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ORIGINAL ARTICLE

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Relationship between ERBB2 and E-cadherin expression in human breast cancer

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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 tomoxifen [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 Ca^{2+}-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-

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tion of E-CD expression in vivo, we conducted an immunohistochemical study of the relationship between the two molecules as regards their expression in a large sample of breast carcinomas.

Materials and methods

Breast tissue was obtained from 247 patients with operable primary breast cancer. Neoplastic and nonneoplastic breast tissue samples were embedded in OCT compound (Miles Laboratory, Naperville, 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 infiltrating 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 tissue 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 streptavidin-alkaline phosphatase complex (Dako). The alkaline phosphatase activity was developed using naphtol AS-MX phosphate as substrate and Fast-Red as the chromogen group (Sigma Chemical Co., St. Louis, Mo.). The sections were finally counterstained with Mayer haematoxylin. In negative controls the primary antibody against ERBB2 or E-CD was omitted or replaced by an irrelevant antibody.

Positive ERBB2 and E-CD expression was only considered to be present when linear membrane staining was observed. A semiquantitative estimation of protein expression based on the staining intensity and relative abundance of immunoreactive cells was performed independently by two pathologists. The intensity of immunostaining was graded 0 to +3, and the percentage of positive cells was assessed by counting at least 100 tumour cells in areas of heterogeneous expression (0=under 5% of cells positive; 1=5–25%; 2=26–50%; 3=51–75%; 4=76–100%). With these data a composite 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-

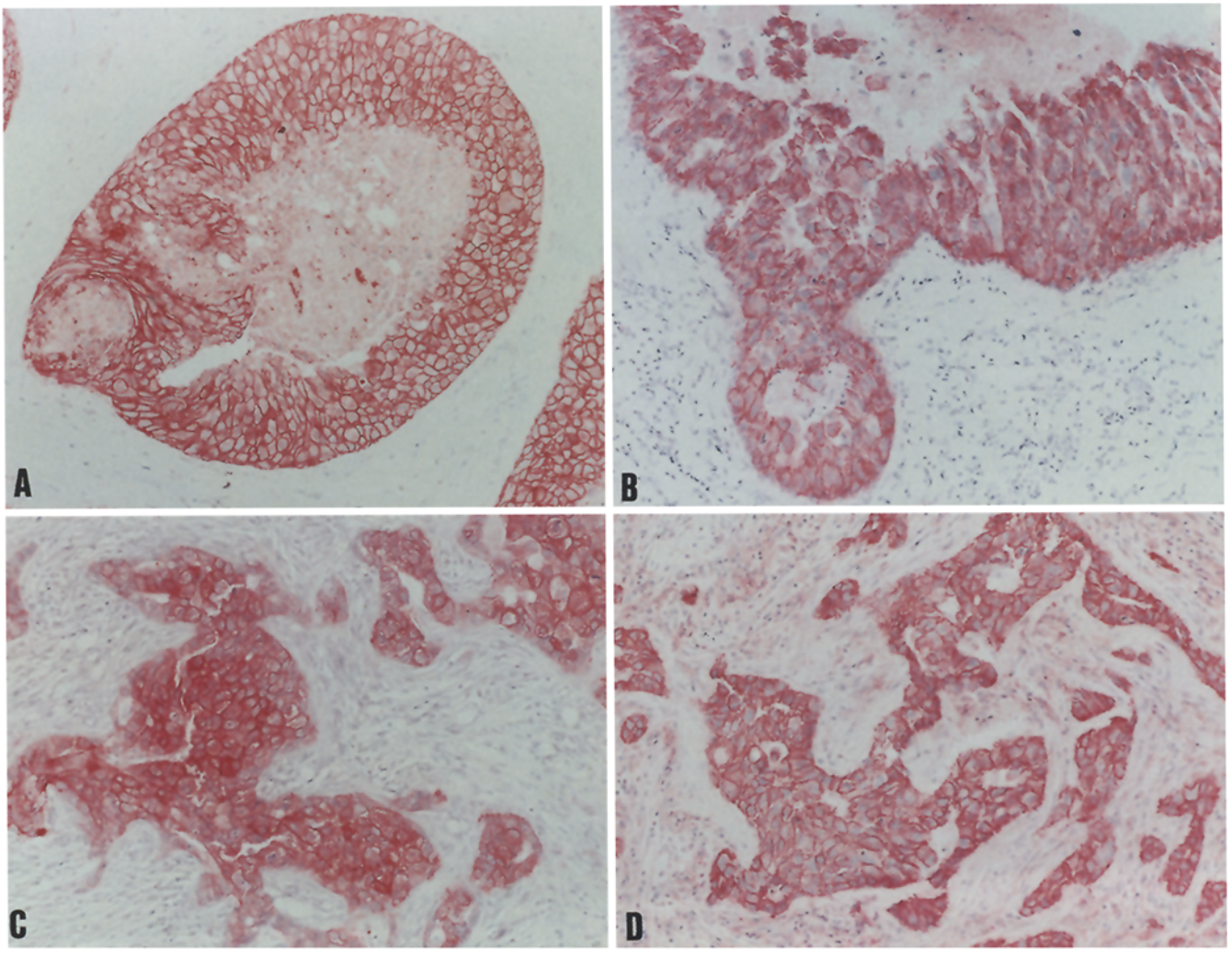

Fig. 1A–D Photomicrographs illustrating ERBB2 overexpression and preserved E-cadherin expression in two breast tumours. **A, B** Comedo ductal carcinoma in situ. **C, D** Grade III infiltrating ductal carcinoma. Note strong membrane immunoreactivity in most cells on immunohistochemical staining with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

Table 1 Relationships between ERBB2 overexpression and tumour grade and E-cadherin expression in infiltrating ductal breast carcinoma (*n.s.* not significant according to Chi-square test)

	No.	Percentage with ERBB2 overexpression	*P*
Tumour grade			
I	47	10.6	
II	82	13.4	
III	97	28.8	0.007
E-cadherin			
Preserved	111	18.0	
Reduced	115	20.8	n.s.

ported [8, 21]. Tumours were considered ERBB2 positive when at least 25% of the cells showed moderate (+2) or intense (+3) immunoreactivity.

The Chi-square test was used to analyse the statistical significance of the relationships between ERBB2 and E-CD expression, and between the expression of each proteins and the histological grade of infiltrating ductal breast carcinomas.

Results

All 5 ductal carcinomas in situ and 44 out of 226 infiltrating ductal carcinomas (19.47%) showed ERBB2 overexpression (Fig. 1A, C). In contrast, none of the 16 infiltrating lobular carcinomas expressed ERBB2. In infiltrating ductal carcinomas, ERBB2 overexpression was significantly more frequent in tumours with high histological grade (Table 1).

Preserved E-CD expression was observed in the 5 ductal carcinomas in situ analysed and in 111 out of 226 infiltrating ductal carcinomas (49.1%) (Fig. 1B, C). Infiltrating lobular carcinomas did not express E-CD. Table 1 shows that ERBB2 overexpression correlated significantly with the tumour grade and that among infiltrating ductal carcinomas with reduced E-CD expression the percentage of tumours with ERBB2 overexpression was similar to that observed among cases with preserved E-CD: the relationship between ERBB2 and E-CD expression was not statistically significant. A statistically significant relationship was observed between E-CD expression and the tumour grade: E-CD expression was preserved in 65.3%, 57.14% and 35% of the grade I, II and III tumours, respectively (Chi-square=14.96%; P=0.0006). A significant relationship between E-CD and tumour grade was also observed in the group of 44 ductal breast carcinomas with ERBB2 overexpression. Thus, E-CD expression was preserved in the 100% (5 out of 5), 45.4% (5 of 11) and 35.7% (10 of 28) of the grade I, II and III tumours that overexpressed ERBB2 (Chi-square=7.07; P=0.0291).

Discussion

Our results agree with previous observations which suggest that ERBB2 overexpression may be involved in human breast carcinoma progression. Thus, ERBB2 was always overexpressed in the comedo type of in situ ductal carcinoma, the subtype of in situ ductal carcinoma that is largest and has the greatest tendency to local recurrence [12]. Approximately 20% of infiltrating ductal carcinomas (IDC) in this series overexpressed ERBB2, a proportion that is in the range of previously reported frequencies [9, 22]. Moreover, ERBB2 overexpression was associated with high histological grade, as previously observed in other series [9, 22, 29].

The manner in which the aberrant expression of ERBB2 promotes malignant progression is still not understood, although several mechanisms have been proposed. Activation of the ERBB2 kinase results in tyrosine phosphorylation and activation of a number of downstream signalling proteins [17]. It has also been observed that there is a cross-regulation between ERBB2 and oestrogen receptor in vitro, since cells lines that overexpressed ERBB2 showed a decrease in mRNA transcript and protein when treated with oestradiol but treatment with a putative ligand for ERBB2 blocked the induction of the oestrogen-responsive progesterone receptor gene [14]. There is some evidence indicating that ERBB2 overexpression may decrease host ability to maintain immune surveillance by inhibition of natural-killer cell activity and by inducing resistance to tumour necrosis factor [10].

A recent in vitro study has shown that ERBB2 overexpression down-regulated E-CD gene expression at the level of transcription in the nontumorigenic human mammary epithelial cell line MTSV1-7. Transfectants expressing higher ERBB2 copy number showed greater reduction in levels of E-CD mRNA and protein. In addition, it was demonstrated that this effect was related to the signal generated by the ERBB2 receptor [5]. Since E-CD down-regulation has been proposed as an important mechanism for invasion and metastasis in human cancer [3, 15, 18, 23], this in vitro observation may explain how ERBB2 overexpression participates in tumour progression. To test this hypothesis in vivo, we have investigated the relationship between ERBB2 and E-CD expression in a large sample of human patients with breast cancer. We used an immunohistochemical approach, but it has already been shown that there is a significant correlation between genomic amplification and ERBB2 oncoprotein overexpression detected by immunohistochemistry in breast cancer [11], and between E-CD mRNA and protein levels in other types of carcinomas [6]. Our observations on different histological types of human breast carcinomas strongly suggest that ERBB2 overexpression and reduced E-CD expression are usually independent alterations in human breast cancer. Thus, ERBB2 overexpression is a constant finding in large-cell pure comedo IDC [1], which in our series also showed preserved E-CD expression. Moreover, a similar percentage of cases with preserved and reduced E-CD expression were found among IDC that overexpressed ERBB2, indicating no statistically significant association between ERBB2 and E-CD expression. Finally, infiltrating lobular carcinoma

is characterized by a complete absence or marked reduction in the expression of E-CD, and it has been suggested that this molecular alteration may be responsible for its typical noncohesive pattern of infiltration [8, 16, 24]; however, ERBB2 overexpression is a very unusual finding in this particular histological type of infiltrating breast carcinoma [9].

In this series, we confirm our previous observation [8, 21] that there is a strong correlation between E-CD expression and the histological grade of infiltrating ductal carcinoma, since a decreased E-CD expression was a frequent finding in grade III tumours. This relationship was also observed in the group of cases that showed ERBB2 overexpression. In fact, 5 out of 5 grade I tumours with ERBB2 overexpression had preserved E-CD expression, suggesting normal functionality of E-CD in this group. However, it would be of interest to explore the functional status of E-CD in breast carcinomas with ERBB2 overexpression further particularly in grade III tumors with preserved E-CD expression. It has been shown that tyrosine phosphorylation of the E-CD-catenin complex by v-src gene induces reduced epithelial differentiation and increased invasiveness in vitro [2], and recent evidence suggests that activation of ERBB2 mediates elevated c-src tyrosine kinase activity by direct interaction between the two molecules [17]. In addition, Ochiai et al. [19] have reported an association of the ERBB2 gene product with β-catenin and plakoglobin in gastric cancer cell lines, indicating that ERBB2 gene product may regulate the cell adhesion and invasive growth of cancer through the E-CD-catenin complex.

Our results demonstrate that ERBB2 overexpression is not associated with down-regulation of E-CD expression at the transcriptional level in human breast carcinoma in vivo. However, we cannot completely exclude the possibility that ERBB2 overexpression might reduce E-CD expression in a particular subset of breast carcinomas in which both features are present or that ERBB2 modifies the cadherin-mediated cell adhesion system in some cancer cells through other mechanisms.

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References

1. Bartkova J, Barnes DM, Millis RR, Gullick WJ (1990) Immunohistochemical demonstration of c-erbB-2 in mammary ductal carcinoma in situ. Hum Pathol 21:1164–1167
2. Behrens J, Vakaet L, Friis R, Vinterhager E, Van Roy F, Mareel MM, Birchmeier W (1993) Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/β-catenin complex in cells transformed with a temperature-sensitive v-SRC gene. J Cell Biol 120:757–766
3. Birchmeier W, Behrens J (1994) Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta 1198:11–26
4. Ciocca DR, Fujimura FK, Tandon AK, Clark GM, Mark C, Lee-Chen GJ, Pounds GW, Vendely P, Owens MA, Pandian MR, McGuire WL (1992) Correlation of HER-2/neu amplification with expression and with other prognostic factors in 1103 breast caners. J Natl Cancer Inst 84:1279–1282
5. D'souza B, Taylor-Papadimitriou J (1994) Overexpression of ERBB2 in human mammary epithelial cells signals inhibition of transcription of the E-CD gene. Proc Natl Acad Sci USA 91:7202–7206
6. Dorudi S, Sheffield JP, Poulsom R, Northover JMA, Hart IR (1993) E-cadherin expression in colorectal cancer. An immunocytochemical and in situ hybridization study. Am J Pathol 142:981–986
7. Elston CW (1987) Grading of invasive carcinoma of the breast. In: Page DL, Anderson TJ (eds) Diagnostic histopathology of the breast. Churchill Livingston, Edinburgh, pp 300–311
8. Gamallo C, Palacios J, Suárez A, Pizarro A, Quintanilla M, Cano A (1993) Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. Am J Pathol 142:987–993
9. Gusterson BA, Gelber RD, Goldhirsch A et al (1992) Prognostic importance of c-erbB-2 expression in breast cancer. J Clin Oncol 10:1049–1056
10. Hoskin K, Weber B (1994) The biology of breast cancer. Curr Opin Oncol 6:554–559
11. Kerns BM, Jordan PA, Huper G, Marks JR, Iglejart D, Layfield LJ (1993) Assessment of c-erbB-2 by immunohistochemistry in paraffin-embedded breast cancer. Mod Pathol 6: 673–678
12. Lagios MD, Margolin FR, Westdahl PR, Rose MR (1989) Mammographically detected ductal carcinoma in situ. Frequency of local recurrence following tylectomy and prognostic effect of nuclear grade in local recurrence. Cancer 63:618–624
13. Lipponen P, Saarelainen E, Ji H, Aaltomaa A, Syrjänen K (1994) Expression of E-cadherin (E-CD) as related to other prognostic factors and survival in breast cancer. J Pathol (Lond) 174:101–109
14. Lupu R, Lippman ME (1993) The role of erbB2 signal transduction pathways in human breast cancer. Breast Cancer Res Treat 27:83–93
15. Mareel M, Bracke M, Van Roy F (1994) Invasion promoter versus invasion suppressor molecules: the paradigm of E-cadherin. Mol Biol Rep 19:45–67
16. Moll R, Mitze M, Frixen UW, Birchmeier W (1993) Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am J Pathol 143:1731–1742
17. Muthuswamy SK, Siegel PM, Dankort DL, Webster MA, Muller WJ (1994) Mammary tumors expressing the neu protooncogene possess elevated c-Src tyrosine kinase activity. Mol Cell Biol 14:735–743
18. Navarro P, Gómez M, Pizarro A, Gamallo C, Quintanilla M, Cano A (1991) A role for the E-cadherin cell-cell adhesion molecule during tumor progression of mouse epidermal carcinogenesis. J Cell Biol 115:517–533
19. Ochiai A, Akimoto S, Kanai Y, Shibata T, Oyama T, Hirohashi S (1994) c-erbB-2 gene product associates with catenins in human cancer cells. Biochem Biophys Res Commun 205:73–78
20. Oka H, Shiozaki H, Kobayashi K, Inoue M, Yahara H, Kobayashi T, Takatsuka Y, Matsuyoshi N, Hirano S, Takeichi M, Mori T (1993) Expression of E-CD cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. Cancer Res 53:1696–1701
21. Palacios J, Benito N, Pizarro A, Suárez A, Espada J, Cano A, Gamallo C (1995) Anomalous expression of P-cadherin in breast carcinoma. Correlation with E-cadherin expression and pathological features. Am J Pathol 146:605–612
22. Paterson MC, Dietrich KD, Danyluk J, Paterson AHG, Lees AW, Jamil N, Hanson J, Jenkins H, Krause BE, McBlain WA,

Slamon DJ, Fourney RM (1991) Correlation between c-erbB-2 amplification and risk of recurrent disease in node negative breast cancer. Cancer Res 51:556–567
23. Pizarro A, Benito N, Navarro P, Palacios J, Cano A, Quintanilla M, Contreras F, Gamallo C (1994) E-cadherin expression in basal cell carcinoma. Br J Cancer 69:157–162
24. Rasbridge SA, Gillett CE, Sampson SA, Walsh FS, Millis RR (1993) Epithelial (E-) and placental (P-) cadherin cell adhesion molecule expression in breast carcinoma. J Pathol (Lond) 169:245–250
25. Schechter AL, Hung MC, Vaidyanathans L, Weinberg RA, Yang-Feng TL, Franke U, Ullrich A, Coussens L (1985) The neu gene: an erb-B-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. Science 229:976–978
26. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177–182
27. Takeichi M (1991) Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251:1451–1455
28. Toikkanen S, Helin H, Isola J, Joensuu H (1992) Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. J Clin Oncol 10:1044–1048
29. Tsuda H, Hirohashi S, Shimoshato Y, Hirota T, Tsugane S, Watanabe S, Terada M, Yamamoto H (1990) Correlation between histologic grade of malignancy and copy number of c-erbB-2 gene in breast cancer. A retrospective analysis of 176 cases. Cancer 65:1794–1800