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Original Article

Malat-1 expression in bladder carcinoma tissues and its clinical significance

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Abstract: Objective: To investigate the expression of metastasis-associated lung adenocarcinoma transcript 1 (Malat-1) in bladder carcinoma and its relationship with clinicopathological characteristics and prognosis. Methods: Specimens were collected from 90 patients with bladder carcinoma who underwent urological surgery in our hospital. Twenty patients diagnosed with benign prostatic hyperplasia were selected as the negative control. The expression of Malat-1 was detected by real-time reverse transcription PCR, and its relationship with clinicopathological factors and prognosis was analyzed. Results: The expression of Malat-1 in bladder carcinoma tissues (2.55±0.31) was higher than that in adjacent tissues (1.62±0.42) and normal bladder mucosa tissues (0.84±0.06); the differences were statistically significant (t=13.647 and 27.302, both P<0.001). The high expression rate of Malat-1 in bladder carcinoma tissues (86.67%) was significantly higher than that in adjacent tissues (22.22%) and normal bladder mucosa tissues (5.00%; P=0.000 and 0.000). The high expression rate of Malat-1 was correlated with age, tumor staging, degree of differentiation and lymph node metastasis (P=0.018, 0.000, 0.000, and 0.000). The median survival time and the 1-year, 3-year and 5-year survival rates of patients with high Malat-1 expression were lower than those with low expression of Malat-1 (P=0.006, 0.011, 0.000 and 0.002). High expression of Malat-1 is an independent risk factor for poor overall survival (OS) in bladder cancer patients. Conclusion: Overexpression of Malat-1 in bladder carcinoma tissues is associated with malignant biological characteristics and poor prognosis of patients.

Keywords: Metastasis-associated lung adenocarcinoma transcript 1, invasion, bladder carcinoma, prognosis

Introduction

Bladder carcinoma is a type of malignant tumor in the urinary system. About 40% of the patients have reached an advanced stage at the time of diagnosis, and their prognosis is often poor [1]. Metastasis-associated lung adenocarcinoma transcript 1 (Malat-1) belongs to the lncRNA family. Studies have found that overexpression of Malat-1 in lung cancer and colorectal cancer is related to poor prognosis and recurrence [2-4]. However, the relationship between Malat-1 and bladder carcinoma is hardly known. This study analyzed the expression of Malat-1 in bladder carcinoma and its relationship with clinicopathological characteristics between them, hoping to search for a new target for the prevention and treatment of bladder carcinoma.

Materials and methods

General information

In this prospective study, samples were collected from 90 patients diagnosed with bladder carcinoma who underwent urological surgery from January 2012 to August 2014. The inclusion criteria were: ① first treatment; ② pathologically confirmed; ③ no serious vital organ dysfunction; ④ complete general information. The exclusion criteria were: ① malignant tumors in other tissues and organs; ② failure to cooperate; ③ complicated with other bladder diseases; ④ low immune function. Fifty-one males and thirty-nine females were enrolled in this study, with an average age of 56.4±13.7 years, ranging from 34 to 78 years. TNM staging: there were 12 cases in stage I, 32 cases in stage II, 36 cases in stage III and 10 cases in

Table 1. Primer sequence

Primer name	Forward Sequence	Reverse Sequence
Malat-1	5'-ATAACCTACCAGGACCA-3'	5'-ACCACCGACTTGTGACA-3'
GAPDH	5'-CACTGTGCCCATCTACGAGG-3'	5'-TAATGTCACGCACGATTTCC-3'

Note: Malat-1: metastasis-associated lung adenocarcinoma transcript 1.

stage IV. Differentiation degree: 51 cases were low, 26 cases were moderate, and 13 cases were high. The bladder mucosa tissues of 20 patients with benign prostatic hyperplasia were collected as the negative control. All enrolled patients agreed to participate in this study, and the study was approved by the Ethics Committee of The People's Hospital of Xinchang.

Reagents and instruments

Primers were prichased from Beijing Huada Gene and Biological Company, China. RNA extraction kit and PCR kit were purchased from Shanghai Chenhao Biological Company, China. The ABI 7900 PCR amplification instrument was from Shanghai INESA Analytical Instrument Co., Ltd., China.

RT-PCR to detect the expression of Malat-1

Total RNA extraction. Firstly, the tissues were sonicated, washed and dissolved with PBS, and then mixed with Trizol reagent of an equal volume. According to the kit, the total RNA was extracted by the one-step method. The total RNA was dissolved in 20 μL DEPC water, and 1 μL was added into 79 μL DEPC water to detect purity by OD260/OD280 value. Reverse transcription: The cDNA was synthesized according to the reverse transcription kit. The total RNA of the sample was 1 μg. One hundred pmol of random primers or oligo-DT (18-20), 1 μL of deoxynucleotide triphosphate (10 mmol/L per dNTP), 4 μL of reverse transcription buffer (5×), 1 μL of RNase inhibitor (20-40 U/μL) and 20 U of M-MLV reverse transcriptase were used. DEPC treated water was supplemented to 20 μL. The target primer sequence is shown in **Table 1**. PCR amplification: The components of the PCR amplification system were prepared according to the kit: 2 μL cDNA, 3 μL forward primers, 3 μL reverse primers, 0.5 μL Taq polymerase, with a total volume of 25 μL. The reaction parameters were set at 92°C 20 s, 96°C 2 s, 85°C 20 s, 80°C 6 s, a total of 40 cycles. The melting curve was constructed, and the relative expression of target mRNA was measured by the $2^{-\Delta\Delta Ct}$ method. Based on the median value of Malat-1 expression (1.11) in all bladder carcinoma tissues, Malat-1 expression values were divided into the high expression group (including the median value) and low expression group.

Follow-up

Follow-up started on the date of discharge, with August 30, 2018 as the deadline. The patients were followed up by telephone or in the outpatient clinic every two months. The follow-up content was patients' survival status after discharge, and death was defined as the endpoint. The overall survival (OS) was from the date of discharge to the occurrence of endpoint events. The OS of patients with different Malat-1 expression levels were calculated and compared.

Statistical methods

SPSS 17.0 software was used in this study. The measurement data were shown as means ± standard deviation ($\bar{x}$ ± sd). One-way ANOVA was adopted to analyze data among the three groups. LSD-t test was used for post-hoc comparison. The enumeration data was shown in (%), and the comparison between groups was performed by Chi-square test. The Kaplan-Meier method and Log-rank test were used for survival analysis. Univariate and multivariate Cox regression analysis was used to analyze the factors affecting the survival of patients. The nonparametric rank-sum test was used for comparison of survival time. $P<0.05$ was considered significantly different.

Results

Expression of Malat-1 in tissues of each group

The expression of Malat-1 in bladder carcinoma tissues (2.55±0.31) was higher than that in adjacent tissues (1.62±0.42) and normal bladder mucosa tissues (0.84±0.06). The differences were statistically significant ($t=13.647$ and 27.302, both $P<0.001$). The high expression rate of Malat-1 in bladder carcinoma tis-

Table 2. Comparison of high expression rate of Malat-1 in each group

Group	n	Malat-1	
		n	%
Normal bladder mucosa	20	1	5.00
Adjacent tissues	90	19	21.11
Bladder cancer tissues	90	79	87.78
F		19.037	
P		0.000	

Note: Malat-1: metastasis-associated lung adenocarcinoma transcript 1.

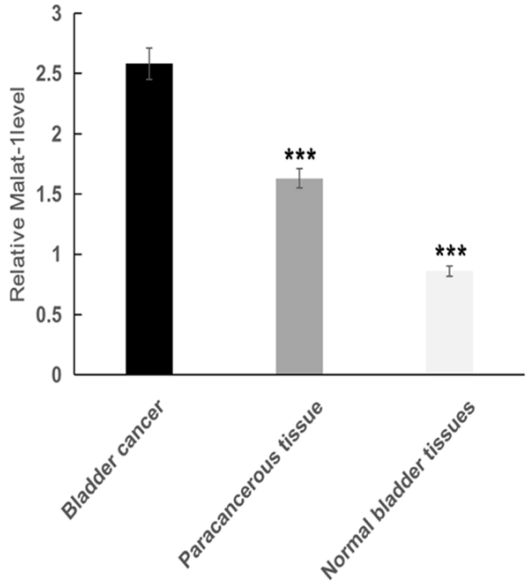

Figure 1. The expression level of Malat-1 in each group. Compared with bladder cancer tissues, ***P<0.001. Malat-1: metastasis-associated lung adenocarcinoma transcript 1.

sues (87.78%) was significantly higher than that in adjacent tissues (21.11%) and normal bladder mucosa tissues (5.00%; P=0.000). See **Table 2** and **Figure 1**.

Relationship between the high expression rate of Malat-1 in bladder carcinoma tissues and the pathological characteristics of the patients

The high expression rate of Malat-1was related to age, tumor staging, degree of differentiation and lymph node metastasis (P=0.018, 0.000, 0.000 and 0.000), while not related to gender and tumor diameter (both P>0.05). See **Table 3**.

Relationship between the expression of Malat-1 and prognosis

The median survival time and the 1-year, 3-year and 5-year survival rates of patients with high Malat-1 expression were lower than those with low Malat-1 expression (P=0.006, 0.011, 0.000, 0.002). See **Table 4** and **Figure 2**.

Cox proportional hazard regression model was used to analyze the risk factors of 5-year survival in patients with bladder carcinoma. The differentiation degree, TNM stage (III+IV) and high expression of Malat-1 were risk factors for poor prognosis. Multivariate Cox regression analysis showed that Malat-1 overexpression was a risk factor for poor prognosis. See **Tables 5**, **6**.

Discussion

The occurrence of bladder carcinoma is associated with multiple genes and multiple factors. It is prone to metastasis in the early stages and has a high degree of malignancy. The pathogenesis of bladder carcinoma is complex, including the activation of oncogenes, the inactivation of tumor suppressor genes, and the regulation of signal transduction pathways [5]. At present, there is no unified indicator for evaluating the prognosis of patients. Thus, it is necessary to explore how to evaluate the prognosis further and find adverse prognostic factors. LncRNA is a type of RNA with lengths no more than 20 nucleotides, which are not translated into protein. It regulates transcriptional and post-transcriptional gene expression after interacting with the target genes [6]. Therefore, exploring the expression of lncRNA in bladder carcinoma and the internal mechanism of regulating cellular biological behavior is of great clinical importance to understand tumor occurrence and find specific intervention targets.

The abnormal expression of lncRNA is essential in the occurrence and progression of carcinomas. Many studies have shown that the differential expression of lncRNA in bladder carcinoma tissues, cell lines and patient serum is relevant to the invasion, migration and drug resistance of bladder cancer cells [7]. For example, Wang et al. found that GAS5 is a tumor suppressor gene for bladder carcinoma and up-

Table 3. Relationship between the high expression rate of Malat-1 and clinicopathological features in bladder cancer (n, %)

Clinicopathological features	Cases	Malat-1		
		High expression rate (n, %)	χ^2	P
Age (years)			5.358	0.014
≥60	61	59 (96.72)		
<60	29	20 (68.97)		
Gender			0.213	0.752
Male	51	46 (90.20)		
Female	39	33 (84.62)		
Tumor diameter (cm)			0.159	0.862
>5	54	48 (88.89)		
≤5	36	31 (86.11)		
Tumor differentiation degree			7.947	0.000
Low differentiation	51	50 (98.04)		
High, medium differentiation	39	29 (74.36)		
Lymph node metastasis			10.786	0.000
Negative	29	18 (62.07)		
Positive	61	61 (100.00)		
TNM stage			8.051	0.000
I+II	44	34 (77.27)		
III+IV	46	45 (97.83)		

Note: Malat-1: metastasis-associated lung adenocarcinoma transcript 1.

Table 4. Relationship between Malat-1 expression level and median survival time, survival rate of patients

Malat-1	Cases	Median survival time (month)	1-year survival rate (%)	3-year survival rate (%)	5-year survival rate (%)
Low expression	12	51.65	91.67	83.33	58.33
High expression	78	36.50	76.92	52.56	35.94
t/χ^2		4.934	4.183	5.824	5.031
P		0.006	0.011	0.000	0.002

Note: Malat-1: metastasis-associated lung adenocarcinoma transcript 1.

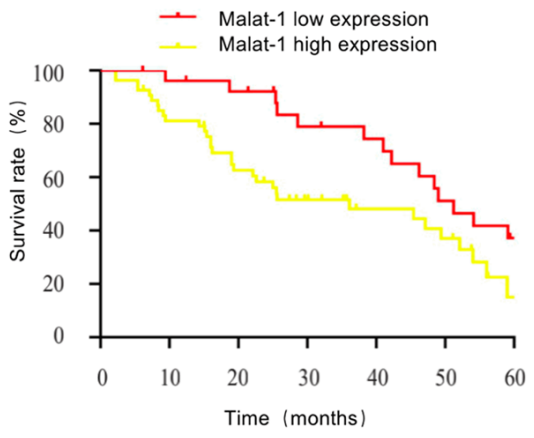

Figure 2. Survival curve of bladder cancer patients with low and high Malat-1 expression. Malat-1: metastasis-associated lung adenocarcinoma transcript 1.

regulating GAS5 can inhibit the proliferation of bladder cancer cells and induce apoptosis [8].

Malat-1 was first discovered in non-small cell lung cancer (NSCLC) in 2003. Overexpression of Malat-1 has been found in a variety of tumor tissues, which is related to poor prognosis and tumor recurrence [9]. A study has shown that Malat-1 is upregulated in prostate cancer tissues, indicating poor prognosis [10]. Another study has suggested that Malat-1 is overexpressed in renal cancer tissues, which is related to malignant pathological characteristics [11]. Malat-1 is also highly expressed in gastric cancer tissues and its expression is positively correlated with the expression of carcino-embryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) [12, 13]. Malat-1 can inhibit

Table 5. Univariate Cox analysis of 5-year survival outcome of bladder cancer patients

Factor	Regression coefficient	Standard error	Wald	HR	P	95% CI
Age	0.232	0.526	0.196	1.259	0.155	0.834-1.652
Gender	0.263	0.325	0.665	1.311	0.100	0.983-1.684
Tumor diameter	0.137	0.294	0.216	1.151	0.143	0.801-1.421
Differentiation degree	0.260	0.227	1.310	1.301	0.044	0.877-1.741
Lymph node metastasis	0.545	0.385	2.013	1.730	0.065	1.235-2.401
TNM stage	1.091	0.424	6.583	2.979	0.023	2.313-3.519
Malat-1 expression	0.917	0.197	15.035	3.278	0.004	2.159-3.430

Note: Malat-1: metastasis-associated lung adenocarcinoma transcript 1.

Table 6. Multivariate Cox analysis of 5-year survival outcome of bladder cancer patients

Factor	Regression coefficient	Standard error	Wald	OR	P	95% CI
Differentiation degree (low differentiation vs high, medium differentiation)	0.499	0.329	1.310	1.646	0.132	0.871-3.327
TNM stage (III+IV vs I+II)	0.099	0.070	2.050	1.114	0.150	0.628-1.894
Malat-1 expression (high expression vs low expression)	0.016	0.031	14.622	1.123	0.000	1.054-1.238

apoptosis gene expression and promote the proliferation and invasion of cervical cancer cells [14]. The results of our study showed that high expression of Malat-1 in bladder carcinoma tissues was related to malignant biological behaviors, indicating that Malat-1 can promote the occurrence and development of tumors. Survival analysis showed that the 5-year OS rate of patients with high Malat-1 expression was lower than those with low Malat-1 expression, consistent with other studies [15-17]. Multivariate and univariate Cox regression analysis showed that high levels of Malat-1 was a risk factor for the poor prognosis of bladder carcinoma patients during the 5-year follow-up period, and it played a vital part in the behavior of the tumor.

A study has shown that Malat-1 can activate the Wnt-EMT pathway to promote tumor cell invasion and metastasis [18]. In addition, another study showed that Malat-1 was highly expressed in renal cancer, which can combine with TFFB to promote tumor occurrence and development [19]. After looking through the relevant literature, we summarized the possible mechanism of Malat-1 affecting the malignant biological behaviors of bladder cancer. By targeting factors such as MDM2, mTOR, Bcl-2 and GSK3, Malat-1 promotes cell invasion, proliferation and migration, and inhibits apoptosis. By inhibiting the tumor microenvironment's immune function, Malat-1 enables the tumor to escape from immune surveillance [20-22]. By promoting the secretion of vascular growth factors by vascular endothelial cells, Malat-1 promotes angiogenesis and tumor invasion [23].

However, this study still has some limitations; the downstream targets and pathways are not analyzed. In the next step, we will analyze and verify related pathways to provide direction for the diagnosis and treatment of bladder carcinoma.

In summary, Malat-1 is highly expressed in bladder carcinoma tissues, which is related to clinicopathological characteristics and poor prognosis. But the pathway through which Malat-1 plays a role needs further *in vivo* and *in vitro* experimentation to be clarified.

Disclosure of conflict of interest

None.

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