ORIGINAL ARTICLE

Localised breast cancers may have systemic influences on skin and hair

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Hypothesis: Biomarkers, commonly expressed in breast cancer cells, may be correlated with their expression in breast skin of the same subjects.

Methods: The expression of biomarkers in specimens from 33 breast tumours and breast skin from the same subject and from 32 normal controls was studied using immunohistochemical techniques.

Results: (1) In normal women, there are significant correlations between the levels of expression of cyclin D1, bcl-2 and p53 in normal breast epithelial cells and breast skin epithelial cells. (2) These patterns of biomarker expression in normal women are similar in breast cancer and breast skin epithelial cells of women with invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS), but are at significantly higher levels in both breast cancer cells and skin from the same subjects. (3) In normal women, human epidermal growth factor receptor 2 (HER-2) is not expressed in either breast epithelial cells or skin epithelial cells. (4) HER-2 is expressed in the breast skin of some subjects with HER-2-positive breast cancer. (5) Positive oestrogen receptor alpha expression occurs significantly more frequently in the breast skin of women with IDC and DCIS than in normal controls.

Conclusion: The influence of localised breast cancer seems to be systemic, and leads to changes in skin and hair.

Abnormal x ray diffraction patterns have been observed in the hair of women with breast cancer.^{1,2} Although this finding has been disputed, it is biologically plausible that the abnormal hormone receptor and oncogene expression commonly associated with carcinogenesis in breast tissues may also occur in skin tissues and are associated with changes in hair.^{3,4} This biological plausibility is based on the following: (1) breasts are specialised sweat glands that originate in skin, (2) hair also originates in skin; and (3) oestrogen and other hormones are metabolised in skin and hair follicles.⁵

Oestrogen receptor alpha (ER α), progesterone receptors, B cell lymphoma (bcl)-2, human epidermal growth factor receptor-2 (HER-2/neu or c-erbB2), cyclin D1 and p53 proteins are abnormally highly expressed in many breast cancer tumours.^{6–12} Oestrogen and progesterone receptors are hormone receptors associated with cell growth; cyclin D1 is associated with cell replication; bcl-2 and p53 are regarded as tumour suppressor genes; and HER-2 is regarded as an oncogene. However, the roles of these receptors and genes are more complex than indicated by these simple role definitions.

We hypothesised that the expression of these biomarkers in breast cancer cells may be correlated with their expression in breast skin of the same subjects. To test this hypothesis, we studied the expression of these biomarkers in breast tissues and breast skin of 23 patients with invasive ductal breast carcinoma (IDC), 10 patients with ductal carcinoma in situ (DCIS) and 32 normal subjects. We have shown that this hypothesis may be correct.

METHODS

Archival formalin-fixed, paraffin-wax-mounted, open breast biopsy specimens of local DCIS and IDC from 33 predominantly Caucasian Australian women were obtained from hospital archives in Sydney. Similar specimens were obtained from 32 normal Australian women who had undergone breast reduction surgery. Both the cancer and normal specimens were selected because the biopsy also contained breast skin. The normal

women are younger than the women with breast cancer, and there may be fluctuations in biomarker expression associated with the menstrual cycle or use of exogenous hormones. These influences are particularly relevant with respect to ER α expression. For these reasons, the specimens from normal women cannot be regarded as exactly matching controls.

The 10% formalin-fixed and paraffin-wax-embedded breast tissue samples were kept at room temperature and sections cut <1 week before staining. The processing and staining of all samples were undertaken by the same investigator (DDT).

The following antibodies were used: (1) anti-ER α —Dako, clone 1D5, (2) anti-PR—Biogenex, clone PR88, (3) cyclin D1—Dako, clone DCS-6, (4) bcl-2—Zymed, clone bcl-2-100, (5) p53—Dako, p53, (6) HER-2—Dako polyclonal erbB-2. Oestrogen receptor, progesterone receptor, cyclin D1 and p53 are nuclear stains. HER-2 and bcl-2 are cytoplasmic and membrane stains.

The antibodies were applied for 30 min and treated with bridging antibody. Antigen unmasking was accomplished by boiling under pressure in a citrate buffer; endogenous peroxidase activity was quenched with 3% hydrogen peroxide. Non-specific epitopes were blocked with 5% normal horse serum. The intensity and clarity of the stains of both the cell nuclei, cytoplasm and membranes with surrounding adipose, fibrous and blood vessel tissues were identical in the sets of cancer and normal specimens, indicating that the fixation and staining procedures were comparable in the two series of specimens. One positive (with specific antibody) and one negative (without specific antibody) control was used for every batch of staining.

The whole of each section was scanned microscopically. For estimating the percentage of positive staining nuclei or, in the case of HER-2 and bcl-2, cytoplasmic and membrane staining,

Abbreviations: bcl-2, B cell lymphoma-2; DCIS, ductal carcinoma in situ; ER α , oestrogen receptor alpha; HER, human epidermal growth factor receptor; IDC, invasive ductal carcinoma

Table 1 Biomarker expression in epithelial cells of skin and cancerous breast epithelial cells from breast and skin biopsy specimens (same subjects) of women with invasive ductal carcinoma and ductal carcinoma in situ

		Average (%) positive cancer cells/skin (SD)	Correlation between (%) positive cancer cells/skin*	Controls	Average (%) positive cells (normal breast/skin controls)	Probability of difference between cancer and controls†
Invasive ductal carcinoma specimens n = 23						
ER α	Cancer	64 (31)	r = 0.29 p = 0.104 NS	Breast	22	0.000 S
	Skin	7 (16)		Skin	2	0.348 NS
Progesterone receptor	Cancer	20 (27)	No computation	Breast	9	0.025 S
	Skin	0.3 (2)		Skin	0	0.268 NS
Cyclin D1	Cancer	29 (18)	r = 0.21 p = 0.303 NS	Breast	22	0.360 NS
	Skin	22 (18)		Skin	39	0.023 S
bcl-2	Cancer	73 (25)	r = 0.42 p = 0.023 S	Breast	32	0.000 S
	Skin	37 (28)		Skin	22	0.001 S
p53	Cancer	57 (33)	r = 0.46 p = 0.012 S	Breast	59	0.821 NS
	Skin	51 (29)		Skin	59	0.721 NS
HER-2	Cancer	22 (38)	r = 0.39 p = 0.068 NS	Breast	0	0.002 S
	Skin	2 (10)		Skin	0	0.057 S
Ductal carcinoma in situ specimens n = 10						
ER α	Cancer	69 (24)	r = 0.41 p = 0.272 NS	Breast	22	0.000 S
	Skin	8 (12)		Skin	2	0.011 S
Progesterone receptor	Cancer	8 (14)	No computation	Breast	9	0.368 NS
	Skin	0		Skin	0	–
Cyclin D1	Cancer	32 (33)	r = 0.28 p = 0.470 NS	Breast	22	0.030 S
	Skin	18 (13)		Skin	39	0.010 S
bcl-2	Cancer	72 (23)	r = 0.58 p = 0.102 NS	Breast	32	0.002 S
	Skin	20 (26)		Skin	22	0.724 NS
p53	Cancer	52 (33)	r = 0.74 p = 0.000 S	Breast	59	0.200 NS
	Skin	43 (31)		Skin	59	0.028 S
HER-2	Cancer	52 (45)	r = 0.297 p = 0.404 NS	Breast	0	0.000 S
	Skin	1 (3)		Skin	0	0.000 S

bcl-2, B cell lymphoma 2; ER α , oestrogen receptor alpha; HER, human epidermal growth factor 2; NS, not significant; S, significant.

* r is Pearson's correlation, p is probability.

†Independent samples two sided t test.

cells were considered positive if the staining was greater than that seen in a negative control. Using these criteria, all breast cancer specimens which were classified as ER α negative showed no nuclear staining in any cells in any part of the specimen. This approach to the assessment of biomarker protein expression is more accurate than the designation of cut-off levels of an arbitrary percentage of positive expressing cells, which is commonly used for clinical purposes.

Because the expressions of all biomarkers used in this study are heterogeneous with variations in expression between breast lobules and between cells, and because there are variations in intensity of stains associated with these techniques, counts were confined to lobular epithelial cells and rounded to 10 percentage points.

Counts were performed at a magnification of $\times 400$ on a minimum of 700 cells/specimen. For all antibodies, the results are expressed as the percentage of positive cells compared with the total number of cells counted.

Statistics

Pearson's correlation (r) was used to determine the strength of the relationship between the expression of biomarkers in breast cancer cells and skin from the same subjects. The Pearson χ^2 value for non-parametric data was estimated to determine whether there were differences in frequencies of ER α expression in the skin of patients with breast cancer as compared with normal specimens.

The SPSS V.11 statistical program was used.

RESULTS

ER α , progesterone receptor, Cyclin D1, bcl-2, p53 and HER-2 were expressed in both breast cancer and breast skin epithelial cells of the same subjects (table 1). ER α , Cyclin D1, bcl-2 and p53, but not progesterone receptor and HER-2, were expressed

in both normal breast and skin epithelial cells of normal controls.

There were significant correlations in the expression of bcl-2 and p53 between IDC and breast skin of the same subjects, and for p53 in DCIS.

As shown in table 2, there were significant correlations in expression of cyclin D1, bcl-2 and p53 between normal breast epithelial cells and breast skin of the same subjects. There was no expression of HER-2 in either breast epithelial cells or breast skin in normal control specimens.

Expression of ER α , progesterone receptor, cyclin D1, bcl-2 and HER-2 (but not p53) was significantly higher in IDC and DCIS breast cancer cells than in breast epithelial cells of normal control specimens.

Expression of cyclin D1, bcl-2 and HER-2 was significantly higher in the breast skin of subjects with IDC than in the breast skin of normal controls. Expression of cyclin D1, p53 and HER-2 was significantly higher in the breast skin of subjects with DCIS than in the breast skin of normal controls.

Positive ER α expression (as distinct from the percentage of positive cells) in the breast skin of subjects with either IDC or DCIS was significantly higher than normal controls (Pearson χ^2 value = 64, $p = 0$). ER α was positively expressed in the skin from 11 of 33 breast cancer specimens as compared with the skin from 3 of 32 normal breast specimens. ER α was expressed in the breast skin from 6 of 23 IDC specimens and from 5 of 10 DCIS specimens.

Images

Figures 1–3 show selected images of ER α , bcl-2 and p53 expression in epithelial cells of breast skin and breast tumours, from the same subject, and images from normal controls. These images have been selected to show the patterns of biomarker expression outlined in tables 1 and 2. ER α and p53 proteins are

Table 2 Biomarker expression in epithelial cells of skin and normal breast acini from breast and skin biopsy specimens (same subjects) of normal women without breast cancer

Normal control specimens n = 32	Average (%) positive cancer cells (SD)	Correlation between normal breast/skin*
ERα		
Normal breast	22 (16)	r = 0.26
Skin	1 (6)	p = 0.20 NS
Progesterone receptor		
Normal breast	9 (9)	Not computed
Skin	0	
Cyclin D1		
Normal breast	22 (16)	r = 0.39
Skin	39 (24)	p = 0.04 S
bcl-2		
Normal breast	32 (27)	r = 0.53
Skin	22 (21)	p = 0.002 S
p53		
Normal breast	59 (26)	r = 0.91
Skin	59 (24)	p = 0.000 S
HER-2		
Normal breast	0	Not computed
Skin	0	

bcl-2, B cell lymphoma 2; ERα, oestrogen receptor alpha; HER, human epidermal growth factor 2; NS, not significant; S, significant.
*r is Pearson's correlation, p is probability.

expressed in cell nuclei. bcl-2 proteins are expressed in cell membranes and cytoplasm. It is of interest that the appearance of the staining in breast cells and breast skin is similar with respect to both cells and connective tissues for specimens from the same subject.

DISCUSSION

In summary, we have shown that

- 1. in normal women, there are significant correlations in the levels of expression of cyclin D1, bcl-2 and p53 in

normal breast epithelial cells and breast skin epithelial cells;

- 2. these patterns of biomarker expression in normal women are similar in breast cancer epithelial cells and breast skin epithelial cells of women with IDC and DCIS, but at significantly higher levels in both breast cancer cells and skin from the same subjects;
- 3. in normal women, HER-2 is not expressed in either breast epithelial cells or skin epithelial cells.
- 4. HER-2 is expressed in the breast skin of some subjects with HER-2-positive breast cancer;

Figure 1 Oestrogen receptor alpha (ERα) expression in breast skin (A) and breast tumour (B) from the same subject with invasive breast ductal carcinoma. ERα expression in normal breast skin (C) and normal breast tissues (D) from a normal subject.

Figure 2 bcl-2 expression (cell membrane and cytoplasm expression) in breast skin (A) and breast tumour (B) from the same subject with invasive breast ductal carcinoma.

Figure 3 p53 expression in breast skin (A) and breast tumour (B) from the same subject with both invasive and breast ductal carcinoma in situ.

- positive ER α expression occurs significantly more frequently in the breast skin of women with IDC and DCIS than in the skin of normal controls.

Reliability of the data

These observations need to be considered with caution as there are several possible biases in this study. The numbers of subjects is small. Information about the presence of metastases was not available. The normal women are younger than those subjects with breast cancer, and there may be fluctuations in biomarker expression associated with the menstrual cycle and the use of exogenous hormones. For these reasons, the specimens from normal women cannot be regarded as exactly matching controls, but these normal specimens are useful for comparative purposes.

The similarity of the staining in breast cells and breast skin of specimens from the same subject is likely to be a consequence of identical fixation and processing of specimens from that subject. This observation adds to the credibility of the immunohistochemistry procedures used in this study, on which the data and discussion are based.

A speculative hypothesis

The expression of abnormal levels of biomarkers seems to be systemic and not confined to malignant breast cells. These observations offer biological plausibility to the abnormal x ray diffraction patterns present in the hair of women with breast cancer.

We offer a speculative hypothesis that malignant breast cells secrete an unknown substance that influences the physiology of fibroblasts, which in turn influence the production of keratins, which form the basic structural skin and hair proteins. Fibroblast cells are scattered through skin, breast and other connective tissues. These cells are involved in the production and physiology of complex sugars and proteins, collectively known as connective tissue ground substance including, keratins, collagen and elastin.¹³ The complex sugars include

hyaluronic acid, which binds water and provides the fluid environment in which the proteins can function. The physiology of hair and its growth can be influenced by any changes in the physiology of fibroblasts. It has been shown that very small changes or mutations in keratin DNA sequences can lead to substantial abnormalities in the formation of keratins in the hair.¹⁴

Skin fibroblasts in patients with breast cancer show markedly different behaviour from those in normal women.¹⁵ The most striking difference is the doubling of their growth potential. Other differences include altered fibroblast ability to organise connective tissue matrix, and the development of DNA repair and processing defects in fibroblast cells.^{15 16}

To metastasise, malignant breast epithelial cells must secrete substances that alter the physiology of connective tissue.

Take-home messages

- Abnormal expression of common biomarkers, such as oestrogen receptor and p53 in breast tumours, may also be abnormally expressed in the skin of the same subjects.
- HER-2 may be expressed in breast tumours and skin of some subjects, but not in the breast or skin of normal controls.
- The influence of breast cancer appears to be systemic and leads to changes in both skin and hair.
- We speculate that to metastasise, malignant breast cells secrete an unknown substance which alters the physiology of connective tissue in skin, hair and other organs.
- These observations add biological plausibility to the prior observation that abnormal x ray diffraction patterns occur in the hair of many women with breast cancer.

Hyaluronan, the product of hyaluronic acid metabolism, is present in most tissues including skin and hair follicles. Hyaluran expression is frequently increased in breast tumours and seems to be directly involved in breast metastases.¹⁷

Oestrogens influence the physiology of fibroblasts, including the stimulation of hyaluronic acid synthesis.¹⁸ Oestrogens are an essential element in breast carcinogenesis.¹⁹ Accordingly, our finding that ER α is expressed more frequently in the skin of patients with breast cancer than in normal controls is of interest.

Malignant breast epithelial cells, and fibroblasts located in tumour and skin, may, under the influence of oestrogens, abnormally metabolise hyaluronic acid, which influences keratin DNA sequences, leading to abnormal growth of hair in patients with breast and other cancers. There may, of course, be other explanations.

In conclusion, we hypothesise that the influence of localised breast cancer may be systemic and may lead to changes in skin and hair.

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Competing interests: None declared.

Ethical approval: This study was conducted with the approval of the Human Ethics Committee of St Vincent's Hospital, Sydney. Approval number H98/097.

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