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Особенности тканевой экспрессии ферментов протеолиза внеклеточного матрикса и их ингибиторов у больных раком мочевого пузыря

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***Цель работы** – определение уровня и интенсивности экспрессии матриксной металлопротеиназы 2 (ММП-2), ММП-9, ММП-14 и тканевого ингибитора металлопротеиназы 1 (ТИМ-1) и ТИМ-2 в ткани опухоли и окружающих тканях в зависимости от характеристик основного заболевания у 73 больных поверхностным раком мочевого пузыря (РМП) стадий TaN0M0 и T1N0M0.*
***Результаты.** Обнаружено, что у пациентов с поверхностным РМП исходно наблюдалось повышение числа опухолевых клеток, экспрессирующих как ММП-2, ММП-9 и ММП-14, так и их ТИМ, по сравнению с гистологически неизмененной тканью и тканью, граничащей с опухолью. Среди изучаемых ММП число опухолевых клеток, экспрессирующих ММП-9, было наибольшим. Тканевая экспрессия ТИМ-1 и ТИМ-2 в опухолевых клетках у больных РМП была выше по сравнению с неизмененной тканью и в биоптате на границе с опухолью. Соотношение выраженности экспрессии ММП и ТИМ было неодинаковым, и протеолитический потенциал ММП был выше, чем ТИМ.*
***Заключение.** Показано, что при поверхностном РМП стадия первичного РМП (от Ta к T1), степень дифференцировки опухоли (от G_1 к G_2), прогноз прогрессирования заболевания (от благоприятного к промежуточному) сказываются на усилении экспрессии ММП-9 и ТИМ-1 в опухолевой ткани.*

***Ключевые слова:** металлопротеиназа 2, 9, 14, тканевый ингибитор металлопротеиназы 1, 2, поверхностный рак мочевого пузыря, тканевая экспрессия*

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Characteristics of tissue expression of extracellular matrix proteolytic enzymes and their inhibitors in patients with bladder cancer

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***Objectives.** Levels and expression of matrix metalloproteases 2 (MMP-2), MMP-9, MMP-14 and tissue inhibitor of metalloproteinases 1 (TIMP-1), TIMP-2 were studied by immunohistochemistry in tumor and surrounding tissues in 73 patients with superficial bladder cancer (BC) TaN0M0 and T1N0M0 depending on the disease characteristics.*
***Results.** The study showed the initial increase in the number of tumor cells expressing MMP-2, MMP-9 and MMP-14 and their tissue inhibitors, compared to histologically unchanged tissues and tumor-adjacent tissues. The number of MMP-9 producing tumor cells was maximal. Tissue expression of TIMP-1 and TIMP-2 in tumor cells in superficial BC patients was higher than in unchanged tissues and in tumor-adjacent tissues. The ratio of MMP and TIMP expression differed, and the proteolytic potential of MMP was higher than that of TIMP.*
***Conclusion.** The study demonstrated the influence of the primary bladder cancer stage (from Ta to T1), tumor differentiation grade (from G_1 to G_2) and prognosis of the disease course (from favorable to intermediate) on the enhancing expression of MMP-9 and TIMP-1 in tumor tissues in superficial BC.*

***Key words:** metalloprotease 2, 9, 14, tissue inhibitor of metalloproteinase 1, 2, superficial bladder cancer, tissue expression*

Background

Proteolytic enzymes, and particularly those belonging to the group of matrix metalloproteinases (MMPs), play a significant role in tumor invasion [1, 2]. The levels of endogenous enzymes is usually determined in biological fluids, which is convenient for both screening and diagnosis

Таблица 1. *Количество клеток с экспрессией ММП и ТИМ в ОТ и окружающих тканях больных поверхностным раком мочевого пузыря*

Table 1. *Number of cells with MMP and TIMP expression in TT and surrounding tissues in patients with superficial bladder cancer*

Ткань, характеристика сопряжения Tissue, coupling characteristic	**<20 % (2 балла)** <20 % (2 points)	**20–40 % (4 балла)** 20–40 % (4 points)	**>40 % (6 баллов)** >40 % (6 points)	**Средний балл (M ± SD)** Mean score (M ± SD)	***p***
ММП-2 *MMP-2*					
ОТ TT	7 (10 %)	15 (20 %)	51 (70 %)	5,2 ± 0,7	<0,001* <0,001**
НТ NT	69 (95 %)	3 (4 %)	1 (1 %)	2,1 ± 0,3	
На границе с ОТ On the border with TT	67 (92 %)	4 (5 %)	2 (3 %)	2,2 ± 0,2	>0,05*
Характеристика Characteristic	χ^2 = 154,9; *p* <0,0001; коэффициент контингенции 0,64 χ^2 = 154,9; *p* <0,0001; contingency coefficient 0,64				
ММП-9 *MMP-9*					
ОТ TT	4 (5 %)	5 (7 %)	64 (88 %)	5,6 ± 0,6	<0,001* <0,001**
НТ NT	64 (87 %)	7 (10 %)	2 (3 %)	2,3 ± 0,4	
На границе с ОТ On the border with TT	53 (73 %)	12 (16 %)	8 (11 %)	2,8 ± 0,2	0,05*
Характеристика Characteristic	χ^2 = 148,6; *p* <0,0001; коэффициент контингенции 0,64 χ^2 = 148,6; *p* <0,0001; contingency coefficient 0,64				
ММП-14 *MMP-14*					
ОТ TT	9 (12 %)	18 (25 %)	46 (63 %)	5,0 ± 0,5	0,001* <0,001**
НТ NT	70 (96 %)	2 (3 %)	1 (1 %)	2,1 ± 0,2	
На границе с ОТ On the border with TT	65 (89 %)	6 (8 %)	2 (3 %)	2,3 ± 0,1	>0,05*
Характеристика Characteristic	χ^2 = 144,6; *p* <0,0001; коэффициент контингенции 0,63 χ^2 = 144,6; *p* <0,0001; contingency coefficient 0,63				
ТИМ-1 *TIMP-1*					
ОТ TT	29 (40 %)	36 (49 %)	8 (11 %)	3,4 ± 0,5	<0,001* <0,001**
НТ NT	67 (92 %)	4 (5 %)	2 (3 %)	2,2 ± 0,3	
На границе с ОТ On the border with TT	55 (75 %)	12 (15 %)	7 (10 %)	2,7 ± 0,2	0,02*
Характеристика Characteristic	χ^2 = 50,8; *p* <0,0001; коэффициент контингенции 0,43 χ^2 = 50,8; *p* <0,0001; contingency coefficient 0,43				
ТИМ-2 *TIMP-2*					
ОТ TT	39 (53 %)	29 (40 %)	5 (7 %)	3,1 ± 0,4	<0,001* <0,001**

Окончание табл. 1
End of table 1

Ткань, характеристика сопряжения Tissue, coupling characteristic	<20 % (2 балла) <20 % (2 points)	20–40 % (4 балла) 20–40 % (4 points)	>40 % (6 баллов) >40 % (6 points)	Средний балл (M ± SD) Mean score (M ± SD)	*p*
НТ NT	70 (96 %)	3 (4 %)	–	2,1 ± 0,1	
На границе с ОТ On the border with TT	62 (85 %)	7 (10 %)	4 (5 %)	2,1 ± 0,2	>0,05*
Характеристика Characteristic	χ^2 = 43,9; *p* <0,0001; коэффициент контингенции 0,41 χ^2 = 43,9; *p* <0,0001; contingency coefficient 0,41				

Примечание. *Здесь и в табл. 2, 3 и 4: ММП – матриксная металлопротеиназа; ТИМ – тканевый ингибитор металлопротеиназ; ОТ – опухолевая ткань; НТ – гистологически неизмененная ткань.*
Note. *Here and in Tables 2, 3, and 4: MMP – matrix metalloproteinase; TIMP – tissue inhibitor of metalloproteinase; TT – tumor tissue; NT – histologically normal tissue.*
**Доверительная вероятность различия с НТ.*
**Confidence probability for difference from NT.*
***Доверительная вероятность различия с тканью, граничащей с ОТ.*
***Confidence probability for difference from the tissue bordering TT.*

of cancer. However, the assessment of MMP levels in tissues has higher diagnostic accuracy [3–5].

Patients with bladder cancer (BC) often have elevated levels of proteinases that correlate with tumor morphological features and clinical manifestations. It can be used as a prognostic marker for predicting tumor progression. Zavalishina et al. reported significantly increased expression of MMP-2 and MMP-9 along with decreased levels of their inhibitors in poorly differentiated carcinomas with vascular invasion and lymph node metastases [6]. High expression of MMPs and low expression of MMP inhibitors was observed in the areas of invasive tumor growth in individuals with BC [7]. Some authors suggest estimating the expression of MMP-2, MMP-14, and tissue inhibitor of metalloproteinases 2 (TIMP-2) to predict survival in patients with invasive BC [8, 9]. Reis et al. demonstrated that 59% of patients with BC after transurethral resection had MMP-9 overexpression; the majority of them also had decreased MMP-14 expression [10]. The highest MMP-9 expression was detected in highly malignant and invasive tumors (pT1–2) rather that in superficial tumors (pTa).

Objective: to evaluate the expression of MMPs and their tissue inhibitors in cancer tissues and surrounding tissues of superficial BC depending on clinical and morphological characteristics.

Material and methods

The study included 73 patients with superficial stage TaN0M0 and stage T1N0M0 BC. Mean age of participants was 62.4 ± 3.4 years.

The diagnosis was based on the results of histological examination in accordance with specific standards and algorithms for the diagnosis and treatment of malignant tumors. The study protocol was approved by the Ethics Committee of the Rostov Research Institute of Oncology. An informed consent for participation in the study was obtained from each patient.

We evaluated BC tissue samples collected during surgery (transurethral resection with subsequent intravesical chemotherapy), samples taken at the visible border of the tumor, and normal tissue samples obtained from the extratumor sites. All tissue samples were frozen and stored at –70 °C.

Specimens underwent immunohistochemical examination according to a standard protocol. We estimated tissue expression of MMP-2, MMP-9, MMP-14, TIMP-1, and TIMP-2 using a semiquantitative scoring system based on the number of cells expressing these markers: 2 points–< 20% of cells with positive staining, 4 points–20–40% of cells with positive staining, 6 points–> 40% of cells with positive staining [11]. The immunohistochemical results were evaluated using the Image Analyzer Leica Q550. The intensity of immunostaining was graded as follows: 0–no staining, 1–weak staining, 2–moderate staining, 3–strong stainig [12].

The parameters of expression were analyzed together with main characteristics of BC.

Statistical data analysis was performed using the software package Statistica 10.0 (StatSoft Inc., USA). We applied the methods of descriptive statistics and assessed the differences between continuous variables using parametric and non-parametric tests (depending on the type of distribution). Distributional differences between categorical variables were assessed by the Mantel-Haenszel χ^2-test. We also constructed conjugacy tables to assess the correlation

Таблица 2. *Интенсивность тканевой экспрессии ММП и ТИМ в ОТ и окружающих тканях больных поверхностным раком мочевого пузыря*
Table 2. *Intensity of MMP and TIMP tissue expression in TT and surrounding tissues in patients with superficial bladder cancer*

Ткань, характеристика сопряжения Tissue, coupling characteristic	Баллы Score				Средний балл (M ± SD) Mean score (M ± SD)	*p*
	0	1	2	3		
ММП-2 *MMP-2*						
ОТ TT	5 (7 %)	48 (66 %)	16 (22 %)	4 (5 %)	1,1 ± 0,03	<0,001* <0,001**
НТ NT	67 (92 %)	6 (8 %)	–	–	0,08 ± 0,001	
На границе с ОТ On the border with TT	64 (88 %)	9 (12 %)	–	–	0,12 ± 0,003	>0,05*
Характеристика Characteristic	χ^2 = 146,2; *p* <0,0001; коэффициент контингенции 0,63 χ^2 = 146,2; *p* <0,0001; contingency coefficient 0,63					
ММП-9 *MMP-9*						
ОТ TT	2 (3 %)	35 (48 %)	31 (42 %)	5 (7 %)	1,53 ± 0,02	<0,001* <0,001**
НТ NT	61 (84 %)	10 (14 %)	2 (3 %)	–	0,19 ± 0,001	
На границе с ОТ On the border with TT	49 (67 %)	23 (32 %)	1 (1 %)	–	0,34 ± 0,001	0,03*
Характеристика Characteristic	χ^2 = 127,1; *p* <0,0001; коэффициент контингенции 0,61 χ^2 = 127,1; *p* <0,0001; contingency coefficient 0,61					
ММП-14 *MMP-14*						
ОТ TT	5 (7 %)	38 (52 %)	28 (38 %)	2 (3 %)	1,37 ± 0,06	<0,001* <0,001**
НТ NT	67 (92 %)	5 (7 %)	1 (1 %)	–	0,1 ± 0,001	
На границе с ОТ On the border with TT	63 (86 %)	10 (14 %)	–	–	0,14 ± 0,001	>0,05*
Характеристика Characteristic	χ^2 = 145,5; *p* <0,0001; коэффициент контингенции 0,63 χ^2 = 145,5; *p* <0,0001; contingency coefficient 0,63					
ТИМ-1 *TIMP-1*						
ОТ TT	22 (30 %)	46 (63 %)	5 (7 %)	–	0,77 ± 0,11	<0,05* <0,05**
НТ NT	66 (90 %)	7 (10 %)	–	–	0,1 ± 0,04	
На границе с ОТ On the border with TT	53 (73 %)	18 (25 %)	2 (3 %)	–	0,3 ± 0,001	0,05*
Характеристика Characteristic	χ^2 = 61,3; *p* <0,0001; коэффициент контингенции 0,47 χ^2 = 61,3; *p* <0,0001; contingency coefficient 0,47					
ТИМ-2 *TIMP-2*						
ОТ TT	39 (53 %)	30 (41 %)	3 (4 %)	1 (1 %)	0,53 ± 0,08	<0,05* <0,05**

Окончание табл. 2
End of table 2

Ткань, характеристика сопряжения Tissue, coupling characteristic	Баллы Score				Средний балл (M ± SD) Mean score (M ± SD)	*p*
	0	1	2	3		
НТ NT	70 (96 %)	3 (4 %)	–	–	0,04 ± 0,001	
На границе с ОТ On the border with TT	62 (85 %)	11 (15 %)	–	–	0,15 ± 0,001	>0,05*
Характеристика Characteristic	χ^2 = 53,6; *p* <0,0001; коэффициент контингенции 0,45 χ^2 = 53,6; *p* <0,0001; contingency coefficient 0,45					

**Доверительная вероятность различия с НТ.*
**Confidence probability for difference from NT.*
***Доверительная вероятность различия с тканью, граничащей с ОТ.*
***Confidence probability for difference from the tissue bordering TT.*

between categorical variables using contingency coefficients.

Results and discussion

The proportion of cells expressing MMP-2, MMP-9, MMP-14, and their inhibitors was significantly higher in tumor tissue than in border or normal tissue (Table 1).

The proportion of MMP-9-expressing tumor cells was higher than the proportion of cells expressing MMP-2 or MMP-14. The vast majority of patients (n = 64; 88%) had MMP-9 expression in over 40% of tumor cells. High proportion (> 40%) of MMP-2 and MMP-14-expressing tumor cells was observed in 51 (70%) and 46 (63%) cases respectively. Thus, among all the markers, MMP-9-overexpression was the most typical of BC tissue. Both healthy tissue and tissue from the tumor border were more likely to have lower proportions (< 20%) of cells expressing MMPs. Only the rate of MMP-9 expression varied between normal (mean score 2.3 ± 0.4) and tumor border (2.8 ± 0.2) tissues (p = 0.05)

Cancer tissue had higher expression of TIMP-1 and TIMP-2 compared to tissues taken from the border of the tumor and healthy tissue (Table 1). Approximately 40% of patients had TIMP-1 expression in less than 20% of tumor cells, whereas 49% of participants had between 20% and 40% of positively stained tumor cells; TIMP-1 overexpression was observed in 11% of cases. Tumor tissues had significantly higher levels of TIMP-1 expression (mean score 3.4 ± 0.5) than normal tissues (mean score 2.2 ± 0.3) and tissues taken from the border of the tumor (mean score 2.7 ± 0.2) (p < 0.001). More than half (53%) of the patients with BC had positive TIMP-2 staining in less than 20% of tumor cells, whereas 40% had TIMP-2 expression in 20%–40% tumor cells, and 7% had overexpression of this marker. The value of TIMP-2 expression was significantly higher in tumor tissue (mean score 3.1 ± 0.4) than in healthy (mean score 2.1 ± 0.1) and tumor border (mean score 2.1 ± 0.2) tissues (p < 0.001).

Despite the high proportion of positively stained cells, the intensity of staining was relatively low (Table 2). Most frequently, BC cells had weak nuclear staining (score 1): in 66% for MMP-2, 48% for MMP-9, and 52% for MMP-14. Mean intensity of MMP-2, MMP-9, and MMP-14 immunostaining in BC cells was 1.1 ± 0.03, 1.53 ± 0.02, and 1.37 ± 0.06 respectively. Significant differences in the staining intensity between normal and tumor border tissues were observed only for MMP-9 (0.19 ± 0.001 vs 0.34 ± 0.001; p = 0.03).

The values of χ^2 and contingency coefficients, characterizing the correlation between the expression of MMPs and tissue status (from normal to tumor) were high, confirming the fact of increased MMP expression in BC.

The level of TIMP-1 and TIMP-2 expression (mean score 3.4 ± 0.5 and 3.1 ± 0.4 respectively) was significantly lower than the rate of MMP-2, MMP-9, and MMP-14 expression (mean score 5.2 ± 0.7, 5.6 ± 0.6, and 5.0 ± 0.5 respectively). Therefore, the increased expression of TIMP-1 and TIMP-2 in superficial BC is more likely to be a compensatory effect aimed to suppress proteolytic activity of MMPs. However, the levels of MMP and TIMP expression were different; MMPs had greater proteolytic potential than TIMPs.

The intensity of TIMP-1 and TIMP-2 staining in tumor tissue was relatively low (Table 2). The mean staining score was 0.77 ± 0.11 and 0.53 ± 0.08 for TIMP-1 and TIMP-2 respectively.

The level of MMP-2 and MMP-14 expression in all types of tissues did not correlate with any clinical

Таблица 3. *Тканевая опухолевая экспрессия ММП-2, ММП-9 и ММП-14 у больных поверхностным раком мочевого пузыря в зависимости от характеристик основного заболевания*

Table 3. *Tumor tissue expression of MMP-2, MMP-9, and MMP-14 in patients with superficial bladder cancer depending on the characteristics of the main disease*

Характеристика Characteristic	Экспрессия, баллы (*p*) Expression, points (*p*)		
	ММП-2 MMP-2	**ММП-9** MMP-9	**ММП-14** MMP-14
Стадия первичного рака мочевого пузыря: Stage of the primary bladder cancer:			
Ta	5,0 ± 0,2	4,8 ± 0,3	4,7 ± 0,1
T1	5,3 ± 0,1 (>0,05)	5,7 ± 0,2 (0,02)	5,1 ± 0,3 (>0,05)
Степень дифференцировки: Differentiation grade:			
G_1	5,1 ± 0,2	4,9 ± 0,1	4,8 ± 0,3
G_2	5,3 ± 0,1 (>0,05)	5,6 ± 0,2 (0,04)	5,1 ± 0,2 (>0,05)
Количество узлов: Number of lesions:			
монофокальные опухоли monofocal tumors	5,1 ± 0,3	5,2 ± 0,2	4,9 ± 0,2
2–3	5,2 ± 0,2 (>0,05)	5,6 ± 0,4 (>0,05)	5,2 ± 0,1 (>0,05)
Размер узлов, см: Lesion size, cm:			
<1	5,2 ± 0,2	5,5 ± 0,4	4,8 ± 0,2
1–3	5,1 ± 0,1 (>0,05)	5,6 ± 0,3 (>0,05)	5,1 ± 0,3 (>0,05)
Прогноз: Prognosis:			
благоприятный favorable	4,9 ± 0,3	4,7 ± 0,2	4,8 ± 0,3
промежуточный intermediate	5,3 ± 0,1 (>0,05)	5,7 ± 0,3 (0,02)	5,2 ± 0,2 (>0,05)

characteristics of the disease (Table 3). The expression of MMP-9 varied depending on the stage of primary BC, differentiation grade, and prognosis (Table 3).

Patients with stage T1 BC had higher levels of MMP-9 expression compared to patients with stage Ta BC (5.7 ± 0.2 vs 4.8 ± 0.3; p = 0.02). Moreover, MMP-9 expression in tumor cells correlated with pathologic differentiation grade (mean score 5.6 ± 0.2 in G2 vs 4.9 ± 0.1 in G1; p = 0.04) and disease prognosis (mean score 5.7 ± 0.3 in intermediate prognosis vs 4.7 ± 0.2 in favorable prognosis; p = 0.02).

The level of TIMP-1 expression correlated with BC stage (mean score 0.79 ± 0.20 in T1 vs 0.68 ± 0.40 in Ta; p < 0.05), pathologic differentiation grade (mean score 0.93 ± 0.40 in G2 vs 0.68 ± 0.30 in G1; p < 0.05), and disease prognosis (mean score 0.91 ± 0.50 in intermediate prognosis vs 0.72 ± 0.30 in favorable prognosis; p < 0.05) (Table 4).

Conclusion

Our results suggest that tumor tissue in patients with superficial BC has significantly higher proportion of cells expressing MMP-2, MMP-9, and MMP-14 than normal tissue or tissue taken at the tumor border. MMP-9 was expressed by a greater proportion of cells than other markers. In both normal and tumor border tissue, the number of MMP-expressing cells usually did not exceed 20%. Only the rate of MMP-9 expression varied between healthy tissue and tissue from the border of the tumor. Cancer tissue had higher expression of TIMP-1 and TIMP-2 compared to normal and tumor border tissues. The levels of MMP and TIMP expression was different; MMPs had greater proteolytic potential than TIMPs

We also demonstrated that the stage of primary BC (from Ta to T1), pathologic differentiation grade (from G1 to G2), and disease prognosis (from favorable to intermediate) correlate with MMP-9 and TIMP-1 expression in tumor tissue.

Таблица 4. *Тканевая опухолевая экспрессия ТИМ-1 и ТИМ-2 у больных поверхностным раком мочевого пузыря в зависимости от характеристик основного заболевания*

Table 4. *Tumor tissue expression of TIMP-1 and TIMP-2 in patients with superficial bladder cancer depending on the characteristics of the main disease*

Характеристика Characteristic	Экспрессия, баллы (*p*) Expression, score (*p*)	
	ТИМ-1 TIMP-1	ТИМ-2 TIMP-2
Стадия первичного рака мочевого пузыря: Stage of the primary bladder cancer: Ta T1	 0,68 ± 0,40 0,79 ± 0,20 (<0,05)	 0,51 ± 0,30 0,55 ± 0,10 (>0,05)
Степень дифференцировки: Differentiation grade: G_1 G_2	 0,68 ± 0,30 0,93 ± 0,40 (<0,01)	 0,51 ± 0,20 0,58 ± 0,30 (>0,05)
Количество узлов: Number of lesions: монофокальные опухоли monofocal tumors 2–3	 0,75 ± 0,40 0,81 ± 0,30 (>0,05)	 0,54 ± 0,20 0,50 ± 0,10 (>0,05)
Размер узлов, см: Lesion size, cm: <1 1–3	 0,75 ± 0,30 0,80 ± 0,50 (>0,05)	 0,55 ± 0,40 0,52 ± 0,30 (>0,05)
Прогноз: Prognosis: благоприятный favorable промежуточный intermediate	 0,72 ± 0,30 0,91 ± 0,50 (<0,05)	 0,50 ± 0,30 0,62 ± 0,20 (>0,05)

Conflict of interest
The authors declare no conflicts of interest related to this manuscript

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