpha\)(+) and women with ER\(\alpha\)(-) BBD, using the \(Q\) test for heterogeneity, the effect estimates were statistically significantly different both for the overall sample (\(P = 0.004\)) and among post/peri-menopausal women (\(P = 0.003\)).

**discussion**

There is convincing evidence that IGF-1, possibly through its effects on cell proliferation and apoptosis, is positively associated with the risk of both pre-menopausal and post-menopausal breast cancer [3, 10]. There is also evidence that this association is limited to ER\(\alpha\)(+) breast cancer on account of a postulated ‘cross-talk’ among estrogens, IGF-1 and their respective receptors in the mammary gland [2, 3, 4, 11]. Important as it is, this evidence does not address a possible role of IGF-1 in the early stages of breast carcinogenesis, that is, at the stages when IGF-1 operates on non-malignant tissue, which is positive or negative for the expression of ER\(\alpha\). We have attempted to fill this gap by examining whether breast cancer [most of which is ER\(\alpha\)(+)] is
more strongly positively associated with IGF-1 when the comparison series comprises women with non-cancerous mammary tissue positive rather than negative for ERα (a). We found that, at least with respect to ERα(+) breast cancer [there were few ERα (–) cases of breast cancer to allow meaningful separate analyses], IGF-1 was positively associated with ERα(+) breast cancer only when the comparison series comprised women with ERα(+) non-malignant mammary tissue. This is particularly evident and statistically significant among post-menopausal women, although suggestive evidence is also apparent among pre-menopausal women (P ~ 0.08). Of note, when all women with breast cancer for whom we had receptor information and hormone measurements were considered, the results were essentially identical to those obtained when only women with ERα (+) breast cancer were examined (data not shown). We interpret our findings as indicating that the expression of ERα is a pre-requisite for the action of IGF-1 in the pathogenesis of breast cancer, at least ERα(+) breast cancer.

Kaaks et al. [4] have reported an overall 17% increase in the risk of ERα(+) breast cancer per 1 SD increase in IGF-1 levels, whereas we found a 95% increase in ERα(+) breast cancer risk per standard deviation increase in IGF-1 levels when the comparison was made with women with ERα(+) BBD. It is possible that the stronger association found in our study could be accounted for by the apparent effect of IGF-1 in the ERα(+) non-cancerous tissue of our comparison group. At this stage, however, this is just speculation, since the study by Kaaks et al. had a very different design and, in their study, expression of both estrogen and progesterone receptors was considered (although expression of ER is required for the expression of progesterone receptors [12, 13]).

Expression of ERα in the non-cancerous tissue that surrounds the malignant one has been investigated in a few studies with inconclusive results. Earlier work provided evidence that ER expression in the non-cancerous tissue may increase the risk of breast cancer by amplifying the influence of circulating steroids [14, 15], whereas later work suggested that expression of ER may reflect terminal differentiation that could counter carcinogenic influences [5]. To our knowledge, however, expression of estrogen receptors in the non-malignant mammary tissue has not been previously studied in conjunction with compounds of the IGF system in epidemiological studies focusing on the aetiology of breast cancer. Yet, there is substantial experimental evidence pointing to interaction of estrogens, IGF-1 and their receptors [1, 2, 11, 16] providing biological plausibility, in a general way, to our findings with respect to breast cancer.

Strengths of our investigation are the introduction of a novel approach in probing the pathogenesis of breast cancer and the simultaneous evaluation of expression of ERα and levels of the two main compounds of the IGF system, that is, IGF-1 and IGFBP3. A limitation of our approach is that our case–control study was not nested in an explicitly defined cohort and the comparison series of BBD does not represent the study base of our breast cancer cases (many of which develop in the absence of an identifiable BBD). However, ethical reasons do not allow mammary biopsies in women with no evidence of breast pathology. The case–control design may be susceptible to biases, but the results with respect to IGF-1 (and IGFBP3) were in line with those reported from large cohort studies [3] and the exclusions of...
study subjects were in no apparent way bias prone, but rather imposed by technical and financial constraints. Finally, the numbers (particularly with respect to breast cancer cases) were not large, although they were adequate to generate strongly suggestive findings; nevertheless, caution is advisable in the interpretation, until our findings are replicated by independent studies.

In conclusion, we found evidence in support of an interaction of IGF-1 with the expression of ERα in the non-malignant mammary tissue in the context of breast cancer pathogenesis. This is in line with previous evidence suggesting that IGF-1 increases the risk of ER(+) breast cancer.

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**disclosure**

The authors have declared no conflicts of interest.

**references**