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Laboratory-Bladder cancer

Validation of survivin and HMGA2 as biomarkers for cisplatin resistance in bladder cancer

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Abstract

Objectives: Cisplatin-based chemotherapy represents the gold standard in the treatment of advanced bladder cancer (BC) both in the neoadjuvant and adjuvant setting. Since novel immunooncologic agents are available for cisplatin-resistant or ineligible patients, biological markers for the prediction of cisplatin resistance become more important in treatment decisions. Therefore, we aimed to assess the therapy predictive value of 8 promising tissue biomarkers with regard to cisplatin therapy.

Methods: Emmprin, survivin, HMGA2, MTA1, RhoGDI, PEG10, TGM2, and TLN1 expressions were analyzed in paraffin-embedded bladder cancer tissue samples of 106 patients who underwent adjuvant or salvage cisplatin-based chemotherapy by using immunohistochemistry. Results were correlated with the clinicopathological and follow-up data by performing both univariable and multivariable survival analyses.

Results: Higher HMGA2 nuclear staining intensity and positive survivin nuclear staining were associated with worse overall survival (OS) ($P = 0.045$ and $P = 0.002$, respectively). In accordance, survivin nuclear staining also significantly correlated with shorter progression free survival (PFS, $P = 0.024$), while HMGA2 nuclear positivity tended to correlate with shorter PFS ($P = 0.069$) after at least 2 cycles of chemotherapy. In the multivariable analyses only survivin remained as an independent predictor of both OS and PFS ($P = 0.008$ and $P = 0.025$). None of the other markers proved to be significant predictors of adjuvant or salvage cisplatin-based chemotherapy.

Conclusions: Our results demonstrate that survivin represents a promising marker for the prediction of cisplatin resistance in BC. In addition the therapy predictive role of HMGA2 should be further investigated. Immunohistochemical analysis of BC samples provides a feasible way for the prediction of cisplatin-resistance and may therefore provide a valuable tool for optimizing treatment decisions in advanced BC.

Keywords: Bladder cancer; Cisplatin; Resistance; Survivin; HMGA2; EMMPRIN

1. Introduction

Bladder cancer (BC) is the most common malignancy affecting the urinary tract. More than 70% of BCs are non–muscle-invasive (NMIBC) at first presentation. These tumors are treated curatively by transurethral resection and exhibit an excellent prognosis with 5-year survival rates of ~95%. In contrast, muscle-invasive BCs (MIBC) are

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commonly associated with poor prognosis with 5-year survival of 50% [1]. MIBC patients are usually treated with radical cystectomy and are at high risk of metastatic tumor progression and cancer-related death. Cisplatin-based chemotherapy provides a survival advantage in the perioperative setting and in advanced disease [2,3]. By the approval of novel checkpoint inhibitor therapies such as atezolizumab (anti-PD-L1) and pembrolizumab (anti-PD-1) in 2017, a novel immunooncological approach became available for the systemic treatment of metastatic BC in the second line setting and for those patients who are ineligible for cisplatin-based therapy [4]. In addition, further drugs including nivolumab, durvalumab, and avelumab with similar mechanisms of action have been currently approved [4–9]. Therefore, to date effective therapies are available for cisplatin-resistant patients, making the prediction of therapy effectiveness of paramount clinical importance. The emerging molecular classification of bladder cancer has recently shown to be predictive for cisplatin-based chemotherapy [10]. However, at present the lack of consensus on different subtype definitions and the complex methodology hinder the widespread use of these tools. Although several immunohistochemical markers such as ERCC1, survivin, and emmprin were found to possess cisplatin-predictive values, no protein markers are used in the current clinical routine [11–13].

Als et al. using a hypothesis-free approach performed a high throughput gene expression profiling on fresh frozen BC tissues and identified a large set of genes which were associated with patients' survival after cisplatin containing chemotherapy [13]. Based on a literature search we selected 8 of these genes (emmprin, survivin, HMGA2, MTA1, RhoGDI, PEG10, TGM2, and TLN1) for immunohistochemical validation. Proteins involved in oncological processes and those with published prognostic or predictive values in other tumors were preferred while those without known functions and without available antibodies were excluded. We analyzed the tissue expression of selected proteins by immunohistochemistry in BC patients who underwent postoperative cisplatin-based chemotherapy. Results were correlated with clinicopathological and follow-up data.

2. Material and methods

2.1. Patients

A total number of 106 patients divided in 3 cohorts were included in this study. The first – "SUSE" – cohort included 57 primary formalin-fixed paraffin-embedded (FFPE) tumor samples derived from a Phase II, prospective, multicenter, randomized, double-blinded trial (SUSE, AB 31/05, RUTT 204) comparing the effect of gemcitabine-cisplatin (GC) with and without sorafenib [14]. Inclusion criteria for this study were: (1) histological confirmed diagnosis of urothelial carcinoma, (2) locally advanced or metastatic urothelial carcinoma UC, (3) written informed consent, (4) patients' age >18 years, (5) ECOG performance status 0 or 1, and (6) at least one measurable lesion on CT or MR. The second and third single institution cohorts (n= 29 and n = 20) included chemotherapy naïve primary FFPE tissue samples from patients who received postoperative cisplatin-based chemotherapy. None of these patients received neoadjuvant chemotherapy. Patients' characteristics for these cohorts are given in Table 1. Time to progression (PFS) and overall survival (OS) were recorded as time from first chemotherapy to the relevant event or censoring. The study was performed according to the Declaration of Helsinki and the institutional ethics committee approved the study protocol.

2.2. Immunohistochemical analysis

For tissue microarray (TMA) construction, hematoxylin, and eosin-stained slides were established from 106 formalin fixed BC-tissue blocks and a pathologist (H.R.) defined representative tumor regions. Tissue cylinders with a diameter of 2 mm were then punched from 3 selected tumor areas of each donor tissue block and brought into a recipient paraffin block. Antigen retrieval was routinely performed with Leica bond retrieval solution (Cat.Nr.: M7228) at 96°C. Emmprin (sc-21746, Santa Cruz, pretreatment: pH: 9.0 for 20 minutes, antibody dilution: 1:100, incubation for 60 minutes), survivin (ab-469, Abcam, pretreatment: pH: 6.0 for 30 minutes, antibody dilution: 1:500, incubation for 30 minutes), HMGA2 (ab-52039, Abcam, pretreatment: pH: 9.0 for 20 minutes, antibody dilution: 1:50, incubation for 60 minutes), MTA1 (sc-17773, Santa Cruz, pretreatment: pH: 9.0 for 20 minutes, antibody dilution: 1:200, incubation for 30 minutes), RhoGDI (ab-133248, Abcam, pretreatment: pH: 9.0 for 20 minutes, antibody dilution: 1:50, incubation for 60 minutes), PEG10 (sc-58899, Santa Cruz, pretreatment: pH: 9.0 for 20 minutes, antibody dilution: 1:500, incubation for 30 minutes), TGM2 (ab-2386, Abcam, pretreatment: pH: 6.0 for 20 minutes, antibody dilution: 1:300, incubation for 30 minutes) and TLN1 (sc-81805, Santa Cruz, pretreatment: pH: 6.0 for 20 minutes, antibody dilution: 1:500, incubation for 60 minutes) IHC staining was conducted on 4 μm thick TMA sections. Automated IHC was performed using the Dako Autostainer Plus System with the anti-mouse IgG EnVision Plus detection kit (Dako) for secondary and tertiary immunoreactions. Negative controls were included in each run. In case of HMGA2, MTA1 and survivin nuclear staining, for emmprin the membranous immunoreaction and for RhoGDI, TGM2, TLN1, PEG10 cytoplasmic localization was considered as the specific immunoreactivity. For survivin and emmprin greater than 10% positivity was considered as positive as in the study by Hemdan et al. [12]. For all other markers, an immunoreactive score was calculated as described earlier [15]. Briefly, staining intensity was scored as 0, 1, 2 or 3, corresponding to negative, weak, moderate and strong intensities. In addition a percentage score was

Table 1
Immunoreactivity and clinicopathological parameters

		EMMPRIN			HMGA2			MTA1			RhoGDI			PEG10			Survivin			TGM2			TLN1		
	All patients	cyt IRS low	cyt IRS high		nucl IRS low	nucl IRS high		cyt IRS low	cyt IRS high		cyt IRS low	cyt IRS high		cyt IRS low	cyt IRS high		nucl neg	nucl pos		cyt IRS low	cyt IRS high		cyt IRS low	cyt IRS high	
	n (%)	*n* (%)	*n* (%)	*P**	*n* (%)	*n* (%)	*P**	*n* (%)	*n* (%)	*P**	*n* (%)	*n* (%)	*P**	*n* (%)	*n* (%)	*P**	*n* (%)	*n* (%)	*P**	*n* (%)	*n* (%)	*P**	*n* (%)	*n* (%)	*P**
Total/[missing] cases]	106 (100)	45	56	[5]	58	46	[2]	85	16	[5]	54	46	[6]	36	64	[6]	55	42	[9]	62	38	[6]	47	51	[8]
Age 64 (37−90)																									
≤65	57 (54)	31 (59)	22 (41)	0.080	29 (53)	26 (47)	0.508	46 (85)	8 (15)	0.762	32 (59)	22 (41)	0.253	17 (32)	36 (68)	0.385	20 (39)	31 (61)	0.170	27 (50)	27 (50)	**0.007**	25 (48)	27 (52)	0.980
>65	49 (46)	36 (75)	12 (25)		29 (59)	20 (41)		39 (83)	8 (17)		22 (48)	24 (52)		19 (40)	28 (60)		12 (26)	34 (74)		35 (76)	11 (24)		22 (48)	24 (52)	
Sex																									
Male	75 (71)	48 (68)	23 (32)	0.678	42 (57)	32 (43)	0.750	61 (84)	12 (16)	0.791	39 (53)	34 (47)	0.849	29 (41)	42 (59)	0.114	26 (37)	44 (63)	0.161	44 (61)	28 (39)	0.769	34 (49)	36 (51)	0.848
Female	31 (29)	19 (63)	11 (37)		16 (53)	14 (47)		24 (86)	4 (14)		15 (56)	12 (44)		7 (24)	22 (76)		6 (22)	21 (78)		18 (64)	10 (36)		13 (46)	15 (54)	
Stage																									
T1−T2	16 (15)	11 (73)	4 (17)	0.483	12 (75)	4 (25)	0.098	10 (67)	5 (33)	0.061	9 (64)	5 (36)	0.512	8 (53)	7 (47)	0.234	6 (40)	9 (60)	0.702	10 (77)	3 (23)	0.211	4 (31)	9 (69)	0.154
T3−T4	56 (53)	35 (63)	20 (37)		29 (52)	27 (48)		48 (87)	7 (13)		30 (55)	25 (45)		20 (36)	35 (64)		18 (35)	34 (65)		32 (58)	23 (42)		28 (53)	25 (47)	
Not available	34																								
Performance status																									
0	46 (43)	28 (38)	17 (62)	0.433	24 (52)	22 (48)	0.511	39 (93)	6 (7)	0.536	28 (65)	15 (35)	0.053	12 (28)	31 (72)	0.143	16 (37)	27 (63)	0.430	25 (44)	20 (56)	0.230	22 (49)	23 (51)	0.865
1-2	60 (57)	39 (70)	17 (30)		34 (59)	24 (41)		46 (82)	10 (18)		26 (46)	31 (54)		24 (42)	33 (58)		16 (30)	38 (70)		37 (67)	18 (33)		25 (47)	28 (53)	
Visceral metastasis																									
Present	37 (35)	20 (59)	14 (41)	0.255	15 (42)	21 (58)	**0.035**	26 (74)	9 (26)	**0.048**	21 (62)	13 (38)	0.263	7 (21)	27 (79)	**0.021**	11 (33)	22 (67)	0.959	19 (54)	16 (46)	0.244	17 (49)	18 (51)	0.928
Absent	69 (65)	47 (70)	20 (30)		43 (63)	25 (37)		59 (89)	7 (11)		33 (50)	33 (50)		29 (44)	37 (56)		21 (33)	43 (67)		43 (66)	22 (34)		30 (48)	33 (52)	
Radiographic response after last cycle																									
CR, PR, SD	22 (41)	18 (82)	4 (18)	0.077	16 (73)	6 (27)	0.076	17 (85)	3 (15)	0.914	14 (70)	6 (30)	0.341	8 (38)	13 (62)	0.917	11 (52)	10 (48)	**0.024**	13 (65)	7 (35)	0.652	8 (42)	11 (58)	0.722
PD	32 (59)	17 (59)	12 (41)		15 (48)	16 (52)		26 (84)	5 (16)		17 (57)	13 (43)		11 (37)	19 (63)		6 (21)	22 (79)		17 (59)	12 (41)		11 (38)	18 (62)	
Not available	52																								

CR = complete response; IRS = immunreactive score; PD = progressive disease; PR = partial response; SD = stable disease.
Numbers in bold indicate significant *P* values.
* Chi-squre test.

defined as follows: 0−10%, 0 points; 11−50%, 1 point; 51−80%, 2 points; and 81−100%, 3 points. Finally, immunoreactive score was calculated by multiplying the intensity score by the percentage score. At the time of examination, the pathologist was blinded to all clinical and follow-up data. Cases with less than 10% of tumor cells with positive immunostaining for survivin were considered as negative.

2.3. Statistical analysis

Associations of emmprin, survivin, HMGA2, MTA1, RhoGDI, TGM2, and TLN1 IHC immunostaining and clinicopathological variables were evaluated using the chi-square test. Univariable survival analyses (OS and PFS) were done using Kaplan−Meier log-rank test and univariable Cox analysis. For multiple Cox analyses, parameters with a P value less than 0.150 in the univariable analysis were considered. In all tests $P < 0.05$ was considered to indicate statistical significance. All statistical analyses were done with the SPSS software package (version 24; SPSS, Chicago, IL).

3. Results

3.1. Clinical background

The main characteristics of the patients are demonstrated in Table 1. The median follow-up time was 8 months ranging from 1 to 123 months. Eighty-one of 106 patients died within the follow-up period. In 72 patients metastatic progression was detected with a median latency of 5 months range (1−102 months). Information on radiographic progression during chemotherapy was available for a subset of 54 patients. In 32 of these patients, a radiographic progression was detected during the treatment.

3.2. Immunohistochemical analyses

HMGA2, RhoGDI, TGM2, and TLN1 showed both cytoplasmatic and nuclear staining. Lymphocytes, centrocytes, and histiocytes showed frequent positivity for HMGA2, RhoGDI, and TLN1. Lymphocytes were negative for TGM2, while endothelial cells and serum stained positive. MTA1 showed a mostly weak nuclear staining in tumor cells. Survivin showed a clear nuclear localization while lymphocytes were mostly negative. Emmprin stained both the membrane and cytoplasm of tumor cells with membranous accentuation (Fig. 1).

Strong cytoplasmic immunostaining of TGM2 was associated with younger age ($P = 0.007$). Other markers showed no significant association with patients' age, sex, ECOG performance status, or tumor stage (Table 1). Stronger HMGA2 nuclear and PEG10 as well as MTA1 cytoplasmic stainings were significantly higher in metastatic patients ($P = 0.035$, $P = 0.021$ and $P = 0.048$, respectively) (Table 1). In accordance, stronger HMGA2 nuclear staining was associated with worse OS ($P = 0.045$) and tended to correlate with shorter PFS ($P = 0.069$) in the subgroup of patients who received at least 2 cycles of chemotherapy (Fig. 2). Similarly, stronger HMGA2 expression tended to correlate with radiographic progression during chemotherapy treatment ($P = 0.076$). Survivin expressions proved to be significantly associated with both OS and PFS regardless of the number of chemotherapy cycles ($P = 0.002$, $P = 0.024$) (Table 2, Fig. 2) and also the rate of survivin positive cases (22/32, 69%) was significantly higher in patients who experienced radiographic progression during cisplatin therapy compared to those who did not (40/22, 45%, $P = 0.024$). The correlation between survivin immunostaining and OS or PFS remained significant also in the multivariable analyses ($P = 0.008$, $P = 0.025$) (Table 3).

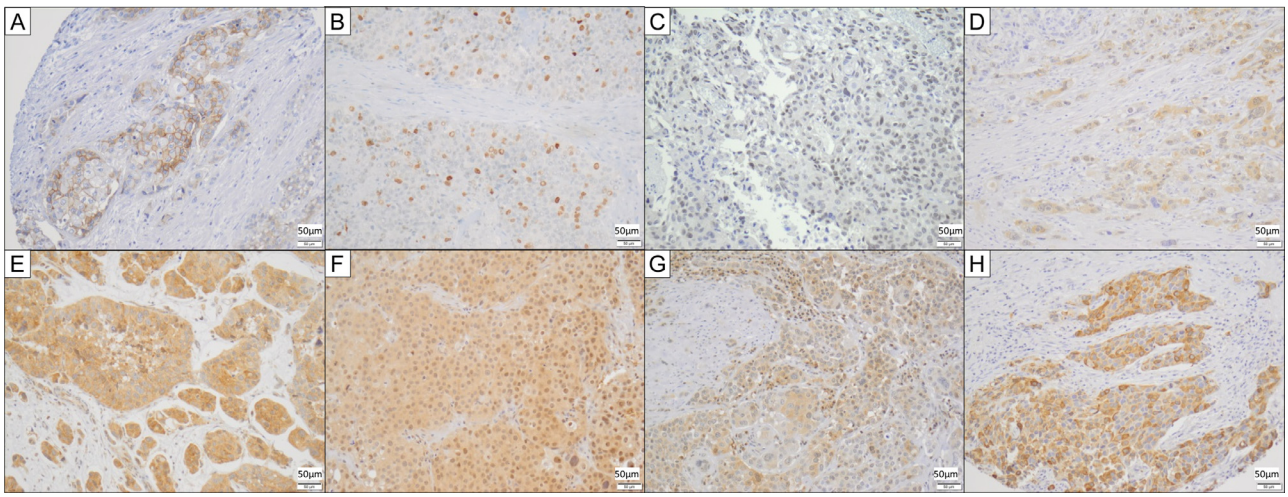

Fig. 1. Examples of immunostainings of the eight different proteins. Typical membranous and cytoplasmatic immunostaining for Emmprin (A). Strong nuclear reactivity in some tumor nuclei for survivin (B) and typical weak nuclear immunostaining for MTA1 (C). Different intensities of cytoplasmic reactivity with or without nuclear overlay for TGM2 (D), PEG10 (E), HMGA2 (F), RhoGDI (G), and TLN1 (H).

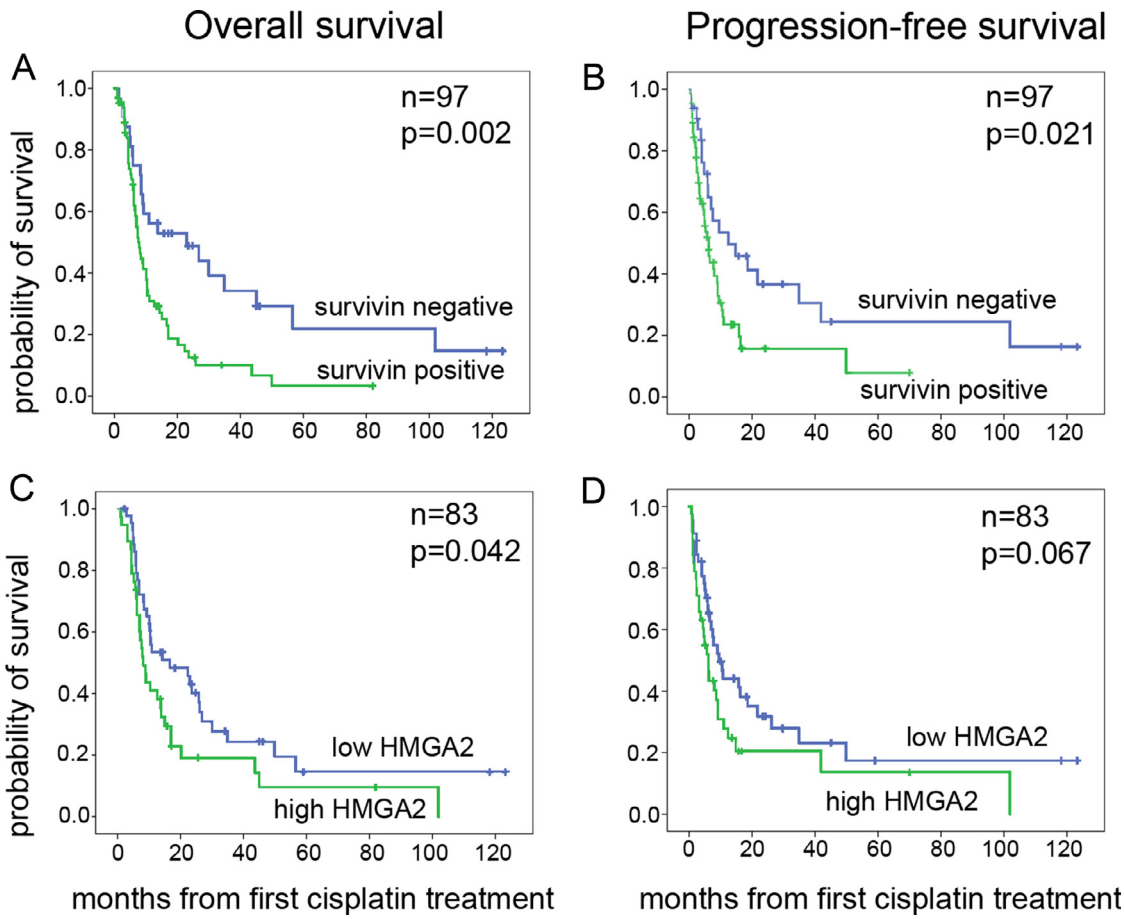

Fig. 2. Kaplan-Meier overall and progression-free survival curves stratified by survivin (A) and (B) and HMGA2 protein expressions (C) and (D) Log rank test has been performed to calculate *P* values. Kaplan-Meier plots for HMGA2 (C) and (D) represents patients with at least 2 cycles of chemotherapy.

4. Discussion

Patients with advanced BC represent a clinically heterogeneous group with different sensitivity to cisplatin-containing chemotherapy. This remarkable heterogeneity cannot sufficiently be resolved by the routine histopathological examination. Recent development in systemic treatment of advanced BC resulted in the approval of novel effective drugs for those patients who are not responsive to cisplatin-based therapy. Therefore, biomarkers are urgently needed for the accurate prediction of cisplatin resistance in order to improve therapy decisions. In the present study, we evaluated 8 formerly identified potential cisplatin therapy-predicting markers and were able to validate survivin as an independent predictive marker of cisplatin-based chemotherapy. Furthermore, we found that HMGA2 expression is associated with survival after cisplatin-therapy. In contrast, neither emmprin nor the other analyzed markers could be validated as predictors of platinum-resistance in BC.

Former studies suggested a series of protein biomarkers to be associated with cisplatin resistance. However, only a few of these markers have been validated in independent patient cohorts. ERCC1 plays a role in nucleotide excision repair and its high expression levels were shown to be associated with poor response to cisplatin-based chemotherapy [16]. This observation has been confirmed in several subsequent studies [11,17,18]. We could formerly also confirm the therapy predicting value of ERCC1 in a subgroup of patients of the present study [19]. In addition, the therapy predicting effect could be further improved when ERCC1 was combined with matrix metalloproteinase 7 (MMP-7) [19].

Two other potentially predictive markers, emmprin and survivin have been assessed in independent patient cohorts, however with partly conflicting results. Emmprin (extracellular matrix metalloproteinase inducer, CD147) is a transmembrane protein which belongs to the immunoglobulin superfamily of receptors. Beside its main function as matrix metalloproteinase inducer, emmprin also promotes angiogenesis, migration, and invasion. Furthermore, emmprin modulates various multidrug transporters of the ABC (ATP-binding cassette) family involved with antiapoptotic signaling and chemotherapy resistance [20]. Survivin is a multifunctional protein which regulates cell division and inhibits apoptosis. In BC survivin has been identified as an antiapoptotic factor, its expression was found to correlate with BC progression and is associated with shorter recurrence-free and progression-free survival [6].

Table 2
Univariable analysis of overall and progression-free survival

Variables	Overall survival			Overall survival minimum 2 cycles			Progression-free survival			Progression-free survival minimum 2 cycles		
	HR	*n* = 106 95% CI	*P*	HR	*n* = 84 95% CI	*P*	HR	*n* = 106 95% CI	*P*	HR	*n* = 84 95% CI	*P*
Age												
≤65	ref.			ref.			ref.			ref.		
>65	1,310	0.840−2.043	0.234	1,246	0.750−2.071	0.396	1,265	0.791−2.026	0.327	1,129	0.672−1.896	0.647
Sex												
Male	ref.			ref.			ref.			ref.		
Female	1,596	0.983−2.593	0.059	1,629	0.935−2.837	0.085	1,124	0.660−1.913	0.668	1,139	0.637−2.036	0.661
Stage												
T1−T2	ref.			ref.			ref.			ref.		
T3−T4	0.934	0.493−1.767	0.833	0.913	0.438−1.901	0.807	1,097	0.547−2.200	0.795	1,283	0.568−2.900	0.549
Performance status												
0	ref.			ref.			ref.			ref.		
1-2	1,393	0.890−2.181	0.147	1,260	0.764−2.077	0.365	1,191	0.743−1.910	0.467	1,118	0.670−1.866	0.670
Visceral metastasis												
Absent	ref.			ref.			ref.			ref.		
Present	1,451	0.922−2.282	0.107	1,659	0.998−2.757	**0.051**	1,820	1.136−2.917	**0.013**	2,011	1.203−3.362	**0.008**
Clinical response												
CR, PR, SD	ref.			ref.			ref.			ref.		
PD	6,870	3.103−15.207	**<0.001**	6,209	2.675−14.414	**<0.001**	8,485	3.590−20.052	**<0.001**	8,448	3.322−21.489	**<0.001**
EMMPRIN cyt. exp.												
Low	ref.			ref.			ref.			ref.		
High	1,265	0.805−1.987	0.309	1,284	0.773−2.134	0.334	1,246	0.771−2.014	0.369	1,216	0.723−2.043	0.461
HMGA2 nucl. exp.												
Low	ref.			ref.			ref.			ref.		
High	1,412	0.907−2.200	0.127	1,661	1.012−2.725	**0.045**	1,313	0.817−2.109	0.260	1,606	0.964−2.676	**0.069**
MTA1 cyt. exp.												
Low	ref.			ref.			ref.			ref.		
High	1,185	0.868−1.618	0.284	1,287	0.900−1.841	0.167	1,138	0.882−1.575	0.437	1,241	0.850−1.809	0.263
RhoGDI cyt. exp.												
Low	ref.			ref.			ref.			ref.		
High	1,120	0.702−1.785	0.635	1,088	0.643−1.841	0.753	1,000	0.617−1.620	0.999	1,619	0.958−2.738	0.072
PEG10 cyt. exp.												
Low	ref.			ref.			ref.			ref.		
High	1,129	0.703−1.812	0.616	1,290	0.751−2.217	0.357	1,025	0.620−1.695	0.923	1,244	0.715−2.166	0.439
Survivin nucl. exp.												
Absent	ref.			ref.			ref.			ref.		
Present	2,255	1.340−3.796	**0.002**	2,981	1.632−5.446	**<0.001**	1,893	1.089−3.290	**0.024**	2,190	1.203−3.988	**0.010**
TGM2 cyt. exp.												
Low	ref.			ref.			ref.			ref.		
High	0.844	0.667−1.068	0.158	0.871	0.673−1.126	0.291	0.957	0.746−1.226	0.726	1,035	0.797−1.344	0.798
TLN1 cyt. exp.												
Low	ref.			ref.			ref.			ref.		
High	1,035	0.659−1.625	0.882	1,076	0.647−1.790	0.778	0.862	0.531−1.400	0.548	0.842	0.497−1.427	0.523

CR = complete response; IRS = immunreactive score; PD = progressive disease; PR = partial response; SD = stable disease.
Numbers in bold indicate significant *P* values.

Table 3
Multivariable analysis of overall and progression-free survival

Variables	Overall survival			Overall survival minimum 2 cycles			Progression-free survival			Progression-free survival minimum 2 cycles		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Gender												
Male	ref.			ref.			ref.			ref.		
Female	1,255	0.737–2.139	0.403	1,137	0.616–2.098	0.681	–	–	–	–	–	–
Performance status												
0	ref.			–	–	–	ref.			ref.		
1-2	1,494	0.899–2.482	0.121	–	–	–	–	–	–	–	–	–
Visceral metastasis												
Absent	ref.			ref.			ref.			ref.		
Present	1,580	0.938–2.661	0.086	1,614	0.940–2.771	0.083	1,624	0.984–2.681	0.058	1,826	1.063–3.138	**0.029**
HMGA2 nucl. exp.												
Low	ref.			ref.			ref.			ref.		
High	1,117	0.699–1.787	0.643	1,195	0.708–2.018	0.504	1,064	0.646–1.755	0.807	1,236	0.724–2.111	0.438
Survivin nucl. exp.												
Absent	ref.			ref.			ref.			ref.		
Present	2,090	1.214–3.600	**0.008**	2,890	1.537–5.434	**0.001**	1,891	1.084–3.298	**0.025**	2,196	1.199–4.022	**0.011**

Abbreviations: CI = confidence interval ; HR = hazard ratio; Ref. = referent.

Formerly, Als et al. identified a set of 55 genes to be associated with shorter survival under cisplatin-based chemotherapy using gene expression profiling. They successfully validated 2 of these genes—emmprin and survivin—at the protein level in an independent set of patients who received adjuvant cisplatin-based chemotherapy [13]. Our present data confirmed the initial observation by Als et al. regarding survivin as in our study, high survivin expression levels were independently associated with patient's OS and PFS. Indeed, when adding survivin to the multivariable basis model (including gender ECOG status and visceral metastatic status), survivin outperformed all of these well-established prognostic factors. In a former validation study, Hemdan et al. found only emmprin but not survivin to be associated with therapy response in terms of downstaging and survival in a neoadjuvant-treated patient cohort [12]. In contrast, our present data using the same antibody as both former studies found no association between emmprin expression and OS or PFS.

HMGA2 belongs to the nonhistone chromosomal high mobility group (HMG) family and is commonly overexpressed in numerous malignant tumors and is associated with increased invasiveness and poor prognosis [21]. In BC, HMGA2 was found to be elevated in tumor cells compared to adjacent normal epithelium. In addition, higher HMGA2 expressions were associated with higher recurrence and progression rates in non−muscle-invasive BC [21]. Silencing of HMGA2 in BC cells resulted in the suppression of tumor cell proliferation, migration, invasion, and epithelial-to-mesenchymal transition [22]. Finally, HMGA2 was found to be involved in gemcitabine resistance of BC [23]. Since its initial identification at the gene expression level, the role of HMGA2 as a predictor of cisplatin resistance has not been evaluated. Our data at the protein level confirmed for the first time the association between HMGA2 expression and OS as well as PFS in patients who underwent at least 2 cycles of cisplatin-based therapy. The fact that HMGA2 proved to be associated with OS only in the subgroup of patients who received at least 2 cycles of chemotherapy but not in the whole cohort suggests HMGA2 as a predictive marker in BC.

Additionally, RhoGDI, TGM2, and TLN1 did not show any correlation with clinicopathological parameters in our study. Although TGM2 − a Ca^{2+}-dependent crosslinking enzyme that regulates multiple cellular processes during normal cell development − was proposed to reduce cisplatin-induced apoptosis in ovarian cancer, our analyses did not show a prognostic role in BC [24]. MTA1 was shown to be upregulated in chemoresistant prostate cancer tissues and also functionally involved in docetaxel resistance of prostate cancer cells [25]. In this study, we observed significantly elevated MTA expressions in higher tumor stages and in metastatic BC but could not observe any association between MTA1 expression and chemotherapy resistance. Paternally expressed gene 10 (PEG10) is an imprinted gene which functions as a transcriptional factor. Recent studies

have indicated that the expression of PEG10 is associated with the prognosis in various cancers [26]. In accordance, we observed significantly elevated PEG10 expressions in metastatic compared to nonmetastatic BC. Furthermore, PEG10 was reported to be associated with resistance to taxane-containing chemotherapy in nasopharyngeal cancer [27]. Our results, showed no correlation between PEG10 expression and OS or PFS.

RhoGDI and TLN1 protein products have not yet been found to be associated with cisplatin resistance, except for the observation at the gene expression level by Als et al. Our results could not confirm the suggested predicting role concerning cisplatin therapy at the protein level for these 2 markers.

Several limitations of this investigation should be noted. Some limitations are inherent from the retrospective nature of this study. Furthermore, as FFPE samples were collected from several hospitals, different tissue handling methods among institutions could be a confounder. However, we did not find obvious differences when comparing results between larger contributors. We used TMAs for our analyses and tumor heterogeneity is a well-known problem for the analysis of protein markers which affect generalizability of results obtained with this technique.

5. Conclusions

In summary, analyzing 8 genes which were formerly suggested as potential markers for poor response to cisplatin-based chemotherapy, we could confirm survivin and HMGA2 as promising markers of cisplatin-resistance in BC at the protein level. In contrast, we could not confirm the therapy-predicting value of emmprin. Further efforts are urgently needed to identify and validate predictors of cisplatin-based chemotherapy to optimize therapy decisions and to save patients from the toxicity of ineffective treatments.

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