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RESEARCH ARTICLE

Evaluation of deleted in malignant brain tumors 1 (*DMBT1*) gene expression in bladder carcinoma cases: preliminary study

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Abstract

This study was undertaken to evaluate the expression of *DMBT1* in bladder cancer and its correlation with clinicopathological parameters analyzed in bladder carcinoma patients. We investigated *DMBT1* in 56 paraffin embedded specimens of transitional cell carcinoma of the urinary bladder. We assessed *DMBT1* gene expression at mRNA level by RT-PCR. Our results show 100% expression of *DMBT1* in bladder carcinoma samples. Due to this preliminary results; gene expression was compared to tumor grade, and a significant difference was detected between grade 1 and 3 ($p = 0.028$). The down-regulation of *DMBT1* gene expression in carcinomas suggests the possible role in bladder cancer.

Keywords: Bladder cancer, *DMBT1* gene, gene expression, real-time online RT-PCR

Introduction

Urinary bladder cancer is among the five most common malignancies worldwide and especially one of the most prevalent cancer diseases in Western countries. Bladder cancer is the seventh most common cancer in males, and seventeenth most common in women. In industrialized countries like United States, Canada, and France; more than 90% of cases originate in the transitional epithelial cells known as TCC (transitional cell carcinoma). In developing countries, 75% of cases are squamous cell carcinomas caused by *Schistosoma haematobium* (parasitic organism) infection. Rare types of bladder cancer include small cell carcinoma, carcinosarcoma, primary lymphoma, and sarcoma (El Sebaie et al., 2005). In the United States, bladder cancer is the fourth most common cancer in men (Jemal et al., 2009), whereas incidence is lowest in Asia and South America, where it is about 70% lower than in the United States. Pathologically, >90% of bladder cancers are transitional cell carcinoma (TCC) (Huang et al., 2010). Eighty percent of the transitional cell carcinomas are confined to epithelium for initial diagnosis (pTa, pT1), and 20% of that are invaded in the muscular layer (muscularis propria-pT2-3-4) (Dinney et al., 2004).

Several factors, including chromosomal markers, genetic polymorphisms, genetic and epigenetic alterations, are involved in carcinogenesis, progression and metastasis of bladder cancer (Theodorescu, 2006). Common approaches for assessing gene and protein expressions identified in bladder carcinomas, which play an important role for prognosis. The identification of this alteration has indicated to understand the genetic mechanism in bladder carcinogenesis and progression. Consequently, bladder cancer includes multiple molecular pathologies, but some of them are still unclear. Recent studies have started to identify panels

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that have multiple markers and shows for prognosis, and researchers have also made studies on new molecular targets for identifying bladder carcinogenesis (Mitra et al., 2009).

Various chromosome alterations, including translocations, deletions, and abnormal amplifications are sources that process some malignant cancer carcinogenesis. Specific cancers have originated from loss of heterozygosity for chromosome 10 (Terada et al., 2002). Prostate cancer, pancreatic cancer, small-cell lung cancer and some brain tumors have this alteration. Recently some researchers have found a candidate tumor-suppressor gene on chromosome 10q and named Deleted in Malignant Brain Tumors 1 (*DMBT1*). It is located at chromosome 10q25.3- q26.1 (*DMBT1*; GenBank NM_004406.2) and consists of 55 exons. *DMBT1* is differentially expressed in various cancer types with majority of these displaying a downregulation. *DMBT1* encodes the scavenger receptor cysteine-rich (SRCR) superfamily that is a secreted high molecular weight glycoprotein. The protein is expressed predominantly by epithelial cells. Variants mostly play a role in the defense against bacterial and viral infections of inflammatory responses (Rosenstiel et al., 2007). Moreover, secretion of the protein to the extracellular matrix (ECM) may trigger processes of epithelial or stem cell differentiation (Mollenhauer et al., 2002; Mollenhauer et al., 1999; Tchatchou et al., 2010). *DMBT1* mutations have determined various types of cancers, and the major inactivation of this gene that affects down regulation of the transcriptional level that is found in some cancer types, play a major role for the period of early carcinogenesis (Ligtenberg et al., 2007). Some of the alterations in *DMBT1* gene are evaluated during proceeding of glioma. First study had been worked on brain tumors and in this study, lack of *DMBT1* mRNA expression, and deletion of the *DMBT1* gene has been reported (Muñoz et al., 2004; Somerville et al., 1998). Loss or reduction of *DMBT1* expression, increased expression and homozygous deletions in *DMBT1* have been demonstrated in gastric cancer, colorectal cancer, brain cancer, lung cancer and esophageal cancer (Braidotti et al., 2004; Mori et al., 1999; Wu et al., 1999). But, there is no *DMBT1* gene expression analysis experiments working with bladder cancer cases in literatures.

In the present study, we intended to probe *DMBT1* expression in the bladder cancer and how its expression could be related to carcinogenesis in bladder. And in this preliminary study, we compared the *DMBT1* expression between patients' sex, age, tumor classification and cellular degree.

Table 1. Patient characteristics and treatment outcome in 56 patients with bladder carcinomas.

Characteristic	*n* (%)
Age (year) mean±SD (range)	28–83 (65.5 ± 10.6)
Sex	
M	49 (87.5%)
F	7 (12.5%)
Tumor classification (%)	
a	14 (25%)
1	23 (41.1%)
2	19 (33.9%)
Cellular grade	
1	5 (8.9%)
2	25 (44.6%)
3	26 (46.4%)

Material and methods

The retrospective cohort comprised 56 patients with a diagnosis of transitional cell carcinoma of the bladder who were to undergo definitive transurethral resection of bladder tumor (TURBT) at Denizli Public Hospital, Department of Pathology in Turkey. Tumor samples taken at TURBT were embedded in paraffin. Among 56 transitional cell carcinomas, 14 were classified as pTa, 23 as pT1 and 19 of them were classified as pT2. In addition, 5 of them were graded as G1, 25 as G2, and 26 as G3 (Table 1). All carcinomas were staged according to the American Joint Committee on Cancer (AJCC)/International Union against Cancer (UICC) tumor-node metastasis (TNM) staging system, graded according to WHO (World Health Organization) criteria. All cases signed a written informed constant statement approved by local ethics committee.

RNA extraction

Fifty micro liters of total RNA were extracted from cases of paraffin-embedded tissue samples using High Pure RNA Paraffin Kit (Roche Applied Science, Germany), according to the manufacturers' instructions.

cDNA synthesis

Reverse transcription procedure was performed for cDNA synthesis by using Transcriptor First Strand cDNA Synthesis Kit according to the manufacturers' instructions. In the first stage, 20 µl complementary DNA (cDNA) was obtained from 10 µl of total RNA (1–5 g final concentration for each case), and in the second stage by using related primers and probes used to multiply the gene expressions of *DMBT1* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) housekeeping gene, with RT-PCR. And the relative quantification was carried out by calculating the ratio of *DMBT1* gene expression to *GADPH*.

Table 2. Primers and probes of genes.

Gene	ForwardPrimer	Reverse Primer	Probe (Roche)
DMBT1	gtccaggaaaccatctatcgac	gaagcctccgcaggaatagt	(cat. no. 04688678001)
GAPDH	gaaggtgaaggtcggagtc	gaagatggtgatgggatttc	FAM-caagcttcccgttctcagcc-TAMRA

Table 3. *GAPDH* and DMBT1 Gene expression and relative ratio of *DMBT1* gene expression in bladder cancer cases.

CASE	AGE	SEX	DEGREE	GRADE	GAPDH	DMBT 1	RR	CASE	AGE	SEX	DEGREE	GRADE	GAPDH	DMBT 1	RR
1	69	F	a	1	9.99E+03	3.34E+00	3.34E+00	29	71	M	a	2	8.16E+03	1.06E+01	1.30E+01
2	55	M	a	1	9.12E+03	4.95E+00	5.43E+00	30	73	M	a	2	8.23E+03	4.81E+01	5.84E+01
3	74	M	a	1	9.76E+03	8.39E+00	8.60E+00	31	60	M	2	3	8.97E+03	1.35E+01	1.51E+01
4	65	F	a	1	9.57E+03	8.00E+00	8.36E+00	32	72	M	1	3	9.33E+03	3.94E+00	4.22E+00
5	60	M	a	1	8.74E+03	5.36E+03	6.13E+03	33	72	M	2	3	8.79E+03	3.64E+02	4.14E+02
6	72	M	1	2	1.04E+04	1.10E+01	1.06E+01	34	79	M	2	3	9.27E+03	2.19E+02	2.36E+02
7	83	M	1	2	9.70E+03	1.83E+00	1.89E+00	35	75	M	1	3	9.42E+03	1.17E+01	1.24E+01
8	59	M	a	2	8.88E+03	1.92E+00	2.16E+00	36	54	M	1	3	9.35E+03	2.27E+00	2.43E+00
9	67	M	1	2	1.05E+04	2.52E+01	2.40E+01	37	61	F	2	3	9.11E+03	1.06E+01	1.16E+01
10	56	M	1	2	9.72E+03	3.75E+00	3.86E+00	38	61	M	2	3	9.80E+03	5.13E+01	5.23E+01
11	68	M	a	2	1.03E+04	7.81E+02	7.58E+02	39	70	M	1	3	1.05E+04	5.29E+01	5.04E+01
12	55	M	1	2	9.58E+03	8.06E+00	8.41E+00	40	73	M	2	3	1.04E+04	2.60E+02	2.50E+02
13	46	M	1	2	1.12E+04	1.69E+02	1.51E+02	41	53	M	2	3	9.75E+03	1.84E+02	1.89E+02
14	73	M	a	2	8.41E+03	6.05E+01	7.19E+01	42	77	M	2	3	9.44E+03	1.10E+01	1.17E+01
15	57	F	1	2	9.31E+03	1.71E-04	1.84E-04	43	62	M	2	3	1.11E+04	6.04E+00	5.44E+00
16	63	F	1	2	9.49E+03	7.05E+00	7.43E+00	44	43	M	1	3	1.00E+04	3.12E+00	3.12E+00
17	74	M	1	2	6.89E+03	4.24E+03	6.15E+03	45	79	M	2	3	9.25E+03	2.01E+02	2.17E+02
18	55	M	1	2	9.06E+03	1.21E+04	1.34E+04	46	57	M	1	3	9.78E+03	6.46E+01	6.61E+01
19	65	M	a	2	8.21E+03	1.21E+02	1.47E+02	47	65	M	2	3	1.02E+04	3.45E+00	3.38E+00
20	53	M	1	2	8.35E+03	1.31E+01	1.57E+01	48	64	M	2	3	8.35E+03	1.79E+01	2.14E+01
21	56	M	1	2	8.91E+03	7.70E+00	8.64E+00	49	67	M	2	3	9.67E+03	9.66E+00	9.99E+00
22	82	M	2	2	8.57E+03	3.00E+02	3.50E+02	50	80	M	2	3	8.81E+03	8.72E+00	9.90E+00
23	28	M	1	2	1.53E+04	1.35E+01	8.82E+00	51	75	M	2	3	7.97E+03	1.11E+01	1.39E+01
24	75	F	a	2	1.07E+04	4.47E+00	4.18E+00	52	59	M	2	3	1.49E+04	4.01E+00	2.69E+00
25	71	M	a	2	8.41E+03	3.00E+03	3.57E+03	53	77	M	2	3	9.24E+03	2.47E+02	2.67E+02
26	70	M	a	2	8.72E+03	1.54E+01	1.77E+01	54	77	F	2	3	1.02E+04	4.07E+00	3.99E+00
27	57	M	1	2	8.80E+03	1.67E+01	1.90E+01	55	63	M	1	3	8.56E+03	4.83E-01	5.64E-01
28	74	M	1	2	1.01E+04	1.80E+01	1.78E+01	56	68	M	1	3	9.17E+03	1.48E+02	1.61E+02

M, male; F, female; RR, relative ratio; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; *DMBT1*, deleted in malignant brain tumors 1.

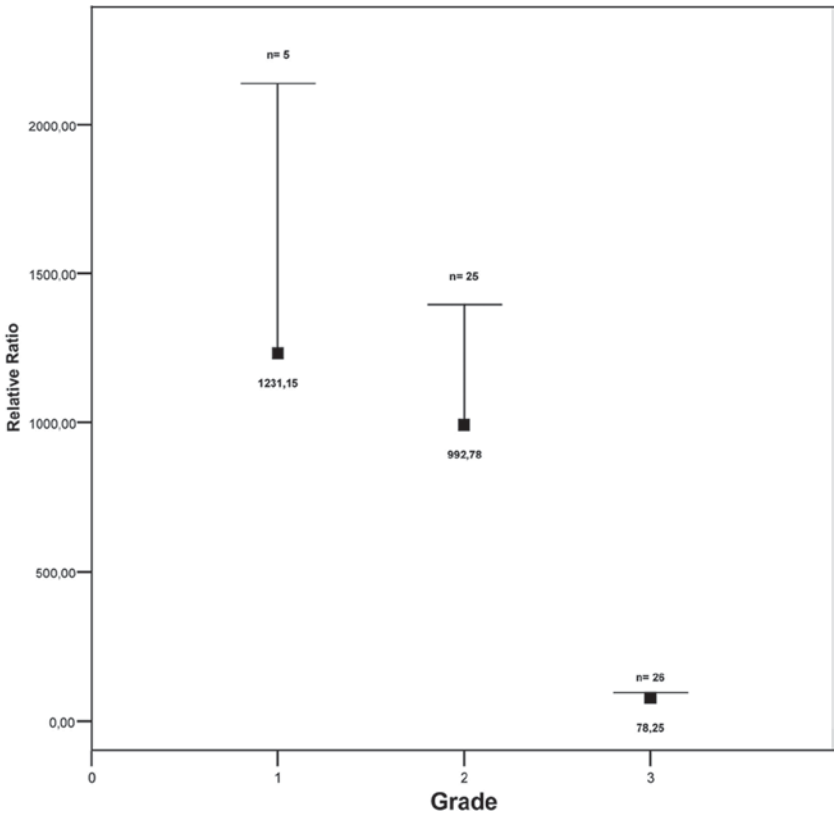

Figure 1. Relative ratio of *DMBT1* gene expression according to tumor grades. Mean relative ratios of Grade 1, Grade 2 and Grade 3 cases were 1231.15, 992.78 and 78.25, respectively. A significant difference was found between Grade 1 and Grade 3 ($p = 0.028$).

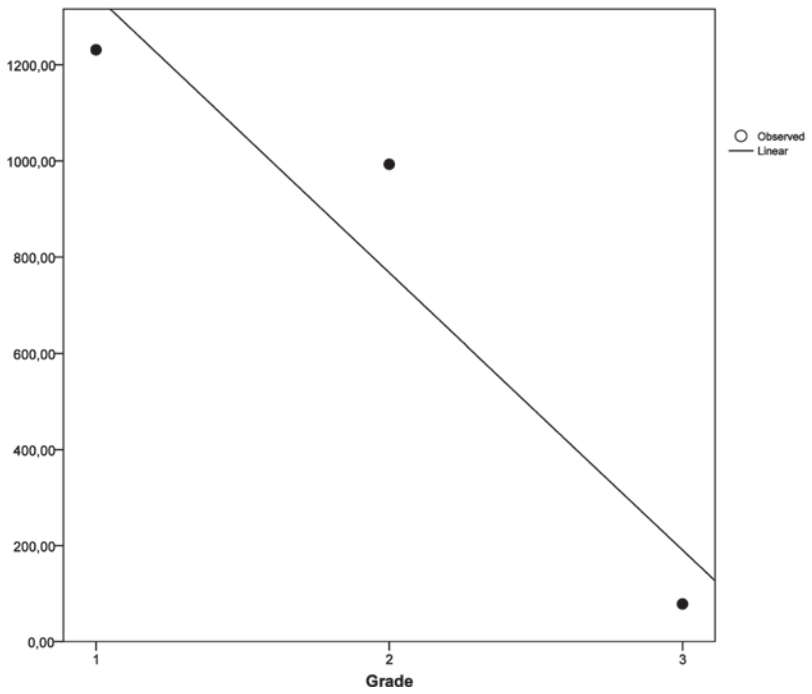

Figure 2. The correlation between tumor grades and *DMBT1* gene expression. Reduced *DMBT1* gene expression was found correlated (94.7%) with high tumor grade in bladder carcinoma.

Relative quantification of *DMBT1*

Real-time quantitative RT-PCR analysis of *DMBT1* was performed with LightCycler instrument and software. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH "housekeeping" gene) was chosen as an internal standard to control variability in amplification. The sequences of primers and probes used are shown in Table-2. PCR was performed by using TaqMan Master Kit (Roche Diagnostics, Germany) according to the instructions of the manufacturer. The *DMBT1* target probe was labeled

at the 5′ end with the reporter dye molecule 6-carboxyfluorescein (FAM). The *GAPDH* target probe was labeled with 6-carboxyfluorescein. Both probes were labeled with the quencher flour 6-carboxytetramethylrhodamine (TAMRA) at the 3′ end. To quantify *DMBT1* total RNA from paraffin block a calibration curve was constructed (Error: 0.100 Efficiency: 1790) using *GAPDH* mRNA as an endogenous control. (RR = Copy number of gene/Copy number of *GAPDH* ×1000)

Statistical Analysis

The *DMBT1* gene expression results compared with patients' sex, age using Student *t*-tests and cellular degrees and grades were evaluated using χ^2 test for independence. The *p* values 0.05 were considered statistically significant. SPSS 10.0 software was used for calculation.

Results

To determine the expression pattern of the *DMBT1* gene in bladder cancer, we analyzed 56 paraffin tissue samples by RT-PCR. *DMBT1* expression was found in bladder cancer samples with TCC. We investigated *DMBT1* gene expression in bladder carcinoma cases and how its expression could be related to carcinogenesis in the bladder. We evaluated the expression results with patients' sex, age, pathologic degree and grade. Total RNA isolation was achieved in all cases. *DMBT1* gene expression was identified in 100% of bladder carcinoma samples [56/56, (Table 3)].

First, we analyzed *DMBT1* gene expression according to sex, age and its pathological degree. No significant association was found in *DMBT1* gene expression when compared to sex [49 M (87.5%), 7 F (12.5%)]. Even no significant association was found in *DMBT1* gene expression when compared to age [28–83 (average 65.5 ± 10.6)].

Next we analyzed the expression of *DMBT1* gene with tumor grade (Figure 1). When *DMBT1* gene expression was compared to tumor grade, a significant decrease was detected between grade 1 and grade 3 ($p = 0.028$), but we didn't find any significant difference between grade 2 and grade 1–3. The correlation between increasing grade levels and reduced *DMBT1* gene expression was found 94.7% (Figure 2). There was no correlation with tumor stage and gene expression level. Comparing the expression profiles between tumor grades and *DMBT1* gene expression, there was a remarkable result found. When tumor grade was increased, the *DMBT1* gene expression was decreased.

Discussion

DMBT1 is evaluated to be a candidate tumor suppressor gene for malignant tumors, including brain, lung, esophagus, stomach and colon, due to the reduction of this gene expression observed in these cancer types (Mollenhauer et al., 1997; Takeshita et al., 1999). However *DMBT1* gene expression levels were found up-regulated in pancreatic, prostatic and salivary gland cancers and these findings were contrary to the other type of cancers, and researchers predicted *DMBT1* gene as a new molecular marker for pancreatic, prostatic and salivary gland cancers (Mori et al., 1999; Bikker et al., 2004).

The presented study in this report is the first demonstrating that *DMBT1* is also expressed frequently in bladder cancer. We performed *DMBT1* gene expression in transitional cell carcinoma parafin embedded tissues of the urinary bladder by using RT-PCR. We found *DMBT1* gene expression in 56 of 56 (100%) bladder carcinoma cases. We assessed these results statistically with age; sex and pathologic degree, but we didn't find any significant correlation with these parameters. There was a significant correlation between *DMBT1* gene expression and cases' grade. When tumor grade was increased, the *DMBT1* gene expression was decreased.

Braidotti et al. found that *DMBT1* expression was down-regulated in breast cancer. They detected *DMBT1* expression in 13 of 35 (37%) infiltrating carcinomas, and in 2 breast cancer cell lines by RT-PCR (Hustinx et al., 2004). Mollenhauer et al. showed similar data for *DMBT1* expression in breast cancer, but they found an indirect correlation between degrees of differentiation of the breast cancers. Also, they indicated that *DMBT1* gene expression may be up-regulated in pathophysiological conditions (Braidotti et al., 2004). Loss or reduction of *DMBT1* expression is commonly found in well-differentiated gastric cancer. An up-regulation of *DMBT1* expression is frequently found across all gastric cancer types. Follow-up studies are required to determine whether this reflects a non-causative role of *DMBT1* expression changes in gastric carcinoma (Mollenhauer et al., 2002).

In addition to down-regulation of *DMBT1*, previous studies were indicative of *DMBT1* homozygous deletion. In a study with primary neuroblastic tumors and 12 neuroblastoma cell lines, homozygous deletion was in 7% of primer tumors and lack of *DMBT1* gene expression was in 16% of cell lines (Conde et al., 2007). In another study with brain tumors, intragenic homozygous deletions were in 33% of brain tumor cell lines, and in 22% of the primary glioblastomas. These results confirmed the observation by finding intragenic homozygous deletion of *DMBT1* in 38% of primary glioblastomas (Muñoz et al., 2004).

Together the mRNA expression and homozygous deletion in oesophageal, gastric and colon cancer cases were detected reduction of *DMBT1* gene expression in 53.5% of oesophageal, 12.5% of gastric, 16.7% of colon cancer cases by RT-PCR (Somerville et al., 1998).

In lung cancer, *DMBT1* gene expression were obtained that 20 of 20 (100%) of small cell lung cancer cell lines and 6 of 14 (43%) of non-small cell lung cancer cell lines lacked *DMBT1* gene expression (Mori et al., 1999). Du et al. stated that the function of *DMBT1* in relation to transitional cell carcinoma of the bladder is still unknown; disruption of *DMBT1* expression is associated with prostate cancer

and *DMBT1* may function as a tumor suppressor gene in prostate carcinogenesis (Du et al., 2011).

In conclusion, we found a significant correlation between *DMBT1* gene expression and the tumor grades of the cases with bladder cancer. Therefore, *DMBT1* gene expression could be used as a biomarker of early detection and prognosis of the bladder cancer. Also present preliminary study will be the first research for assessing *DMBT1* gene expression in bladder cancer and we think that this article will contribute to further studies for new researches. Furthermore, detailed studies about *DMBT1* gene should be performed in protein level in a large scale study, and we also think that clinical significance will be verified with increasing number of grade 1 patients.

Declaration of interest

The authors report no conflicts of interest.

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