RESEARCH ARTICLE

Expression of Aquaporin 1 in Bladder Uroepithelial Cell Carcinoma and its Relevance to Recurrence

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Abstract

Objectives: To explore the expression of aquaporin 1 (AQP_1) in bladder uroepithelium cell carcinoma (BUCC) and its relevance to recurrence. Materials and Methods: Tissue samples from 45 BUCC patients who underwent total cystectomy or transurethral resection of bladder tumor (TURBT) and from 40 patients with non-bladder cancers who underwent special detection or treatments were collected. The level of expression of AQP_1 in BUCC tissues and normal bladder tissues was assessed by immunohistochemistry so as to analyze the relevance to pathological patterns and time of recurrence in BUCC patients. Results: The expression levels of AQP_1 normal bladder tissues and BUCC tissues were 2.175±0.693 and 3.689±0.701, respectively, and the difference was significant (t=9.99, $P<0.0001$). Marked increase was noted with BUCC histological grade and pathological stage ($P<0.01$). Moreover, the expression of AQP_1 was evidently higher in cancerous tissues with lymph node metastasis than in those without ($P<0.01$). With short-term recurrence, the positive cell expression rate of AQP_1 was higher in primary tissues, which increased obviously after recurrence. Additionally, the recurrent time of BUCC was negatively associated with the positive cell expression rate of AQP_1 and the difference between the expression of AQP_1 before and after recurrence ($r=-0.843$, F=39.302, $P=0.000$; $r=-0.829$, F=35.191, $P=0.000$). Conclusions: AQP_1, which reflects the grade, stage, lymph node metastasis and recurrence of BUCC, has potential guiding significance in the diagnosis and treatment of bladder cancarcinoma.

Keywords: Bladder uroepithelium cell carcinoma - recurrence - aquaporin 1 - biological marker

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Introduction

Bladder uroepithelium cell carcinoma (BUCC), which accounts for more than 90% of bladder cancers, is one of the malignant tumors commonly seen in urinary and reproductive systems. Its lesions are marked by phases, and the whole urinary tract (from pelvis to urethra)-adhered system is the predilection site of diseases, in which BUCC is the most typical one. Due to its multi-centered biological patterns in space and time, BUCC is polygenetic in characteristic and is more likely to recurrent after the local resection of visible tumors. It was demonstrated in a study that there was significant difference in the 5-year survival rate between patients with BUCC in different pathological stages (Taylor et al., 2009), indicating that early diagnosis and immediate discovery of recurrence could prolong patients' survival time, which was of great significance in the treatment of BUCC. However, so far, no index with both high sensitivity and specificity has been found that can be used to predicate the morbidity and postoperative recurrence of BUCC patients (Azemar et al., 2011).

Aquaporins (AQPs), which is extensively distributed in cells, can specifically transport the water molecules and some other small molecules synchronously. At present, a total of 13 members of AQPs have been found. It is reported that AQPs, which can participate in the development, progression and metastasis of tumors through multiple pathways, exerted great functions in the proliferation and differentiation of cancerous tissues (Verkman et al., 2008; Shi et al., 2012). As tumor cells change dramatically in morphology and sizes in the metastatic process and their metabolizing speed is quicker than normal cells, thus water is required increasingly and AQPs are demanded to finish its rapid trans-membrane transportation. A study revealed that Aquaporin 1 (AQP_1) was in close association with the tumor angiogenesis, suggesting that it could be a potential index for predicating the development and prognosis of tumors (Deb et al., 2012). In order to search a non-invasive marker that had both high sensitivity and specificity so as to detect the recurrence of BUCC, this study analyzed the relevance between the expression of AQP_1 with the clinical pathological patterns (tumor stages and grades, etc.) and recurrence, so as to verify the function and mechanism of AQP_1 in the development and progression of BUCC, hoping to provide basis for the diagnosis and treatment of BUCC patients.

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Materials and Methods

General data

After all informed consent forms were signed by the patients and (or) their families, tissue samples from 45 patients diagnosed as BUCC pathologically who were treated with total cystectomy or transurethral resection of bladder tumor (TURBT) from June, 2013 to December, 2014 in *Henan Provincial People's Hospital* were selected (BUCC group). There were 28 males and 17 females, aged 34~76 years with average age of (61.51±18.74) years; 27 were with non-muscle invasive urothelial carcinoma (including 17 in phase Ta, 2 in phase Tis and 8 in phase T1) and 18 muscle invasive urothelial carcinoma (including 13 in phase T2 and 5 in phase T5); 26 with high-grade mamillary urothelial carcinoma and 19 with low-grade mamillary urothelial carcinoma; 11 with lymph node metastasis and 34 without; and 18 with postoperative recurrence and 27 without. The tissue samples from 40 healthy people with non bladder cancer undergoing special detections or treatments served as control group, and all tissues were diagnosed as normal bladder tissues by pathology. There were 24 males and 16 females, aged 31~77 years with average age of (60.09±19.38) years.

Methods

Histoimmunochemical method was adopted to detect the expression of in BUCC tissues and normal bladder tissues. The detailed procedures were as follows: All samples were fixed subsequently with 4% paraformaldehyde and 5%, 10% and 20% sucrose solutions, embedded by OTC gel and cut into 4 μm continuous sections. Endogenous peroxidase (EGPO) was sealed by 3% hydrogen peroxide solution. Antigens were repaired by warming with sodium citrate at 92~98°C. Non-immunological goat serum was added and incubated for 20 min at room temperature. Primary antibodies (rabbit antibodies AQP_1 polyclonal antibody was bought from Santa Cruz company) were added, incubated at 4°C overnight and washed for 3 times by 0.1 mol/L phosphate buffer solutions (PBS). Second-antibody working solution (goat anti-rabbit IgG was purchased from Santa Cruz company) marked with horseradish peroxidase (HRP) was added and incubated at room temperature for 2h. Diaminobenzidine (DAB) (DAB coloring kits were purchased from Santa Cruz company) was added for color development. The sections were re-stained with hematoxylin and then sealed. The normal bladder tissues were collected as positive controls and PBS was used as the replacement of primary antibody.

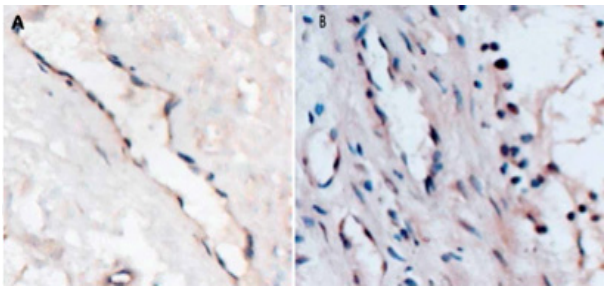

Figure 1. Expression of AQP1 in Normal Bladder Tissues and BUCC Tissues. A) Normal bladder tissues; B) Non-muscle invasive BUCC tissues

Observational indexes

The expressions of AQP_1 in cancerous tissues and normal bladder tissues were observed so as to analyze the relationship between the expressions of AQP_1 the clinical pathological characteristics. The expressions of AQP_1 in cancerous tissues before and after recurrence were compared to analyze the relevance between the positive expression rate of AQP_1 and the recurrent time.

Evaluation criteria

All sections were independently observed and diagnosed by two pathologists uninformed of the patients' clinical data. Cells with claybank granules in membrane or cytoplasm were considered to be positive. Ten high-fold folds (×400) were randomly selected from each section to calculate the total tumor cell count and positive cell count. Positive cell expression≤5% was recorded with 0 score, 6%~25% with 1 score, 26%~50% with 2 scores, 51%~75% with 3 scores, and >75% with 4 scores. According to the coloring severity, free of color was recorded with 0 score, light yellow with 1 score, claybank with 2 scores and brown with 3 scores. If the total score of above two programs was 0~1 score, the section was regarded to be negative, otherwise to be positive. The average value of the scores was recorded as the expression value of positive cells.

As to samples before and after recurrence, 5 fields were selected from each section and 200 cells of each field were counted. Expression rate of positive cells=positive cell count/total counted tumor cells×100%.

Statistical data analysis

SAS 9.3 software package was applied for all data analysis. Test of normality was conducted on measurement data, and those with normal distribution were expressed by mean±standard deviation (X±S) and detected with t test between two groups. The relevance analysis between the positive cell expression rate of AQP_1 and recurrent time was detected by Spearman correlation test. Two-tailed test was applied for all statistical detection, with significant level being α=0. 05.

Results

Expression of AQP_1 in tumor tissues

Positive response of AQP_1 showed claybank and expressed in the membrane and cytoplasm of microvascular endothelial cells in both normal bladder tissues and BUCC tissues. The expression of AQP_1 in normal bladder tissues and BUCC tissues were (2.175±0.693) and (3.689±0.701) respectively and there was significant difference (t=9.99, P<0.0001) (Figure 1).

Relevance between expression of AQP_1 in BUCC tissues and clinical pathological characteristics

The expression level of AQP_1 increased apparently along with the increase of BUUC histological grade and pathological stage (P<0.01), which was higher in BUCC

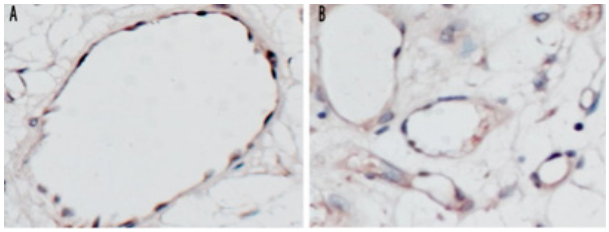

Figure 2. Expression of AQP1 in Bladder Cancer Before and After Recurrence. A) Expression of AQP1 in primary tissues; B) Expression of AQP1 in recurrent tissues

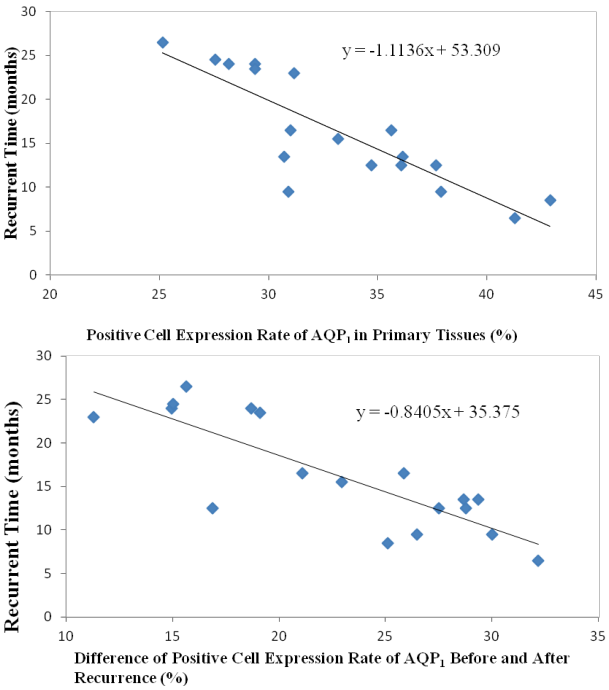

Figure 3. Relevance Between Positive Expression Rate of AQP1 and Recurrent Time

tissues with lymph node metastasis than those without, and the difference was significant (P<0.01) (Table 1).

Comparison of AQP_1 expression before and after recurrence

AQP_1 expressed in the membrane and cytoplasm of microvascular endothelial cells in both BUCC primary tissues and recurrent tissues, whose expression was higher in primary tissues than in recurrent tissues (Figure 2&3).

Relevance between positive expression rate of AQP_1 and recurrent time

In tissues with short-term recurrence, the expression of AQP_1 positive cells was higher in tissues with primary tissues samples, which increased obviously after recurrence than recurrence before. Additionally, the recurrent time of BUCC was in negative association with the positive cell expression rate of AQP_1 and the difference of the expression of AQP_1 before and after recurrence (r=-0.843, F=39.302, P=0.000; r=-0.829, F=35.191, P=0.000).

Discussion

Bladder cancers are high in morbidity and rank the

Table 1. Relevance Between AQP1 Expression and Clinical Pathological Characteristics

N	AQP1 expression level	T or F	*P*
Non-muscle invasive carcinoma			
27	3.259±0.748	4.56	<0.0001
Muscle invasive carcinoma			
18	4.333±0.811		
Low-grade urothelial carcinoma			
19	3.053±0.772	4.14	0.0002
High-grade urothelial carcinoma			
26	4.115±0.901		
With lymph node metastasis			
11	4.182±0.898	5.87	<0.0001
Without lymph node metastasis			
34	2.676±0.684		

11th and 7th in malignant tumors and female tumors, respectively. The pathological patterns include UCC, adenocyte carcinoma and squamous-cell carcinoma, in which BUCC is the most common one (Torre et al., 2015). As BUCC is easy to develop, relapse and metastatize, for which the single surgical therapy is poor in clinical efficacy, it has become one of the primary diseases severely decreasing the quality of life of elderly patients (Huang et al., 2013; Qin et al., 2013). Therefore, targets with high sensitivity and specificity are needed to promote the diagnostic level of BUCC.

In AQPs, 13 members have been discovered. Each AQP is special in the distribution and location of cell expression, and has different functions on different sites in order to meet the requirement of body metabolism and maintain the fluid equilibrium in and out of cells. In recent years, some studies demonstrated that in the development and progression of malignant tumors, there were abnormally active metabolism and increased frequency of osmotic pressure changes in and out of cells, in which AQPs were critical and might participate in the development and progression of tumors via multiple pathways (Warth et al., 2011; Shi et al., 2014; Shi et al., 2014). Some scholars found that AQP_1 was in close relationship with the development of tumor angiogenesis (Zou et al., 2013), whose positive expression rate was 35.8% in patients with colorectal cancer in phase II~III and was closely associated with lymph node metastasis, vascular infiltration and vessel infiltration. Moreover, the 5-year survival rate of patients with positive expression of AQP_1 was evidently lower than those with negative expression (Yoshida et al., 2013). Kang et al detected the expressions of AQP_1, AQP3 and AQP5 in the cancerous tissues of patients with colorectal cancer after radical resections, which concluded similar results that the expressions of above 3 indexes were in obvious connection with lymph node metastasis (Kang et al., 2015). Additionally, AQP_1, which expressed excessively in multiple tumors, such as breast cancer, adenoid cystic carcinoma, renal cell carcinoma and glioblastoma multiforme, etc., could be used as a potential biological marker for the early diagnosis and prognostic predication of tumors (Shi et al., 2012; El Hindy et al., 2013; Morrissey et al., 2014; Morrissey et al., 2015; Tan et al., 2014). In addition, the application of AQP inhibitor or RNA interference to interfere the over-expression of

AQP_1, which can inhibit the development and progression of tumors through suppressing the tumor cell metabolism, may provide new developmental direction for the treatment of malignant tumors, e.g., Stigliano et al adopted chitosan small-interfering RNA naonparticle specificity to inhibit the expression of AQP_1 in tumor cells (Stigliano et al., 2013). However, this technique is still in the stage of basic research and preclinical study, so further research is needed.

AQP_1 is also in close association with the recurrence of tumors. From the aspect of histology, AQP_1 is closely connected with tumor angiogenesis that can promote the invasive growth of tumor cells through multiple pathways, leading to the endless proliferation of a small amount of residual tumor cells after lumpectomy that can develop into recurrent tumors. In this study, it was proved that AQP_1 expressed highly in BUCC tissues and the expression level was in close connection with the tumor histological grade, pathological stage and lymph node metastasis. The expression level of AQP_1 in BUCC tissues, which could be used to predicate the invasive and metastatic severity of tumors primarily, was of certain significance in guiding the clinical diagnosis and treatment as well as prognostic evaluation. In addition, the further detection of AQP_1 expression level in BUCC tissues before and after recurrence showed that the expression level of AQP_1 was in negative association with the recurrent time of BUCC, and patients with higher expression level of AQP_1 in primary tissues were higher in malignant severity and recurrent rate after operation, short in recurrent intervals and poor in prognosis.

This study provided a new pathway for the early diagnosis, prognostic predication and surveillance for patients with BUCC. However, because of the low amounts of samples in this study, the research results required large sample trial to be further verified. And as a biological marker for tumor recurrence, the sensitivity and specificity as well as clinical application value of AQP_1 still need to be deeply explored.

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