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.

References

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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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Table 1
Dual fluorescent label IHC analysis of epithelial progesterone receptor (PR), $p27^{KIP1}$ and Ki67 proliferation antigen expression in 20 samples of normal human breast tissue obtained at surgery

n	Total cells	$p27^{KIP1}$ + ve (%)	PR + ve (%)	Ki67 + ve (%)	% PR + expressing p27	% Ki67 + ve expressing p27
20	21 259	9878 (46)	–	366 (2)	–	10 (+/−SEM 0.3)
20	21 795	9895 (45)	2255 (10)	–	92 (+/−SEM 2.6)	

SEM, standard error of the mean.

1. Introduction

The receptors for oestrogen and progesterone (ER and PR) are co-expressed in approximately 10–20% of normal human breast epithelial cells, but expression of the Ki67 proliferation-associated antigen segregates to a separate population [1]. Loss of this separation between cell proliferation and steroid receptor expression may be an early event in breast tumorigenesis as it occurs in atypical ductal hyperplasia and ductal carcinoma *in situ*, as well as in invasive carcinoma [2]. Our working hypothesis is that in the normal breast epithelium, the steroid receptor-positive cells are a differentiated population derived from the proliferative cells to act as steroid sensors and to control proliferative activity via paracrine mechanisms. To test this hypothesis we tracked the fate of proliferating breast epithelial cells over time and in relation to the expression of the $p27^{KIP1}$ inhibitor of cyclin-dependent kinase activity, which has been shown to be a differentiation marker in other tissues such as the ovaries and testes [3].

2. Experiments

Normal breast tissue was implanted into athymic nude mice which were treated with oestradiol for 3 weeks at concentrations known to stimulate proliferation. One week after the start of oestradiol treatment, four injections of [^{3}H]-dT (100 μCi intraperitoneally) were administered over an 18-h period to intensively label the proliferating cells, and pieces of breast tissue were harvested 1 h, 12 h and 2, 3, 4, 7 and 14 days later. $p27^{KIP1}$ expression and proliferation were co-localised on the same sections by immunohistochemistry (IHC) followed by autoradiography.

One hour after injection, radiolabel was present in 5.1% of luminal epithelial cells declining to 1.5% after 14 days suggesting that, although most of the radiolabelled cells had continued to proliferate during the 2-week period halving their DNA label at each division until it was undetectable, a small number had divided just once. The proportion of ^{3}[H]dT-labelled cells that were also $p27^{KIP1}$ positive was 7.4% at 1 h but nearly all of the label retaining cells were $p27^{KIP1}$ + ve (97 and 86% at 1 and 2 weeks, respectively) suggesting that they were a terminally differentiated population. Dual fluorescent label IHC was then used to examine the co-expression of PR, $p27^{KIP1}$ and Ki67 in normal breast tissue obtained from 20 women at surgery. $p27^{KIP1}$ was expressed in 45% of all normal breast epithelial cells, but 92% of PR + ve cells were $p27^{KIP1}$ + ve, whereas only 10% of Ki67 + ve cells contained $p27^{KIP1}$ (Table 1).

3. Conclusion

These data suggest that we have observed the process of terminal differentiation in the normal human breast epithelium whereby some proliferating cells undergo a final round of division before becoming quiescent and switching on $p27^{KIP1}$ and steroid receptor expression. These data support the hypothesis developed to explain the dissociation between steroid receptor expression and proliferation seen in the normal breast. Further studies on the mechanisms by which steroid receptor expression and proliferation become associated during breast tumorigenesis should be informative.

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