Transforming growth factor α in epithelial proliferative diseases of the breast

D M Parham, J Jankowski

Abstract

Aims: To determine at what stage there is increased expression of transforming growth factor alpha (TGF α) in pre-neoplastic diseases of the breast and to determine if this would assist in the histological diagnosis of different intraduct epithelial proliferations.

Methods: Specimens were retrieved from the archives of 17 cases of ductal hyperplasia, six cases of atypical ductal hyperplasia and 13 cases of ductal carcinoma in situ together with 12 'normal' breast biopsy specimens. Sections were stained immunohistochemically for TGF α . The staining was assessed semi-quantitatively taking into account both the staining intensity and the proportion of cells stained.

Results: Minimal expression of TGF α was observed in normal breast tissue. Increased levels of expression were seen in ductal hyperplasia, atypical ductal hyperplasia, and ductal carcinoma in situ. Increased levels of expression of TGF α were also found in morphologically normal ducts immediately adjacent to areas of intraduct epithelial proliferation.

Conclusion: Increased expression of TGF α occurs in the early stages of intraduct epithelial proliferation and will not help the histopathologist distinguish atypical ductal hyperplasia from either ductal hyperplasia or ductal carcinoma in situ. The molecular changes within a cell may precede the morphological changes observed by light microscopy thereby reflecting the biological potential of the epithelium.

Intraduct epithelial proliferations of the breast are a complex group of disorders. Changes range from simple epithelial hyperplasia (ductal hyperplasia, epitheliosis) to ductal carcinoma in situ (DCIS). The simple and complex changes have well described histological criteria for diagnosis, but the intermediate form—atypical ductal hyperplasia (atypical epitheliosis)—remains poorly defined.¹ The histological features overlap with both ductal hyperplasia (epitheliosis) and DCIS making it difficult to diagnose and as a result this diagnosis is rarely made.

The relation between these intraduct epithelial proliferations and malignancy is

unclear. There is a small risk of developing a subsequent invasive carcinoma with ductal hyperplasia (epitheliosis), a high risk with DCIS, and intermediate risk atypical ductal hyperplasia (atypical epitheliosis).¹ It is not clear whether ductal hyperplasia (epitheliosis) transforms directly into carcinoma through an intermediate phase of atypical ductal hyperplasia (atypical epitheliosis) or whether malignant change occurs de novo.²⁻⁴

In an attempt to improve the diagnosis of the different intraduct epithelial proliferations and determine the relevance of each of these states to the others, we have examined the role of transforming growth factor (TGF α), one of a family of growth factors. TGF α has recently been shown to be important in the control of cell proliferation and in the development of neoplasia.⁵ The recent availability of a monoclonal antibody to TGF α , for use in paraffin fixed tissue sections, has made it possible to examine archival breast material.

Methods

Breast specimens collected between 1988 and 1990 and considered typical examples, at diagnosis and review, of ductal hyperplasia (17 cases, mean age 48 years, range 28-64), atypical ductal hyperplasia (six cases, mean age 52 years, range 21-63) and ductal carcinoma in situ (DCIS, 13 cases, mean age 58 years, range 29-76) were retrieved from the archives of the Pathology Department, Ninewells Hospital. Six of the 13 cases of DCIS had areas of stromal invasion. Breast tissue from four necropsies, four breast reduction specimens, and four surgical breast biopsies reported as normal were used as controls (mean age 54 years, range 20-96). The term ductal hyperplasia (epitheliosis) will be used to refer solely to the benign intraluminal proliferation described by Azzopardi.⁶

Formalin fixed, paraffin wax embedded material was sectioned at 4 μ m. An IgG monoclonal antibody was used against TGF α (Oncogene Science, Manhasset, NY). This antibody shows no cross-reactivity with epidermal growth factor.⁷ The antigen-antibody reaction was visualised by the modified streptavidin technique⁸ and sections were counterstained with alum haematoxylin. Examples of the resulting tissue sections are shown in figs 1 and 2.

Negative control sections included sections of ductal hyperplasia and DCIS which were incubated overnight with an excess of TGF α peptide 1 μ g/ml (Sigma) and TGF α antibody

Department of Pathology, Ninewells Hospital and Medical School, Dundee DD1 9SY

D M Parham

Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee DD1 9SY

J Jankowski

Correspondence to: Dr D M Parham

Accepted for publication 1 November 1991

Figure 1 Photomicrograph of a tissue section of DCIS (comedo subtype; necrosis top left) exhibiting variation in staining of cells within ducts for TGF α .

Figure 2 Photomicrograph of a tissue section of ductal hyperplasia exhibiting uniform staining of cells for TGF α .

(Oncogene Science). This technique is designed to assess the specificity of the antibody by staining proteins other than TGF α alone (absorbance reaction). It showed negligible staining of other cellular proteins.

TGF α staining was graded by a method similar to that previously described.⁹ An assessment of both staining intensity and the proportion of cells stained was made. One hundred consecutive nucleated cells were assessed at high power magnification with the assistance of an eyepiece graticule. The intensity of cytoplasmic staining in each cell was graded 0–3. The number of cells exhibiting each grade of staining was counted and the mean staining intensity calculated as in the following example. If 100 cells are counted and the scores are obtained: grade 0, 4 cells; grade 1, 14 cells; grade 2, 78 cells; grade 3, 4 cells, the mean staining intensity of the section would be $[(0 \times 4) + (1 \times 14) + (2 \times 78) + (3 \times 4)]/100 = 182/100$, i.e. 1.82.

STATISTICAL ANALYSIS

Statistical analysis (non-parametric, Mann-Whitney U test) was performed on an IBM compatible personal computer (Statgraphics Ver 3.0, Statistical Graphics Corporation, USA).

Results

A preliminary study showed that assessing more than 50 cells would give a representative value for monoclonal antibody staining (fig 3). Intra-observer error is shown in fig 4. No significant difference was observed between the degree of staining in each of the classes of intraduct epithelial proliferation or the invasive tumour cells, but in each of these categories the staining was significantly greater than the normal controls (fig 5, table). No difference in the staining of the in situ cells was observed between cases of DCIS which showed evidence of invasion and those which did not. The degree of staining of TGF α in the nine cases of comedo DCIS covered the range of values for the other DCIS subtypes (one case solid, one

Figure 3 Cumulative average of monoclonal antibody staining in a case of ductal hyperplasia which showed a degree of heterogeneity in staining. MC score, mean staining intensity.

Histological Type	MC Score
DCIS	2.0
DCIS	1.75
DCIS	1.8
Atypical ductal hyperplasia	2.1
Atypical ductal hyperplasia	1.6
Atypical ductal hyperplasia	1.95
Ductal hyperplasia	1.75
Ductal hyperplasia	1.4
Ductal hyperplasia	1.3
Normal	0.1
Normal	0.05
Normal	0.2

In the sections of intraduct epithelial proliferations, the normal mammary duct epithelium did not stain for TGF α and thus acted as an internal control. However, apparently normal epithelium within a few millimetres of the epithelial proliferation showed TGF α staining. This was assessed and it was found that within each category of abnormality the degree of staining of this apparently normal epithelium tended to be a little lower than the adjacent intraduct epithelial proliferation. The staining of this apparently normal epithelium was nevertheless higher than that of the normal control cases (fig 6).

Control of normal cell proliferation and differentiation is complex and our understanding of the mechanisms involved is incomplete. It is becoming apparent that normal cells can synthesise and respond to specific growth signals.¹⁰ These growth factors may act locally (paracrine) or on the producing cell (autocrine). Of particular interest in the regulation of cell proliferation are the transforming growth factors (TGF) for which both paracrine and autocrine mechanisms have been postulated.

The average staining scores for each category of intraduct epithelial proliferation and the invasive cells in the predominantly DCIS lesions

<i>Sample</i>	<i>Average staining score (MC score)</i>	<i>Probability that the result is not statistically different from normal</i>
Normal	0.06	—
Ductal hyperplasia	2.05	$p < 0.000\ 006$
Atypical ductal hyperplasia	1.33	$p < 0.006$
DCIS	2.35	$p < 0.0001$
Invasive cells	2.33	$p < 0.0005$

appears to be differential processing of the receptor depending on the ligand.¹⁴ TGF α has been shown to be present in normal cells and in non-neoplastic keratinocytes, and both EGF and TGF α were able to stimulate significant increases in TGF α mRNA concentrations in these cells, suggesting that autocrine growth control mechanisms exist.¹⁵

A role for TGF α in the development of neoplasia was suggested by the induction of anchorage independence growth in normal rat kidney cells.^{16,17} More recently TGF α has been detected in human mammary cancer cells¹⁸ and in transgenic mice. TGF α overexpression has been implicated in the development of mammary epithelial hyperplasia and carcinoma.¹⁹

We have examined TGF α expression in examples of intraductal epithelial proliferations of the human breast. Each case has been chosen carefully to reflect the different stages between

Figure 6 Staining with TGF α of apparently normal duct epithelium immediately adjacent to DCIS, atypical ductal hyperplasia, and ductal hyperplasia. Normal controls also shown. MC score, mean staining intensity.

normality and malignancy. Minimal TGF α expression was found in normal breast whereas significantly higher expression was found in proliferative breast disease. No statistical difference was observed between TGF α expression in simple, atypical and "malignant" intraduct epithelial proliferations. The single case of atypical epitheliosis with low concentrations of TGF α is an interesting finding. Re-evaluation of the histology of this case showed that while showing atypical features it was distinct from the other cases of atypical ductal hyperplasia and other intraduct proliferations, in that there was marked clearing of the cytoplasm. The significance of this finding is uncertain at present. It may represent a fixation artefact or be a genuine feature, possibly a degenerative change.

The findings show that altered TGF α expression occurs early in proliferative disease of the breast but the staining in invasive tumour cells tends to be a little lower suggesting that proliferation in these cells may become more independent of TGF α and that other mechanisms are involved in maintaining growth. It is of interest that Barrett-Lee *et al*²⁰ found little difference in the degree of TGF α mRNA synthesis in malignant or benign breast disease or in normal breast tissue. The sensitivity of their methods was low but if their findings are correct then the differences in TGF α expression between normal and proliferative breast disease as observed in our study could be due to alterations in the half-life of TGF α , possibly due to alterations in the structure of the molecule. It has been shown that TGF α exists in different states and that its precursor, a transmembrane protein (pro-TGF α), is biologically active and may function as a highly localised/prolonged autocrine growth signal.²¹

The absence of any observable difference in TGF α expression between the different intraduct epithelial proliferations suggest that this molecule has only a limited role in the development of neoplasia. However, the recent demonstration that immortalised mouse mammary epithelial cells (cells which have already moved one step in the direction of malignancy) are susceptible to transformation by TGF α ^{22,23} indicates that the situation is more complex. It is possible that other components in the pathway, for example the concentrations of EGF and EGF receptor, can play an important part in limiting or amplifying any stimulus for neoplastic transformation. Thus TGF α can act as one step in the multi-step theory of carcinogenesis.

Evaluation of TGF α expression in epithelial proliferations of the breast will not help the histopathologist in distinguishing atypical ductal hyperplasia from ductal hyperplasia or DCIS. However, the demonstration of increased TGF α expression in morphologically normal ducts immediately adjacent to ducts exhibiting epithelial proliferation is an important finding. Firstly it questions the validity of studies using tissue taken from mastectomy specimens as normal control material, especially if the tissue has been taken from the vicinity of a tumour. It is possible that the changes observed in these apparently normal

ducts could be due to a local field (juxtacrine) effect, but they could indicate the molecular events that precede the development of changes seen morphologically by light microscopy and hence reflect the biological potential of seemingly normal epithelium. This emphasises the importance of studying the molecular mechanisms involved in the development of neoplasia and the possible limitations of conventional histopathology. In the future it could be possible to determine the potential behaviour of tissues based on biological parameters rather than on the empirical factors currently studied by conventional light microscopy.

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