Modern Pathology (2010) 23, 644–653

EGFR and HER-2/neu expression in invasive apocrine carcinoma of the breast

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This study was undertaken to investigate epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER-2)/neu expression in a cohort of apocrine carcinomas of the breast with emphasis on the classification of the breast tumors with apocrine morphology. In total, 55 breast carcinomas morphologically diagnosed as apocrine were evaluated for the steroid receptor expression profile characteristic of normal apocrine epithelium (androgen receptor positive/estrogen receptor (ER) negative/progesterone receptor (PR) negative), and for the expression of EGFR and Her-2/neu proteins, and the copy number ratios of the genes *EGFR/CEP7* and *HER-2/CEP17*. On the basis of the results of steroid receptors expression, 38 (69%) cases were classified as pure apocrine carcinoma (androgen receptor positive/ER negative/PR negative), whereas 17 (31%) were re-classified as apocrine-like carcinomas because they did not have the characteristic steroid receptor expression profile. Her-2/neu overexpression was observed in 54% of the cases (57% pure apocrine carcinomas *vs* 47% apocrine-like carcinomas). *HER-2/neu* gene amplification was demonstrated in 52% of all cases (54% pure apocrine carcinomas *vs* 46% apocrine-like carcinomas). EGFR protein (scores 1 to 3+) was detected in 62% of all cases and was expressed in a higher proportion of pure apocrine carcinomas than in the apocrine-like carcinomas group (76 *vs* 29%, $P=0.006$). In the pure apocrine carcinoma group, Her-2/neu and EGFR protein expression were inversely correlated ($P=0.006$, $r=-0.499$). *EGFR* gene amplification was observed in two pure apocrine carcinomas and one apocrine-like carcinoma. Polysomy 7 was commonly present in pure apocrine carcinomas (61 *vs* 27% of apocrine-like carcinomas; $P=0.083$) and showed a weak positive correlation with EGFR protein expression ($P=0.025$, $r=0.326$). Our study showed that apocrine breast carcinomas are molecularly diverse group of carcinomas. Strictly defined pure apocrine carcinomas are either HER-2-overexpressing breast carcinomas or triple-negative breast carcinomas, whereas apocrine-like carcinomas predominantly belong to the luminal phenotype. Pure apocrine carcinomas show consistent overexpression of either EGFR or HER-2/neu, which could have significant therapeutic implications.
Modern Pathology (2010) **23,** 644–653; doi:10.1038/modpathol.2010.50; published online 5 March 2010

Keywords: apocrine carcinoma; breast; HER-1/EGFR; HER-2/neu; gene amplification; polysomy

Apocrine carcinomas of the breast, defined as breast tumors composed of epithelium with apocrine differentiation in >90% of the tumor cell population, represent a rare subtype, constituting <5% of all breast cancers.[1–3] Apocrine differentiation is defined by the presence of large cells with prominent eosinophilic, flocculent cytoplasm, with sharply defined cell borders, and with large nuclei containing prominent macronucleoli. Importantly, a characteristic steroid receptor expression profile further defines these tumors as consistently estrogen receptor (ER) negative, progesterone receptor (PR) negative and androgen receptor (AR) positive.[4–8]

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Received 29 July 2009; revised and accepted 25 January 2010; published online 5 March 2010

Although AR expression has been variably observed in up to 60–70% breast carcinomas,[9,10] consistent AR expression tends to be a feature of apocrine breast lesions including invasive apocrine carcinomas.[4,5,8,11,12] Moreover, recently published gene expression microarray studies defined a characteristic 'molecular apocrine' gene expression profile found in apocrine carcinomas. These studies showed apocrine tumors to be different from common luminal and basal cell breast carcinoma subtypes.[13–16] This molecular apocrine group was characterized by increased AR signaling along with increased human epidermal growth factor receptor 2 (*HER-2*)*/neu* gene signaling.[13,16] A study using an apocrine cell line model also demonstrated the existence of a functionally significant cross-talk between AR and *HER-2/neu* pathways through ERK1/2 in ER-negative breast carcinomas.[17] This cross-talk affects cell proliferation and apoptosis and could have a significant therapeutic impact.[17] Although apocrine carcinoma exhibits distinctive histopatological and molecular features, the lack of standardized diagnostic criteria has produced controversial and heterogeneous results in the scientific literature in terms of its immunohistochemical profile and molecular classification.[2,18–20]

The erbB (HER) family is comprised of four homologous transmembrane receptors involved in growth factor cellular signaling.[21] Epidermal growth factor receptor (*EGFR*) (or *HER-1*) and *HER-2/neu* genes are of particular importance in breast cancer pathogenesis as their activation and coexpression are associated with an aggressive clinical course and a poor outcome.[22] Both proteins can be targeted by specific therapeutic modalities. However, these tyrosine kinase receptors have not been systematically studied in invasive apocrine carcinomas of the breast.

We studied EGFR and HER-2/neu in apocrine breast carcinomas meeting strict morphological and immunophenotypic criteria with regard to both protein expression and gene copy number. We identified significant differences between pure apocrine carcinoma (apocrine morphology and a characteristic AR+/ER−/PR− steroid receptor profile) and apocrine-like breast carcinomas (apocrine morphology without characteristic apocrine steroid receptor profile), which could have important diagnostic and therapeutic implications.

Materials and methods

Specimens

The formalin-fixed paraffin-embedded tumor samples were obtained from 55 female patients with invasive apocrine carcinomas (52 surgical and 3 core biopsy specimens). Mean age of patients was 62 years (range: 32–92 years). The cases were retrieved from the files of Creighton University Medical Center (Omaha, NE, USA), Kansas University Medical Center (Kansas City, KS, USA), Thomas Jefferson University Hospital (Philadelphia, PA, USA), The University of Texas Medical Branch (Galveston, TX, USA) and Clinical Center of the University of Sarajevo (Bosnia and Herzegovina). Routinely stained hematoxylin and eosin tumor sections were re-examined (ZG and SV) and the diagnoses were confirmed. Institutional review board of the Creighton University approved the study.

Immunohistochemistry

Immunohistochemical assays for ER-alpha (ER-α; clone 6F11, Ventana Medical Systems, Tucson, AZ, USA), PR (clone 16, Ventana Medical Systems), AR (Clone AR441, DakoCytomation, Carpinteria, CA, USA), EGFR (DAKO EGFR PharmDX diagnostic kit; DakoCytomation) and Her-2/neu (Clone CB11, Ventana Medical Systems) expression were performed on the formalin-fixed paraffin-embedded sections using the commercially available detection kits and automated staining procedures.

The tumor was regarded as positive for ER and PR if $>5\%$ of the cells showed nuclear staining, whereas a 10% cutoff was applied for AR staining.[8,23]

Her-2/neu protein expression results were scored according to the American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations.[24] Briefly, cases showing no membrane immunostaining or membrane immunostaining in $<10\%$ tumor cells were scored $0+$; cases with weak and incomplete membrane staining in $>10\%$ of tumor cells were scored $1+$; cases with complete membrane staining that was either nonuniform or weak in intensity but with obvious circumferential distribution in $>10\%$ of cells were scored $2+$; and cases with strong membrane staining in $>30\%$ tumor cells were scored $3+$.[24]

EGFR scoring was carried out according to the manufacturer's (Dako) recommendation: only membranous staining is considered as a specific positive result; weak ($1+$) intensity is defined as faint and incomplete membrane positivity; moderate ($2+$) intensity and strong ($3+$) staining are both varying degrees of circumferential staining of membranes. The tumor was considered positive if a proportion of stained cells exceeded 1% at any intensity.

Automated Cell Imaging System (ChromaVision Medical Systems, San Juan Capistrano, CA, USA) was used for measuring the percentage of cells with the nuclear staining for ER, PR and AR, and the extent and intensity of membranous staining of EGFR and Her-2/neu. Pathologists reviewed the images and selected tumor-rich areas of the sections for the analysis.

FISH Analysis

Fluorescent *in situ* hybridization (FISH) was performed to evaluate copy number at *EGFR* and *HER-2/neu* loci. Chromosome enumeration probes CEP7 and CEP17 were used as positive controls and

indicators of chromosome ploidy (Abbott Molecular, Des Plaines, IL, USA). Probe signals were enumerated in predominant tumor cell populations. At least 30 nuclei were scored per sample. A ratio of *HER-2/CEP17* >2.2 was defined as gene amplification; a ratio 1.8–2.1 was interpreted as borderline, and a ratio <1.8 was defined as negative. The same criteria were used for interpretation of EGFR/CEP7 ratios. Equivocal FISH results (ratio of 1.8–2.2) were considered as negative for *HER-2/neu* and *EGFR* gene amplification, respectively.[24] Polysomy 7 and 17 were defined as three or more *CEP* signals per cell.[21,25,26] Stromal cells and normal breast epithelial cells served as an internal control.

SNP Array Karyotyping

SNP array karyotyping was performed on selected cases. Following tumor enrichment through manual microdissection, DNA was obtained from 10-μm paraffin sections according to a previously described protocol for de-paraffinization and DNA extraction.[27] Samples were processed with the 250K Nsp Assay Kits (Affymetrix, Santa Clara, CA, USA). Briefly, 1 μg of gDNA was digested with *Nsp* restriction enzyme, ligated to the adaptors and amplified by PCR using a universal primer. After purification of PCR products with SNP Clean magnetic beads (Agencourt Biosciences, Beverly MA, USA), amplicons were quantified, fragmented, labeled and hybridized to 250K *Nsp* arrays. After washing and staining, the arrays were scanned to generate CEL files for downstream analysis.

Data acquired from the Affymetrix GeneChip Operating System v4.0 (GCOS) was analyzed using Affymetrix Gene-Chip Genotyping Analysis Software (GTYPE) 4.1. Copy number analysis was performed with Copy Number Analyzer for Affymetrix GeneChip arrays (CNAG 3.0), as described before.[28]

Statistical Analysis

Where appropriate, χ^2-test/Fisher's exact test or nonparametric tests (Mann–Whitney *U*-test) were used for comparisons of the groups. Spearman's correlation rank was applied for the correlation between the variables. All statistical analysis was carried out using the Statistical Package for the Social Sciences version 17.0 (SPSS, Chicago, IL, USA). *P*-values of <0.05 were considered significant.

Results

Classification and Steroid Receptor Profile of Apocrine Carcinomas

Morphologically, all 55 cases fulfilled the criteria for apocrine carcinoma and were characterized by large cells with prominent eosinophilic, flocculent cytoplasm, sharp cell borders and large nuclei with prominent macronucleoli (Figures 1a and b). Of these, 38 cases (69%) also fulfilled immunophenotypic diagnostic requirements for pure apocrine carcinoma: ER and PR negative, AR positive (Figures 1c and e, Table 1). The 17 remaining cases (31%) were then termed 'apocrine-like' carcinomas because they lacked the specific apocrine immunophenotypic profile (Figures 1d and f). These were further subcharacterized as apocrine-like carcinomas with ER+/AR− immunophenotype (three cases), apocrine-like carcinomas with ER−/AR− immunophenotype (four cases) and apocrine-like carcinomas with ER+/AR+ immunophenotype (10 cases) (Table 1).

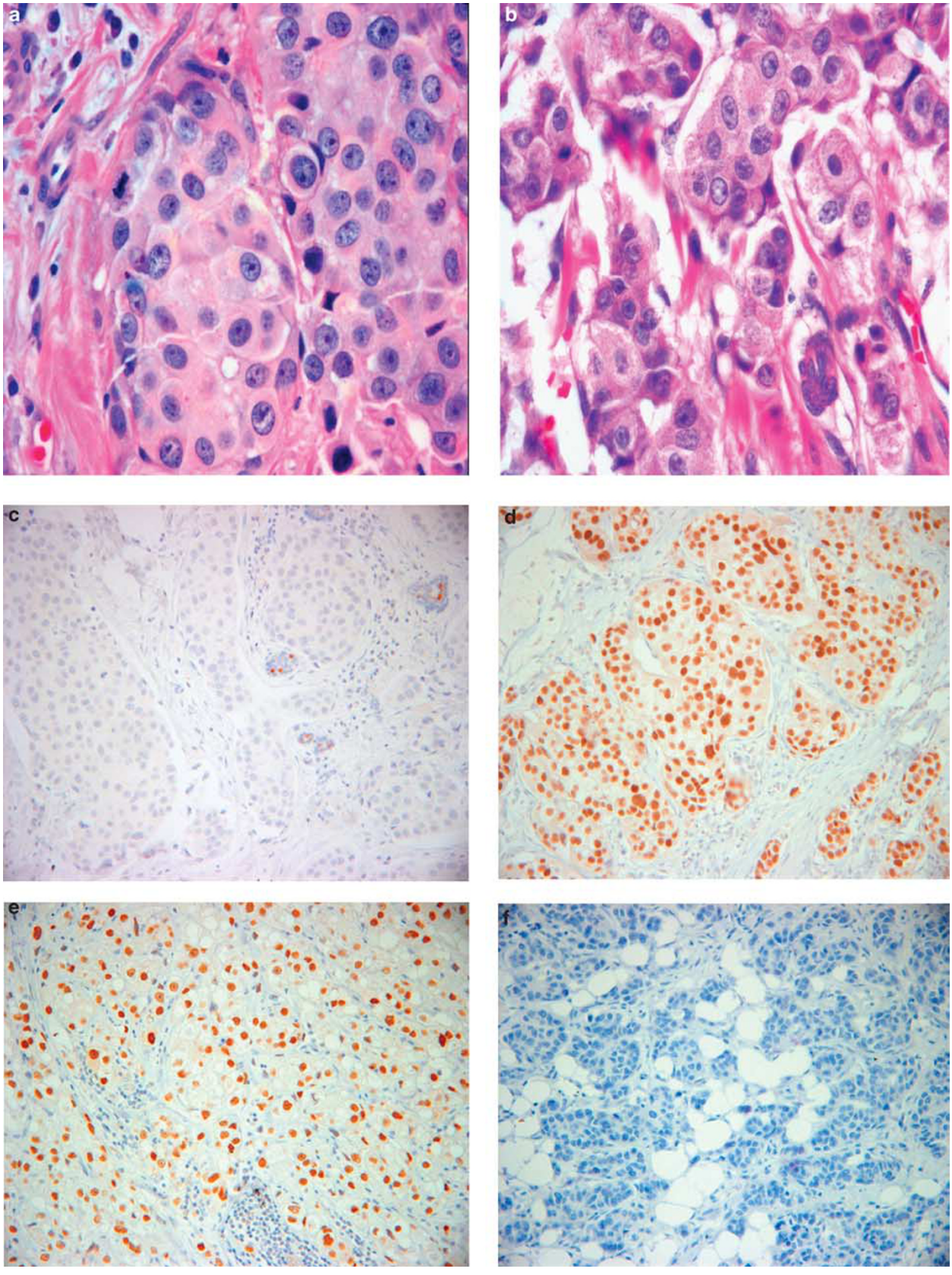
The mean tumor AR positivity was significantly higher in the pure apocrine carcinoma subgroup in comparison with the apocrine-like carcinoma AR+/ER+ subgroup (76 *vs* 59%, $P=0.037$). Pure apocrine carcinomas exhibited a diffuse and strong nuclear staining of AR (16 of 30 or 53% of pure apocrine carcinomas had a 100% cells expressing AR). In contrast, none of the 17 apocrine-like carcinoma cases exhibited such complete AR expression.

HER-2/neu Expression in Apocrine Carcinomas

Her-2/neu protein overexpression (score 3+) was observed in 54% of the cases in the entire cohort without significant difference between the pure apocrine carcinoma and apocrine-like carcinoma groups (57 *vs* 47%, $P=0.81$) (Figure 1g).

HER-2/neu gene amplification was detected in 28 of 54 tested cases (52%) without significant differences between the pure apocrine carcinoma and the apocrine-like carcinoma group (54 *vs* 46%, $P=0.42$) (Figure 2a). The average *HER-2/neu* gene signal number per cell ranged from 1.67 to 50 (mean: 9.57). *HER-2/neu* FISH results were concordant with *Her-2/neu* immunohistochemistry results in 49 of 53 available cases (92%). Four positive immunohistochemistry Her-2/neu results (score 3+) were discordant with *HER-2* FISH results (negative for *HER-2/neu* gene amplification). Three of eight cases (38%) with equivocal immunohistochemistry (score 2+) had *HER-2/neu* gene amplification.

Figure 1 (**a** and **b**) Hematoxylin and eosin -stained sections of two cases of breast carcinomas with apocrine features: pure apocrine carcinoma (**a**) and apocrine-like carcinoma (**b**) ($\times$ 40 magnification). (**c** and **d**) Immunohistochemistry showing negative estrogen receptor expression in a case of pure apocrine carcinoma with a positive staining of normal epithelium (**c**), and strongly positive expression in an apocrine-like breast carcinoma (**d**) ($\times$ 10 magnification). (**e** and **f**) Immunohistochemistry showing diffusely positive androgen receptor expression in a case of a pure apocrine carcinoma (**e**), and negative expression in an apocrine-like carcinoma (**f**) ($\times$ 20 magnification). (**g** and **h**) Immunohistochemistry showing strong membrane expression of EGFR protein in a case of pure apocrine carcinoma (**g**), and 3+ membrane expression of Her-2/neu protein in a case of apocrine-like breast carcinoma (**h**) ($\times$ 20 magnification).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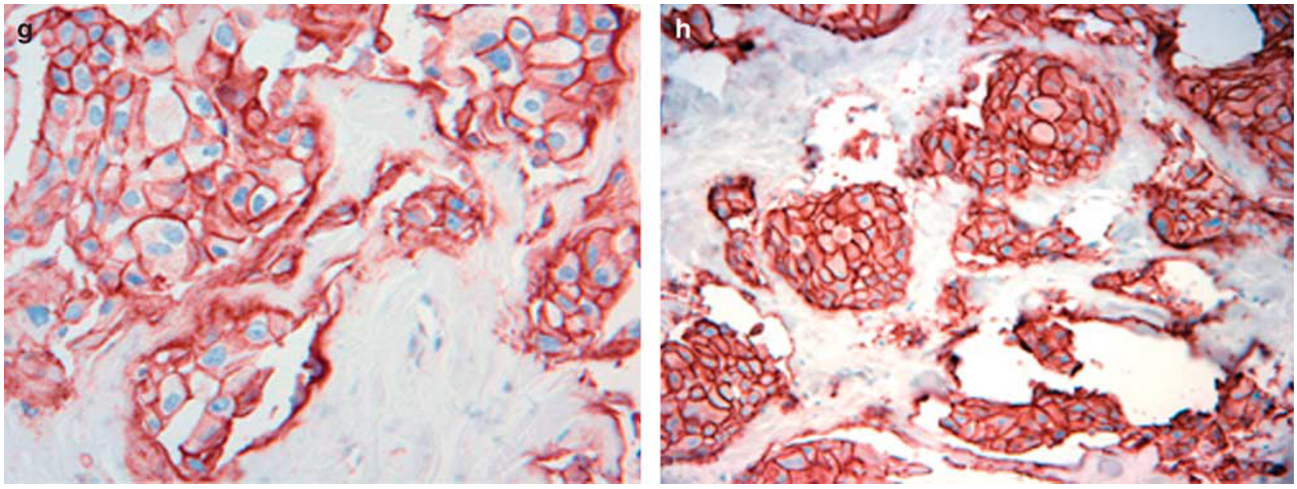

Figure 1 Continued.

Table 1 Status of EGFR and Her-2/neu protein expression and gene amplification in pure apocrine carcinomas and subgroups of apocrine-like carcinomas

Category	*Androgen r eceptor (AR)*[a]	*Her-2/neu*[b]	*HER-2/neu (FISH)*[c]	*EGFR*[d]	*EGFR (FISH)*[c]
Pure apocrine carcinomas	38/38 (100%) Mean: 76 Range: 10–100	21/37 (57%)	20/37 (54%)	29/38 (76%)	2/35 (6%)
Apocrine-like carcinomas (ER+, AR+)	10/10 (100%) Mean: 59 Range: 15–90	2/10 (20%)	4/10 (40%)	3/10 (30%)	1/7 (14%)
Apocrine-like carcinomas (ER+, AR−)	0/3 (0%)	3/3 (100%)	3/3 (100%)	0/3 (0%)	0/1 (0%)
Apocrine-like carcinomas (ER−, AR−)	0/4 (0%)	3/4 (75%)	1/4 (25%)	2/4 (50%)	0/4 (0%)

[a]Positivity defined if >10% cells exhibited nuclear staining.
[b]Defined by the 3+ score by immunohistochemistry.
[c]Defined by the gene to centromere ratio >2.2.
[d]Scores 1 to 3+ by immunohistochemistry.

Six samples had fewer *HER-2/neu* signals per cell than signals for chromosome 17 centromere (ratio: 0.72–0.99). One of these cases had a Her-2/neu protein overexpression.

Polysomy of chromosome 17 (defined as three or more copies of CEP17 signals per nucleus) was observed in 10 pure apocrine carcinomas (32%) and 8 apocrine-like carcinomas (50%). Polysomy 17 was seen without *HER-2/neu* gene amplification in 8 cases (Figure 2c) and with *HER-2/neu* gene amplification in 10 cases. The polysomy 17 rate was low: mean 3.55 CEP17 signals; (range: 3.0–6.0). Two pure apocrine carcinomas and three apocrine-like carcinomas (5 of 8, 63%) with polysomy 17 alone had Her-2/neu protein expression scores of 2 to 3+ by immunohistochemistry.

EGFR Expression in Apocrine Carcinomas

In all, 34 out of 55 (62%) cases expressed EGFR protein (scores 1 to 3+). A significantly higher proportion of pure apocrine carcinomas was positive for EGFR protein in comparison with the apocrine-like carcinoma subgroups (76 *vs* 29%, $P=0.006$) (Figure 1h). A diffuse (>50% of positive cells) and strong (intensity scores 2 to 3+) EGFR expression was seen in 20 of 29 (69%) of the pure apocrine carcinoma-positive cases and in 5 out of 5 (100%) of the apocrine-like carcinoma-positive cases.

EGFR gene amplification was a rare event present only in three (two pure apocrine and one apocrine-like tumors) of 44 studied cases (7%) (Figure 2b). All three cases exhibited EGFR protein overexpression. The average *EGFR* gene signal number per cell ranged from 1.6 to 20 (mean: 5.76).

Polysomy of chromosome 7 (defined as three or more copies of CEP7 signals per nucleus) was detected in 20 of 33 pure apocrine carcinomas (61%) and in 3 of 11 apocrine-like carcinomas (27%) either alone (21 cases) (Figure 2d) or in association with the *EGFR* gene amplification (two cases). Polysomy 7 was more frequently observed in the pure apocrine carcinoma subgroup compared with

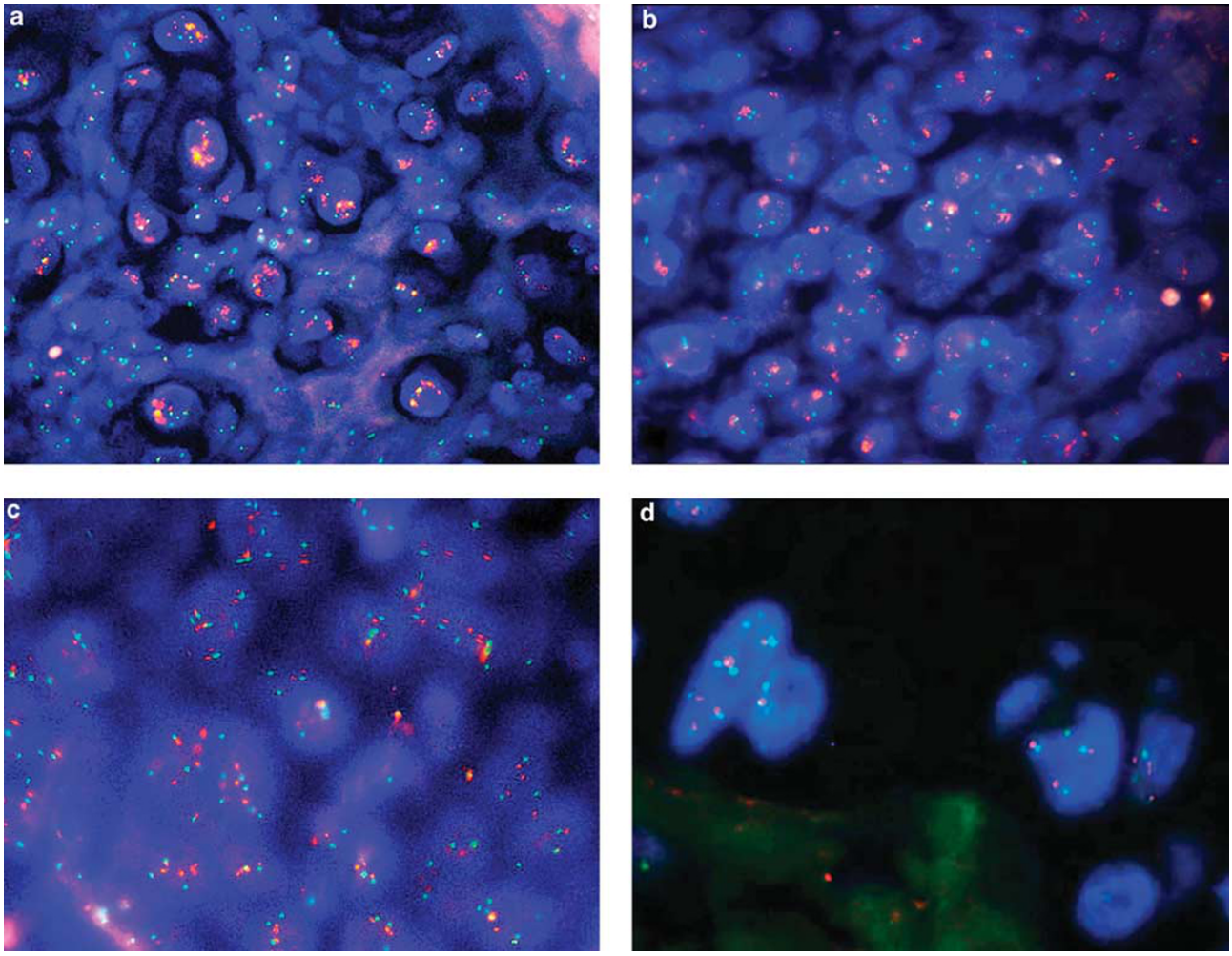

Figure 2 Pure apocrine carcinomas showing *HER-2/neu* gene amplification (**a**), *EGFR* gene amplification (**b**), polysomy 17 (CEP17) without *HER-2/neu* gene amplification in a case of an apocrine-like carcinoma (average: 6.06 signals per cell) (**c**) and polysomy 7 (CEP7) without *EGFR* gene amplification (average: 3.62 signals per cell) (**d**).

the apocrine-like carcinoma subgroup ($P=0.083$). Overall, the level of polysomy 7 was low (mean: 4.09, range: 3.0–7.06). A weak positive correlation between polysomy 7 and the EGFR protein expression was also present ($P=0.025$, $r=0.326$).

Chromosomal Analysis Using Conventional Cytogenetics and SNP Array Assay

Corroborative genetic evidence for FISH results was obtained in three cases, which were further studied by conventional cytogenetics and SNP arrays. One case of pure apocrine carcinoma (displaying polysomy 7 (4.37 *CEP7* copies on average) and *HER-2/neu* gene amplification) was analyzed by conventional cytogenetic analysis (see Acknowledgement) and showed complex cytogenetic alterations (Figure 3) described as: 65–69,XXX,+i(1)(q10),−2,−3,add(3)(p12),add(6)(q27),+7,−8,−10,−11,add(11)(p15),add(11)(q23),−12,−13,add(14)(p11.2),−15,+16,−17,−18,−19,add(19)(q13.4),−520,−21,−22,

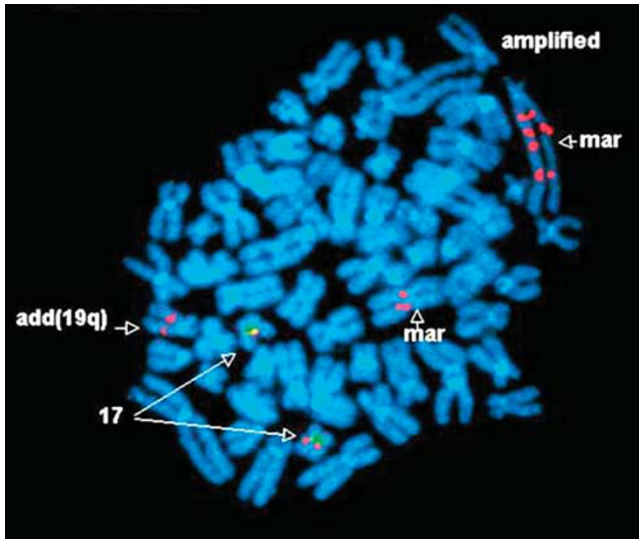

Figure 3 Metaphase FISH analysis of the apocrine carcinoma showing amplification of the *HER-2/neu* gene (red). One of the larger marker chromosomes contains homogeneously staining region (hsr) of *HER-2/neu* gene amplification.

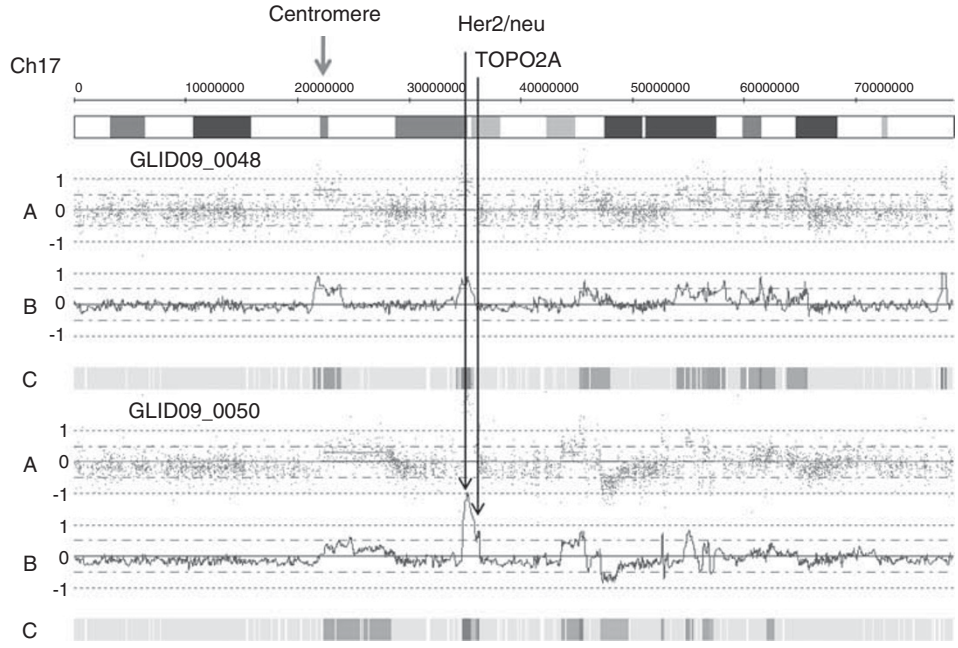

Figure 4 SNP array karyotypes of chromosome 17 for two samples with amplification of *HER-2/neu*. The first one (Sample GLID09_0048) had amplification of both *CEP17* and *HER-2/neu* amplification without polysomy 17, whereas the second one (Sample GLID09_0050) had coamplification of *HER-2/neu* and *TOP2A* along with a gain of *CEP17*. Plots are as follows: (A) the raw log 2 ratio of tumor/normal for each probe on the array; (B) smoothing average over 10 probes; and (C) Hidden Markov Model of copy number with aqua = 1, yellow = 2, pink = 3, pink-red = 4, red-pink = 5 and red > 5.

+mar1, + mar2, + mar3, + mar4, + mar5, + 5-8mar[5]/130–138, idemx2[2]/46,XX[13]. Another two cases (one pure apocrine carcinoma and one apocrine-like carcinoma, both with *HER-2/neu* gene amplification) were studied by SNP arrays, which confirmed FISH results and further revealed amplification of *CEP17* without polysomy 17 in the first one, whereas the second one (Sample GLID09_0050) had coamplification of *HER-2/neu* and *TOP2A* along with a gain of *CEP17* (Figure 4).

Molecular Subclassification of Carcinomas with Apocrine Morphology

We found a statistically significant inverse correlation between EGFR and Her-2/neu expression in the pure apocrine carcinoma subgroup ($P = 0.006$, $r = -0.499$). Therefore, 20 of 37 (54%) pure apocrine carcinoma cases can be classified as HER-2-overexpressing, whereas the remaining 17 cases (46%) as triple-negative breast carcinomas. In all, 16 out of 17 triple-negative pure apocrine carcinomas (94%) overexpressed EGFR and would accordingly be classified as basal-like breast carcinomas[29] (Table 2). None of the pure apocrine carcinomas fulfilled the criteria for luminal tumors.[29–31]

In contrast, a large proportion of apocrine-like carcinomas belonged to the luminal group (13 of 17 cases, 76%). Only three cases (18%) could be classified as triple-negative breast carcinomas and one case only as HER-2-overexpressing breast carcinoma.

Table 2 Molecular subclassification of apocrine carcinoma subtypes

Apocrine carcinoma subtype	*Molecular phenotype*
Pure apocrine carcinoma	HER-2 (20/37, 54%)[a] Triple negative (17/37, 46%) Basal-like breast carcinoma[b] (16/17, 94%)
Apocrine-like carcinoma	Luminal (13/17, 76%) Triple negative (3/17, 18%) Basal-like breast carcinoma[b] (2/3, 67%) HER-2 (1/17, 6%)

[a]*HER-2/neu* gene amplification was used as a criterion.
[b]On the basis of EGFR protein expression (Nielsen *et al*[29]).

Discussion

The diagnosis of apocrine carcinoma of the breast has been controversial because of the lack of strict diagnostic criteria. With the increasing use of immunohistochemistry, apocrine breast cancer differentiation has shown a consistent pattern of steroid receptor expression irrespective of grade[8] and this method should be applied for unequivocal

definition of this special carcinoma type. With such consistency, additional correlations between the histological phenotype and biological potential become more meaningful.

In this study, we applied strict morphological and immunohistochemical criteria to correctly classify and characterize apocrine carcinoma of the breast. Consequently, our results clearly separated breast tumors with apocrine cytomorphology into two different groups: the pure apocrine carcinomas with consistent lack of ER and overexpression of AR, and morphologically apocrine-like carcinomas that did not exhibit the protein expression profile associated with the true apocrine phenotype.[4,5,13,32] Similarly, Celis *et al*[8] using another set of morphological and immunohistochemical criteria for classification of apocrine carcinoma defined and confirmed the existence of a distinct apocrine carcinoma group with a consistent steroid receptor profile (ER−, AR+). Together, these results strongly support the recent advances in molecular classification of breast carcinoma that have revealed the existence of a specific 'molecular apocrine' gene expression profile among ER-negative breast carcinomas characterized primarily by increased AR signaling, along with a common Her-2/neu gene amplification.[13] The pure apocrine carcinoma subgroup from our study seems to be equivalent to the 'molecular apocrine' group from Farmers' study although that cohort was not entirely compatible with pure apocrine carcinomas.[13] Our findings showing coexpression of AR and Her-2/neu proteins in pure apocrine carcinomas also support results of other studies that highlighted a functional cross-talk and association between AR and *HER-2/neu* in a subset of breast carcinomas and breast carcinoma cell lines.[17,33]

Pure apocrine carcinomas were further characterized by nearly mutually exclusive expression of Her-2/neu and EGFR proteins. Thus, a majority of HER-2-negative cases (that is, triple-negative apocrine carcinomas) overexpressed EGFR and accordingly could be classified as basal-like breast carcinomas.[29–31] On the other hand, HER-2-overexpressing pure apocrine carcinomas were mostly negative for EGFR protein expression.

The apocrine-like carcinomas were much more heterogeneous with various combinations of steroid receptor expression including AR. Apocrine-like carcinomas are characterized by a common ER expression and *HER-2/neu* gene amplification but significantly less common EGFR overexpression, thus mainly belonging to the luminal phenotypes (A and B) according to the molecular classification of breast carcinomas.[30,31] It is noteworthy that of the remaining four ER-negative apocrine-like carcinomas, only one case had *HER-2/neu* gene amplification.

Our results suggest that a strict definition of pure apocrine carcinomas could clarify some of the previous contradictions in the classification of apocrine carcinoma (variable and heterogeneous gene expression profiles of morphologically defined apocrine tumors) leading some investigators to challenge its existence.[18]

Overexpression of Her-2/neu protein has been reported in up to 25% of invasive breast carcinomas and has been associated with a worse clinical outcome.[34] In most cases, this can be attributed to amplification of the *HER-2/neu* gene located on the long arm of chromosome 17 (17q12).[35] Our study revealed *HER-2/neu* gene amplification in ~52% of the cases, similar to the rate of *HER-2/neu* gene amplification in invasive apocrine carcinomas of the breast observed in two previously published small cohorts (44 and 50%, Moinfar *et al*[12] and Varga *et al*,[36] respectively). The pure apocrine carcinoma subgroup exhibited slightly higher rate of *HER-2/neu* gene amplification in comparison with the apocrine-like carcinoma subgroup, but the difference was not statistically significant. This is in line with previous studies, which demonstrated a strong association between *HER-2/neu* status and apocrine differentiation.[13,37] Although we found a high degree of concordance between immunohistochemistry and FISH results, four cases were negative for *HER-2/neu* gene amplification despite high protein expression on immunohistochemistry, which was previously explained by various preanalytical and analytical factors including tissue fixation, a choice of the anti-Her-2/neu antibody and scoring system.[37,38]

Aneusomy 17, including polysomy 17, has been a common observation in breast carcinomas,[39,40] although the definition of polysomy 17 is not universally defined.[24] Therefore, we followed the arbitrary cutoff of three or more copies of CEP17 applied in previous publications.[22,25,26] Our FISH analysis revealed polysomy 17 in a proportion of apocrine carcinomas, either as the sole finding or in combination with *HER-2/neu* gene amplification. *CEP17* polysomy without concomitant *HER-2/neu* gene amplification was seen in eight cases of which five had Her-2/neu protein overexpression (scores 2+ and 3+). Several investigators previously considered polysomy 17 a potential cause of equivocal HER-2/neu results by FISH or immunohistochemistry.[25] However, Vanden Bempt *et al*[22] found neither increased Her-2/neu protein nor increased *HER-2 mRNA* in polysomy 17 cases and concluded that the tumors displaying unamplified polysomy 17 probably represented more Her-2/neu-negative than Her-2/neu-positive breast tumors. Some investigators recently questioned the interpretation of the *CEP17* copy number as a reliable predictor of the entire chromosome 17 polysomy,[41] and our whole genome analysis using SNP arrays in two cases also supports this observation.

EGFR is a 170-kDa transmembrane glycoprotein encoded by the *HER-1* protooncogene, located at 7p11.2-p12.[42] High expression of EGFR in a variety of epithelial tumors has led to the development of a number of drugs specifically targeting the EGFR that are now in use for treatment of advanced colorectal carcinoma, non-small cell lung carcinoma, head and

neck squamous cell carcinoma and pancreatic carcinoma.[43] EGFR protein expression has also been a common finding in breast carcinoma, particularly in a subgroup of triple-negative, basal-like breast carcinomas (>50%) leading some investigators to use it as a surrogate marker for a basal-like breast carcinoma.[29] However, *EGFR* gene alterations (activating mutations and gene amplification) tend to be a rare event in breast carcinoma and were found in <8% of the cases.[44,45] In this study, we demonstrated EGFR protein expression in 62% of the cases. The expression pattern was predominantly strong (scores 2 to 3+) and diffuse (>50% of positive cells) in both subgroups and was not accompanied by the *EGFR* gene amplification, similar to the results of a study by Park *et al*.[45] Polysomy of chromosome 7 (*CEP7*), which we found associated with the pure apocrine carcinoma subgroup, is a novel finding, not previously associated with apocrine breast cancer.[46–49] It also correlated and might be responsible for the EGFR protein overexpression in the pure apocrine carcinoma.

In summary, our study indicates that breast carcinomas with apocrine differentiation are heterogeneous in molecular terms. The combination of morphological and immunohistochemical criteria are essential for the proper identification of pure apocrine carcinomas. When strictly defined, these carcinomas express either Her-2/neu or EGFR in a nearly exclusive manner, resulting in their classification as either HER-2-overexpressing or triple-negative types of breast carcinomas. In contrast, apocrine-like carcinomas predominantly belong to the luminal molecular phenotype (both A and B). Our findings also demonstrate that *EGFR* and *HER-2/neu* have important roles in the pathogenesis of apocrine carcinomas and these findings may have significant therapeutic implications.

Acknowledgements

The conventional cytogenetic study was performed at the Human Genetics Laboratory, Munroe Meyer Institute, University of Nebraska Medical Center, Omaha, NE, USA; we thank Dr Warren Sanger, Dr Julia Bridge and Ms Marilu Nelson for the excellent report and providing us with the Figure 3. We are also indebted to Kay M Krogman, Department of Pathology, Creighton University School of Medicine, for technical assistance. Dr Semir Vranic was a research fellow at the Creighton University Medical Center, Omaha, NE, USA, and had been supported by the UICC American Cancer Society Beginning Investigators Fellowship (ACSBI) award (ACS/08/004) funded by the American Cancer Society. The preliminary results of the study were presented at the 96th Annual Meeting of the United States and Canadian Academy of Pathology, San Diego, CA, USA, 2007.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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