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Transferrin receptor (TrfR) expression in breast carcinoma and its possible relationship to prognosis

An immunohistochemical study

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Summary. TrfR, a primitive membrane protein was demonstrated by immunohistochemistry in 87,6% of 105 cases of breast carcinoma, predominantly on the cell surface and in a strong and rather uniform pattern. Sporadic staining in a patchy fashion was observed. No difference between individual tumour types was seen, neither in cytomorphological staining pattern nor in staining intensity. Exceptionally, mucoid carcinomas showed weaker intensity for receptor expression.

Because of the heterogenous expression of TrfR within most of the tumours the extent of staining reaction was determined by semiquantitative grading (low, moderate, high). These results were compared with grade of anaplasia, tumour staging and nodal status of the axilla. The extent of immunoreactivity revealed significant correlation with grade of anaplasia, whereas no correlation was found with staging and status of axillary lymph nodes. Tumours with higher degree of malignancy (GII–GIII) showed a higher extent of staining. The presence of TrfR in a high degree of expression thus implies some prognostic value. Its quantitative determination can provide kinetic data on the neoplasm.

Key words: Breast carcinoma – Transferrin receptor – Immunohistochemical study

Introduction

Transferrin receptor (TrfR) mediates iron uptake via internalization of the iron carrying serum protein transferrin (reviewed by Newman et al. 1982). The receptor is an integral membrane protein composed of two identical subunits of 95,000 daltons which are linked by a disulfide bridge to form a dimer (Schneider et al. 1981; Omary and Trowbridge 1981). Expression of this receptor on the cell surface correlates with cellular proliferation, being highest on rapidly dividing cells and much lower on resting and most terminally differentiated cells (Larrick and Cresswell 1979). Because of its association with cell proliferation TrfR may be expressed selectively on some tumour cells (Shindelman et al. 1981) and act as a marker for dividing cells. In respect of these facts the detection of TrfR in breast cancer may be useful in tumour prognosis (Page Faulk et al. 1980) if correlation with morphological prognostic criteria such as grading of anaplasia, tumour staging and the status of axillary lymph nodes can be shown. With regard to this we examined 105 primary breast carcinomas immunohistochemically for TrfR in order to look for its expression and distribution histo- and cytomorphologically, to compare the extent of expression of individual tumours with grade of anaplasia, tumour staging and the status of axillary lymph nodes. For semiquantitative estimation of staining extent a grading of low, moderate and high degree was performed.

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Materials and methods

Histomorphology. The tumours were classified according to Azzopardi (1979). Non-lobular carcinomas were graded using a modification of the method described by Bloom and Richardson (1957), taking into account the formation of tubules, nuclear pleomorphism and mitotic activity. The tumour staging corresponded to TNM classification, UICC (Scheibe 1970). Nodal status of the ipsilateral axilla was determined by examination of a minimum of two slides of each detectable lymph node.

For routine diagnosis haematoxylineosin staining was performed.

Immunohistochemistry. Frozen sections of 105 primary breast carcinomas were prepared on a cryostat. The slides were fixed in absolute acetone for 5 min and stored at −20 °C until use. In addition, the indirect method was carried out in the usual manner. After incubation with normal rabbit serum, diluted 1:10 in Tris buffered saline (TBS), pH 7.5, the sections were labelled with the first antibody (monoclonal antibody to TrfR, Hybritech, San Diego, CA) diluted 1:250 in 3% normal rabbit serum for $^1/_2$ h. Further, immunoreactivity was tested with a two step peroxidase technique, using horseradish peroxidase conjugated rabbit anti-mouse IgG as second antibody and horseradish peroxidase conjugated swine anti-rabbit IgG as third antibody (DAKO-Immunoglobulins, Copenhagen, Denmark). The second and third antibodies were incubated for $^1/_2$ h each with working dilution 1:100 in 3% normal rabbit serum. As chromogen 3,3-diaminobenzidine-tetrahydrochloride (DAB, 0.05%, Sigma Chemical Co) and H_2O_2 (0.01%) in TBS was added for 5 mins. The excess dye was removed, the slides were immersed in running tab water for 10 min and counterstained with Mayer's haemalaun.

Control experiments omitting the first antibody yielded negative results.

The extent of immunoreaction was graded as low (+) when single ≥10% of tumour cells showed positivity, moderate (++) when >10% ≤50% and high (+++) when >50%–100% were positive for immunoreaction.

Statistics. To compare the variables of interest Chi-square method was performed (Sachs 1982) P values <0.05 were considered significant.

Referring to Table 2 the individual dates of low and medium degree of staining extent were summarized for each group of grading of anaplasia to one single row (graphically figured by an interrupted line, sums presented behind parenthesis), so that 6 groups of dates obtained by this means could be compared.

Results

87.6% of the tumours (92 cases) showed a positive immunoreaction for TrfR, predominantly on the tumour cell surface in a fine granular pattern. In some areas where the cells showed tubular formation a tendency to a concentrated immunoreaction on the basal oriented cell pole was observed (Fig. 5). The majority of the tumour cells positive for TrfR whether lying detached or in clusters, displayed a similar intense staining without any essential differnce in all histological types investigated (Fig. 1, 2, 3, 6). Exceptionally, in mucoid carcinomas, staining intensity seemed to be weaker (Fig. 4).

The histomorphological classification of 105 primary breast carcinomas and the relationships to the results of immunohistochemistry are summarized in Table 1. Tumours of lobular type showed in a slighly lower immunoreactivity (76.5%) positivity than tumours of the other groups.

Table 1. TrfR expression in various histological types of 105 primary breast carcinomas

Histological type	Number of cases	TrfR pos (%)
Total	105	92 (87.6%)
Invasive Ductal NOS	67	59 (88.0%)
Mucoid	5	4/4
Medullary with Lymphoid infiltration	4	4/4
Infiltrating Comedocarcinom	8	8/8
Lipid-rich	2	2/2
DCIS (Mb Paget)	2 (1)	2/2
Invasive Lobular	17	13 (76.5%)

Table 2. TrfR expression and grading of staining extent in relation to grading of anaplasia of 88 primary breast carcinomas

TrfR pos Grading of staining extent	Grading of anaplasia (number of cases)		
	GI (21)	GII (36)	GIII (31)
+	5 (8)	2 (8)	0 (3)
++	3	6	3
+++	6	26 S	27 S

S = significant $P<0.01$

Table 3. TrfR expression and grading of staining extent in relation to tumour staging of 99 primary breast carcinomas

TrfR pos Grading of staining extent	Tumour staging (number of cases)				
	pT1 (47)	pT2 (26)	pT3 (1)	pT4 (25)	
+	5	2	0	0	
++	7	5	0	4	
+++	29	15	1	19	NS

NS not significant

Table 4. TrfR expression and grading of staining extent in relation to axillary lymph node status of 89 breast carcinomas

TrfR pos Grading of staining extent	Lymph node status (number of cases)		
	Nodal pos (45)	Nodal neg (44)	
+	3	2	
++	8	8	
+++	32	27	NS

NS not significant

88 tumours were subjected to grading of anaplasia. 21 cases were allocated to the low grade group GI, 36 to the moderate grade group GII and 31 to the high grade group GIII. The frequency and extent of the expression of the TrfR

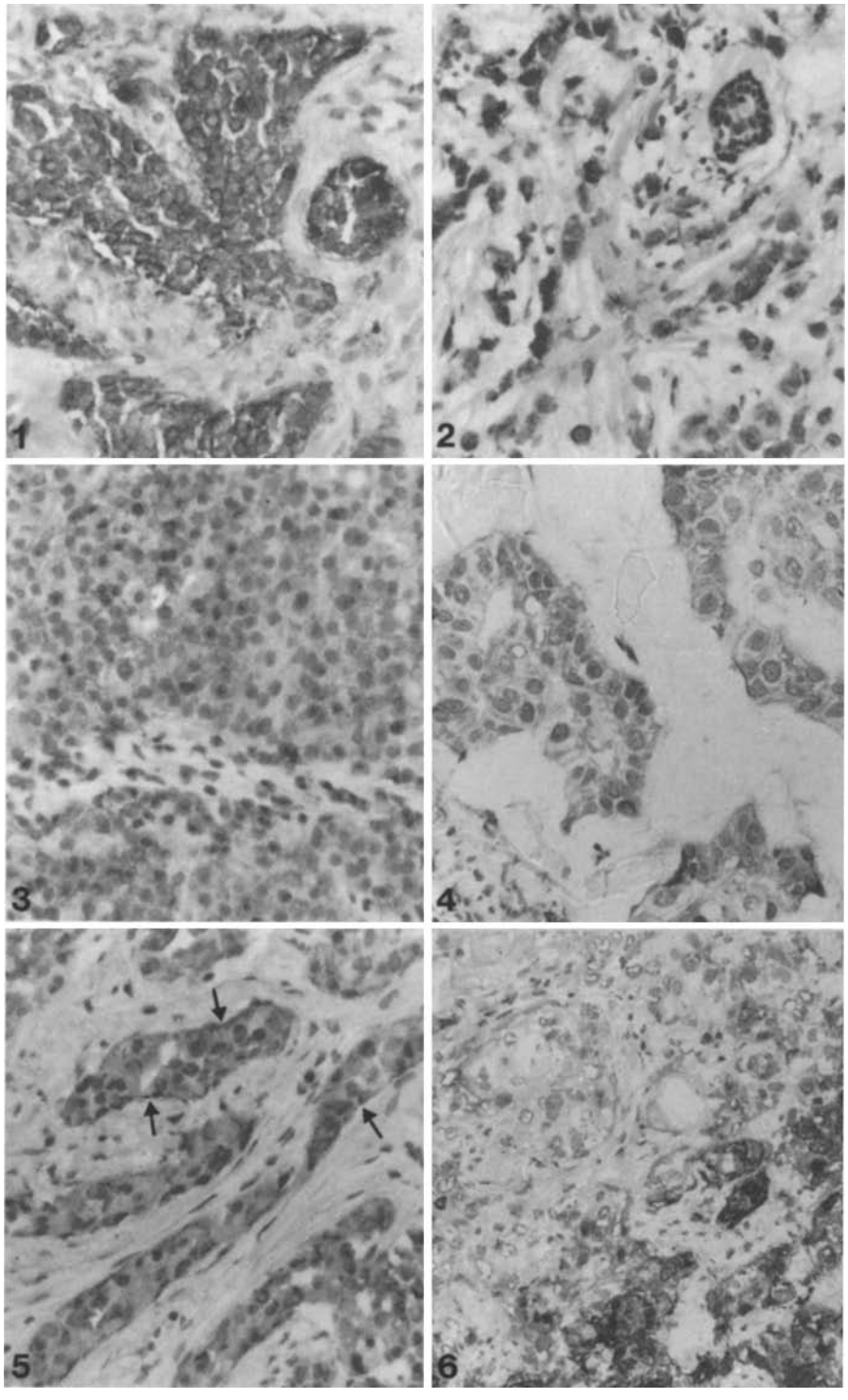

Fig. 1. Ductal carcinoma, NOS, GIII. All tumour cells give a strong staining reaction for TrfR (×400)

Fig. 2. Lobular carcinoma presenting tumour cells with strong immunoreactivity. (×400)

Fig. 3. Medullary carcinoma. All tumour cells exhibit a strong staining for TrfR. (×400)

Fig. 4. Mucoid carcinoma. Tumour cells with weakly positive immunoreaction. (×400)

Fig. 5. Ductal carcinoma, NOS, GII. Tumour cells in tubular arrangement showing concentration of TrfR expression on the basal oriented cell pole. (*arrows*, ×400)

Fig. 6. Ductal carcinoma, NOS, GIII. Tumour cell clusters with strong immunoreactivity (right side of the picture) opposite to an area with no reaction. (×250)

in relation to tumours grading can be seen in Table 2. The number of cases with immunostaining of high extent (+++) was increased in the GII and GIII tumour groups (χ^2:11.5361 $p<0.01$).

99 cases were staged according UICC. 47 cases were grouped as pT 1; 26 as pT 2; 1 as pT 3 and 25 as pT 4. Table 3 showes the extent of immunoreaction in relation to tumour staging. The distribution of these variables revealed no correlation (χ^2:3.5833).

89 tumours were examined for axillary nodal involvement. 45 presented with positive and 44 with negative lymph nodes. The immunoreactivity in relation to the nodal status is outlined in Ta-

ble 4. There was no apparent correlation between TrfR positivity and the content of nodal metastases (χ^2:0.2106).

Discussion

TrfR is a glycoprotein expressed on the surface in coated pits of various proliferating cells. It is distinct from the well known biological markers of breast carcinoma, described as appropriate or inappropriate to breast tissue (Bussolati et al. 1975; Harris et al. 1975; Walker 1978, 1979; Horne et al. 1979) and according to some reports dealing with specificity of tumour markers (Yu et al. 1980; Thompson et al. 1983). The presence of TrfR has been established in normal or malignant cells of epithelial, lymphatic, bone marrow and mesenchymal origin of man and various animals. It serves predominantly as a model for receptor mediated endocytosis (Harding et al. 1983; Iacopetta et al. 1983; Hopkins et al. 1983; Hanover et al. 1984; Watts 1985). Binding of the ligand leads to rapid endocytosis and a subsequent intracellular pathway results in divorce of the complex and recycling of TrfR to the cell surface.

The scheme of evolution for transmembrane and secreted proteins proposed by Sabatini et al. (1982) suggests that TrfR may have arisen very early in cellular evolution since its structure is that of a proposed primitive form of membrane proteins (Mc Clelland et al. 1984). It takes part in a fundamental iron transport system required for cell growth. For that reason and according to published results referring to TrfR expression in breast tissue (Shindelman et al. 1981; Page Faulk et al. 1980) we expected a large number of reacting tumour cells and thus a semiquantitative subdivision of the extent of staining reaction was performed.

TrfR was observed in 87,6% of primary breast carcinomas in various histological subtypes. There was no correlation between immunoreactivity in general and tumour classification. The cytomorphological findings of the fine granular distribution on the cell surface are consistent with the location of TrfR predominantly in coated pits. Intracytoplasmic staining should display a more patchy pattern due to the appearance of the receptor in certain compartments and vesicles and in those cells where immunoreactivity revealed a concentration on the basal cell pole a particular distribution is imaginable, but ultrastructural studies will be necessary to support this thesis.

The reason for the heterogeneity of TrfR expression within a tumour is not clear. Nor can the negative tumours or the cases of lobular carcinomas which stained positive less extensively than tumours of the other histological groups be readily explained. If we exclude methodological errors, the supposition may arise that the expression of TrfR is impossible either partially or totally in tumour cells. This may be due to insufficiency of the competent gene and disarrangement of protein synthesis or due to the fact that cells of the tumour area accessible to investigation are in a phase where induction of TrfR is not required. Another possibility may be the masking by autoantibodies blocking the binding site for the anti-TrfR antibody used, leading to totally negative tumours. Suppression of cell proliferation caused by masking is possible. Trowbridge and Lopez (1982) reported that blocking of TrfR in vitro will lead to an arrest of cell division and accumulation of cells in S phase.

One of the most important tumour variables with prognostic value is the grade of anaplasia (Bloom and Richardson 1957, Wallgren et al. 1976). The comparison between tumour grading and the extent of immunoreaction was significant in so far as extensive positivity (+ + +) showed a distinct preponderance in G II and GIII tumours. Good differentiation indicates a low malignant potential and tumours expressing extensive TrfR may have a less favourable prognosis than tumours immunoreactive in a low or moderate degree. Recently published results of tumour markers described as appropriate and inappropriate to breast cancer did not show similar results (Lee et al. 1985). Despite this finding immunoreactivity in relation to tumour staging and status of axillary nodal involvement did not appear to be significant. Although in the group of pT 4 and nodal positive cases the number of tumours with extensive staining (+ + +) revealed a slight predominance over the other groups for tumours of low (+) and moderate (+ +) extent the distribution of all variables was regular. The possibility exists therefore, that efforts to achieve early diagnosis are detecting an increasing number of patients harbouring lesions expressing TrfR extensively in a relatively early stage. There is clearly a need for additional information especially about the period of tumour detection by the patient and medical intervention. In conclusion, these results suggests that the presence of extensive TrfR implies a stage of moderate or less good tumour differentiation, corresponding to elevated proliferative activity and therefore having some prognostic value. Secondly, the demonstration of TrfR, its distribution pattern within a tumour as well as its quantitative determination, can provide kinetic data helpful for both an addi-

tional understanding of tumour biology and as an approach for planning therapy.

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