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Report

Loss of co-ordinate expression of progesterone receptors A and B is an early event in breast carcinogenesis

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Summary

Progesterone receptor (PR) mediates the effects of progesterone in mammary tissues and plays a crucial role in normal breast development and in breast cancer. PR proteins are expressed as two isoforms, PRA and PRB, that have different capacities to activate target genes, yet it is unknown whether progesterone action in normal and malignant breast is mediated by PRA and/or PRB. This study determines the relative expression of PRA and PRB in normal breast and in benign, premalignant and malignant archival breast lesions by dual immunofluorescent histochemistry.

In normal breast and in proliferative disease without atypia (PDWA) PRA and PRB were co-expressed within the same cells in comparable amounts, implicating both isoforms in progesterone action. In atypical lesions, however, there was a significant increase in predominant expression of PRA or PRB, with lesion progression from the normal state to malignancy. PR isoform predominance, especially PRA predominance, was evident in a high proportion of ductal carcinomas *in situ* (DCIS) and invasive breast lesions.

In the normal breast and in PDWA, the relative expression of PRA and PRB in adjacent cells was homogenous. There was a significant increase in cell-to-cell heterogeneity of PR isoform expression in ADH and DCIS lesions and in the majority of breast cancers. Heterogeneous cell-to-cell expression of PR isoforms occurred prior to overall predominant expression of one isoform in premalignant breast lesions, demonstrating that loss of control of relative PRA:PRB expression is an early event in the development of breast cancer. PRA:PRB ratios within a breast lesion are likely to be important as both markers and effectors of tumor growth and development, and progressively aberrant PR isoform expression may play a role in the etiology of breast cancer.

Introduction

Estrogen and progesterone are essential for normal breast development, and studies of ER or PR null mice have shown that these hormones mediate their effects via their cognate receptors. Female mice lacking ER have undeveloped mammary glands with only rudimentary ducts present at the nipples [1], and female PR null mice fail to establish the lobular-alveolar system essential for lactation [2]. The roles of estrogen and progesterone on adult mammary gland function during the menstrual cycle are less clear, although, as morphological alterations observed in the breast during the menstrual cycle are related to levels of cycling hormones [3], there is little doubt that the adult human breast is a target organ for ovarian steroid hormones.

PR is implicated in breast cancer in addition to its role in normal breast development and function. Primary breast cancers that are PR positive are more likely to be of smaller size, less proliferative and better differentiated [4, 5], and patients with PR positive tumors are considered to have a more favorable prognosis. Moreover, PR expression in breast cancers is associated with better response to endocrine agents. In postmenopausal women, primary breast tumors which lack PR are more likely to progress to secondary sites

than tumors which express PR [6], suggesting that PR may also play a role in limiting breast cancer progression.

The mechanism of progesterone action in breast cancer is not known. Progesterone is known to inhibit the mitotic actions of estrogen in the normal uterus by down-regulating expression of ER and inducing estrogen metabolism [7, 8], and hyperplasia, consistent with unopposed estrogen action, is observed in the uterus of estrogen-treated PR null mice [2]. However, it is not known whether progesterone has anti-estrogenic effects in the breast. Cell line studies support such a role [9, 10] and ER expression in both the normal breast and breast carcinomas is down-regulated during the luteal phase of the menstrual cycle at a time when serum progesterone levels are high [11].

The progesterone receptor is expressed as two isoforms, PRA and PRB, which are identical except that the shorter A protein is N-terminally truncated by 164 amino acids. PRA and PRB are co-expressed in the same target cells in the human [12], and have different capacities to activate target genes [13–19]. In all cell types examined PRB exhibited hormone-dependent transactivation, whereas the transcriptional activity of PRA was cell- and reporter- specific [14, 16, 17]. Interestingly, PRA acted as a transdominant inhibitor of PRB where PRA had little or no transactivational activity [14, 17] and moreover PRA regulated the transcriptional activity of other nuclear receptors such as glucocorticoid, mineralocorticoid, androgen and estrogen receptors [14, 19, 20], suggesting that PRA may play a central role in regulating the activity of a number of nuclear receptors in addition to PRB.

The different transcriptional activities of PRA and PRB, and the inhibitory activity of PRA *in vitro*, suggest that tissues that express different relative levels of the two proteins, and in particular high levels of PRA, may have impaired responsiveness to progesterone and other nuclear receptor ligands. This is supported by the observation that transgenic mice over-expressing PRA exhibited features in their mammary glands that were abnormal and commonly associated with neoplasia [21]. In breast cancers, high levels of PRA can occur: previous studies from this laboratory using immunoblot analysis documented very high levels of PRA in a subset of breast tumors [22].

Despite the importance of PR in normal breast development and its association with features of good prognosis in breast cancer, it is still unknown whether progesterone action in normal and malignant breast is mediated by PRA and/or PRB. Given the different transcriptional activities of the two PR isoforms, analysis of PRA and PRB expression in normal breast and breast lesions is fundamental to understanding the respective roles of these PR isoforms in this tissue. This study was designed to determine whether both PR isoforms are expressed in normal breast tissue, and whether there are any changes in the relative expression of PRA and PRB in normal tissue linked to hormonal fluctuations during the menstrual cycle. In addition, to compare PR isoform expression in the normal breast and breast lesions, as variation in PRA and PRB expression is likely to be associated with aberrant response to ovarian steroid hormones.

Materials and methods

Patient material

Paraffin-embedded normal breast tissues ($n = 13$) from autopsies were dated during the menstrual cycle by dating of the endometrium from the same woman [3]. The cases were evenly distributed between the follicular ($n = 7$) and luteal ($n = 6$) phases of the menstrual cycle. Archival specimens of formalin-fixed, paraffin-embedded blocks of premalignant and malignant breast lesions were obtained from the Department of Tissue Pathology, Westmead Hospital, Westmead, Australia. The women were aged between 21–80 years. Premenopausal status was arbitrarily assigned in women who were aged 50 years or less. Based on the pathology reports the specimens were divided into four categories:- proliferative disease without atypia (PDWA, $n = 15$); atypical ductal hyperplasia (ADH, $n = 15$); ductal carcinoma *in situ* (DCIS, $n = 15$), and malignant carcinoma ($n = 39$). Where more than one type of lesion was present within a specimen, this was noted. Most of the DCIS lesions (13/15) were adjacent to an area of breast carcinoma within the same section. In two DCIS specimens (2/15) there were adjacent benign lesions, with no evidence of tumor. The malignant breast cohort was comprised of various tumor types that were predominantly infiltrating ductal carcinomas ($n = 33$) but also included infiltrating lobular carcinomas ($n = 2$); invasive cribriform ductal adenocarcinoma ($n = 1$); infiltrating tubulo-lobular carcinoma ($n = 1$); carcinoid-like tumor ($n = 1$); and infiltrating ductal papillary carcinoma ($n = 1$). Details of tumor grades and mitotic rates (number of

cells undergoing mitosis per 10 high power fields) of the malignant breast cohort were obtained from the Department of Tissue Pathology, Westmead Hospital. This project was approved by the Human Research Ethics Committees of the Western Sydney Area Health Service and the University of Sydney, under the guidelines of the National Health and Medical Research Council of Australia.

Section preparation and antigen retrieval

Formalin-fixed, paraffin-embedded sections were cut at 2 μm using a standard rotary microtome, mounted onto Superfrost Plus slides (Lomb Scientific, NSW, Australia) to which Mayer Albumen adhesive [23] had been applied, and dried at 37°C for 72 h. This was followed by storage at 4°C for no longer than 3 weeks. A combination of heat and pressure was used for antigen retrieval as previously described [24]. Briefly, immediately prior to staining, sections were deparaffinized, rehydrated to distilled water, placed in 0.01 M sodium citrate solution (pH 6.0), and heated in a Tuttnauer 2540 EKA autoclave at 121°C, 15 psi for 30 min. After autoclaving, the sections were allowed to remain in the sodium citrate solution for a minimum of 30 min, followed by washing in phosphate buffered saline (PBS).

Immunoperoxidase staining

Following antigen retrieval, endogenous peroxidase activity was reduced by incubation of sections in 3.0% (v/v) hydrogen peroxide. All incubations were performed at room temperature in a moist chamber. Non-specific background staining was blocked with normal goat serum (Hunter Antisera, NSW, Australia) (1:1 in PBS, 30 min). Sections were incubated with a mixture of the primary mouse anti-human PR monoclonal antibodies hPRa7 and hPRa6 which recognize PRA and PRB, respectively in archival tissue [12, 25], (1:10 and 1:5, respectively in PBS/0.5% triton-X 100, overnight), a biotinylated goat anti-mouse antibody (Dako, NSW, Australia, 1:100 in PBS, 30 min), and a streptavidin-biotin-horseradish peroxidase complex (Zymed, USA). PR proteins were visualized using diaminobenzidine (DAB) (Dako, NSW, Australia) (1 mg/ml DAB, 0.02% hydrogen peroxide in PBS). Sections were lightly counterstained with haematoxylin, dehydrated and mounted in Normount (Fronine, Australia). In control sections the primary antibody was replaced with antibody diluent. PR content was estimated by analysis of the entire section for both staining intensity and the number of cells PR positive, and scored according to a four point scale: very high (4); high (3); moderate (2); low (1).

Dual immunofluorescent staining

Following antigen retrieval, sections were stained sequentially for PRB then PRA as described previously [12]. Briefly, to detect PRB, sections were incubated with a mouse anti-human PR monoclonal antibody that detects PRB only (hPRa 6) [26] and with a biotinylated goat anti-mouse antibody (Dako, NSW, Australia), and Texas red (TXR)-avidin (Vector Laboratories, CA, USA). To reveal PRA, sections were incubated with a mouse monoclonal antibody to detect PRA (hPRa7) [12] and with a biotinylated goat anti-mouse antibody (Dako, NSW, Australia) and fluorescein isothiocyanate (FITC)-avidin (Calbiochem, Australia). Sections were mounted with Vectashield mountant for fluorescence (Vector Laboratories, CA, USA) and stored in the dark at 4°C.

Using the dual immunofluorescent staining technique described, under dual fluorescent excitation PRB proteins were labeled with TXR and stained orange; PRA proteins were labeled with FITC and stained green, and cells expressing both PRA and PRB appeared yellow. Control sections were treated and stained in the same way as the test sections. Controls included adjacent sections to each breast sample stained using antibody diluent (i) in place of both primary antibodies to control for non-specific staining and (ii) to replace the second sequence primary antibody to ensure no cross-reactivity between the two staining sequences. Human colon tissue was also used as a negative control. Dual staining of T-47D clones known to be expressing approximately equal amounts of PRA and PRB [27], and a PR positive breast tumor were also included as controls to confirm the specificity and reproducibility of the dual immunofluorescent staining technique.

Fluorescent analysis

PR staining was examined using an Olympus BX 40 fluorescence microscope fitted with filters to detect both TXR (band pass 545–580 nm) and FITC (band pass 450–480 nm) fluorescence simultaneously, and each of the two fluorochromes separately. The whole section was examined in detail under individual

fluorochrome excitation and also using the dual filter by two observers, and intensity per field recorded.

The relative expression of PRA and PRB was assessed by determination of the level of FITC and TXR fluorescence over the entire lesion under single and dual excitation. Given the limitations in quantitation of fluorescent images, cases were scored conservatively: tumors which expressed both PRA and PRB at a similar intensity were described as PRA = PRB; tumors which contained both isoforms but in which one isoform was clearly expressed predominantly, or tumors that contained only one isoform, were described as PRA > PRB or PRB > PRA. In addition to the conservative scoring method described above, the breast tumor sections were also scored by individual analysis of each PR positive cell and scoring the relative intensity (scale 0–5) of each fluorochrome per cell, under dual excitation, to derive a FITC and TXR score for each tumor (a minimum of 1000 cells were scored per tumor). An overall green:orange (PRA:PRB) ratio was determined for each tumor from the FITC and TXR scores: ratios falling between 0.8 and 1.2 were determined to reflect approximately equivalent PRA and PRB expression. Comparison of this and the conservative method of scoring revealed the results to be similar.

Statistical analyses

Paired Student *t*-Test was used to compare the levels of PR expressed between pre- and postmenopausal women, between DCIS and adjacent tumor lesions, and between premalignant and invasive lesions. Spearman rank correlation was used to assess correlation of relative PRA and PRB expression in DCIS and adjacent lesions. Significant associations of PR isoform predominance or inter-cell heterogeneity of PRA and PRB expression between normal, premalignant and malignant breast lesions were determined by logistic regression analysis.

Results

PRA and PRB expression in normal breast during the menstrual cycle

When PRA and PRB were revealed by dual immunofluorescence, PR positive cells were detected with moderate to high intensity amidst cells that were negative for PR (Figure 1(A)). In the majority of normal breast samples (10/13), PR positive cells were green/yellow or yellow under dual fluorescent excitation, demonstrating that PRA and PRB were co-expressed at similar levels throughout the menstrual cycle (Figure 1(A)). Single wavelength excitation confirmed that both PR isoforms were present at significant levels (not shown). The proportion of PR positive epithelial cells in the normal breast averaged 10–20%, but there was marked variability throughout the section, with PR positivity in individual ducts or lobules ranging from 0 to 90%. All PR staining of epithelial cells was nuclear and no cytoplasmic staining was detected.

Total PR expression in PDWA and ADH breast lesions

Compared to normal breast tissue, there was a marked increase in the number of epithelial cells expressing PR among PDWA (Figure 1(B)) and ADH (Figure 1(C)) cases, with greater than 70% of cells in the majority of ducts and lobules being PR positive. This was in contrast to normal breast tissue where PR expression was observed in 10–20% of epithelial cells. In normal breast tissue, PR negative ducts and lobules were also more frequently observed than in PDWA lesions (not shown). There were no significant differences between PDWA and ADH lesions with respect to total levels of PR expression (not shown).

PRA and PRB expression in PDWA, ADH, DCIS and invasive breast lesions

In all PDWA cases PRA and PRB proteins were co-expressed at similar levels in PR positive cells (Figure 1 (B) and Table 1). In 10/15 (67%) ADH lesions, both isoforms were represented at similar levels (Figure 1(C)), with the remaining cases showing a predominance of either PRA or PRB (Table 1). The relative expression of PRA and PRB proteins in PDWA and ADH lesions was unrelated to menopausal status of the patient, or to the presence of any particular type of adjacent abnormal lesion (not shown).

In contrast to the normal breast and PDWA and ADH lesions, the majority of DCIS lesions (53%) expressed a predominance of one PR isoform, with a predominance of PRA accounting for 6/15 (40%) of the total samples tested (Table 1). In the majority of cases (9/13, 69%) the total level of PR expressed in a DCIS lesion was the same as the PR expression level in the adjacent tumor and there was good correlation

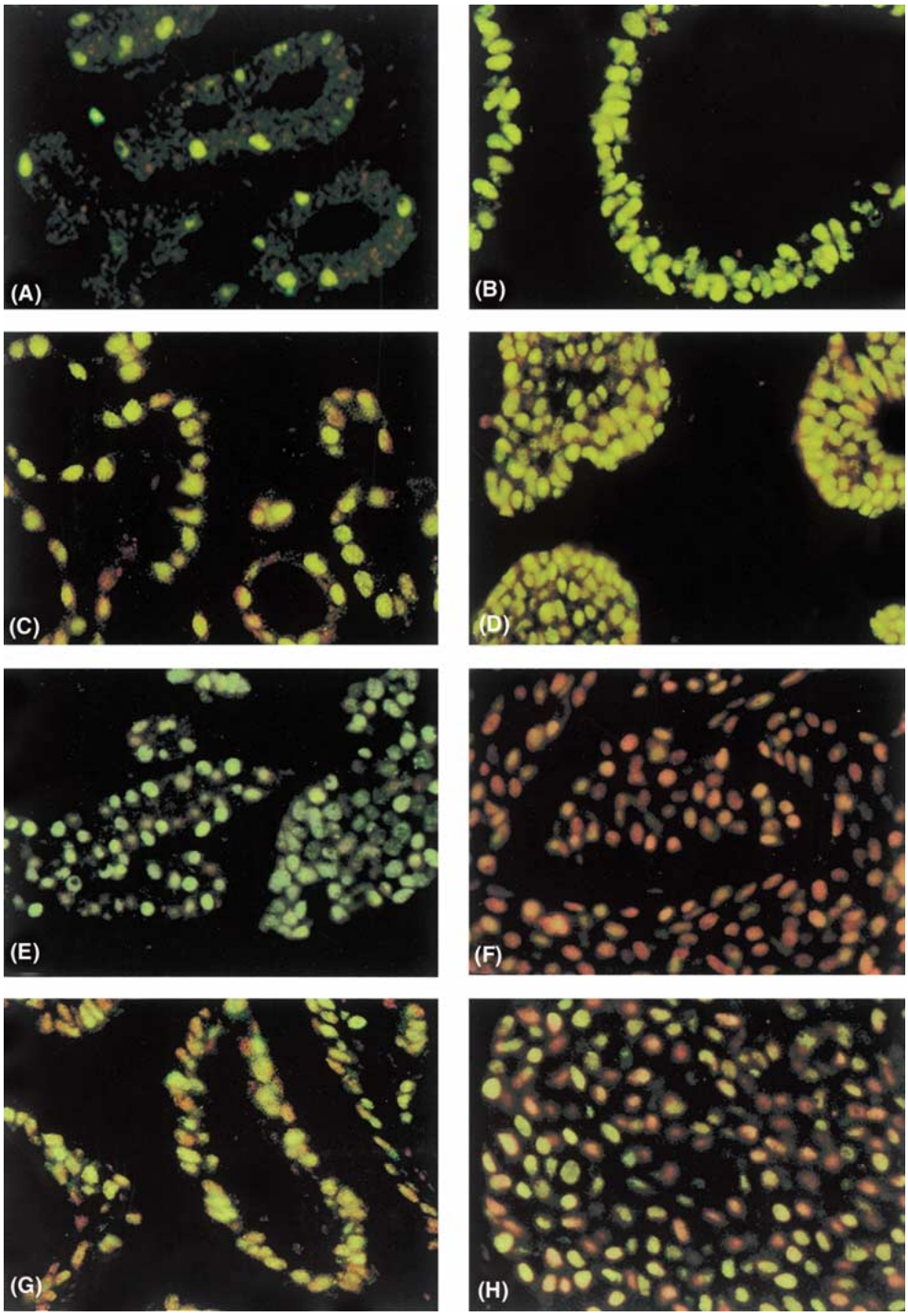

Figure 1. Expression of PRA and PRB in breast lesions. Expression and relative levels of PRA and PRB were determined by dual immunofluorescent histochemistry. Similar, homogeneous expression of PRA and PRB proteins in (A) normal breast, (B) PDWA, and (C) ADH breast lesions. Expression of PR isoforms in invasive breast lesions showing similar levels of PRA and PRB (D); predominance of PRA (E), and predominance of PRB (F). Cell to cell heterogeneity in PRA and PRB levels in ADH (G) and invasive (H) breast lesions. Original magnification $\times$ 400.

Table 1. PRA and PRB expression in normal, premalignant and invasive breast lesions

	Normal (%)	PDWA (%)	ADH (%)	DCIS (%)	Invasive (%)
PRA > PRB	0 (0)	0 (0)	1 (7)	6 (40)	15 (39)
PRA ≡ PRB	13 (100)	15 (100)	10 (67)	7 (47)	20 (51)
PRB > PRA	0 (0)	0 (0)	4 (26)	2 (13)	4 (10)

Number of cases (%) of normal breast and breast lesions showing the indicated expression of PRA and PRB.

between relative PRA and PRB expression in DCIS lesions and their adjacent tumors (not shown, Spearman rank correlation coefficient = 0.6, $p = 0.007$).

Fifty one percent of invasive breast cancers expressed similar levels of the two PR isoforms (Figure 1(D); Table 1) and the remainder (49%) expressed a predominance of one PR isoform. PRB predominance was noted in 10% of cases (Figures 1(F); Table 1), and a predominance of PRA in 39% of cases (Figure 1(E) and Table 1). Expression of a predominance of one PR isoform was not related to the overall level of PR expressed (not shown).

Relative PRA:PRB expression in breast carcinogenesis

There were no differences in the total levels of PR proteins expressed between benign or premalignant (PDWA, ADH or DCIS) and malignant breast lesions (paired Student *t*-Test, $p = 0.3$), although PR expression was invariably greater than that observed in normal tissue. However, the percentage of cells expressing PR was more variable in the malignant cohort than in the PDWA, ADH or DCIS tissues, and, as determined in a subcohort of cases, ranged from 40 to 83% (malignant) and 79 to 98% (PDWA, ADH or DCIS), respectively (not shown).

There was a significant increase, overall, in the number of lesions expressing a predominance of one PR isoform associated with lesion progression from the normal to the invasive state with the odds of isoform predominance increasing by a multiplicative factor of 1.7 (logistic regression analysis, $p = 0.002$, 95% CI 1.2–2.25) (Figure 2). Predominant expression of PRA or PRB was not observed in normal or PDWA cases and ADH lesions were not significantly different to normal in predominant expression of one PR isoform (logistic regression analysis, $p = 0.08$, Figure 2). The odds of a predominant expression of one PR isoform were 30 times higher in DCIS and malignant lesions than in normal breast ($p = 0.02$, Table 2).

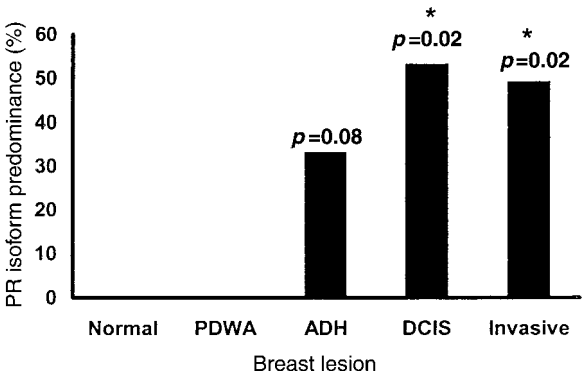

Figure 2. PR isoform predominance in breast lesions. Expression and relative levels of PRA and PRB were determined by dual immunofluorescence in normal, premalignant and invasive breast lesions, and the data expressed as percent of cases showing a predominance of PRA or PRB expression. (p value = statistical difference compared to normal breast tissue; *logistic regression, significance at $p \leq 0.05$).

Cell-cell heterogeneity of PRA:PRB expression in breast carcinogenesis

In normal breast and PDWA lesions there was remarkable homogeneity in relative PR isoform expression in PR positive cells (Figures 1(A) and (B)) and in the relative expression of PRA and PRB between areas of the same section (not shown). In contrast, in ADH, DCIS (not shown) and invasive lesions there was marked heterogeneity between adjacent cells in the relative concentrations of PRA and PRB (Figures 1(G), and (H)) and cells in close proximity were green, orange or yellow under dual immunofluorescence depending on the ratio of PRA:PRB expressed in each cell. Almost half (47%) of the DCIS lesions and the majority (72%) of malignant breast cases demonstrated marked adjacent cell heterogeneity of PRA:PRB expression (Figure 3). Overall, there was a highly significant increase in inter-cell heterogeneity of PRA and PRB expression associated with breast lesion progression to malignancy with the odds of heterogeneity increasing by a multiplicative factor of 2.5 for each stage of progression (logistic regression analysis, $p < 0.00001$;

Table 2. The odds of observing PR isoform predominance or inter-cell heterogeneity in breast lesions compared to normal breast

Breast lesion	Odds of PR isoform predominance	*p*-value	Odds of PRA/B inter-cell heterogeneity	*p*-value
PDWA	9.9	0.13	4.4	0.40
ADH	14.4	0.08	19.3	0.05
DCIS	32.8	0.02	25.3	0.03
Invasive	27.3	0.02	73.8	0.004

Logistic regression analysis of the association between PR isoform predominance, or inter cell heterogeneity of PRA:PRB expression in PDWA, ADH, DCIS and invasive breast lesions compared to normal breast.

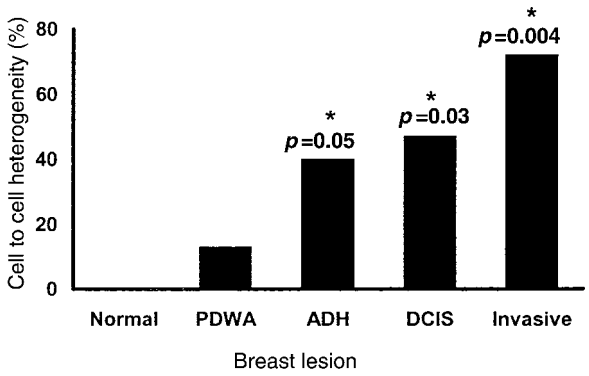

Figure 3. Inter-cell heterogeneity of PR isoform expression in breast lesions. Expression and relative levels of PRA and PRB were determined by dual immunofluorescence in normal, premalignant and invasive breast lesions, and the data expressed as percent of cases showing marked adjacent cell heterogeneity of PRA:PRB expression. (p value = statistical difference compared to normal breast tissue; *logistic regression, significance at $p \leq 0.05$).

95% CI 1.7–3.6). The odds of observing inter-cell heterogeneity were 74 times higher in malignant lesions than in normal breast (Table 2).

Discussion

PR expression in normal breast during the menstrual cycle

In this study, PRA and PRB expression was determined in normal breast tissue derived from autopsies. This allowed precise dating of the breast tissue in the menstrual cycle and also avoided the limitations inherent in using normal tissue adjacent to breast lesions. PRA and PRB proteins were co-expressed within the same cells of normal breast epithelium. The relative levels of PRA and PRB were similar throughout the menstrual cycle, consistent with the absence of a marked effect of circulating ovarian hormones either on total PR levels or on relative PR isoform expression. The lack of hormonal regulation of PR in the normal human breast contrasts with the known regulation of PR by E and P in the uterus, in other tissues and in breast cancer cells [28–35] and suggests that PR expression may be maintained in the breast by mechanisms other than hormonal regulation. However, studies on hormonal treatment of normal breast implanted into nude mice suggest that PR in this tissue may be regulated by low levels of estrogen [36]. These studies were carried out using normal breast adjacent to benign lesions but there are suggestions from our study and others [37] that such tissue may not be directly comparable to the normal breast which has no adjacent lesions. Nevertheless, the presence of both PR isoforms in the normal breast throughout the cycle implicates both PRA and PRB in progesterone action in this tissue.

PR expression in benign, premalignant and malignant breast tissues

Breast malignancies are likely to evolve from normal mammary epithelium through a series of increasingly irregular premalignant changes [38–40], associated with a concomitant increased risk of the patient subsequently developing breast cancer [38, 40]. There is evidence that premalignant lesions have increased sensitivity to estrogen and increased proliferation, which may contribute to development of increasingly disordered lesions in the breast [41]. This study showed a higher PR expression in PDWA and ADH breast lesions, compared to PR expression in normal breast tissue. Increased amounts of PR concomitant with proliferation have previously been reported in proliferative breast disease [41–43] and suggest that the level of hormone dependence of a lesion may cor-

respond to increasing mammary hyperplasia/dysplasia and an overall hypersensitivity to hormone. Furthermore, high expression of ER in histologically normal breast epithelium is considered a marker of increased tissue sensitivity to the effects of estrogen and therefore increased risk for the development of breast cancer [44, 45].

The increased PR levels in PDWA were associated with maintenance of comparable levels of PRA and PRB, as was seen in the normal breast. However, there was a significant increase in cases expressing a predominance of one isoform in ADH, DCIS and invasive cancer. Predominance of PRA was noted in nearly half the DCIS and invasive cancer cases examined. These results show that although loss of either PR isoform is noted in breast cancer, loss of PRB resulting in predominance of PRA is more common overall than loss of PRA.

Predominance of a PR isoform, in particular PRA, may have important implications for the biology of breast cancer. A tumor expressing a predominance of PRA may be more resistant to endocrine treatment, most of which target ER, if PRA is acting in a dominant negative manner on ER activity as suggested by *in vitro* studies [20, 46] and this may provide one explanation for the reported 25% of ER+PR+ breast tumors that fail to respond to hormone treatment [47]. Alternatively, overexpression of PRA may increase expression of genes that are associated with tumor growth. Work in our laboratory, on breast cancer cell lines, has shown that overexpression of the PRA isoform is associated with loss of adherent properties, and may play a role in loss of adhesion observed in malignancy [27]. On the other hand, tumors expressing a predominance of PRB may have increased sensitivity to the effects of estrogen. There is evidence in the literature for preferential up-regulation of PRB by estrogen, in T-47D human breast cancer cells [48]; in human endometrial tissue [49]; in chicken spleen and lung [50], and in the freshwater turtle oviduct [51]. There are no studies as yet linking PRA:PRB ratios with tumor response to endocrine treatment, or determining the potential prognostic value of relative PRA:PRB expression with patient outcome, and these will form part of our investigations proposed for the future.

Heterogeneity of PR isoform expression

In the normal breast, the relative expression of PRA and PRB in adjacent cells was remarkably homogenous and this was also observed in hyperplasias (PDWA). There was a significant increase in cell-to-cell heterogeneity of PR isoform expression in ADH and DCIS lesions and the majority of breast cancers displayed heterogeneous cell-to-cell levels of PR isoforms. If loss of one PR isoform is a feature of breast cancer development, as suggested in this study, then heterogeneous cell-to-cell expression of both PR isoforms may reflect the asynchronous loss of one isoform from adjacent cells, leading eventually to predominance of one isoform. In support of this, there was significant heterogeneity of cell-to-cell PR isoform expression in ADH lesions, whereas predominance of one PR isoform was not statistically significantly observed in these lesions, suggesting that dysregulation of relative PRA:PRB expression occurs prior to overall predominant expression of one isoform. These data demonstrate that loss of control of relative PRA:PRB expression is an early event in the development of breast cancer.

It is not known whether tumor expression of PR isoforms remains stable within a tumor or alters with time or under changing hormonal influences, as there is limited access to sequential samples taken from the same primary tumor. It has previously been shown in studies from this laboratory that PR expression between primary breast tumors and subsequent secondary deposits from the same patient show a significant correlation in PR positivity [6]. This suggests that total PR expression is generally not lost with tumor progression beyond the primary site and that PR expression is stable upon progression. Currently there are no data available on relative PRA and PRB expression in matched primary and secondary tumors and it is not known if this ratio is stable upon progression.

In summary, the results of this study show that there is co-ordinate regulation of PR isoform expression in cells of the normal breast, that allows these cells to respond to systemic hormonal and other signals in unison and supports a role for both PR isoforms in the normal response to progesterone in the human breast. The cellular homogeneity of PRA:PRB expression observed in normal breast is replaced by an increasing frequency of cellular heterogeneity in the relative expression of PR isoforms in benign and malignant breast lesions. Breast cancers commonly express a predominance of one PR isoform and these changes in the ratio of PRA:PRB proteins within a cell are likely to result in aberrant hormonal responses. In support of this, as over-expression of PRA in cultured breast cancer cells results in altered cell response to

progestins [27], expression of PRA predominance in carcinomas may alter hormone action in the breast and contribute to the evolution of the malignant phenotype.

The mechanisms that control the relative expression of PRA and PRB are not known, but this study has revealed an association between disruption of these mechanisms and progression to malignancy. The etiology of breast cancer remains unknown, but it is clear that progression of a breast lesion from the normal state to malignancy is accompanied by progressively aberrant PR isoform expression. PRA:PRB ratios within a breast lesion are likely to be important as both markers and effectors of tumor growth and development.

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