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Oestrogen receptor expression in normal breast epithelium

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Abstract

Higher levels of oestrogen receptor (ER) expression in normal breast epithelium may compound the increase in breast cancer risk seen with prolonged estrogen exposure. In prior studies, we have used immunohistochemical ER assays on fresh frozen samples of benign breast tissue. Future studies will be more feasible on paraffin-embedded samples, and newer, more sensitive antibodies are now available. We examined 30 samples of paraffin-embedded breast epithelium from postmenopausal women with two antibodies, 6F11 and TE111. We find that the median labelling indices for ER are significantly higher using these antibodies, compared with previous results. The threshold for ER positivity will, therefore, have to be reset in future studies, since there are still many issues that remain to be resolved in this area.

Keywords: Oestrogen receptor; Normal breast; Breast cancer risk

1. Background

Lifetime oestrogen exposure is a major risk factor for breast cancer. Increased oestrogen responsiveness of breast epithelium may enhance this effect. In previous studies [1,2] we examined the relationship between breast cancer diagnosis and the presence and level of oestrogen receptor (ER) expression in benign breast epithelium. We found that the median ER labelling index was 7.7 in the cases and 3.2 in the controls ($P = 0.001$). When adjusted for age and other known breast cancer risk factors, the odds ratio (OR) of carrying a diagnosis of breast cancer in ER-positive women overall was 2.63 (95% confidence interval (CI) = 1.47–4.70). For premenopausal women, it was 2.04 (95% CI = 0.97–4.3), and for postmenopausal women the association was stronger, with an OR of 3.8 (95% CI = 1.5–9.8). The level of ER expression was higher in breast cancer patients than in control subjects (i.e. those undergoing breast biopsy for benign conditions), and it was related to breast cancer risk in postmenopausal women (P trend < 0.005). Expression declined as expected in premenopausal control subjects as the menstrual cycle progressed but rose in breast cancer patients (P trend < 0.015). This work was performed on frozen sections by immunohistochemistry (IHC) using the monoclonal antibody (MAb) H222, since this was the gold standard for IHC at the time the study was initiated. Samples expressing ER in at least 1% of epithelial cells were designated ER-positive, based on data from a comparative study of breast cancer cryostat sections and ligand binding assays for ER [3]. Future confirmatory studies will most likely be performed on paraffin sections, and we, therefore, tested two different antibodies on paraffin-embedded sections, to assess the importance of antibody performance in the measurement of ER expression in normal breast tissue.

2. Methods

We measured ER expression (as a per cent of positive cells) by immunohistochemistry in normal breast epithelium from women undergoing breast surgery. Sections of formalin-fixed, paraffin-embedded breast epithelium from 30 women (15 breast cancer cases and 15 age-matched controls) were processed. We included only postmenopausal women (mean age: 57 years) to avoid the menstrual cycle variation seen in premenopausal women. Sections were immunostained with ER-TE111 and ER-6F11 antibodies and counterstained with haematoxylin. Positively stained epithelial cells were counted, and all cells were estimated by field coverage. Ductal and lobular structures were counted

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Table 1
Median labelling indices for the two oestrogen receptor (ER) antibodies by case–control status, for ductal and lobular tissue

	Cases		Controls	
	TE111	6F11	TE111	6F11
Ducts	26.4	34.0	27.3	26.5
Lobules	18.0	13.8	13.0	17.5
Total	28.6	30.2	23.8	26.1

separately. Labelling indices (LIs) were calculated as (number of positive cells/number of total cells)×100.

3. Results

In general, 6F11 stained sections demonstrated stronger and crisper staining than TE111 sections. The labelling indices for the two antibodies was well correlated, $R^2 = 0.7$, $P < 0.0001$. Median values of the ER LIs are shown in Table 1.

4. Discussion

ER labelling on paraffin sections was significantly higher than seen in our prior study using frozen sections, where the median ER LI was 3.2 for controls and 7.7 for cases. The two antibodies used in this small study showed no significant differences in the ER LI, with median values being generally slightly, but not significantly higher for cases than for controls.

5. Conclusions

The overexpression of ERs in normal breast epithelium may augment oestrogen sensitivity and hence the risk of breast cancer. The proportion of epithelial cells positive for ER is markedly higher using IHC on paraffin sections with both of the antibodies tested in this study compared with results obtained using H222 on frozen sections. The threshold for ER positivity will have to be redefined when studies are done using paraffin embedded tissue and these newer antibodies.

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P27^{KIP1} expression indicates that steroid receptor-positive cells are a non-proliferating, differentiated subpopulation of the normal human breast epithelium

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Abstract

To test the hypothesis that steroid receptor-expressing cells are derived from the proliferative population, we examined expression of the p27^{KIP1} inhibitor of cyclin-dependent kinase activity (a differentiation marker) while tracking the fate of proliferating cells in normal human breast tissue implanted into athymic nude mice using tritiated thymidine [^{3}H]-dT. We identified a small number of cells that appeared to have divided just once before switching on p27^{KIP1} expression. p27^{KIP1} + ve cells also expressed steroid receptors, but not the Ki67 proliferation-associated antigen. These data support the hypothesis that steroid receptor-expressing cells are a differentiated population within the normal human breast epithelium.

Keywords: Steroid receptors; Normal breast epithelium; Differentiation; p27^{KIP1}

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