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The metastasis-associated gene MTA3 is downregulated in advanced endometrioid adenocarcinomas

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Summary. The metastasis-associated gene MTA3 has an important function in invasion and metastasis of human cancer cells. Therefore, the aim of this study was to investigate the expression of this protein in endometrial adenocarcinomas and to analyse potential correlations between this nuclear transcription factor and estrogen receptors in endometrial adenocarcinomas. Additionally, we evaluated whether MTA3 might be a prognostic parameter in endometrioid adenocarcinomas. Endometrioid adenocarcinomas were obtained from 200 patients and immunohistochemically analysed for MTA3 and estrogen receptor alpha and beta (ER-alpha and ER-beta) expression. Overall, endometrioid adenocarcinomas of histological differentiation grade 3 demonstrated a significantly lower expression of MTA3 compared to carcinomas of histological grade 1 and 2 ($p<0.05$). MTA3 expression is reduced in endometrioid adenocarcinomas of poor differentiation, though without any correlation to ER-alpha and ER-beta expression. Furthermore, the expression of MTA3 did not affect progression-free, cause-specific and overall survival. Overall, MTA3 did not constitute an independent prognostic factor in this study, suggesting that MTA3 is not a useful marker to assess and identify high-risk patients with endometrial adenocarcinomas. Still, the downregulation of MTA3 predispose this cell type to be of high metastatic potential after malignant transformation, playing an essential, but as yet unknown role in human endometrial carcinogenesis.

Key words: MTA3, Endometrioid adenocarcinomas, Immunohistochemistry, Estrogen receptors, ER-alpha, ER-beta, Survival, Prognosis

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Introduction

Endometrial cancer has become the most frequent gynaecologic malignancy in the Western World (Abeler and Kjorstad, 1991; Rose, 1996; Prat, 2004; Amant et al., 2005; Chan et al., 2007). An incidence of 15-20 cases/100.000 women/ year has been estimated with a life time risk to develop this type of cancer being approximately 2.5% (Gloeckler Ries et al., 2003). Meanwhile, several prognostic factors, such as histological type, histologic grade, surgical stage, pelvic lymph node involvement and myometrial invasion have been established (Abeler and Kjorstad, 1991; Rose, 1996; Prat, 2004; Amant et al., 2005; Chan et al., 2007). Although endogenous and exogenous sources of unopposed estrogen increase the risk of endometrial adenocarcinoma, the molecular pathogenesis of endometrial carcinoma remains unclear (Sherman, 2000; Prat, 2004). Furthermore, although more than 50% of patients with endometrial carcinoma are diagnosed at an early stage, as many as 20% die of their disease (Jereczek-Fossa et al., 1999). The reason for this unusual situation compared to other solid tumours is still unclear.

Major characteristics of cancer progression are thought to be invasion into connective tissues, transmigration through blood vessels and the capability of neoangiogenesis (Hanahan and Weinberg, 2000). These changes are often accompanied by the so called epithelial-mesenchymal transition (EMT) that has been described to play an essential role during cancer cell progression (Hugo et al., 2007). The EMT is accompanied by a shift in gene expression, most apparently by that of cell adhesion molecules (Vicovac and Aplin, 1996; Moustakas and Heldin, 2007). The expression of these cell adhesion proteins is predominantly regulated by nuclear transcription factors such as MTA1, MTA3, and SNAIL. These transcription regulators are nuclear proteins that mediate gene silencing by binding to histone deacetylases (Yao and

Yang, 2003).

The metastasis-associated gene 1 (MTA1) has been described to be upregulated in several types of human cancer tissues (Manavathi et al., 2007). In contrast, the expression of MTA3 has been found to be reduced in breast cancer (Fujita et al., 2003) and ovarian cancer (Dannenmann et al., 2008). We have recently demonstrated that MTA1 is upregulated in advanced ovarian cancer and regulates the expression of E-cadherin, in addition to that of angiogenic cytokines (Dannenmann et al., 2008). A significant upregulation of MTA1 in endometroid carcinomas has recently been described (Balasenthil et al., 2006) and suggests an important function of MTA proteins in endometroid cancer development, although no studies on MTA3 expression in endometrial cancer have been performed yet. Additionally, MTA3 expression in breast cancer cells is enhanced by estrogen receptor activity (Fujita et al., 2003), linking MTA3 and steroid hormone receptors. Therefore, we investigated the expression of MTA3 and the correlation between this nuclear transcription factor and estrogen receptors alpha (ER-α) and beta (ER-ß) in endometrial adenocarcinomas. Additionally we evaluated whether MTA3 might be a prognostic parameter in endometrioid adenocarcinomas.

Materials and methods

Tissue samples

Pathological and surgical records of 200 patients who had been operated in the 1st Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University Munich between 1990 and 2002 were reviewed for this retrospective analysis. Only specimens with an endometrioid adenocarcinoma were included, while other histological types, including that of non-endometrioid histology, mucinous adenocarcinoma and mixed adenocarcinomas were excluded in this analysis. The evaluated patient group has been previously well characterised and an evaluation for several prognostic markers has been performed (Shabani et al., 2007a; Mylonas et al., 2009). Patients with endometrial adenocarcinoma received modified radical hysterectomy, salpingo-oophorectomy or selective pelvic lymph-adenectomy, with or without para-aortic lymph-adenectomy.

All hematoxylin and eosin–stained slides were re-reviewed by a gynaecological pathologist to verify the diagnosis, histological grade, histological type, FIGO stage, lymphangiosis, adnexal or cervical involvement as previously described (Shabani et al., 2007a; Mylonas et al., 2009). Pathological stage and histological subtype were determined for each surgical specimen according to the 1988 International Federation of Gynecology and Obstetrics (FIGO) criteria (FIGO, 1989).

Patients with endometrial carcinoma received modified radical hysterectomy, salpingo-oophorectomy or selective pelvic lymphadenectomy, with or without para-aortic lymphadenectomy. Lymph node sampling or dissection was generally performed in patients having tumours with deep myometrial invasion and/or high-grade or aggressive histological features. Obesity, advanced age and excessive comorbidity were factors against full surgical staging.

Patient data were obtained from three sources: hospital tumour registry, automated database and chart review as previously described (Shabani et al., 2007a; Mylonas et al., 2009). All cases of recurrence had radiographic evidence of disease or biopsy-proven progression of disease. Only the records of patients who died of disease were considered to be uncensored; the records of all patients who were alive at follow-up or who did not die of disease (or a related cause) were considered to be censored. Additionally, censored cases were also considered those cases where the exact cause of death was unknown but died within two years after the diagnosis of a metastatic lesion (Shabani et al., 2007a; Mylonas et al., 2009).

Immunohistochemistry

Immunohistochemistry was performed using a combination of microwave-oven heating and the standard streptavidin-biotin-peroxidase complex using the mouse-IgG-Vectastain Elite ABC kit (Vector Laboratories, Burlingame, California, USA) as previously described for steroid receptors (Mylonas et al., 2004, 2005, 2007; Shabani et al., 2007a,b) and rabbit-IgG-Vectastain Elite ABC kit (Vector Laboratories, Burlingame, California, USA) for MTA3 (Brüning et al., 2009). For positive controls, sections of human breast cancer tissue and normal colon were used, while human ileum served as negative control tissue.

Briefly, paraffin-fixed tissue sections were dewaxed using xylol for 15 min, rehydrated with decreasing alcohol-water mixtures, and subjected to antigen retrieval on a high setting for 10 min in a pressure

Table 1. Antibodies used for immunohistochemical characterization of endometrial adenocarcinomas.

Antibody	Clone	Isotype	Dilution	Source
MTA3		rabbit polyclonal antibody	1:500	Calbiochem, Darmstadt, Germany
ER-α	1D5	mouse IgG_1	1:150	Immunotech, Hamburg, Germany
ER-ß	PPG5/10	mouse IgG_{2a}	1:50	Serotec, Oxford, United Kingdom

ER: estrogen receptor

cooker in sodium citrate buffer (pH 6.0), containing 0.1 M citric acid and 0.1 M sodium citrate. After cooling, the slides were washed twice in PBS. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (Merck, Darmstadt, Germany) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with diluted horse serum (10 ml PBS with 150 μl horse serum, provided by Vectastain Elite ABC kit) for 20 min at room temperature. Sections were then incubated at room temperature for 60 min with the primary antibodies (Table 1). ER-α and MTA3 were diluted in dilution-medium (Dako, Glostrup, Denmark) while ER-ß was diluted in PBS. After washing with PBS, the slides were incubated in diluted biotinylated anti-serum secondary antibody for a further 30 min at room temperature (10 ml PBS, 50 μl horse serum). After incubation with the avidin–biotin peroxidase complex (diluted in 10 ml PBS, provided by Vectastain Elite ABC kit) for another 30 min and a repeated washing step with PBS, visualisation was performed with ABC substrate (Vectastain Elite ABC kit) and chromogenic 3,3ß-diaminobenzidine (DAB; Dako, Glostrup, Denmark) for 8–10 min. The slides were further counterstained with Mayer's acidic haematoxylin and washed in an alcohol multiple-row (50–98%). After xylol treatment the slides were embedded. Negative controls were performed by replacing the primary antibody with normal mouse serum. Positive controls for ER-α and MTA3 include human invasive breast cancer. The ER-ß antibody was tested positive on human colon tissue. Positive cells showed a brownish colour, and negative controls, as well as unstained cells, were blue.

Immunohistochemical evaluation

The intensity and distribution patterns of specific MTA3, ER-α and ER-ß immunohistochemical staining reaction was evaluated by two blinded, independent observers, including a gynaecological pathologist, using a semi-quantitative score as previously described, and used to assess the expression pattern of steroid receptors in normal and pathological endometrial tissue (Mylonas et al., 2000, 2004, 2005, 2007; Shabani et al., 2007a,b).

The immunoreactive score (IRS) score was calculated by multiplication of optical staining intensity (graded as 0 = no, 1 = weak, 2 = moderate and 3 = strong staining) and the percentage of positive stained cells (0 = no staining, 1 = <10% of the cells, 2 = 11-50% of the cells, 3 = 51-80% of the cells and 4 = >81% of the cells) as previously described (Remmele and Schicketanz, 1993). Sections were examined using a Leica (Solms, Germany) photomicroscope. Digital images were obtained with a digital camera system. The IRS-scores of MTA3, ER-α and ER-ß were compared using the non-parametric Mann-Whitney-U test. Correlations were assessed using the Spearman rank correlation test. Significance of differences was assumed at $p \leq 0.05$ at the two-sided test. The Statistical Package for the Social Sciences computer software (version 16.0; SPSS Inc., Munich, Germany) was used.

Statistical analysis

For the purposes of statistical survival analysis, MTA3 expression in tumor samples was considered to be elevated if the immunoreactive score was >3 (median for MTA3=3). ER-α expression in tumour samples was considered to be elevated if >10% positive staining was

Table 2. Clinicopathological characteristics of the analyzed endometrial adenocarcinomas.

Clinicopathological characteristics			Total (n= 200)
Age	≤65 years		98 (49%)
	>65 years		102 (51%)
FIGO	FIGO I	FIGO Ia 25 (12,5%)	160 (80%)
		FIGO Ib 94 (47%)	
		FIGO Ic 41 (20,5%)	
	FIGO II	FIGO 2a 2 (1%)	15 (7,5%)
		FIGO 2b 13 (6,5%)	
	FIGO III	FIGO 3a 8 (4%)	18 (9%)
		FIGO 3b 3 (1,5%)	
		FIGO 3c 7 (3,5%)	
	FIGO IV		7 (3,5%)
Grading (WHO)	Grade 1		122 (61%)
	Grade 2		54 (27%)
	Grade 3		24 (12%)
LN status	negative		133 (66,5%)
	positive		9 (4,5%)
	unknown		58 (29%)
LVSI	negative		183 (91,5%)
	positive		17 (8,5%)
Lymphangiosis		negative 183 (91,5%)	
		positive 17 (8,5%)	
Haemangiosis		negative 194 (97%)	
		positive 6 (3%)	
myometrial invasion	Only endometrium		27 (13,5%)
	<50% myometrial invasion		108 (54%)
	>50% myometrial invasion		65 (32,5%)
Cervical Invasion	negative		177 (88,5%)
	positive		23 (11,5%)
Ovarial invasion	negative		185 (92,5%)
	positive		15 (7,5%)
Adipositas	negative		125 (62,8%)
	positive		74 (37,2%)
Diabetes	negative		174 (87%)
	positive		26 (13%)
Hypertension	negative		119 (59,5%)
	positive		81 (40,5%)
Radiotherapy	negative		127 (63,5%)
	positive		73 (36,5%)
Anti-hormone therapy	negative		193 (96,5%)
	positive		7 (3,5%)

LVSI: lymphovascular space invasion.

observed (IRS>2) (Shabani et al., 2007a; Jongen et al., 2009), while positive ER-ß expression was suggested if IRS>1 (median for ER-ß = 0) as previously suggested (Shabani et al., 2007a). For the evaluation of the MTA3 and ER-ß staining intensity the median for all tumour samples was used. Increased/positive versus not increased/negative immunostaining in tumour samples was compared using the χ^2 test and the exact Fisher's test where applicable.

The outcomes analyzed were progression-free survival, cause-specific survival and overall survival. Univariate analysis was performed with Kaplan-Meier life-table curves to estimate survival (Kaplan and Meier, 1958) and were compared using the log-rank test. Prognostic models used multivariate Cox regression analysis for multivariate analyses of survival. The data were adjusted for age (≤65 years vs. >65 years), surgical stage (FIGO I/II vs. III/IV), histological grade (grade 1/2 vs. 3), lymph node status (negative vs. positive), lymphovascular space invasion (negative vs. positive), myometrial invasion (<50% vs. >50%), cervical invasion (negative vs. positive), ovarial invasion (negative vs. positive), ER-α (negative vs. positive), ER-α (negative vs. positive) and MTA3 (negative vs. positive) status. The variables were entered in a forward stepwise manner (Cox, 1972). Significance of differences was assumed at p≤0.05 (SPSS version 16.0; SPSS Inc., Chicago, IL).

Results

Clinicopathological characterization

The clinicopathological characteristics of the patients with endometrioid adenocarcinomas are summarized in Table 2. The median patient age at the

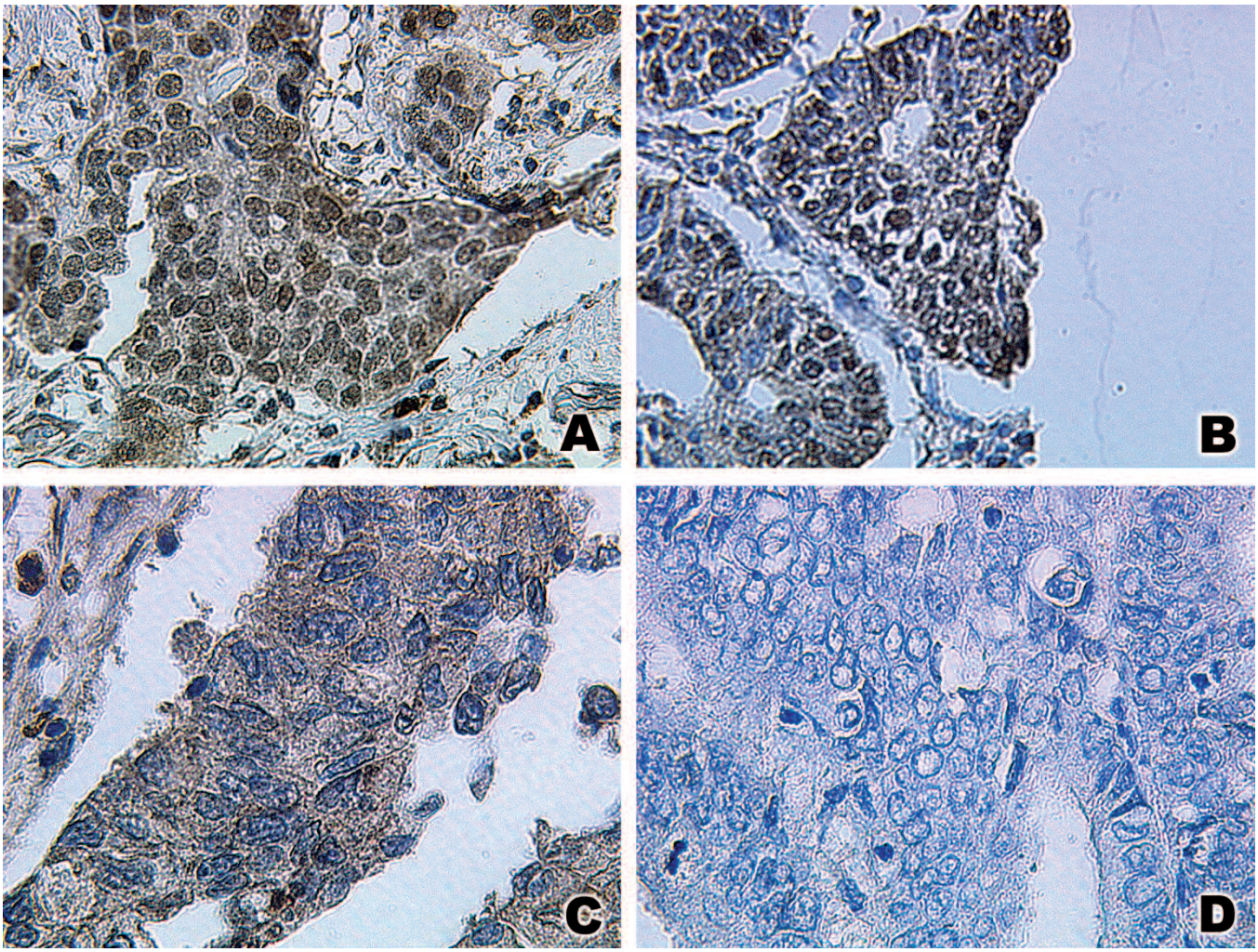

Fig. 1. Immunohistochemical staining reaction of MTA3 in endometrioid adenocarcinomas **(A)**. Strong positive immunohistochemical staining reaction in mammary carcinoma that served as positive control. Endometrioid adenocarcinoma grade 1 expressed MTA3 with moderate to strong intensity, similar to grade 2 adenocarcinomas **(B, C)**. However, endometrioid adenocarcinoma grade 3 showed minimal to no expression of MTA3 **(D)**. A, B, x 250; C, D, x 400

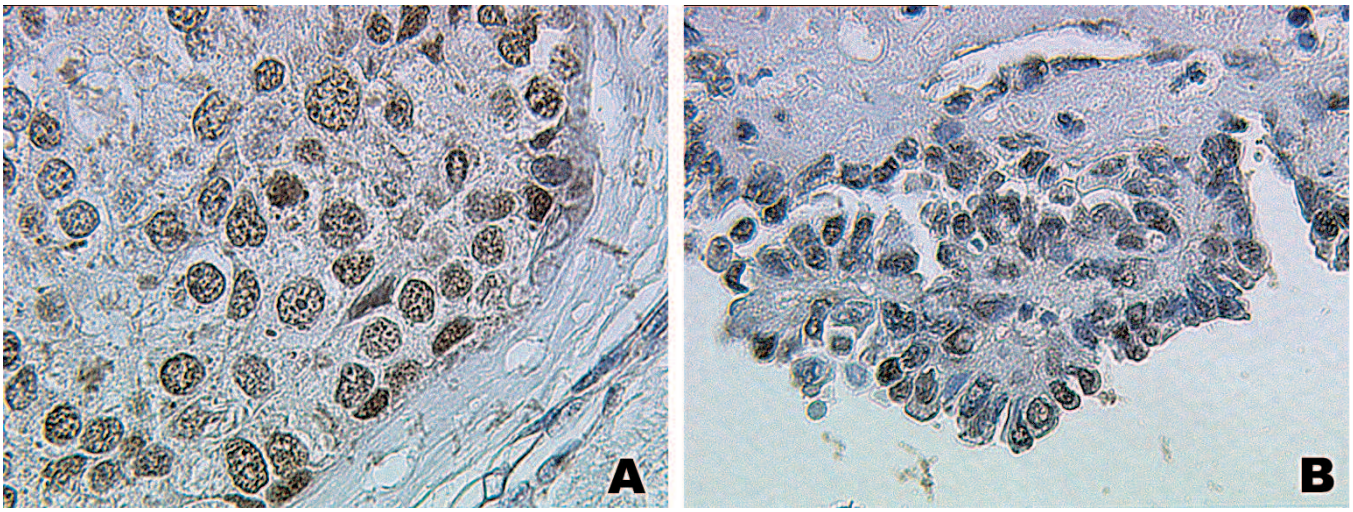

Fig. 2. Immunohistochemical staining reaction of ER-α and ER-ß in endometrioid adenocarcinomas. Positive immunohistochemical nuclear staining reaction in endometrioid adenocarcinoma for ER-α was observed **(A)**. ER-ß however demonstrated minimal to no expression in endometrioid adenocarcinoma **(B)**. x 400

Table 3. Univariate statistical analysis for positive MTA3, ER-α and ER-ß according to various clinicopathological features.

			MTA 3			ER-α			ER-ß		
		Total	negative	positive	P (χ^2)	negative	positive	P (χ^2)	negative	positive	P (χ^2)
Age	≤ 65 years	98 (49%)	53 (54.1%)	45 (45.9%)	N.S.	57 (58.2%)	41 (41.8%)	N.S.	86 (87.8%)	12 (12.2%)	N.S.
	> 65 years	102 (51%)	59 (57.8%)	43 (42.2%)		56 (54.9%)	46 (45.1%)		88 (86.3%)	14 (13.7%)	
FIGO	FIGO I and II	175 (87.5%)	97 (55.4%)	78 (44.6%)	N.S.	96 (54.9%)	79 (45.1%)	N.S.	153 (87.4%)	22 (12.6%)	N.S.
	FIGO III and IV	25 (12.5%)	15 (60%)	10 (40%)		17 (68%)	8 (32%)		21 (84%)	4 (16%)	
Grading (WHO)	Grade 1 and 2	176 (88%)	92 (52.3%)	84 (47.7%)	0.004	95 (54%)	81 (46%)	0.077	153 (86.9%)	23 (13.1%)	N.S.
	Grade 3	24 (12%)	20 (83.3%)	4 (16.7%)		18 (75%)	6 (25%)		21 (87.5%)	3 (12.5%)	
LN status	negative	133 (66.5%)	73 (54.9%)	60 (45.1%)	N.S.	72 (54.1%)	61 (45.9%)	N.S.	116 (87.2%)	17 (12.8%)	N.S.
	positive	9 (4.5%)	6 (66.7%)	3 (33.3%)		7 (77.8%)	2 (22.2%)		8 (88.9%)	1 (11.1%)	
	unknown	58 (29%)	33 (56.9%)	25 (43.1%)		34 (58.6%)	24 (41.4%)		50 (86.2%)	8 (13.8%)	
LVSI	negative	183 (91.5%)	101 (55.2%)	82 (44.8%)	N.S.	103 (56.3%)	80 (43.7%)	N.S.	158 (86.3%)	25 (13.7%)	N.S.
	positive	17 (8.5%)	11 (64.7%)	6 (35.3%)		10 (58.8%)	7 (41.2%)		16 (94.1%)	1 (5.9%)	
Myometrial invasion	< 50%	135 (67.5%)	75 (55.6%)	60 (44.4%)	N.S.	74 (54.8%)	61 (45.2%)	N.S.	117 (86.7%)	18 (13.3%)	N.S.
	> 50%	65 (32.5%)	37 (56.9%)	28 (43.1%)		39 (60%)	26 (40%)		57 (87.7%)	8 (12.3%)	
Cervical Invasion	negative	177 (88.5%)	99 (55.9%)	78 (44.1%)	N.S.	100 (56.5%)	77 (43.5%)	N.S.	154 (87%)	23 (13%)	N.S.
	positive	23 (11.5%)	13 (56.5%)	10 (43.5%)		13 (56.5%)	10 (43.5%)		20 (87%)	3 (13%)	
Ovarial invasion	negative	185 (92.5%)	103 (55.7%)	82 (44.3%)	N.S.	103 (55.7%)	82 (44.3%)	N.S.	163 (88.1%)	22 (11.9%)	N.S.
	positive	15 (7.5%)	9 (60%)	6 (40%)		10 (66.7%)	5 (33.3%)		11 (73.3%)	4 (26.7%)	
Adipositas	negative	125 (62.8%)	70 (56%)	55 (44%)	N.S.	71 (56.8%)	54 (43.2%)	N.S.	105 (84%)	20 (16%)	N.S.
	positive	74 (37.2%)	41 (55.4%)	33 (44.6%)		41 (55.4%)	33 (44.6%)		68 (91.9%)	6 (8.1%)	
Diabetes	negative	174 (87%)	94 (54%)	80 (46%)	N.S.	95 (54.6%)	79 (45.4%)	N.S.	153 (87.9%)	21 (12.1%)	N.S.
	positive	26 (13%)	18 (69.2%)	8 (30.8%)		18 (69.2%)	8 (30.8%)		21 (80.8%)	5 (19.2%)	
Hypertension	negative	119 (59.5%)	67 (56.3%)	52 (43.7%)	N.S.	65 (54.6%)	54 (45.4%)	N.S.	103 (86.6%)	16 (13.4%)	N.S.
	positive	81 (40.5%)	45 (55.6%)	36 (44.4%)		48 (59.3%)	33 (40.7%)		71 (87.7%)	10 (12.3%)	
Radiotherapy	negative	127 (63.5%)	69 (54.3%)	58 (45.7%)	N.S.	68 (53.5%)	59 (46.5%)	N.S.	111 (87.4%)	16 (12.6%)	N.S.
	positive	73 (36.5%)	43 (58.9%)	30 (41.1%)		45 (61.6%)	28 (38.4%)		63 (86.3%)	10 (13.7%)	
Anti-hormone therapy	negative	193 (96.5%)	109 (56.5%)	84 (43.5%)	N.S.	109 (56.5%)	84 (43.5%)	N.S.	167 (86.5%)	26 (13.5%)	N.S.
	positive	7 (3.5%)	3 (42.9%)	4 (57.1%)		4 (57.1%)	3 (42.9%)		7 (100%)	0 (0%)	

N.S.: not significant; LVSI: lymphovascular space invasion.

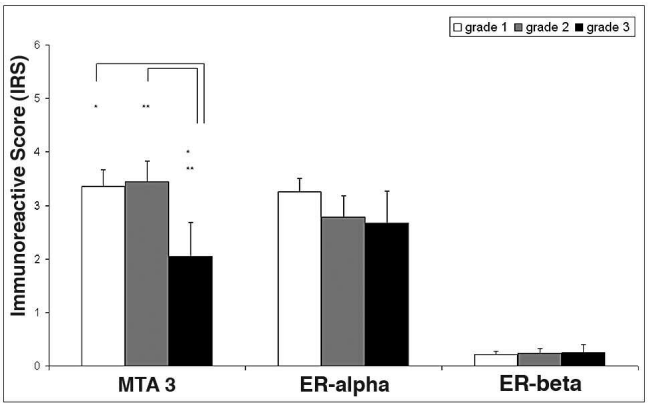

Fig. 3. Immunohistochemical expression analysis of MTA3, ER-α and ER-β in endometrial cancer depending on pathological differentiation. The IRS scores determined for MTA3 expression in endometrial cancer were related to the histological grading and plotted as mean +/-SEM. Statistical significant difference: *: p=0.033; **: p=0.006.

Table 4. Correlation of the immunohistochemical score of MTA3, ER-α and ER-β in human endometrioid adenocarcinomas.

	MTA 3 (IRS)	ER A (IRS)	ER-beta (IRS)
MTA 3 (IRS)			
Correlation Coefficient		-.041	.041
Significance		N.S.	N.S.
ER-α (IRS)			
Correlation Coefficient	-.041		-.065
Significance	N.S.		N.S.
ER-β (IRS)			
Correlation Coefficient	.041	-.065	
Significance	N.S.	N.S.	

N.S.: not significant.

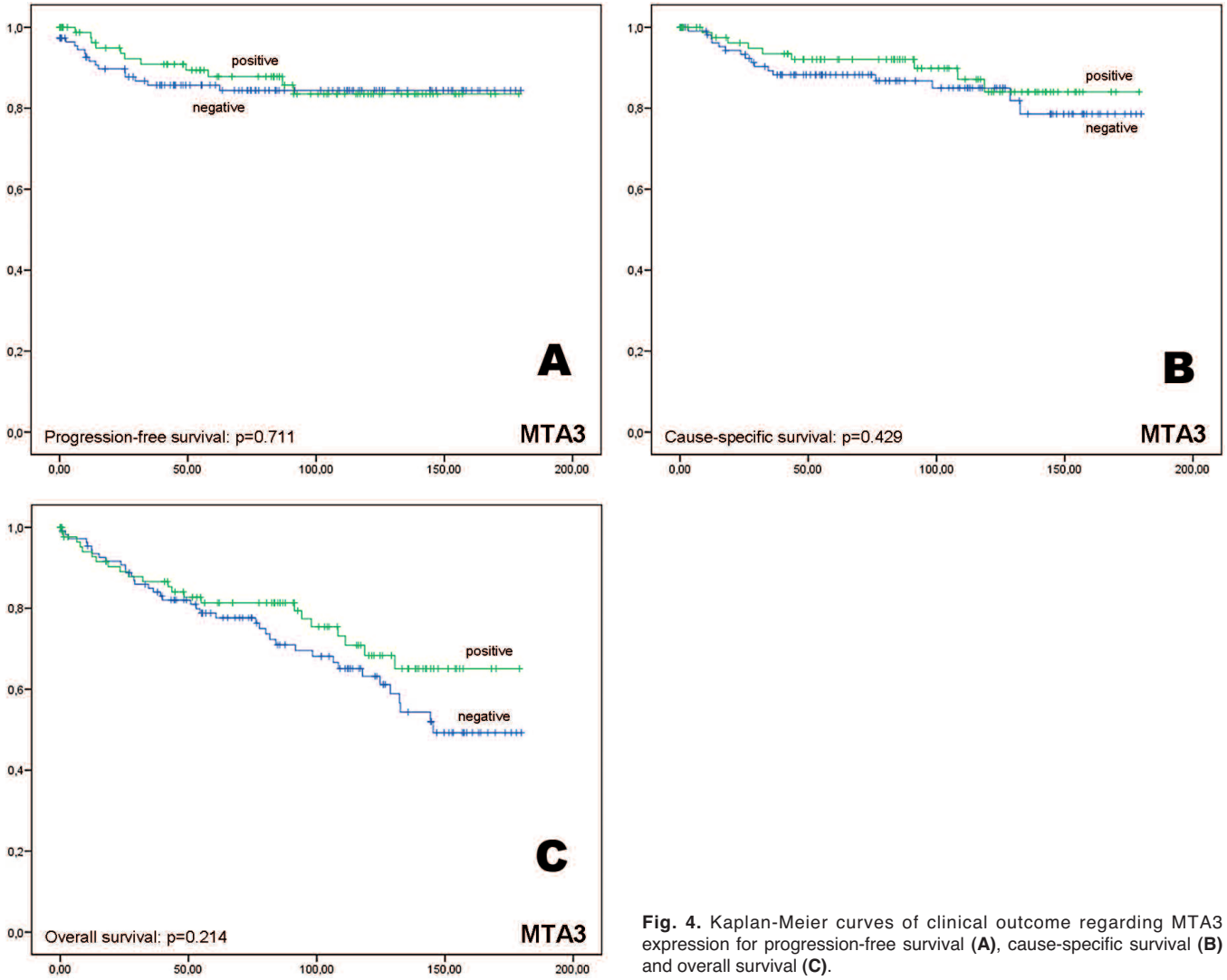

Fig. 4. Kaplan-Meier curves of clinical outcome regarding MTA3 expression for progression-free survival **(A)**, cause-specific survival **(B)** and overall survival **(C)**.

Table 5. Hazard ratios by multivariate Cox regression analysis.

	Progression-free Survival			Cause-Specific Survival			Overall Survival		
	RR	CI (5%-95%)	p	RR	CI (5%-95%)	p	RR	CI (5%-95%)	p
age (>65years)							3.195	1.703-5.996	<0.001
WHO Grading (G1/G2 vs. G3)	2.676	1.023-7.00	0.045				2.086	1.089-3.997	0.027
FIGO stage (I/II vs. III/IV)	7.544	3.236-17.589	<0.001	2.955	1.125-7.763	0.028	3.044	1.623-5.692	<0.001
cervical invasion (pos. vs. neg.)	2.912	1.212-7.00	0.017	4.037	1.579-10.321	0.004	2.756	1.419-5.351	0.003
myometrial invasion (>50% vs. <50%)				3.464	1.336-8.981	0.011			
LN status (pos. vs. neg.)				1.615	1.036-2.519	0.035	1.623	1.703-2.139	0.001

time of diagnosis was 66.44 years (range, 36.18-89.35 years). 160 (80%) and 15 (7.5%) patients were diagnosed in FIGO stage I and II, respectively, while 18 (9%) patients had FIGO stage III and 7 patients (3.5%) presented with metastatic disease (FIGO IV). Lymph node sampling or dissection was generally performed in patients having tumours with deep myometrial invasion and/or high-grade or aggressive histological features. Pelvic and/or para-aortic lymph node sampling was performed for 142 patients (71%) while 9 patients (4.5%) demonstrated lymph node metastasis. A low FIGO stage (FIGO Ia), obesity, advanced age and excessive comorbidity were factors against a full surgical staging in 58 patients (29%). Obesity was observed in 74 (37.2%) cases, while 81 (40.5%) and 26 (13%) patients presented with high blood pressure and diabetes respectively. Of the analyzed 200 patients, 73 patients (36.5%) received radiation therapy, while seven patients (3.5%) received anti-hormone therapy. Tumour progression was observed in 27 patients (13.5%), and 61 patients (30.5%) died during the follow-up interval, of whom 25 patients (12.5%) died of their cancer disease.

Expression of MTA3, ER-α and ER-ß in human endometrioid adenocarcinomas

The specificity of the MTA antibodies has previously been confirmed by us on ovarian cancer (Dannenmann et al., 2008) and placental tissues (Brüning et al., 2009). Positive MTA3 immunostaining was observed in 88 (44%) of 200 endometrial carcinoma samples respectively. Immunohistochemical staining reaction for MTA3 demonstrated immunostaining in the nuclei of malignant cells (Fig. 1a-d). Overall, endometrioid adenocarcinomas of histological grades 3 differentiation demonstrated a lower expression of MTA3 (Fig. 1d). Additionally, when using ER-α and ER-ß antibodies, 87 (43.5%) and 26 (13%) patients demonstrated a positive immunohistochemical staining reaction in the nuclei of malignant cells (Fig. 2a-b).

A significant decrease was noted from endometrioid adenocarcinomas grade 1 to grade 3 (p=0.033), as well as from grade 2 to grade 3 (p=0.006), while no significant differences could be observed between grade 1 and grade 2 endometrioid adenocarcinomas (Fig. 3). No significant difference in the MTA3 staining reaction was found among the various analysed clinicopathological characteristics, with the exception of grading in the univariate analysis (χ^2) (p=0.012) (Table 3). Additionally, ER-α and ER-ß also demonstrated no significant difference among the various analysed clinicopathological characteristics, with an tendency to significance for ER-α and grading in the univariate analysis (χ^2) (p=0.077) (Table 3). Moreover, no correlation was demonstrated between MTA3, ER-α and ER-ß expression (Spearman test: p>0.05 each) (Table 4).

Survival analysis

Univariate survival analysis with the Kaplan-Maier test revealed no significant differences of the MTA3 immunohistochemical staining reaction for progression-free survival, cause-specific survival and overall survival (Fig. 4a-c).

Prognostic factors were also analyzed by the multivariate Cox proportional-hazard model. Forward stepwise elimination according to Cox regression results led to a model containing three independent terms that were predictive of progression-free survival: WHO grading (p=0.045, FIGO stage (p<0.001), cervical invasion (p=0.017). Independent prognostic factors for cause-specific survival were FIGO stage (p=0.028), cervical invasion (p=0.004), myometrial invasion (p=0.011) and lymph node involvement (p=0.035). Overall survival was influenced by age (p<0.001), FIGO stage (p<0.001), tumour grade (p=0.027), cervical invasion (p=0.003) and lymph node involvement (p=0.001) (Table 5).

Discussion

Endometrial cancer is the most frequent gynaecologic malignancy in the Western World with several established prognostic factors, such as histological type, histological grade, surgical stage, pelvic lymph node involvement and myometrial invasion (Abeler and Kjorstad, 1991; Rose, 1996; Prat, 2004; Amant et al., 2005; Chan et al., 2007). However, although more than 50% of patients with endometrial carcinomas are diagnosed with FIGO stage I, as many as 20% die as a result of their disease (Jereczek-Fossa et al., 1999). This unusual situation might reflect that the

currently used diagnostic technology is insufficient to identify endometrial cancer patients with poor prognosis. Therefore, immunohistochemistry of different specific markers might be an interesting alternative to select high risk patients, leading to a more patient-specific risk profile and treatment (Oreskovic et al., 2004; Jeon et al., 2006; Shabani et al., 2007a; Jongen et al., 2009).

Because MTA3 has been shown to play an important function in invasion and metastasis of human cancer cells, the aim of our study was to investigate the expression of this protein in endometrial adenocarcinomas. We here demonstrate for the first time that MTA3 expression is reduced in endometrial adenocarcinomas of poor histological differentiation. Moreover, no association between estrogen receptors and MTA3 expression could be observed in endometrioid adenocarcinomas. Additionally, MTA3 expression was significantly associated with histological grading, although the expression of this nuclear transcriptional factor did not affect survival. Moreover, MTA3, as well as ER-α and ER-ß, did not constitute an independent prognostic factor in this study.

The function and role of MTA3 in human cancer cells, and in particular in endometrial cancer, is as yet unclear. MTA3 is part of a transcriptional regulation network and acts as a repressor of SNAIL (Fujita et al., 2003), which is associated with a lower overall survival of ovarian cancer patients (Blechschmidt et al., 2008). Moreover, the SNAIL-expressing Ishikawa estrogen receptor negative endometrial carcinoma-cell line showed a higher migration potential than Ishikawa estrogen receptor positive cell line with SNAIL expression level (Blechschmidt et al., 2007), linking SNAIL, and indirectly MTA3, to estrogen receptors in human endometrium. Moreover, MTA3 expression in breast cancer cells is enhanced by estrogen receptor activity (Fujita et al., 2003), establishing an association of MTA3 and estrogen receptor and thus to invasion and metastasis. Since estrogen receptors play several substantial roles in human diseases, including normal and pathological human endometrium (Herynk and Fuqua, 2004; Mylonas et al., 2004; Deroo and Korach, 2006; Leader et al., 2006; Shabani et al., 2007a), it might be possible that MTA3 also has important roles during endometrial carcinogenesis, especially within the view that MTA1/MTA3/SNAIL and E-cadherin are part of a transcriptional regulation network. However, we could not observe any correlation between MTA3 and ER-α and ER-ß expression, implicating that the MTA3 regulation might be independent of steroid receptors in endometrial endometrioid adenocarcinomas. Interestingly, four analysed endometrial cell lines demonstrated expression of the MTA3 protein, although no correlation with ER-α could be observed (Blechschmidt et al., 2007). Therefore, an as yet unknown regulation mechanism of MTA3 might be suggested in this tumour identity.

MTA3 expression is reduced in endometrial adenocarcinomas of poor histological differentiation, suggesting an important function in human endometrial malignant transformation. Although histological grading constitutes an important prognostic factor in endometrial cancer patients (Prat, 2004; Amant et al., 2005), the expression of MTA3 did not affect survival. Moreover, MTA3 did not constitute an independent prognostic factor in this study, suggesting that MTA3 is not a useful marker to assess and identify high-risk patients with endometrial adenocarcinomas.

The exact target genes of MTA3 in endometrial cancer remain unclear, and it can only be speculated that MTA3 is involved in the regulation of similar transcription clusters, as recently shown for other human cancer cells (Manavathi et al., 2007; Dannenmann et al., 2008). Known target genes comprise the nuclear transcription factors SNAIL and SLUG (Dannenmann et al., 2008), known to be overexpressed in human carcinoma cells (Castro Alves et al., 2007). MTA3 acts as a repressor of SNAIL, a transcriptional repressor of E-cadherin (Fujita et al., 2003) and thus links the expression of nuclear MTA3 to the expression of the metastasis-relevant cell adhesion protein E-cadherin (Beavon, 2000). However, additional gene silencing mechanisms for MTA3 are also possible, as observed for example for E-cadherin, as well as other tumour suppressor proteins and the estrogen receptor, which can be silenced by promoter hypermethylation (Auerkari, 2006; Giacinti et al., 2006). However, if and to what extent MTA3 modulates carcinogenesis in endometrioid cancer is still unclear and warrants further research.

In summary, our observations indicate that MTA3 is expressed in endometrioid adenocarcinomas of human endometrial cancer and becomes downregulated in poorly differentiated carcinomas, predisposing this cell type to be of high metastatic potential after malignant transformation. Although histological grading constitutes an important prognostic factor in endometrial cancer patients, the expression of MTA3 did not affect progression-free, cause-specific and overall survival. Moreover, MTA3 did not constitute an independent prognostic factor in this study, suggesting that MTA3 is not a useful marker to assess and identify high-risk patients with endometrial adenocarcinomas.

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