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Co-expression and prognostic value of gross cystic disease fluid protein 15 and mammaglobin in primary breast cancer

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Summary. Gross cystic disease fluid protein (GCDFP-15) and mammaglobin are both widely used and accepted markers for epithelia of breast origin. We aimed to evaluate their relation of expression on parallel whole tissue sections in primary breast cancer by immunohistochemistry and also to correlate it with clinico-pathological parameters including patient survival. Primary breast carcinomas from 165 patients with a mean clinical follow-up of 73 months were immunostained using commercially available antibodies against GCDFP-15 and mammaglobin. An immunoreactive score (IRS) was calculated based on the cytoplasmic staining intensity and the number of cells stained. Cytoplasmic expression of GCDFP-15 and mammaglobin was observed in 73.3% and 72.1% of invasive breast carcinomas respectively. 91.8% of breast cancer cases expressed at least one of both markers. Both markers strongly correlated with each other and were significantly associated with lower tumour grading. Additionally, GCDFP-15 negativity was significantly associated with shortened disease-free survival times in univariate and multivariate analyses. We demonstrated the strong correlation of GCDFP-15 and mammaglobin with each other and showed that only very few primary breast cancers are completely negative for both markers. The significantly longer disease free survival times for patients with GCDFP-15 positive tumours clearly warrants further study.

Key words: Breast cancer, GCDFP-15, Mammaglobin, Prognostic marker, Immunohistochemistry

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Introduction

Commonly used immunohistochemical tissue markers of breast cancer comprise cytokeratins (CK7 positivity, CK20 negativity), hormone receptors (estrogen/progesterone receptor) as well as carbohydrate antigens, e.g. CA15.3 (Huang et al., 2004), none of which are entirely specific for breast cancer. As more breast specific markers, Gross cystic disease fluid protein 15 (GCDFP-15) and mammaglobin have been suggested. Apart from being both expressed in the majority of breast cancers, there is no known biological link between these markers so far.

Gross cystic disease fluid protein 15 (synonyms: prolactin-inducible protein (PIP), extra parotid glycoprotein (EP-GP), secretory actin-binding protein (SABP) and glycoprotein 17 (gp17)) is a 15 kDa glycoprotein, first described by Haagensen et al. (Haagensen et al., 1979) is regarded as a specific marker of apocrine cells (Haagensen et al., 1990) and is particularly strongly expressed in apocrine breast cancer (Honma et al., 2005). Diagnostically, GCDFP-15 has been proposed, but is not generally accepted as a tissue marker for breast cancer (Clark et al., 1999; Kaufmann et al., 2002). A large immunohistochemically based study encompassing 680 malignancies, including 105 breast cancers, demonstrated a specificity of GCDFP-15 expression of 96%, with a sensitivity of 74% (Wick et al., 1989).

Another recently proposed breast cancer biomarker, mammaglobin or secretoglobin, first described by Watson in 1996 (Watson and Fleming, 1996), is a small (10kDa) secretory, rarely glycosylated protein. It is a member of the uteroglobin family, is of unknown function and is localized on chromosome 11q12.2 (Ni et al., 2000). Mammaglobin has immunohistochemically been shown to be expressed in about 80% of primary

breast cancers (Fleming and Watson, 2000; Han et al., 2003), independently of tumour grade (Watson et al., 1999). Controversially, Span et al., who examined mammaglobin on the mRNA level in 280 patients, found it significantly associated with low-grade tumours, positive hormone receptor status and longer relapse free survival times (Span et al., 2004). This is in line with former results from Nunez-Villar et al. demonstrating univariate significant associations of mammaglobin expression with hormone receptor expression, lower nuclear grade, low proliferation index and other markers less aggressive tumour biology, some of them also significant in multivariate testing (Nunez-Villar et al., 2003). We recently described the expression patterns of mammaglobin in several other gynecological malignancies (Zafrakas et al., 2006) which was further supported by the data of Bhargava et al. (Bhargava et al., 2007).

In this study we aimed to evaluate the rates of expression of GCDFP-15 and mammaglobin immunohistochemically on parallel sections on a large, clinically well characterised cohort of primary breast cancer to analyze the relation of both markers. Further, we carefully looked for associations with clinicopathological parameters, including patient follow-up data.

Materials and methods

Patients

Our study included 165 patients diagnosed with primary breast cancer at the Institute of Pathology, Charité-Universitätsmedizin, Berlin, between 1991 and 1997 with institutional review board approval. Patient age at the time of diagnosis ranged from 30 to 87 with a median of 58 years (mean 59). Follow-up data including overall survival and disease recurrence or progression times were available for all cases. The average observation time for overall survival was 73 months for patients still alive at the time of analysis, and ranged from one to 165 months. Twenty-seven patients (16%) died during follow-up and 61 patients (37%) experienced disease progression defined by either metastatic or local recurrent disease.

Adjuvant therapy was administered as follows: The

Table 1. Clinicopathological parameters and association with GCDFP-15 expression of the tumour set.

	No. of patients (%)			
Variable	Patients	GCDFP-15 neg.	GCDFP-15 pos.	p value
Patient age				0.450
< 60 years	89	21 (23.6)	68 (76.4)	
≥ 60 years	76	14 (18.4)	62 (81.6)	
Histology				0.541
ductal	147	30 (20.4)	117 (79.6)	
lobular	18	5 (27.8)	13 (72.2)	
pT-status				0.523*
pT1	98	21 (21.4)	77 (78.6)	
pT2	53	13 (24.5)	40 (75.5)	
pT3/4	14	1 (7.1)	13 (92.9)	
pN-status				0.181
pN0	76	20 (26.3)	56 (73.7)	
pN1+	89	15 (16.9)	74 (83.1)	
Histological grade				0.069*
G1	41	8 (19.5)	33 (80.5)	
G2	82	12 (14.6)	70 (85.4)	
G3	42	15 (35.7)	27 (64.3)	
Estrogen receptor				0.174
negative	43	12 (27.9)	31 (72.1)	
positive	108	18 (16.7)	90 (83.3)	
C-erbB2 expression				0.820*
0, 1+	103	25 (24.3)	78 (75.7)	
2+	12	4 (17.4)	19 (82.6)	
3+	16	4 (25.0)	12 (75.0)	
Therapy				0.698
none/local/CTx	69	16 (23.2)	53 (76.8)	
Tamoxifen±CTx	90	18 (20.0)	72 (80.0)	
Estrogen receptor and c-erbB2 combined			0.050	
both negative	23	9 (39.1)	14 (60.9)	
one or both positive	108	20 (18.5)	88 (81.5)	

*Chi square test for trends.

first group received either no or local therapy/ radiotherapy (n=41), or systemic chemotherapy excluding hormone agents (n=28). The second group (n=90) received tamoxifen (TAM) with or without an additional systemic or local therapy. For 6 patients, no data on adjuvant therapy was available.

The selection of cases for this study was based on availability of tissue. Cases with systemic disease (pM1) at the time of diagnosis were excluded. Tumour histology was determined according to the criteria of the World Health Organization (Tavassoli and Devilee, 2003). Only invasive ductal and invasive lobular carcinomas were included. Tumours were graded according to Bloom and Richardson, as modified by Elston and Ellis (1993) Data regarding estrogen receptor status and the expression of c-erbB2 were taken from archival pathology reports. For several cases c-erbB2 analysis was performed separately at the implementation of the cohort. Estrogen receptor positivity was defined as an immunoreactive score (IRS) >3. Over-expression of c-erbB2 was defined according to the clinical trial assay (3+) as recommended in the HercepTest® (DAKO). The clinico-pathological characteristics are described in Table 1 and 2.

Immunohistochemistry

Formalin-fixed paraffin embedded tissue was freshly cut (4 µm). The sections were mounted on superfrost slides (Menzel Gläser, Braunschweig, Germany), dewaxed with xylene and gradually hydrated. We used monoclonal antibodies for GCDFP-15 (Signet BRST-2, dilution 1:200) and mammaglobin (BioPrime, NY, USA, MAM001-05, dilution 1:100). Antigen retrieval for mammaglobin was achieved by heat and citrate buffer by the Ventana immunostainer. Antigen retrieval for GCDFP-15 was achieved by denaturation with proteinase K. All slides were stained with the BenchMark® XT autostainer (Ventana, Tucson AZ, USA).

Evaluation of the immunohistochemical stainings

The immunostainings were independently examined by two pathologists experienced in breast pathology, who were blinded to patient outcome. The scoring

Table 2. Clinico-pathological parameters and association with mammaglobin (MGB) expression of the tumour set.

Variable	No. of patients (%)			p value
	Patients	MGB neg.	MGB pos.	
Patient age				0.187
< 60 years	83	26 (31.3)	57 (68.7)	
≥ 60 years	63	13 (20.6)	50 (79.4)	
Histology				1.000
ductal	130	35 (26.9)	95 (73.1)	
lobular	16	4 (25.0)	12 (75.0)	
pT-status				0.357*
pT1	87	26 (29.9)	61 (70.1)	
pT2	46	10 (21.7)	36 (78.3)	
pT3/4	13	3 (23.1)	10 (76.9)	
pN-status				0.137
pN0	67	22 (32.8)	45 (67.2)	
pN1+	79	17 (21.5)	62 (78.5)	
Histological grade				0.094*
G1	39	8 (20.5)	31 (79.5)	
G2	73	18 (24.7)	55 (75.3)	
G3	34	13 (38.2)	21 (61.8)	
Estrogen receptor				0.078
negative	35	14 (40.0)	21 (60.0)	
positive	99	23 (23.2)	76 (76.8)	
C-erbB2 expression				0.968*
0, 1+	91	23 (25.3)	68 (74.7)	
2+	20	6 (30.0)	14 (70.0)	
3+	13	3 (23.1)	10 (76.9)	
Therapy				1.000
none/local/CTx	57	15 (26.3)	42 (73.7)	
Tamoxifen±CTx	81	22 (27.2)	59 (72.8)	
Estrogen receptor and c-erbB2 combined				0.083
both negative	19	8 (42.1)	11 (57.9)	
one or both positive	96	21 (21.9)	75 (78.1)	

*Chi square test for trends.

system for GCDFP-15 and mammaglobin staining was relatively simple to minimize inter-observer variability and enhance reproducibility in future studies. We evaluated the cytoplasmic staining intensity and the percentage of cells stained. An immunoreactive score (IRS) was applied, as described by Remmele and Stegner (Remmele and Stegner, 1987). The IRS is the product of staining intensity (graded between negative=0 to strong=3) and the percentage of positively stained cells (categorised between 0 and 4, being 0 = 0%, 1 = <10%, 2 = 10–50%; 3 = 51–80%, and 4 = >80%, respectively). Inter-observer variability was minimal (<5%). There was no disagreement concerning positivity or negativity of cases but only in the estimation of percentage of cells stained. Cases with discrepancies in IRS score among pathologists were discussed at a multi-headed microscope until consensus was reached.

Statistical analysis

The data were analyzed with the software package SPSS, version 14.0 (SPSS Inc., Chicago, USA). Fisher's exact, chi-square tests for trends and binary logistic regression analysis (backward wald) were used to assess the statistical significance of associations between clinico-pathological parameters and GCDFP-15 and mammaglobin expression respectively. IRS values were compared using the Wilcoxon-signed rank test. Bivariate correlations according to Spearman were applied to the immunoreactive scores of GCDFP-15 and mammaglobin. Univariate survival analysis was performed according to Kaplan-Meier and differences in survival curves were assessed with the Log rank test. P values < 0.05 were considered significant. Multivariate analyses were performed according to the Cox regression model. Statistics were accredited by the head biostatistician of the Tumor Center, Charité – Universitätsmedizin Berlin.

Results

Immunostaining of GCDFP-15 and mammaglobin in breast tissues and primary breast cancer

Normal breast tissues adjacent to invasive tumours generally showed a moderate to strong expression of mammaglobin (90% positive) and GCDFP-15 (100% positive), as shown in Fig. 1A,B.

Cytoplasmic expression of GCDFP-15 was seen in 78.8% (130/165) of breast cancer cases. Mammaglobin was seen in 73.3% (107/146) of cases (Fig. 2, Table 3). Interestingly, many carcinomas showed a marked heterogeneity in the staining pattern for both markers with some areas exhibiting a strong and continuous immunoreactivity, whereas other areas had no or only a minimal marker expression in single cells (evaluated in the x100 magnification). The immunoreactive score of GCDFP-15 and mammaglobin correlated significantly with each other (Spearman's rho=0.226, p=0.006). In these 146 cases, for which both markers were immunostained, only 8.2% (12/146) of breast carcinomas were completely negative for both markers. In cross-tables, stratifying cases for GCDFP-15 and mammaglobin expression (completely negative vs. positive), no significant associations with clinico-pathological parameters were found (Tables 1, 2). GCDFP-15 expression correlated positively with age (p=0.024) and negatively with tumour grading (p=0.014). Mammaglobin expression correlated positively with estrogen receptor status (p=0.045) and negatively with tumour grading (p=0.019) (Table 4). In repeated cross-table analyses, stratifying each marker according to its median IRS (3 vs. 4 (GCDFP-15)) and 2 vs. 3 (mammaglobin)), we could further confirm these

Table 3. Distribution of the Immunoreactive Scores (IRS) of GCDFP-15 and mammaglobin in Breast Cancer.

IRS	GCDPF-15 number of cases (%)	Mammaglobin number of cases (%)
0	35 (21.2)	39 (26.7)
1	15 (9.1)	11 (7.5)
2	29 (17.6)	37 (25.3)
3	12 (7.3)	10 (6.8)
4	29 (17.6)	24 (16.4)
6	22 (13.3)	10 (6.9)
8	13 (7.9)	5 (3.4)
9	5 (3.0)	6 (4.1)
12	5 (3.0)	4 (2.7)

Table 4. Correlation of GCDFP-15 and mammaglobin expression (IRS) in breast cancer with conventional clinico-pathological parameters.

Protein IRS	Statistic	pT-status	pN-status	Grading	ER-status	Age
GCDFP 15	SR	0.011	0.027	-0.191	0.064	0.176
	p	0.889	0.734	0.014	0.436	0.024
	N	165	165	165	151	165
Mammaglobin	SR	0.141	0.126	-0.195	0.173	0.157
	p	0.089	0.130	0.019	0.045	0.058
	N	146	146	146	134	146

SR: Spearman´s rho; p: p-value; N: number of cases.

significant associations (data not shown). Furthermore, in logistic regression analyses (backward wald) for both markers, positivity for GCDFP-15 (p=0.021, odds ratio (OR) 0.507) and mammaglobin (p=0.036, OR 0.540) was each significantly associated with lower tumour grade. Additionally, for both markers (GCDFP-15: p=0.039, OR 2.351; mammaglobin: p=0.046, OR 2.280) a significant relation with positive nodal status was found.

Survival analyses

Tumour size, nodal status, histological grade, tumour stage and estrogen receptor status were significant predictors of both overall and disease-free survival (n=165) (Table 5).

The Kaplan-Meier curves (Fig. 3) showed GCDFP-15 negativity of the invasive carcinomas to be significantly associated with shortened disease-free survival (median 59 vs. 140 months, p=0.014). The Cox regression model further confirmed this prognostic value of GCDFP-15 for disease free survival (Table 6). Univariate analyses were repeated for several subgroups and analogous results could be shown for nodal positive, locally treated, cerbB2 negative, large (pT2-4) and intermediate graded carcinomas (data not shown).

Mammaglobin expression was not significantly associated with either disease free or overall patient survival (Fig. 3 C,D), even in a stratified analysis of patient subgroups considering tumour size, nodal status, tumour grade, cerbB2-status, estrogen receptor status or patient age.

The univariate survival analyses were repeated for mammaglobin and GCDFP-15 with a different cut-off value, each group being stratified according to their median IRS value to define low and high levels of expression. The results did not relevantly differ from the initial analyses, showing a prognostic impact of GCDFP-

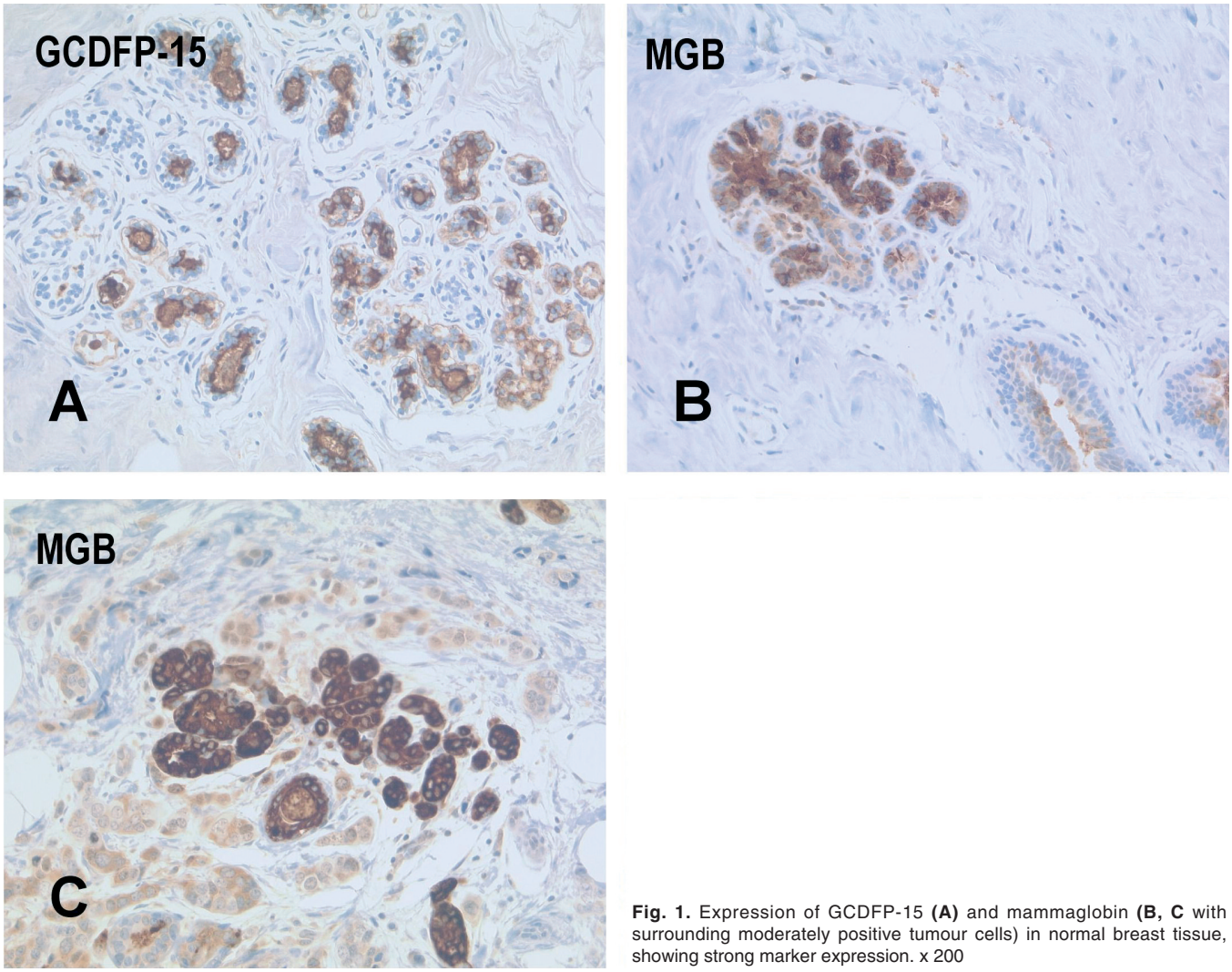

Fig. 1. Expression of GCDFP-15 **(A)** and mammaglobin **(B, C** with surrounding moderately positive tumour cells) in normal breast tissue, showing strong marker expression. x 200

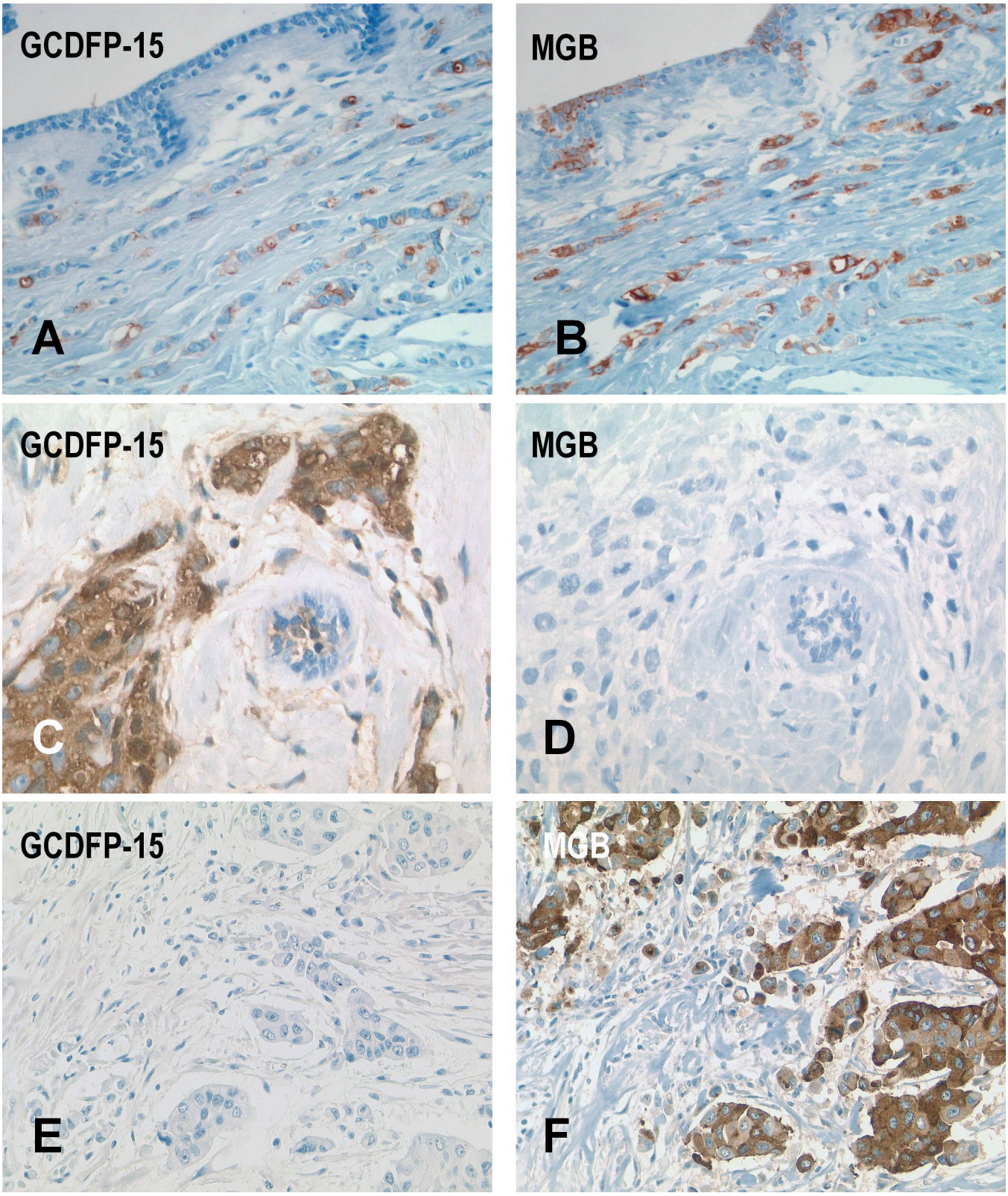

Fig. 2. Expression of GCDFP-15 and mammaglobin in breast cancer. **A, B.** We found a significant co-expression of both markers. **C-F.** Some cases were only positive for either GCDFP-15 (C/D) or mammaglobin (**E, F**). x 400

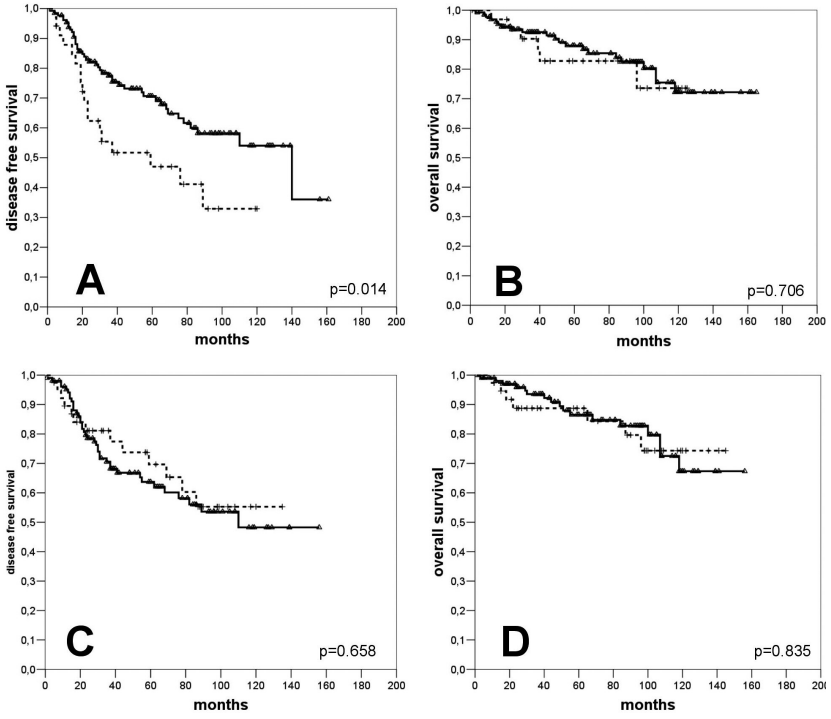

Fig. 3. Kaplan-Meier curves with univariate analyses (Log Rank) for patients without GCDFP-15 expression (dotted line) versus GCDFP-15 expressing tumours (bold line). **A, B.** Disease free survival time **(A)** and overall survival time **(B)** analyses showing a significantly longer disease free survival time of tumours with GCDFP-15 expression. **C, D.** Disease free survival time **(C)** and overall survival time **(D)** analyses for patients without mammaglobin expression (dotted line) versus mammaglobin expressing tumours (bold line).

Table 5. Associations of clinico-pathological parameters and expression data with survival times.

Characteristic	Disease free survival				Overall survival			
	No. of cases	No. of events	5-year survival rate (±SE)	p-value	No. of cases	No. of events	5-year non-progression rate (±SE)	p-value
Mammaglobin expression			0.626				0.921	
negative	39	13	69.7 ± 8.3		39	7	88.8 ± 5.3	
positive	107	40	63.3 ± 5.1		107	18	85.7 ± 3.9	
GCDFP-15 expression				0.014				0.706
negative	35	18	47.0 ± 9.4		35	6	82.8 ± 7.1	
positive	130	43	70.7 ± 4.4		130	21	88.0 ± 3.2	
Age				0.299				0.488
<60 years	89	36	61.0 ± 5.8		89	16	90.0 ± 3.5	
≥60 years	76	25	71.3 ± 5.6		76	11	83.6 ± 4.8	
Histology				0.463				0.509
ductal	147	56	64.9 ± 4.3		147	25	86.0 ± 3.2	
lobular	18	5	72.4 ± 12.0		18	2	93.3 ± 6.4	
pT status				0.001				<0.001
pT1	98	27	80.0 ± 4.4		98	6	95.1 ± 2.4	
pT2	53	29	39.8 ± 7.8		53	16	78.2 ± 6.6	
PT3/4	14	5	62.3 ± 13.4		14	5	62.3 ± 13.4	
Nodal status				0.001				<0.001
pN0	76	19	78.5 ± 5.2		76	3	95.0 ± 2.9	
pN1+	89	42	55.0 ± 5.8		89	27	80.3 ± 4.6	
Histological grade				<0.001				0.027
G1	41	7	81.9 ± 6.9		41	5	88.9 ± 6.1	
G2	82	30	71.8 ± 5.5		82	10	91.0 ± 3.6	
G3	42	24	37.7 ± 8.6		42	12	76.9 ± 6.9	
Estrogen receptor				0.037				0.039
negative	43	20	61.1 ± 8.2		43	11	79.3 ± 6.6	
positive	108	35	69.6 ± 4.9		108	13	89.3 ± 3.4	
c-erbB2 expression				0.678				0.493
0, 1+	103	38	70.2 ± 5.0		103	17	87.0 ± 3.7	
2+,	23	7	66.2 ± 10.5		23	2	94.7 ± 5.1	
3+	16	6	60.9 ± 12.5		16	3	80.8 ± 10.0	

Table 6. Cox regression model (disease free survival) for conventional parameters and GCDFP-15, categorised as in Table 5.

Variable	Disease free survival (61 events)		
	RR	95% Conf. Int.	p-value
GCDFP-15	0.519	0.276-0.977	0.042
pT-status	1.041	0.648-1.671	0.869
Nodal status	2.065	0.989-4.312	0.054
Histological grade	2.009	1.275-3.168	0.003
Estrogen receptor	1.048	0.565-1.945	0.882

RR: relative risk; 95% Conf.; Int: 95% confidence interval.

15 (median 86 vs. 140 months, p=0.05), whereas no differences in disease-free survival times were seen for mammaglobin.

An additional univariate survival analysis using the combined GCDFP-15/mammaglobin status and three different cut-off points (IRS = 0, IRS = 0-1, IRS = 0-3) did not reveal any significant results either (data not shown).

Discussion

In the present study, we carefully analysed the parallel expression of GCDFP-15 and mammaglobin on whole slides in primary breast cancer on protein level to evaluate these markers in terms of their potential diagnostic value. This study is the first to describe a significant co-expression of GCDFP-15 and mammaglobin in human breast cancer on a larger whole tissue section cohort and also this is the first report to describe a significant prognostic value of GCDFP-15, which retained validity in a multivariate analysis. Pagani et al. investigated the expression of GCDFP-15 in primary breast carcinomas by Northern Blot analysis (n=17), in situ hybridisation (n=26) and immunohistochemistry (n=33). Expression of GCDFP-15 on mRNA level as well as on protein level was significantly associated with longer disease free survival times. Additionally, a statistically significant association of GCDFP-15 mRNA expression with nodal negativity and the level of progesterone receptors was found (Pagani et al., 1994). Surprisingly, although these interesting results clearly indicated a potential prognostic value for GCDFP-15, they have not been further validated so far. Mazoujian et al. did not find any association of the GCDFP-15 expression with grading, tumour size, estrogen receptor status, risk of recurrence or survival times. However, concerning the last two points they presented cross-tables, which is inadequate to assess survival data. There was also no correlation of GCDFP-15 with tumour size or nodal status as proposed by Honma et al. (Honma et al., 2005). The significant association of GCDFP-15 and mammaglobin in the regression analysis with positive nodal status in our cohort seems rather inconclusive as this would be the first association of this kind for both markers. It is also in contrast to the wealth of published data supporting the notion of that mammaglobin and GCDFP-15 are markers for favourable rather than unfavourable tumour biology. Possibly, the high rate of nodal metastases in combination with the high expression rate of both markers could have influenced this result. The rate of GCDFP-15 positivity (78.8%) found in our study ranges rather in the upper field of formerly published data. This could be due to a high sensitivity of our immunohistochemical detection system employing a proteinase induced antigen retrieval, the low cut-off point to define positivity, as any specific immunoreactivity was interpreted as positive, but could also result from using conventional tissue slides (1 slide per case) and not only a tissue micro array (TMA), which allows to appreciate the high heterogeneity of immunoreactivity of both markers, which might be missed on small tissue spots of TMAs, as also recently shown by others (Bhargava et al., 2007).

This study also confirms the high expression rate of mammaglobin in primary breast cancers reported by Fleming and Watson (Watson et al., 1999; Fleming and Watson, 2000). Mammaglobin was significantly correlated with lower tumour grade and with positive estrogen receptor status as already shown by Nunez-Villar et al. (2003) and Span et al. (2004) on mRNA level. Watson et al. 1999) did not find a significant correlation with the tumour grade in his study group (n=100) by immunohistochemistry, albeit 78% of the highly differentiated and only 63% of the low differentiated carcinomas showed mammaglobin expression. The significance demonstrated in our study might result from the higher discriminative power due to the larger number of cases. The significant association between absence of mammaglobin mRNA and poor differentiation in breast cancer further supports this notion (Roncella et al., 2006).

Using the combination of GCDFP-15 and mammaglobin only 8% of the primary breast carcinomas were negative for both markers. Still, the heterogeneity in staining for both markers may cause false negative interpretations in very small samples, e.g. punch biopsies or tissue micro-array spots which might by chance be negative for GCDFP-15 and mammaglobin even if the primary tumour would be positive for either marker. Since the molecular portrait of marker expression in a primary tumour is often preserved in its metastasis (Weigelt et al., 2005; Bhargava et al., 2007), it is tempting to assume that the GCDFP-15/mammaglobin profile could be helpful to identify a mammary origin in metastases of yet unknown primaries. However, as this study did not evaluate the expression of GCDFP-15 and mammaglobin in breast cancer metastases, it would be methodically incorrect to directly deduce such a diagnostic use in metastases. This study was not designed to evaluate the specificity of GCDFP-15 and mammaglobin as no other tumours than primary breast cancers were included and this was already done by

others (Monteagudo et al., 1991; Satoh et al., 2000; Kaufmann et al., 2002; Han et al., 2003; O'Connell et al., 2005; Sasaki et al., 2007; Bhargava et al., 2007).

Bhargava et al. (2007) found a stronger and more frequent expression of mammaglobin and GCDFP-15 in lobular versus ductal carcinomas with only four of 63 carcinomas positive for GCDFP-15 on the microarray but they did not find a significant association between GCDFP-15 and mammaglobin expression on the microarray (p=0.19). An association of both markers with lower tumour grade, as already described by others and verified in the present study, was not found by Bhargava et al. which might be due to the small group size, the staining heterogeneity causing problems in the valid evaluation of microarrays and unconventional lumping of groups (G1 vs G2/3) in the analysis, since from their raw data (G1 57%, G2 32% and G3 40% positivity for mammaglobin) a trend is visible.

In summary, this is the first comprehensive immunophenotypical description of a highly significant co-expression of GCDFP-15 with mammaglobin on a large well characterized whole slide cohort in primary breast cancer. Depending on the cut-off level, positivity for both markers ranged from about 50% to 75%, reflecting their heterogeneity of expression, but still supports their role as breast cancer markers. We found both markers associated with low grade breast carcinomas and further validated the prognostic value of GCDFP-15. Mammaglobin protein expression alone as well as the combined expression level of GCDFP-15 and mammaglobin was of no prognostic value.

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