Expression of E-cadherin and c-erbB-2/HER-2/neu Oncoprotein in High-Grade Breast Cancer

Turashvili G.[1], Bouchalova K.[2], Bouchal J.[1], Kolar Z.[1]
[1]Laboratory of Molecular Pathology, Department of Pathology, Palacky University, Olomouc, Czech Republic
[2]Laboratory of Experimental Medicine, Department of Pediatrics, Palacky University and University Hospital, Olomouc, Czech Republic

Summary

E-cadherin (E-CD) is an epithelial-specific cell adhesion molecule, whose expression is lost in invasive lobular (ILC) but not in invasive ductal carcinoma (IDC) of the breast. This cell adhesion system can be disrupted by tyrosine kinase c-erbB-2/HER-2/neu. We examined 106 cases of high-grade invasive breast cancer, including 91 IDCs, 12 ILCs and 3 pleomorphic lobular carcinomas (PLCs). We determined Nottingham histological grade and performed immunohistochemistry for estrogen and progesterone receptors (ER/PR), Ki-67, E-CD and c-erbB-2/HER-2/neu with subsequent fluorescence in situ hybridization. Amplification of c-erbB-2/HER-2/neu gene was observed in 55/91 (60.4%) of IDCs, 3/12 (25%) of ILCs and 1/3 (33.3%) of PLCs, and associated with positive axillary lymph nodes. E-CD expression was lost in 14/91 (15.4%) of IDCs, 10/12 (83.3%) of ILCs and 2/3 (66.7%) of PLCs. The loss of E-CD immunoreactivity in IDCs appeared to be associated with c-erbB-2/HER-2/neu gene amplification, negative ER/PR status and positive lymph nodes, whereas E-CD-positive ILCs tended to be HER-2/neu-positive. The biological significance of E-CD expression seems to be different in high-grade IDC and ILC. Oncogenic pathway mediated by c-erbB-2/HER-2/neu may affect the E-CD expression in most invasive ductal breast carcinomas in vivo.

Key words: ductal breast carcinoma - lobular breast carcinoma - E-cadherin - c-erbB-2/HER-2/neu - immunohistochemistry

Souhrn

Exprese E-cadherinu a c-erbB-2/HER-2/neu onkoproteinu v málo diferencovaných karcinomech prsu

E-cadherin (E-CD) je pro epitel specifická adhezní molekula, jejíž exprese se, na rozdíl od invazivních duktálních karcinomů, snižuje nebo zcela ztrácí u invazivních lobulárních karcinomů. O tyrozin kináze c-erbB-2/HER-2/neu je známo, že může expresi adhezního systému, jehož součástí je E-CD, narušit. Vyšetřili jsme 106 případů málo diferencovaných (G2-G3) invazivních karcinomů prsu, z toho 91 IDC, 12 ILC a 3 pleomorfní lobulární karcinomy (PLC). Provedli jsme imunohistochemické barvení estrogenového a progesteronového receptoru (ER/PR), Ki-67, E-CD a c-erbB-2/HER-2/neu a vyšetřili amplifikaci genu pro c-erbB-2/HER-2/neu pomocí fluorescenční in situ hybridizace. Amplifikace genu pro c-erbB-2/HER-2/neu byla pozorována u 55/91 (60,4 %) IDC, 3/12 (25 %) ILC a 1/3 (33,3 %) PLC a byla asociována s pozitivitou axilárních lymfatických uzlin. Ke ztrátě exprese E-CD došlo u 14/91 (15,4%) IDC, 10/12 (83,3 %) ILC a 2/3 (66,7 %) PLC. Ztráta imunoreaktivity E-CD v IDC byla asociována s amplifikací genu pro c-erbB-2/HER-2/neu, negativitou ER/PR a pozitivními lymfatickými uzlinami, zatímco ILC s pozitivním E-CD byly obvykle HER-2/neu pozitivní. Exprese E-CD má pravděpodobné odlišný biologický význam v málo diferencovaných IDC a ILC. Signální dráha c-erbB-2/HER-2/neu může ovlivnit expresi E-CD ve většině invazivních duktálních karcinomech prsu in vivo.

Klíčová slova: duktální karcinom prsu – lobulární karcinom prsu – E-cadherin - c-erbB-2/HER-2/neu - imunohistochemie

Čes.-slov. Patol., 43, 2007, No. 3, p. 87–92

E-cadherin (E-CD) is a calcium-dependent, epithelial-specific cell-cell adhesion molecule, whose reduced or lost expression is associated with tumor dedifferentiation and increased metastatic potential in human carcinomas (8). Many investigations suggest that E-CD protein expression is lost in invasive lobular (ILC) but not in invasive ductal carcinomas (IDC) of the breast (10, 16, 25, 29). It has been shown that loss of E-CD expression is an early event in the formation of ILC. The absence of E-CD induces a partial loss of epithelial differentiation and may account for the extended spread of lobular carcinoma *in situ* (LCIS) and the peculiar diffuse

invasion mode of ILC (20). E-CD expression correlates with histological type and grade in breast carcinomas. None of ILCs expressed E-CD in infiltrating tumor cells, whereas they showed only weak immunostaining in areas of atypical lobular hyperplasia and LCIS (6). E-CD and high molecular weight cytokeratins in combination have been shown to be useful in distinguishing lobular and ductal intraepithelial lesions (LIN and DIN, respectively). All samples of LIN showed complete negativity for E-CD, whereas the DIN lesions displayed cytoplasmic positivity, often in a distinct perinuclear pattern (3).

It has been suggested that the loss of normal E-CD expression is an indicator of increased invasiveness and dedifferentiation in breast carcinoma and that E-CD is a potentially important prognostic factor in primary IDCs. The proportion of tumors with reduced or lost E-CD expression increased significantly from pure intraductal carcinomas (20%) through invasive ductal (52%) to recurrent carcinomas (64%). None of the ILCs retained normal E-CD expression in contrast to 48% of the IDCs (26). The loss of E-CD expression was related to an increase in diffuse growth pattern in both IDC and ILC, and the differential proportions of growth patterns caused the tendency for lower E-CD expression in ILC. In 60% of ILC the diffuse growth pattern and in 72% of IDC the compact growth pattern predominated. E-CD expression was significantly lower in diffuse than in compact tumor area without relation to carcinoma type when it was considered in tumor areas with either diffuse or compact growth pattern (4). E-CD staining in >10% of ILC cells was seen in 7% mixed, predominantly lobular carcinomas, 61% mixed carcinomas, and 67% mixed, predominantly ductal carcinomas. Lobular carcinoma-type systemic metastases were identified in 84% pure lobular, 11% mixed and 4% pure ductal carcinomas. No E-CD staining was found in 98% of ILC, all cases of ILC-type systemic metastases and all cases of ILC systemic metastases (7).

A few papers report that E-CD mediated cell adhesion system can be disrupted by tyrosine kinase c-erbB-2/HER-2/neu in breast carcinomas despite ductal or lobular type (11-14, 27), however, other studies failed to confirm this finding (15, 21, 22, 24, 32). Protein c-erbB-2/HER-2/neu is a member of the human epidermal growth factor receptors with tyrosine kinase activity which regulates cell growth and proliferation (19). Overexpression of this receptor, typically caused by amplification of the HER-2 gene, is present in approximately 10-30% of invasive breast cancers, and is associated with an aggressive disease course and decreased disease-free and overall survival in lymph node positive patients (17). In this study we examined the relationship between c-erbB-2/HER-2/neu and E-cadherin expression and their association with lymph node positivity in high-grade invasive ductal and lobular breast carcinomas.

Methods

Specimens. Formalin-fixed and paraffin-embedded samples were obtained by mastectomy from a total of 106 breast cancer patients aged >35 years in University Hospital Olomouc. The lesions included 91 IDCs no special type (NST), 12 ILCs and 3 PLCs. Histopathological diagnosis was confirmed by an experienced pathologist using haematoxylin and eosin (H&E) stained sections. Tumors were graded using the Nottingham combined histologic grading system (5). In accordance with this grading system, each tumor was assessed and scored numerically for the percentage of tubule formation, the degree of nuclear pleomorphism and the mitotic count in 10 high-power fields (field diameter 0.44 mm). All IDCs were Grade 3 (G3), ILCs were Grade 2 (G2), and PLCs were considered G3. These cases were submitted to our laboratory for suspicion of c-erbB-2/HER-2/neu overexpression. Clinical data including the status of axillary lymph nodes were recorded.

Immunohistochemistry. The expression of estrogen and progesterone receptors (ER and PR), marker of proliferating cells Ki-67, E-cadherin and c-erbB-2/HER-2/neu was determined by indirect immunohistochemistry on paraffin sections using a microwave antigen retrieval method. The monoclonal antibodies used were: mouse anti-E-cadherin (clone NCH-38, Dako, Glostrup, Denmark; dilution 1:100), mouse anti-ER (clone 1D5, Dako, dilution 1:20) and mouse anti-Ki-67 (clone MIB-1, Dako, dilution 1:75). The rabbit anti-PR polyclonal antibody (Dako; dilution 1:50) and HercepTest (Rabbit Anti-Human HER-2 Protein, Ready-to-use, Dako) were also used.

Sections were deparaffinized in xylene, rehydrated and endogenous peroxidase was blocked in 5% hydrogen peroxide for 15 min. Following antigen retrieval procedures (10 mM citrate buffer, pH 6.0, 100°C for 15 min in a microwave generator, 750 W), the sections were kept in a container for 30 min to reach room temperature. After washing in distilled water (1 min) and PBS (1 min) sections were incubated in blocking milk buffer (5% dry skimmed milk in PBS, pH 7.6 for 30 min) and incubated with primary antibody at 4°C overnight. Following washing in PBS (3 @ 5 min) sections were incubated with horseradish peroxidase (HRP)-labelled polymer conjugated with secondary antibody for 60 min at room temperature (mouse and/or rabbit EnVision System HRP, Dako). Visualization was performed by the standard diaminobenzidine (DAB) reaction. The nuclei were counterstained by haematoxylin. Positive and negative controls were included in all

Table 1. Clinical and histopathological features of 106 breast cancer patients

Features		IDC (n=91)	ILC (n=12)	PLC (n=3)
LN status	LN+	53 (58.2%)	6 (50%)	2 (66.7%)
	LN-	38 (41.8%)	6 (50%)	1 (33.3%)
HER-2	HER-2+	55 (60.4%)	3 (25%)	1 (33.3%)
	HER-2-	36 (39.6%)	9 (75%)	2 (66.7%)
E-CD	E-CD+	77 (84.6%)	2 (16.7%)	1 (33.3%)
	E-CD-	14 (15.4%)	10 (83.3%)	2 (66.7%)
ER	ER+	46 (50.5%)	7 (58.3%)	2 (66.7%)
	ER-	45 (49.5%)	5 (41.7%)	1 (33.3%)
PR	PR+	39 (42.9%)	6 (50%)	2 (66.7%)
	PR-	52 (57.1%)	6 (50%)	1 (33.3%)
Ki-67	Ki-67+	77 (84.6%)	6 (50%)	2 (66.7%)
	Ki-67-	14 (15.4%)	6 (50%)	1 (33.3%)

runs. The primary antibodies were omitted as negative controls and sections from selected tissue specimens were used as positive controls.

The immunohistochemical results were estimated by two independent histopathologists. For ER and PR, only the nuclear staining was considered positive. Scoring was based on examining all tumor cells on the slide. A proportion score (PS) was assigned representing the estimate proportion of positive staining cells. An intensity score (IS) was assigned representing the estimated average staining intensity of positive cells. A total score (TS) was calculated from the sum of PS and IS (ranging from 0-8). A positive result for both ER and PR was defined as TS ≥ 3 (2).

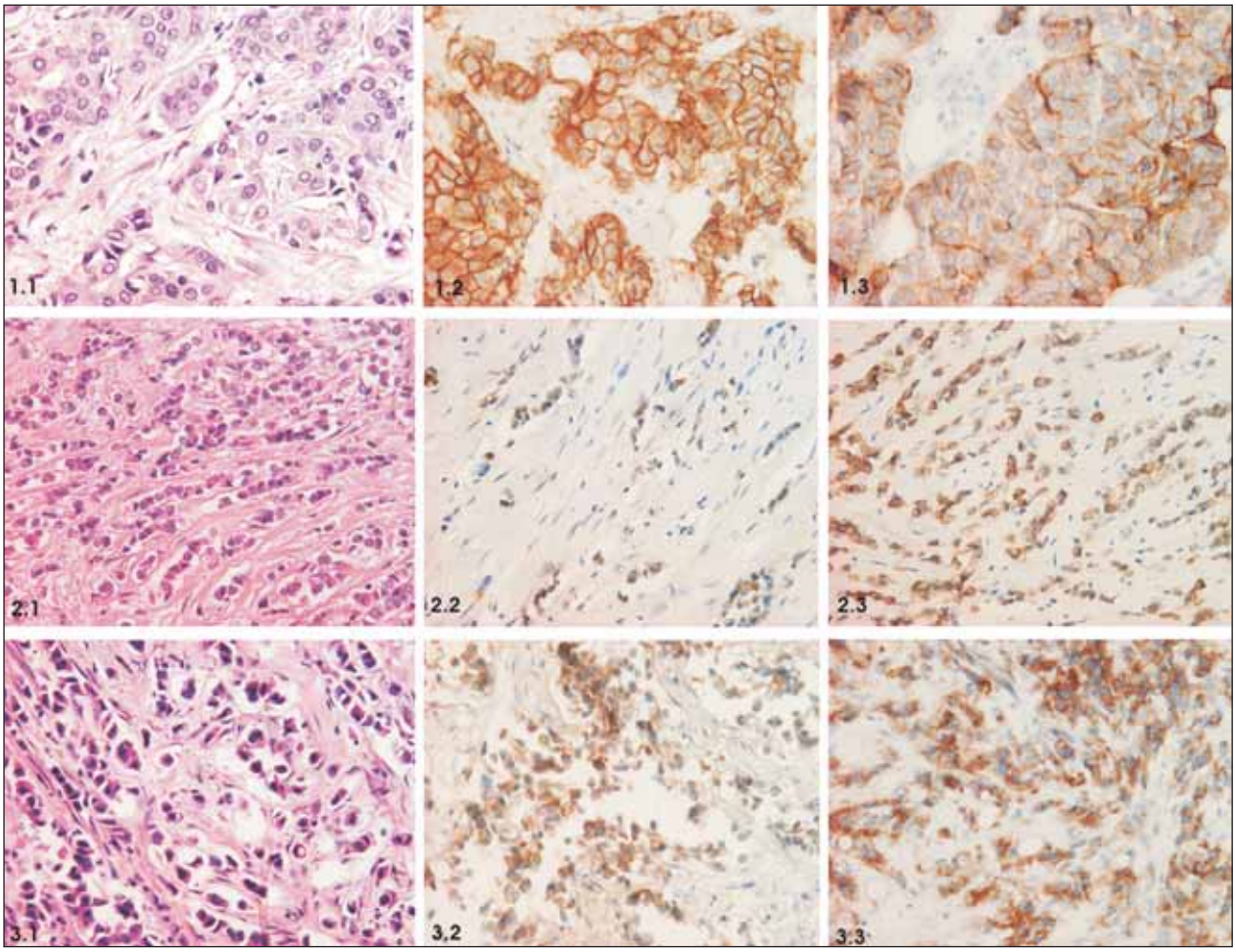

Figure 1. 1. Invasive ductal carcinoma, x400. 1.1. H&E stain, 1.2. HER-2/neu is positive, 1.3. E-cadherin is positive; 2. Invasive lobular carcinoma, x400. 2.1. H&E stain, 2.2. HER-2/neu is positive, 2.3. E-cadherin is positive; 3. Pleomorphic lobular carcinoma, x400. 3.1. H&E stain, 3.2. HER-2/neu is positive, 3.3. E-cadherin is positive.

For the determination of c-erbB-2/HER-2/neu protein overexpression, only the membrane staining intensity and pattern were evaluated using the following scale: 0, no staining is observed or membrane staining is observed in <10% of the tumor cells; 1+, a faint/barely perceptible membrane staining is detected in >10% of the tumor cells. The cells are only stained in part of their membrane; 2+, a weak to moderate complete membrane staining is observed in >10% of the tumor cells; 3+, a strong complete membrane staining is observed in >10% of the tumor cells. HercepTest™ was interpreted as negative for HER-2 protein overexpression (0 and 1+ staining intensity), weakly positive (2+ staining intensity), and strongly positive (3+ staining intensity). All tumors were submitted for fluorescence in situ hybridization (FISH) to determine HER-2 gene amplification. FISH analyses were performed using the FISH pharmDx™ Kit (Dako), according to the supplied instructions and using reagents, probes, and positive controls. Signal enumeration was performed following the criteria recommended by the supplier.

For E-CD and Ki-67, we regarded cells as immunoreactive when an obvious membranous or nuclear staining was seen, respectively. The cutoff to define positive immunoreactivity for Ki-67 was ≥10% positive nuclei. Frequencies of positive and negative stainings were compared and association with lymph node status was determined by Fisher`s exact test (28). All *p* values were two tailed and considered significant when 0.05.

Results

Of 91 IDC cases, 53 patients (58.2%) were lymph node-positive, whereas metastases could not be seen in routine H&E sections of remaining 38 patients (41.8%). Of 12 ILC cases, 6 patients (50%) were lymph node-positive, and remaining 6 patients (50%) showed no metastases in axillary lymph nodes. Of 3 PLCs, 2 patients (66.7%) were lymph node-positive compared with one lymph node-negative patient (33.3%) (Table 1). All IDCs were Grade 3, ILCs were Grade 2 and PLCs were considered Grade 3. These cases were submitted to our laboratory for suspicion of HER-2/neu overexpression. HER-2/neu gene amplification using FISH method was observed in 55 cases (60.4%) out of 91 IDCs, 3 cases (25%) out of 12 ILCs, and one case (33.3%) of PLC. Of 55 HER-2/neu-positive IDCs, HER-2 protein expression was 2+ in 33 cases (60%) and 3+ in remaining 22 cases (40%). Of 3 HER-2/neu-positive ILCs, HER-2 protein expression was 2+ in one case (33.3%), 3+ in remaining 2 cases (66.7%), and finally 3+ in one PLC (Figure 1).

Table 2. Clinical and histopathological features of HER-2/neu+ breast cancer patients

Features		IDC (n=55)	ILC (n=3)	PLC (n=1)
LN status	LN+	53	3	1
	LN-	2	0	0
E-CD	E-CD+	41	2	1
	E-CD-	14	1	0
Ki-67	Ki-67+	55	3	1
	Ki-67-	0	0	0
ER	ER+	10	0	0
	ER-	45	3	1
PR	PR+	4	0	0
	PR-	51	3	1

HER-2/neu-positive 55 patients with IDCs included all 53 lymph node-positive cases and 2 lymph node-negative cases, also all three HER-2/neu-positive patients with ILC and one patient with PLC had positive axillary lymph nodes, suggesting the association between HER-2/neu gene amplification and lymph node positivity ($p<0.001$).

The majority of ILCs (10 cases, 83.3%) and 2 cases of PLC (66.7%) showed negative staining for E-CD. E-CD expression was lost in 14 cases (15.4%) of IDC and associated with HER-2/neu gene amplification, negative hormone receptor status and positive lymph nodes ($p<0.001$) (Table 2). Two cases of ILC and one case of PLC were immunoreactive for E-CD and shown in Figure 1. Interestingly, all E-CD-positive ILCs and PLC were HER-2/neu-positive and also had positive axillary lymph nodes.

Strong positivity for Ki-67 was observed in 77 cases (84.6%) of IDCs and 6 (50%) cases of ILCs and positively correlated with HER-2/neu positivity ($p<0.001$). IDCs were negative for ER in 49.5% (45 patients), and PR in 57.1% of cases (52 patients) of cases. ILCs were negative for ER in 41.7% (5 patients), and PR in 50% (6 patients) of cases, and 33.3% (one case) of PLC (Table 1). ER/PR-negativity in both IDC and ILC was associated with HER-2/neu gene amplification and positive lymph nodes (Table 2).

Discussion

E-cadherin (E-CD) is considered to be the most important cell adhesion molecule in mammary gland. The gene encoding this protein acts as a tumor suppressor inhibiting invasion and metastasis. Its mutations are correlated with gastric, breast, colorectal, thyroid and ovarian cancer. During tumor progression, E-CD can be functionally inactivated or silenced by different mechanisms such as post-translational control, somatic mutations, downregulation of gene expression through promoter hypermethylation,

histone deacetylation, and transcriptional repression (9, 23). In our study we have found E-CD expression in 84.6% of IDCs, whereas its expression was lost in 83.3% of ILCs, explaining the specific histological appearance of ILC such as "Indian file" arrangement of tumor cells.

E-CD has been described as a useful diagnostic tool for differentiation of ILC and IDC (10, 16), however, various types of immunoreactivity have been reported. IDCs have been shown to express E-CD in a similar peripheral-predominant immunostaining pattern, while ILCs were negative for E-CD, suggesting a role for E-CD in the architectural organization of the cytoskeletal scaffolding within the tumor cells (18). Invasive carcinomas with both ductal and lobular features showed 3 staining patterns: complete or almost complete lack of membrane staining similar to that seen in ILC, uniform membrane expression throughout the tumor similar to IDC, and focal loss of E-CD staining, which correlated well with the focal lobular features histologically (1). We have noted complete membrane staining in all E-CD-positive cases including ILC and PLC.

In our study the loss of E-CD expression in IDCs was associated with c-erbB-2/HER-2/neu gene amplification, negative hormone receptors and positive lymph nodes which agrees with the literature (12, 13, 26). Treatment of c-erbB-2/HER-2/neu-overexpressing breast cancer cell lines with anti-HER-2 antibody Trastuzumab resulted in a number of phenotypic changes including downmodulation of the c-erbB-2/HER-2/neu receptor, and restored E-CD expression levels (27). Using two-dimensional cultures it has been demonstrated that activated c-erbB-2/HER-2/neu induced breakdown of cell-cell junctions involving E-CD, increased cell motility and dispersal of epithelial colonies (14). Transfection of MCF-10A breast cancer cell line with individual c-erbB-2 containing episomes induced a high number of c-erbB-2/HER-2/neu gene copies, highly expressed c-erbB-2 protein, and lost E-CD expression (31).

The scenario seems to be different in ILC. All E-CD-positive ILCs and one PLC appeared to be c-erbB-2/HER-2/neu-positive and had positive axillary lymph nodes, suggesting the association between c-erbB-2/HER-2/neu and E-CD expressions. The significant tendency toward expression of E-CD in conjunction with c-erbB-2/HER-2/neu overexpression in breast cancer was originally reported in 2004. Expression of E-CD was positively associated with c-erbB-2/HER-2/neu overexpression, and high levels of c-erbB-2/HER-2/neu occurred with strongly E-CD-positive tumors (11). E-CD mediated cell-cell adhesion has been suggested to be disrupted by c-erbB-2/HER-2/neu (11-14, 27), however, controversial results have also been reported (15, 21, 22, 24, 32). Furthermore, ILCs usually tend to be both E-CD-negative and c-erbB-2/HER-2/neu-negative (30). This suggests that biological significance of E-CD expression is different in high-grade IDC and ILC and may depend on HER-2 amplification.

Conclusion

The loss of E-cadherin expression is not an exclusive feature of invasive lobular carcinoma of the breast. It can also be seen in high-grade invasive ductal tumors. The loss of E-cadherin immunoreactivity in invasive ductal carcinoma appeared to be associated with c-erbB-2/HER-2/neu gene amplification, negative hormone receptor status and positive lymph nodes, whereas E-cadherin-positive invasive lobular carcinomas tended to be c-erbB-2/HER-2/neu-positive. This suggests that c-erbB-2/HER-2/neu-mediated pathway may affect the E-CD expression in most invasive ductal breast carcinomas in vivo.

List of abbreviations

DAB – diaminobenzidine
DIN – ductal intraepithelial neoplasia
E-CD – E-cadherin
ER – estrogen receptor
FISH – fluorescence in situ hybridization
G3 – grade 3
G2 – grade 2
H&E – haematoxylin and eosin
HRP – horseradish peroxidase
IDC – invasive ductal carcinoma
ILC – invasive lobular carcinoma
IS – intensity score
LCIS – lobular carcinoma in situ
LIN – lobular intraepithelial neoplasia
NST – no special type
PR – progesterone receptors
PS – proportion score
TS – total score

References

1. **Acs, G., Lawton, T.J., Rebbeck, T.R., et al.:** Differential expression of E-cadherin in lobular and ductal neoplasms of the breast and its biologic and diagnostic implications. Am. J. Clin. Pathol., 115, 2001, p. 85–98.
2. **Allred, D.C., Harvey. J.M., Berardo, M., Clark, G.M.:** Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod. Pathol., 11, 1998, p. 155–168.
3. **Bratthauer, G.L., Moinfar, F., Stamatakos, M.D. et al.:** Combined E-cadherin and high molecular weight cytokeratin immunoprofile differentiates lobular, ductal, and hybrid mammary intraepithelial neoplasias. Hum. Pathol., 33, 2002, p. 620-627. –
4. **Brinck, U., Jacobs S., Neuss M., et al.:** Diffuse growth pattern affects E-cadherin expression in invasive breast cancer. Anticancer Res., 24, 2004, p. 2237–2242.

5. **Elston, C.W., Ellis, I.O.:** Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology, 19, 1991, p. 403–410.
6. **Gamallo, C., Palacios, J., Suarez, A., et al.:** Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. Am. J. Pathol., 142, 1993, p. 987–993.
7. **Goldstein, N.S.:** Does the level of E-cadherin expression correlate with the primary breast carcinoma infiltration pattern and type of systemic metastases? Am. J. Clin. Pathol., 118, 2002, p. 425–434.
8. **Goldstein, N.S., Bassi, D., Watts, J.C., et al.:** E-cadherin reactivity of 95 noninvasive ductal and lobular lesions of the breast. Implications for the interpretation of problematic lesions. Am. J. Clin. Pathol., 115, 2001, p. 534–542.
9. **Grille, S.J., Bellacosa, A., Upson, J., et al.:** The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res., 63, 2003, p. 2172–2178.
10. **Gupta, D., Croitoru, C.M., Ayala, A.G., et al.:** E-cadherin immunohistochemical analysis of histiocytoid carcinoma of the breast. Ann. Diagn. Pathol., 6, 2002, p. 141–147.
11. **Howard, E.M., Lau, S.K., Lyles, R.H., et al.:** Correlation and expression of p53, HER-2, vascular endothelial growth factor (VEGF), and e-cadherin in a high-risk breast-cancer population. Int. J. Clin. Oncol., 9, 2004, p. 154–160.
12. **Howard, E.M., Lau, S.K., Lyles, R.H., et al.:** Expression of e-cadherin in high-risk breast cancer. J. Cancer Res. Clin. Oncol., 131, 2005, 14–18.
13. **Jones, J.L., Royall, J.E., Walker, R.A.:** E-cadherin relates to EGFR expression and lymph node metastasis in primary breast carcinoma. Br. J. Cancer, 74, 1996, p. 1237–1241.
14. **Khoury, H., Dankort, D.L., Sadekova, S., et al.:** Distinct tyrosine autophosphorylation sites mediate induction of epithelial mesenchymal like transition by an activated ErbB-2/Neu receptor. Oncogene, 20, 2001, p. 788–99.
15. **Kleer, C.G., van Golen, K.L., Braun, T., Merajver, S.D.:** Persistent E-cadherin expression in inflammatory breast cancer. Mod. Pathol., 14, 2001, p. 458–64.
16. **Kowalski, P.J., Rubin, M.A., Kleer, C.G.:** E-cadherin expression in primary carcinomas of the breast and its distant metastases. Breast Cancer Res., 5, 2003, p. R217–222.
17. **Laudadio, J., Quigley, D.I., Tubbs, R., Wolff, D.J.:** HER-2 testing: a review of detection methodologies and their clinical performance. Expert Rev. Mol. Diagn., 7, 2007, p. 53-64. –
18. **Lehr, H.A., Folpe, A., Yaziji, H., et al.:** Cytokeratin 8 immunostaining pattern and E-cadherin expression distinguish lobular from ductal breast carcinoma. Am. J. Clin. Pathol., 114, 2000, p. 190-196. –
19. **Meric-Bernstam, F., Hung, M.C.:** Advances in targeting human epidermal growth factor receptor-2 signaling for cancer therapy. Clin. Cancer Res., 12, 2006, p. 6326–6330.
20. **Moll, R., Mitze, M., Frixen, U.H., Birchmeier W.:** Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am. J. Pathol., 143, 1993, p. 1731–1742.
21. **Nagae, Y., Kameyama, K., Yokoyama, M., et al.:** Expression of E-cadherin catenin and C-erbB-2 gene products in invasive ductal-type breast carcinomas. J. Nippon Med. Sch., 69, 2002, p. 165–171.
22. **Palacios, J., Benito, N., Pizarro, A., et al.:** Relationship between ERBB2 and E-cadherin expression in human breast cancer. Virchows Arch., 427, 1995, p. 259–263.
23. **Peinado, H., Portillo, F., Cano, A.:** Transcriptional regulation of cadherins during development and carcinogenesis. Int. J. Dev. Biol., 48, 2004, p. 365–375.
24. **Pierceall, W.E., Woodard, A.S., Morrow, J.S., et al.:** Frequent alterations in E-cadherin and alpha- and beta-catenin expression in human breast cancer cell lines. Oncogene, 11, 1995, p. 1319–1326.
25. **Rieger-Christ, K.M., Pezza, J.A., Dugan, J.M., et al.:** Disparate E-cadherin mutations in LCIS and associated invasive breast carcinomas. Mol. Pathol., 54, 2001, p. 91–97.
26. **Siitonen, S.M., Kononen, J.T., Helin, H.J., et al.:** Reduced E-cadherin expression is associated with invasiveness and unfavorable prognosis in breast cancer. Am. J. Clin. Pathol., 105, 1996, p. 394–402.
27. **Sliwkowski, M.X., Lofgren, J.A., Lewis, G.D., et al.:** Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). Semin. Oncol., 26, 1999, p. 60–70.
28. **Uitenbroek, Daan G.:** Binomial. SISA. 1997. http://home.clara.net/sisa/binomial.htm. (1 Jan. 2004).
29. **Vos, C.B., Cleton-Jansen, A.M., Berx, G., et al.:** E-cadherin inactivation in lobular carcinoma in situ of the breast: an early event in tumourigenesis. Br. J. Cancer, 76, 1997, p. 1131–1133.
30. **Weidner N., Cote R., Suster S.:** Modern Surgical Pathology. Elsevier Science, 2003, p. 891–892.
31. **Yu, B.W., Bowers, M., Witkowski, G., et al.:** Episomally mediated overexpression of wild-type erbB-2 transforms MCF-10A breast epithelial cells. Breast Cancer Res. Treat., 76, 2002, p. 181–193.
32. **Zschiesche, W., Schonborn, I., Behrens, J., et al.:** Expression of E-cadherin and catenins in invasive mammary carcinomas. Anticancer Res., 17, 1997, p. 561–567.

Corresponding author:
Gulisa Turashvili, MD
Lab. of Molecular Pathology, Department of Pathology
Palacky University, Hnevotinska 3
Olomouc 77515, Czech Republic
Phone: +420 585 632460
Fax: +420 585 632966
E-mail: guliat@gmail.com