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The Role of Metastasis-Associated Protein 1 in Prostate Cancer Progression

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Abstract

Distinguishing aggressive prostate cancer from indolent disease represents an important clinical challenge, as current therapy requires over treating men with prostate cancer to prevent the progression of a few cases. Expression of the metastasis-associated protein 1 (MTA1) has previously been found to be associated with progression to the metastatic state in various cancers. Analyzing DNA microarray data, we found MTA1 to be selectively overexpressed in metastatic prostate cancer compared with clinically localized prostate cancer and benign prostate tissue. These results were validated by demonstrating overexpression of MTA1 in metastatic prostate cancer by immunoblot analysis. MTA1 protein expression was evaluated by immunohistochemistry in a broad spectrum of prostate tumors with tissue microarrays containing 1940 tissue cores from 300 cases. Metastatic prostate cancer demonstrated significantly higher mean MTA1 protein expression intensity (score = 3.4/4) and percentage of tissue cores staining positive for MTA1 (83%) compared with clinically localized prostate cancer (score = 2.8/4, 63% positive cores) or benign prostate tissue (score = 1.5/4, 25% positive cores) with a mean difference of 0.54 and 1.84, respectively ($P < 0.00001$ for both). Paradoxically, for localized disease, higher MTA1 protein expression was associated with lower rates of prostate specific antigen recurrence after radical prostatectomy for localized disease. In summary, this study identified an association of MTA1 expression and prostate cancer progression.

Introduction

Although effective surgical and radiation treatment exist for clinically localized prostate cancer, hormone refractory metastatic prostate cancer remains incurable. Distinct sets of genes and proteins dictate the progression from precursor lesions to localized disease and finally to metastatic disease (1). Identifying and characterizing key genes that regulate the metastatic ability of prostate cancer may help identify which tumors are on an aggressive path from the outset and treat them adequately before the development of metastases.

Through gene expression profiling studies, we have recently identified metastasis-associated gene 1 (*MTA1*), a gene involved in the transcriptional silencing machinery of mammalian cells, to be significantly overexpressed in metastatic prostate cancer when compared with clinically localized disease (2). *MTA1* was first identified by differential cDNA library screening of metastatic breast cancer cell lines (3) and confirmed in breast cancer tissue (4). *MTA1* overexpression at the transcript level was also observed in other cancers like gastrointestinal cancers and associated with tumor invasiveness and metastasis (5, 6). Also, inhibition of MTA1 protein expression resulted in growth inhibition of cancer cell lines (7). In the current study, we characterize for the first time the role of *MTA1* in prostate cancer progression. Expression array data were used to determine *MTA1* expression levels in a large number of samples containing benign prostate tissue, clinically localized and metastatic prostate cancer. These observations are validated by Western blot analysis in frozen tissue samples and in a wide range of prostate tissues with immunohistochemistry using tissue micoarrays (TMAs). In addition, associations of MTA1 expression and indicators of poor prognosis were explored to test the potential use of MTA1 as a biopsy biomarker.

Materials and Methods

Patient Population and Tissue Collection. Prostate tissue samples were taken from the radical prostatectomy series and the rapid autopsy program at the University of Michigan Prostate Cancer Specialized Program of Research Excellence Tissue Core with Institutional Review Board approval. Clinically localized prostate cancer samples were taken from a cohort of men who underwent radical retropubic prostatectomy as a monotherapy (*i.e.*, no hormonal or radiation therapy) between January 1995 and December 2001. Tumors were staged using the Tumor-Node-Metastasis system (8) and graded according to the system described by Gleason (9). The median age at time of surgery was 60.4 years (range, 40–84 years) with a median presurgical prostate-specific antigen (PSA) level of 13.5 ng/ml (range, 0.5–43.3 ng/ml), and Gleason scores ranged from 4–10. Snap frozen samples used for cDNA expression array and immunoblot analysis were all evaluated by the study pathologist. All samples were trimmed to ensure >95% of the sample used represented the desired lesion. Areas of benign prostate tissue from prostates with prostate cancer were used as normal adjacent tissue in these experiments. Metastatic prostate cancer samples from 15 autopsy cases performed from 1997 to 2000 were also collected from the rapid ("warm") autopsy program (10). The patient's ages ranged from 40 to 84 years with a median age of 67.5 years. In total, the TMAs contained tissue from 300 patients with benign prostate tissue, tissue containing proliferative inflammatory atrophy (PIA), high-grade prostatic intraepithelial neoplasia (PIN), clinically localized, and metastatic prostate cancer. A subset of 114 patients with clinically localized prostate cancer, as determined by pretreatment parameters, was used to evaluate for associations between MTA1 expression and parameters of poor prognosis.

Immunoblot Analysis. Protein extracts were prepared from normal and prostate cancer tissues using NP40 lysis buffer containing 50 mM Tris-HCl (pH 7.4), 1% NP40, and mixture of protease inhibitors (Roche Applied Science, Indianapolis, IN). Fifteen μg of proteins were boiled in sample buffer, separated by SDS-PAGE, and transferred onto nitrocellulose membrane. After incubation in blocking buffer [Tris-buffered saline with 0.1% Tween (TBS-T) and 5% nonfat dry milk] for 1 h, the membrane was incubated overnight at 4°C with anti-MTA-1 mouse monoclonal antibody [anti-MTA1 monoclonal (A11),

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sc-17773; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:50 in blocking buffer. The secondary antibody was horseradish peroxidase conjugated, and the signals were visualized by enhanced chemiluminescence system as described by the manufacturer (Amersham Biosciences, San Francisco, CA). The blot was reprobed with glyceraldehyde-3-phosphate dehydrogenase to confirm equal loading of the different tissue samples. Ratios of MTA1 to glyceraldehyde-3-phosphate dehydrogenase were calculated to normalize the samples.

Immunohistochemistry. Sections of 4-μm thick paraffin-embedded TMAs were dewaxed and rehydrated with xylene and ethanol. After immersion in 10 mM citrate buffer (pH 6.0), the slides underwent microwave pretreatment for 10 min for optimal antigen retrieval. The primary antibody against MTA1 [anti-MTA1 monoclonal (A11), sc-17773; Santa Cruz Biotechnology] was incubated overnight in a 1:5 dilution at 4°C. The secondary antibody was biotin labeled and was applied for 30 min. Streptavidin-LSA amplification method (DAKO K0679) was carried out for 30 min followed by peroxidase/diaminobenzidine substrate/Chromagen. The slides were counterstained with hematoxylin. Nuclear protein expression was determined by the study pathologist, and immunohistochemistry was scored as negative (score = 1), weak (score = 2), moderate (score = 3), or strong (score = 4) using a system that has been validated previously (2, 11–14).

Statistical Analysis. Pertinent clinical information about patients (clinical stage, pretreatment PSA, tumor stage, surgical margin status, and Gleason score) was prospectively collected and stored in the TMA database. Clinical postprostatectomy follow-up was also ascertained and stored prospectively in this database, including an annual patient assessment by clinic visit, phone, or mail contact to ascertain overall, cancer-specific, and PSA recurrence-free survival. All patients undergo annual serum PSA testing. A PSA level of >0.2 ng/ml is considered biochemical evidence of micrometastatic recurrence or progression. The association of clinical, pathology, and TMA parameters with recurrence-free survival was first evaluated by bivariate (univariate) analysis. The relationship between preoperative variables and recurrence-free survival were then examined using Cox proportional-hazard regression models. For each outcome, a backward model selection procedure was used to choose the most parsimonious model. All decisions were made using a 0.05 significance level, and all analyses were run using SPSS 11.0.1 (SPSS, Inc., Chicago, IL). PSA recurrence-free survival curves were established using Kaplan-Meier analysis.

Results

MTA1 Transcript and Protein Expression Are Elevated in Hormone Refractory Metastatic Prostate Cancer. In our previous work to identify key genes involved in prostate cancer progression (2), we reported that MTA1 transcript was significantly up-regulated in metastatic prostate cancer as compared with clinically localized prostate cancer or benign prostatic tissue. Fig. 1, *A* and *B*, demonstrates the results of previous significance analysis of microarrays (SAM) analysis of cDNA microarray data comparing 7 benign prostate tissues, 9 clinically localized, and 6 metastatic prostate cancer samples (2) showing a heatmap, demonstrating genes that most highly distinguish metastatic from clinically localized prostate cancer tissue. Expression levels are with respect to the reference pool of benign prostate tissues as described previously (14). *MTA1* is selectively over expressed in metastatic prostate cancer compared with clinically localized prostate cancer and benign prostate tissue with a 1.7-fold increase (d-score = 2.5, q-value 0.81). Other genes sharing this trend included the previously described *EZH2* gene (2-fold increase, d-score = 4.6, q-value = 0.12) and *TRAF2* (1.6-fold increase, d-score = 2.8, q-value = 0.52), a central regulator of cellular response to stress and cytokines, as well as *PNUTL1* (2.1-fold increase, d-score = 2.86, q-value = 0.53), a human septin gene.

To confirm that MTA1 was elevated at the protein level in metastatic prostate cancer, we analyzed normal prostate, clinically localized prostate cancer, and metastatic prostate cancer frozen tissue extracts by semiquantitative Western blot analysis using glyceraldehyde-3-phosphate dehydrogenase expression as reference. Consistent with the transcript data, we observed an increase in MTA1 protein expression in metastatic and clinically localized prostate cancer samples as compared with benign prostate tissue (Fig. 1*C*). To characterize MTA expression *in situ*, we evaluated a large spectrum of prostate tissue by immunohistochemistry using 7 TMAs, allowing to analyze 1940 prostate TMA samples from >300 patients. We detected MTA1 protein expression confined to the cell nucleus (Fig. 1*D*). The staining intensity was scored using a 4-tier system [*e.g.*, 1 (absent), 2 (weak), 3 (moderate), or 4 (strong)]. The strongest MTA1 staining intensity was observed in metastatic prostate cancer samples. Metastatic prostate cancer had a significantly higher mean MTA1 protein expression intensity [score = 3.4 (SE = 0.04)] when compared with clinically localized prostate cancer [score = 2.8 (SE = 0.04)] or benign prostate tissue [Score = 1.5 (SE = 0.05)] with a mean difference of 0.54 (SE = 0.055) and 1.84 (SE = 0.066), respectively (ANOVA posthoc Scheffé, $P < 0.00001$ for both). Interestingly, increased expression of MTA1 protein was detected in prostate cancer precursor lesions. There was no significant difference in MTA1 expression between high-grade PIN and clinically localized prostate cancer (mean difference 0.4, SE = 0.2, ANOVA posthoc Scheffé, $P = 0.4$). PIA, a putative prostate cancer precursor lesion (15–17), had a mean score of 1.8, demonstrating MTA1 protein expression between benign prostate tissue and high-grade PIN with differences of 0.3 (SE = 0.1, ANOVA posthoc Scheffé, $P = 0.4$) and 0.6 (SE = 0.2, ANOVA posthoc Scheffé, $P = 0.082$), respectively. These results are consistent with the cDNA expression array data and show up-regulation of MTA1 most strikingly in metastatic prostate cancer (Table 1).

In addition to analyzing the mean staining intensity in each tissue type, we performed an analysis of our data measuring the frequency of MTA1 expression by determining the percentage of MTA1 positive cores in each tissue type. This analysis shows similar results: in metastatic prostate cancer, 83% of the cores expressed MTA1. In clinically localized prostate cancer, 63% of cores expressed MTA1, and in high-grade PIN, 48% were positive. In PIA, 20% of the tissue cores showed MTA1 expression, and only 15% of benign tissue cores were MTA1 positive. This analysis shows that MTA1 is expressed most in metastatic prostate cancer, followed by clinically localized prostate cancer, and high-grade PIN. In benign prostate tissue, MTA1 expression was detected rarely.

To assess the possibility of heterogeneous MTA1 expression, we analyzed clinically localized prostate cancer cases ($n = 49$), each having a minimum of 5 but as many as 11 tissue cores. Absent and weak staining intensity was counted as negative and moderate and strong staining intensity as positive for MTA1 expression. Perfect agreement of the staining intensity among the cores was seen in 37% (18 of 49 cases), an agreement of 80–99% was seen in 32% of cases (16 of 49), and an agreement of 60–79% was seen in 22% (11 of 49). Agreement of <60% was seen in just 8% (4 of 49) cases. These results show a homogeneous MTA1 expression in prostate cancer as the large majority of cases (69%) showed a >80% agreement of either a presence or absence of MTA1 protein expression.

MTA1 Protein Expression Is Inversely Associated with Prostate Cancer Recurrence after Radical Prostatectomy for Clinically Localized Disease. To determine whether MTA1 expression might be a useful biomarker to help distinguish clinically indolent tumors from more aggressive prostate cancer, we looked for associations between MTA1 protein expression and clinicopathological parameters in a subset of the patients included in the TMA experiment. All patients were treated with surgery for clinically localized prostate cancer. We excluded patients with lymph node metastasis. The remaining 108 patients had a PSA failure rate of 20% (21 of 108) as of March 1, 2003. These patients were representative for the total cohort. Patient demographics are presented in Table 2.

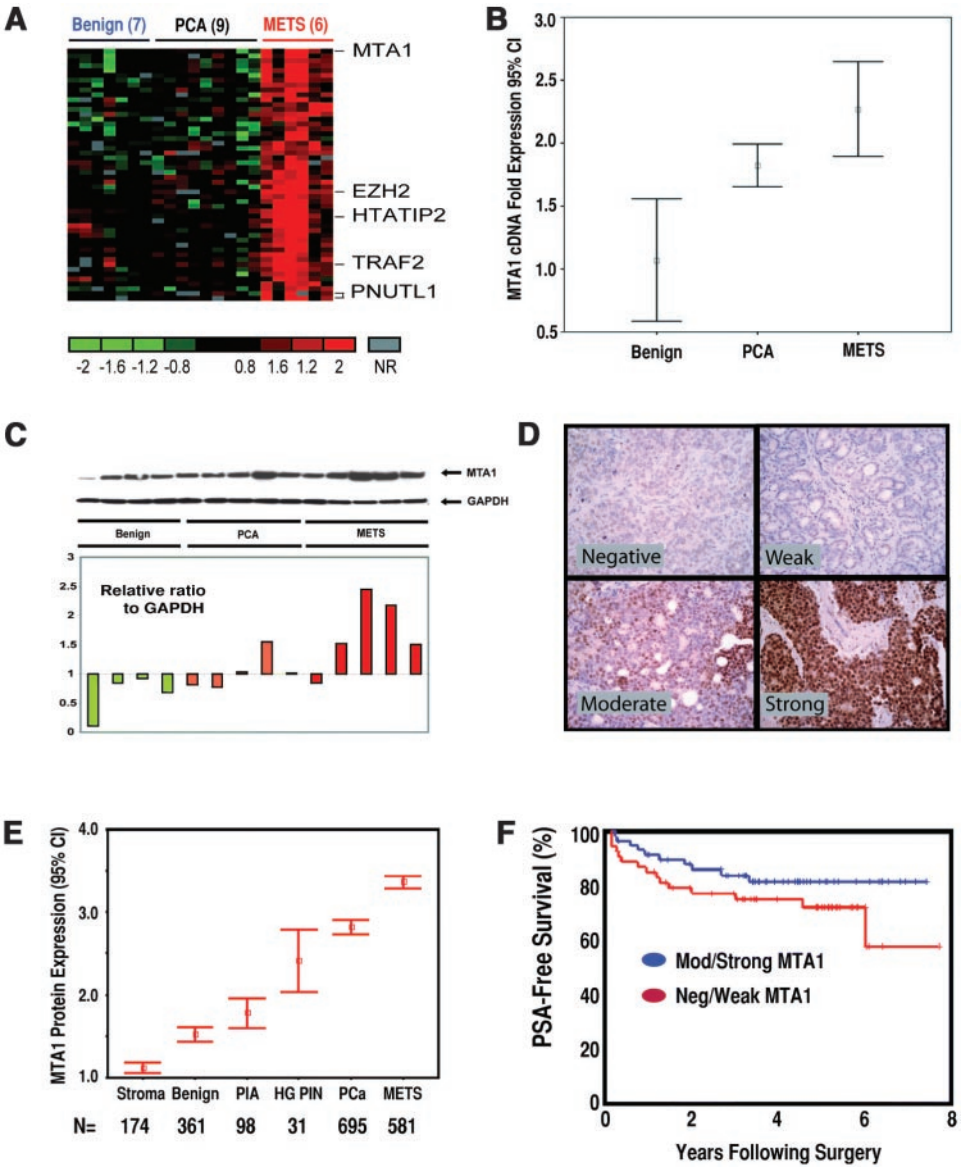

Fig. 1. *A*, heatmap of the cDNA microarray data for benign prostatic tissue (Benign), localized prostate cancer (PCA), and metastatic prostate cancer (METS) samples [modified from Varambally *et al.* (2)]. Metastasis-associated protein 1 (MTA1) was among several genes up-regulated in METS when compared with PCA or benign prostate tissue. *B*, *error bars* with 95% confidence intervals (CIs) demonstrating the expression level of MTA1 in benign prostatic tissue, PCA, and METS cases. The level of MTA1 expression in PCA was significantly increased compared with benign prostate tissue. METS showed the highest level of MTA1 expression. *C*, Western blot analysis demonstrating MTA1 protein expression. Fifteen μg of protein extract of normal, PCA, and METS tissue was separated in a 10% polyacrylamide gel. Incubation with anti-MTA1 antibody 1:50 overnight at 4°C. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to demonstrate equal protein loading. The *bar graph* shows normalized expression as MTA1/GAPDH ratio. Normal samples showed only little MTA1 expression, whereas the majority of localized prostate cancer and almost all of the metastatic prostate cancer samples have a strong increase in MTA1 expression. *D*, representative images of tissue microarray samples staining absent, weak, moderate, and strong for MTA1 expression. MTA1 expression was confined to the nuclei of cells as demonstrated by immunohistochemistry (×200 magnification). *E*, *error bars* with 95% CIs demonstrating MTA1 protein expression levels in a wide spectrum of prostate tissue samples. MTA1 staining intensity was evaluated along a scale ranging from negative expression (score = 1) to strong staining (score = 4). *F*, Kaplan-Meier analyses of MTA1 status and prostate-specific antigen (PSA)-defined recurrence-free survival after radical prostatectomy for clinically localized prostate cancer. Biochemical failure was defined as a PSA elevation > 0.2 ng/ml after radical prostatectomy. Negative MTA1 expression was associated with a 2.8 relative risk (95% CI: 1.2–6.7, $P = 0.02$) of PSA recurrence as compared with moderate/strong MTA1 expression.

Multiple TMA samples were available for each patient (>4/patient on average) and allowed us to explore for the strongest possible associations in the effort to develop the most robust statistical model as we have described previously (11). Specifically, we examined models that took greatest staining intensity, median staining intensity, and mean staining intensity. The remaining analysis is based on the strongest staining intensity (Table 3). As expected, preoperative PSA level, tumor dimension, tumor stage 3 (American Joint Committee on Cancer), extraprostatic extension, seminal vesicle invasion, and positive surgical margins status were all significantly associated with PSA failure (Table 3). Gleason score failed to significantly predict PSA recurrence in this cohort. Surprisingly, the univariate analysis revealed that higher MTA1 expression was associated with a longer PSA-free survival time after radical prostatectomy. Negative MTA1 expression (absent and weak MTA1 expression level) was associated with a 2.8 relative risk [95% confidence interval (CI): 1.2–6.7, $P = 0.02$) for developing PSA recurrence. These results are presented graphically using Kaplan-Meier analysis (Fig. 1*F*). PSA recurrence was more likely to appear in patients with absent/weak MTA1 expression than in patients with moderate MTA1 expression. Patients with strong MTA1 expression had the best prognosis.

In multivariable analysis (Table 3), the parameters that best explain PSA failure after radical prostatectomy for clinically localized prostate cancer included decreased MTA1 expression (3.8 relative risk, 95% CI: 1.5–9.6, $P = 0.005$), preoperative PSA (1.1 RR, 95% CI: 1–1.1, $P = 0.014$), extraprostatic extension (2.1 RR, 95% CI: 1.2–3.7, $P = 0.006$), and positive surgical margins (4.1 RR, 95% CI: 2–8.1, $P < 0.00001$).

Table 1 *Frequency of metastasis-associated protein 1 protein expression in prostate tissue sample as determined by immunohistochemistry*[a]

Prostate tissue	*n*	Staining intensity 1	2	3	4	Mean intensity
Stroma	174	159 (91%)	9 (5%)	6 (3%)	0	1.12
Benign	361	248 (69%)	58 (16%)	34 (9%)	21 (6%)	1.52
PIA[a]	98	46 (47%)	32 (33%)	15 (15%)	5 (5%)	1.79
PIN	31	7 (23%)	9 (29%)	10 (32)	5 (16%)	2.42
PCa	695	145 (21%)	113 (16%)	161 (23%)	276 (40%)	2.82
Mets	581	43 (7%)	54 (9%)	134 (23%)	350 (60%)	3.36
Total	1940					2.53

[a] Because of rounding percentages may not equal 100%.
[b] PIA, proliferative inflammatory atrophy; PIN, high-grade prostatic intraepithelial neoplasia; PCa, clinically localized prostate cancer; Mets, hormone refractory metastatic prostate cancer.

Table 2 *Patient demographics of 108 men with clinically localized prostate cancer*[a]

	n	%
Median age (yrs)	59 (range, 43–80)	
Preoperative prostate-specific antigen (PSA)		
≤4	22	20
and <10	64	59
≥10	22	20
Gleason score		
≤6	40	37
7	64	59
>8	4	4
Tumor stage (American Joint Committee on Cancer)		
T_2	87	81
T_3	21	19
Surgical margin status		
Negative	87	81
Positive	29	19
Seminal vesicle invasion		
Negative	105	97
Positive	3	3
Extraprostatic extension		
Negative	87	80
Positive	21	19
PSA failure		
Yes	22	20
No	86	80
Metastasis-associated protein 1 expression		
Absent	10	9
Weak	11	10
Moderate	22	20
Strong	65	60

[a] Because of rounding percentages may not equal 100%.

We also looked at a correlation between absent MTA1 expression and clinicopathological parameters in the study cohort. Negative MTA1 expression was only correlated with PSA recurrence [Pearson correlation 0.216, $P = 0.025$ (two-tailed)]. No significant correlation between MTA1 expression level and Gleason score, preoperative PSA level, tumor dimension, extraprostatic extension, seminal vesicle invasion, positive surgical margin status, and tumor stage was found.

Discussion

The goal of this study was to characterize *MTA1* expression in a wide range of prostate cancer samples and to determine its role as a putative biomarker for prostate cancer progression. This study is significant in that *MTA1* has not been previously characterized in prostate cancer and that its potential as a biomarker has not been determined.

MTA1 is physiologically expressed at only low levels in human tissue, except the testis (3). Its expression has been found to be associated with progression in solid cancers of various organs and cancer cell lines with high invasive potential (3, 5–7, 18–20) and thus believed to play a role in cancer progression to the metastatic state. Functional studies supported this by showing that experimental overexpression of *MTA1* in keratinocytes is associated with increase in migration and invasion and survival in the anchorage-independent state (21). Interestingly, *MTA1* appeared as one of the most highly dysregulated genes in metastatic prostate cancer as compared with clinically localized prostate cancer using SAM analysis on expression array data (2). This observation initiated this current study. Studying *MTA1* expression in prostate tissue and at the largest cancer patient cohort thus far, we found it to be only weakly expressed in benign prostate tissue, whereas moderate to strong expression was observed in neoplastic prostate tissue. Furthermore, we are able to show that *MTA1* expression levels are associated with the degree of malignant potential as the level of expression increases from PIN over localized to metastatic prostate cancer. These results are consistent with previous descriptions of *MTA1* expression being associated with cancer progression (3, 5–7, 18–20). *MTA1* expression demonstrated an increasing progression moving through various stages of prostate cancer development. For example, although the total number of samples was small, MTA1 was found to be expressed at a lower rate in PIA than high-grade PIN. PIA, believed to represent one of the earliest recognizable prostate cancer precursor lesions, had higher MTA1 expression than benign prostate tissue. Also, we observed a homogeneity of MTA1 expression within tumors.

To test the clinical use of MTA1 as a biomarker in prostate cancer, we evaluated the associations between MTA1 protein expression and biochemical failure after radical prostatectomy in a group of 108 patients with localized prostate cancer. At both the univariate and multivariate level, MTA1 appears to play a protective role with regards to prostate cancer progression. Patients with clinically localized prostate cancer with high MTA1 expression levels showed an association with better outcome than patients with low MTA1 protein expression measured as PSA recurrence-free survival time.

The inverse relationship between MTA1 expression and PSA recurrence-free survival time is a perplexing result as we would have anticipated the reverse. This observation may be because of other interrelated parameters not examined in the multivariable model. Also, higher PSA levels or larger tumor size could account for early detection. However, no significant associations were detected between MTA1 expression and these or other clinical parameters. Still, other parameters not assessed could account for these results. The potential role of using MTA1 as a protective biomarker needs to be validated in a separate clinical cohort.

MTA1 is part of the nucleosome-remodeling-and-deacetylation complex that is involved in transcriptional repression (22) and is

Table 3 *Parameters associated with prostate-specific antigen recurrence after radical prostatectomy for clinically localized prostate cancer*[a]

			95% confidence interval	
	P	Relative risk	Upper bound	Lower bound
Univariate analysis				
Gleason score	0.09	1.9	0.9	4
Preoperative prostate-specific antigen (PSA)	0.03	1.1	1	1.1
Tumor dimension	0.04	1.9	1	3.4
Extraprostatic extension	<0.00001	3	1.7	5.2
Seminal vesicle invasion	0.001	7.4	2.2	25.1
Positive surgical margins	<0.00001	3.8	2.1	6.9
Tumor stage (American Joint Committee on Cancer, T_3 *versus* T_2)	0.001	2.7	1.5	4.7
Metastasis-associated protein 1 (MTA1) negative	0.02	2.8	1.2	6.7
Multivariable analysis				
Preoperative PSA	0.014	1.1	1	1.1
Extraprostatic extension	0.006	2.1	1.2	3.7
Positive surgical margins	<0.00001	4.1	2	8.1
MTA1 negative	0.005	3.8	1.5	9.6

[a] Gleason scores (GS) were divided into three categories: GS ≤ 6, GS = 7, and GS ≥ 8. Tumor stage: progression from AJCC tumor stage T_2 to AJCC tumor stage T_3. MTA1: negative staining defined as weak and absent staining.

mainly dependent on the function of two histone deacetylases, *HDAC1* and *HDAC2*. Just recently, the use of inhibitors of histone deacetylase in the drug treatment of neoplasm such as breast, bladder, and prostate cancer has been proposed (23, 24). Our cDNA microarray data did not show a significant up-regulation of histone deacetylases in MTA1 expressing prostate cancers, and a possible use of histone deacetylase inhibitors in MTA1 overexpressing prostate cancer still needs to be evaluated.

In summary, this study is the first to characterize MTA1 expression in a wide range of prostate tissue samples and suggests that MTA1 expression level may be a useful tissue biomarker.

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