Abstract A recent in vitro study has suggested that overexpression of ERBB2 may mediate breast tumour progression and metastasis by inhibiting the transcription of the E-cadherin (E-CD) gene. To test this hypothesis in human breast cancer in vivo, we studied the relationship between the expression of both molecules in 247 breast carcinomas immunohistochemically. Five ductal carcinomas in situ overexpressed ERBB2 and showed preserved E-CD expression. Forty-four of 226 infiltrating ductal carcinomas (19.47%) showed ERBB2 overexpression, and a statistically significant relationship was found between ERBB2 overexpression and high histological grade. E-CD expression was preserved in 111 cases (49.1%) and correlated with the histological grade. However, no significant relationship was found between ERBB2 and E-CD expression. None of the 16 infiltrating lobular carcinomas expressed ERBB2 or E-CD. These observations in different histological types of breast carcinoma strongly argue against a role for ERBB2 as a transcriptional regulator of E-CD expression in most human breast carcinomas in vivo.

Key words Breast carcinoma • ERBB2 • E-cadherin

Introduction

The human ERBB2 gene (c-erbB-2, HER-2/neu) encodes a transmembrane 185-kDa protein, which is a member of the type 1 family of receptor tyrosine kinases [14, 25]. This receptor protein is expressed at low levels in normal breast tissue, but gene amplification and protein overexpression occur in 10–40% of breast cancers and are reported to correlate with poor prognosis [1, 4, 9, 22, 26, 28, 29]. In addition, it has been observed that tumours that overexpress ERBB2 have a lesser response rate to conventional chemotherapeutic agents [9] and tamoxifen [10]. Although the activation of the ERBB2 receptor tyrosine kinase appears to play an important part in mammary tumour progression and metastasis, the mechanisms by which ERBB2 promotes these events are unclear. Recently, it has been shown that the ability of ERBB2 transfectants of a human mammary epithelial cell line to undergo morphogenesis in vitro is reduced, and this phenomenon has been related with decreased expression of the E-cadherin (E-CD) gene [5]. Based on this in vitro observation, the authors suggested that ERBB2 overexpression would mediate tumour progression and metastasis in human breast carcinoma by inhibition of E-CD expression [5].

E-CD is a Ca²⁺-dependent cell-cell adhesion molecule, which usually mediates homophilic and homotypic intercellular adhesion between epithelial cells [27]. Observations in experimental and human carcinomas have suggested that reduced E-CD expression induces dedifferentiation, tumorigenicity and invasiveness in carcinoma cells [3, 15, 18, 23]. E-CD is expressed in breast epithelial luminal cells, and a relationship between E-CD expression and the histological type and/or tumour grade has been observed in breast carcinomas by ourselves and others [8, 13, 16, 20, 24]. In addition, Oka et al. have reported an association between reduced E-CD expression and tumour size and metastasis [20]. These authors also observed that the expression of epidermal growth factor receptor, another member of the type 1 family of receptor tyrosine kinases, which is partially homologous with ERBB2, tended to be positive in tumours with preserved E-CD [20].

To investigate whether or not ERBB2 overexpression could mediate breast carcinoma progression via inhibi-
tion of E-CD expression in vivo, we conducted an immuno-
histochemical study of the relationship between the
two molecules as regards their expression in a large sam-
ple of breast carcinomas.

Materials and methods

Breast tissue was obtained from 247 patients with operable prima-
ry breast cancer. Neoplastic and nonneoplastic breast tissue sam-
ple were embedded in OCT compound (Miles Laboratory, Nap-
erville, IL.) snap-frozen in liquid nitrogen-cooled isopentane, and
stored at -70 ° C. The remaining breast tissue was routinely fixed
in 10% formalin for 24 h and embedded in paraffin. Histological
typing was performed on formalin-fixed and paraffin-embedded
samples. The combined histological grade (I, II, and III) of infil-
trating ductal carcinomas was obtained as described by Elston [7].

Immunostaining for ERBB2 and E-CD was performed by the
avidin-biotin-alkaline phosphatase method. Immunostaining for
ERBB2 was performed on formalin-fixed paraffin-embedded tis-
sue sections. E-CD expression was studied in cryostat sections as
previously reported [8, 21]. To analyse ERBB2 overexpression the
rabbit anti-human c-erbB-2 oncoprotein polyclonal antibody
(Dako, Glostrup, Denmark) was used. ECCD-2 (a generous gift
from M. Takeichi, Kyoto University, Japan) is a rat monoclonal
antibody against mouse E-CD, which also recognizes human E-
CD [8, 21]. The primary antibodies were applied at dilutions of
1:400 and 1:200, respectively. After washing in Tris-buffer, tissue
sections were incubated with biotinylated goat anti-rabbit or rabbit
anti-rat immunoglobulins (Dako), and then incubated with strepta-
vidin-alkaline phosphatase complex (Dako). The alkaline phos-
phatase activity was developed using naphthol AS-MX phosphate
as substrate and Fast-Red as the chromogen group (Sigma Chemi-
cal Co., St. Louis, Mo.). The sections were finally counterstained
with Mayer haematoxylin. In negative controls the primary anti-
body against ERBB2 or E-CD was omitted or replaced by an irrel-
evant antibody.

Positive ERBB2 and E-CD expression was only considered to
be present when linear membrane staining was observed. A semi-
quantitative estimation of protein expression based on the staining
intensity and relative abundance of immunoreactive cells was per-
formed independently by two pathologists. The intensity of immu-
nostaining was graded 0 to +3, and the percentage of positive cells
was assessed by counting at least 100 tumour cells in areas of het-
erogeneous expression (0=under 5% of cells positive; 1=5–25%;
2=26–50%; 3=51–75%; 4=76–100%). With these data a compos-
te score was obtained by adding the values of the immunoreaction
intensity and relative abundance. Preserved E-CD expression was
estimated when the composite score was 6 or 7, as previously re-

Fig. 1A-D Photomicrographs illustrating ERBB2 overexpression
and preserved E-cadherin expression in two breast tumours. A, B
Comedo ductal carcinoma in situ. C, D Grade III infiltrating duct-
al carcinoma. Note strong membrane immunoreactivity in most
cells on immunohistochemical staining with antibodies against
ERBB2 (A, C) and E-cadherin (B, D)