ISSN 2618-1584 Vol. 5, Issue 4: 62-66, DOI: 10.25236/FMSR.2023.050411

Expression of TMEFF2 gene and significance of initiation methylation in bladder cancer

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Abstract: *Objective: To analyze the expression and significance of TMEFF2 gene methylation in bladder cancer. Methods: The promoter region methylation level of the MEFF 2 gene was analyzed by applying the expression database and gene methylation.64 urine samples were detected by demethylated drug-nitrogen-2 deoxycytidine to observe the changes of cell invasion and apoptotic valence, and its application value in bladder cancer was analyzed by applying high expression of TMEFF2 gene. Results: Drug treatment of bladder cancer cells showed that the appreciation and migration of bladder cancer were inhibited, but the number of apoptosis increased. Conclusion: TMEFF2 gene promoter methylation is one of the markers of bladder cancer and plays an important role in the diagnosis of bladder cancer.*

Keywords: *TMEFF2 gene; expression of bladder cancer; initiating methylation level*

1. Introduction

Objective: Bladder cancer is a urinary system disease, and it is easy to appear in men. More than 80,000 new bladder cancer patients are reported in China every year, of which more than three quarters of patients are non-primary invasive, and a quarter of patients are primary invasive. Therefore, screening tumor biomarkers to prevent the emergence of bladder cancer is of great significance for the prevention and diagnosis of bladder cancer[1-2]. According to some research and analysis, genetic changes will have a certain impact on gene expression, which is mainly associated with tumorigenesis. Hyperplastic polyposis has follistatin and transmembrane proteins. Nowadays, a large number of clinical experiments show that TMEFF2 initiates hypermethylation in both lung cancer and breast cancer. However, in patients with bladder cancer, whether it is involved in the development of the disease has not been clearly defined[3]. On the effects of the demethylation of bladder cancer.

2. Data and methods

2.1. General information

As Gene methylation expression database belongs to a public database, which focuses on tumor gene expression and DNA methylation, focusing on 18 human cancers, 6,000 samples, and more than 12,000 sequencing data. In the gene database, a bladder cancer item is selected and TMEFF2 is entered into the database for lookup. In the stage of case selection, the urine of patients in the whole year in 2022 was 64, of which 32 were healthy urine, and 32 were bladder cancer. Urine samples from bladder cancer patients were collected 3 days before the operation, and the selected samples were dissolved in an environment of minus 80 degrees Celsius and then used. 64 specimens in age equal baseline data and no significant difference.

2.2. Methods

Human normal bladder epithelial cells were selected, selected bladder transitional cell lines were from the cell bank, methylase inhibitor 5-nitrogen-2 deoxycell liver from S igma, and fluorescent PCR kit from Shanghai Biomedical Technology Co., Ltd. All the selected antibodies are produced by Abcam, USA.

Methylation-specific fluorescence PCR was performed for patient urine at 50 ml, centrifugal at 3000 rpm, and the supernatant was removed and calm fluid kept for DNA extraction. During the nucleic acid extraction, the automatic nucleic acid extraction instrument is used. After lysis, the nucleic acid can be

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converted with bisulfite and then purified. The amplification treatment was implemented by applying methylation specificity with acute amplification by fluorescent PCR. The reaction conditions are as follows: in 50 degrees Celsius environment, amplify for 2 minutes, in 95 degrees Celsius environment, amplify for 10 seconds, in 60 degrees Celsius environment, amplify for 40 seconds, cycle about 42 times. The gene sequence was obtained, the number of fluorescence domain cycles was obtained, the amplification curve was observed, and the analysis was performed. For bladder cancer T24 cell culture and 5-Aza-d for treatment, the selected medium required 10% FBS and double antibody RPMI-1640 for paving on a 6-well plate by culture and passage. If cells were grown to 75%, medium with 5-Azadc could be added, with the final drug concentration set to 8 μ mol per liter. The control group was set to add the same volume of DMSO for 3 days, requiring the patient to be changed daily.

T24 cells were transfected in bladder cancer, and the T24 cells were routinely cultured and paved in a 6-well plate. Cells were grown to 85% in 6-well plates, according to the reagent instructions, and the control group was transfected with an empty plasmid.

Western blot Test, add 40 μ g protein sample, using the concentration of 10% SDS-PAGE wet turn power to nitrocellulose membrane, the use of 5% concentration of nonfat milk powder closed treatment, time for 1 hour, using the concentration of 5% nonfat milk powder for dilution, in about 4 degrees Celsius environment overnight, the sheep anti rabbit secondary antibody, using room temperature, incubation time is 2 hours, development.

For CCK-8 experiments, the cell increment rate was measured, cells were seeded into 96 wells and drug added for 5 days. CCK 08 reagent was added to all six wells and cells were placed in a 37 ° C environment for 2 hours. Was measured by an applied microplate spectrophotometer with an absorbance value of 450 nm.

Migrating cell were scratched, streaked on the right, middle and left side of the 6-well plate using a 20 μ l gun head. Cells were removed by using PBS. At this time, serum-free medium was applied to replace the medium to observe cell migration. During photography, the migration capacity was recorded at 0 hours, 24 hours, and 48 hours.

For invasion assay, cell invasion was measured, domedicated cells were seeded into medium without FBS and FBS cell medium containing concentrations of 10% was placed into the bottom chamber. Medium was placed in a 37 ° C incubator for 16 hours. After 16 hours, use FBS in the chamber for 3 times, use a wet cotton swab to wipe the unmigrated cells, should filter another layer, cell invasion through, using a concentration of 70% ethanol solution, processing time for 30 minutes, after the use of a concentration of 0.1% crystal violet dye, staining time for 10 minutes, with the help of a microscope, observe cell invasion.

2.3. Observed indicators

(1) Analyze the methylation expression in the database;

(2) Analysis of the increased methylation level in human urine.

2.4. Statistical methods

(± s) was used to show numerical variables, test t, and qualitative data was shown using% to test X2; 0.05 For the middle bound point, the weak P is smaller than the middle bound point, which represents the obvious difference in the data. The software tool: SPSS23.0.

3. Results

3.1 Analyzed the methylation expression status in the database

Table 1: Analyzes the methylation expression in the patient database [n (%)]

group	n	tumor of bladder	Normal bladder tissue
Urine of bladder cancer	32	31(96.88)	1(3.13)
Healthy urine	32	8(25.00)	24(75.00)
t	-	-	34.724
P	-	-	<0.001

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There were differences in methylation expression in the database (P <0.05) (Table 1).

3.2 Analysis of the increase of human urine methylation level

In the urine of bladder cancer patients, the number of patients with hypermethylation was 29, or 90.63%. In the urine of healthy population, the number of patients with hypermethylation was 12, with a proportion of 37.50, and the 19.614.P value of 0.000 was obtained.

4. Discussion

Bladder cancer is a tumor that occurs at the bladder, occurring at any age, and, or smoking can increase the risk of bladder cancer. Bladder cancer can be classified, including bladder squamous cell cancer, bladder sarcoma, with a high probability of incidence. At present, the clinical etiology is not clear, but there is a basic etiology. Genetic, environmental factors, smoking, chronic infections and so on can all lead to diseases. Most patients experience hematuria, urine pain, dysuria and urgency. If invaded, or metastasis occurs, it can also cause a variety of clinical symptoms. Patients will appear the following typical symptoms, haematuria is the first symptom, often appear intermittent, lasting for a long time, the disease gradually develops, the patient hematuria interval will become shorter, after taking anti-inflammatory drugs, the condition will be improved. Bladder irritation symptoms are the second clinical feature. Some patients will have dysuria, and even urinary retention if the patient's condition worsens. Upper urinary tract obstruction symptoms is also one of the clinical symptoms, tumor in the ureter mouth, can cause hydronephrosis, also can lead to ureter expansion, some patients will appear waist ache, if obstruction event is longer, obstruction degree is more serious, can lead to patients with kidney function injury, cause renal insufficiency. During the medical treatment, patients often appear after hematuria. During the implementation of examination for patients, cystoscopy can be used to diagnose bladder cancer and pathological biopsy, which belongs to the gold standard of bladder cancer examination. Urine examination can be used. During the examination of the patients, the imaging examination method, including the X-ray examination, or the ultrasound examination method can be used. Using the ultrasound examination method, the application in the diagnosis of bladder cancer, has a certain effect. The CT urography examination can evaluate the disease stage and provide information for the diagnosis and treatment of medical staff. During the diagnosis for the patient, differentiation is required from the following diseases, including upper urinary tract tumor, urolithiasis, radiation cystitis, prostate cancer and other symptoms. During the treatment of patients, surgical treatment is usually used, which includes epirubicin and doxorubicin. Radiation therapy can be used as an adjuvant therapy. If the patient is refractory to surgery, the surgical unresectable lesion can be used as an alternative treatment. Once the patient gets sick, there will be various complications, usually after the treatment, adverse symptoms such as postoperative bleeding and infection. After the implementation of chemotherapy, patients are prone to symptoms such as bone marrow suppression, and patients are prone to adverse symptoms such as frequent urination after radiotherapy. Because after patients get sick, it is easy to cause various complications, which seriously affect the health of patients, so it is necessary to carry out effective diagnosis for patients to avoid the aggravation of the patient's condition, leading to the adverse impact on the treatment effect.

Since the first sequence that TMEFF2 was differentially methylated in 2001, it is now found in a variety of cancers. Bladder cancer is a malignant tumor with high incidence probability and a common urinary tumor in China. Nowadays, after the full development of molecular biology, epigenetics and other basic disciplines in China, the development and discovery of the development of malignant tumors are related to genetics and molecular biology. According to some studies, CPG methylation in the promoter region of the tumor suppressor gene is an important reason for the inheritance of the gene, and it also suppresses the transcription of the gene, which is confirmed to be closely related to the occurrence and development of several malignant tumors. CPG island abnormalities, is the focus of genetic research. DNA methylation occupies an important component in genetics, which is the corresponding concept of imagery genetics, which refers to the stable change of gene expression and cell phenotypic heritability. But it doesn't change the gene sequence. The mammalian genome becomes one of the genetic events, by changing DNA methylation, although genetic, but prone to reversal, so it can be used as a target for targeted therapy. The epigenetic mechanism is closely related to DNA methylation, which means that DNA transfers related methyl groups to specific bases catalyzed by methyltransferase, and DNA methylation may occur in related parts of cytosine adenine in the human body. In mammals, it occurs mainly at the C-5 position of the cytosine, and eventually has methylcytosine production. The main regions of DNA methylation are cytosine and uracil. If they are normal cells, more than 80% are in the

ISSN 2618-1584 Vol. 5, Issue 4: 62-66, DOI: 10.25236/FMSR.2023.050411

non-methylated state. The methylation state will lead to the inhibition of patient gene transcription. Be able to. Protein deacetylase aggregates, and according to the action characteristics, the main mechanism of action is the conversion of semi-methylated double-stranded DNA into fully methylated double-stranded DNA. Some studies have analyzed DNA methylation for genetic imprinting and embryonic development.

DNA methylation is an important mechanism of gene expression silencing, including the following contents: (1) DNA methylation can lead to the inhibition or termination of gene transcription, through a direct way, inhibit the binding of deliberately transcription factors and the recognition site on the promoter, and can also interfere with it.(2) After the methylation of the regulatory sequences at the gene end, it can bind to the methylated CPG sequences, thus preventing the complex formation between genes and transcription factors.(3) During the expression, DNA demethylation can promote a better chromatin environment. DNA itself is transcriptionally active, and after methylation, it binds to methylated proteins, thus promoting changes in chromatin structure and inhibiting transcription. Some studies associated the methylation status of genes with the early progression of cancer. In malignant tumor species, methylation of gene promoters is a common form, which is also closely associated with tumor prognosis and disease progression. In a variety of tumors, the methylation of gene promoter has a certain role, so it needs to be paid attention to by medical staff.

By selecting 64 samples, the bladder cancer cells and the database proved that TMEFF2 showed moderate and low expression conditions, but from the analysis of methylation levels, it showed a high expression status. Through the application of methylation drugs, prompting TMEFF2 hypermethylation state for demethylation, when the drug concentration in 10 μ mol per liter, through CCK-8 experiment and scratch experiment, after processing T24 cell biology changes, shows that after drug treatment, patients TMEFF2 expression level improved, methylation level will reverse, lead to bladder cancer appreciation, migration, etc[4]. At the same time, the TMEFF2 is in a high expression state, indicating that this gene interferes with bladder cancer. Hypermethylation levels also play a role. After TMEFF2, found in 2001, hypermethylation levels can now be found in many cancers. In bladder cancer, the expression of TMEFF2 was analyzed, but the specific occurrence mechanism has still not been deeply explored[5]. Demethylation drugs were selected to disturb the appreciation and invasion of bladder cancer cell lines and provide a direction for the pathogenesis of bladder cancer. During the expression of human normal tumor cell genes, epigenetic changes in DNA can regulate cell expression. Around the promoter region of most genes, they can form CpG. In many malignant tumors, the high methyl group of CPG island has restrictions on tumor suppressor genes, which is genetic silencing. If DNA is abnormal in the early stage of tumors, demethylation is more frequent, and methylated genes belong to biomarkers in tumor patients compared to healthy populations[6]. Urine needs to be stored in the patient's bladder, and gene methylation in urine is able to observe the patient's bladder changes to predict whether bladder cancer will progress to primary invasive bladder cancer. For patients with bladder cancer, it has high sensitivity by using methylation level. However, there are still shortcomings in this study. Due to the small sample size, methylation can be used as a diagnostic indicator in the future, but more books are needed to prove the above points[7-8]. The TMEFF2 gene contains 11 exons, located in human chromosomes, which contains not only initiation factors, but also multiple turnover factor binding sites such as specific proteins, which is prone to hypermethylation in human tumor cells. Therefore, this gene can be selected during screening for bladder cancer tumors. In this study, there are still some shortcomings. Due to the small number of selected cases, the number of study cases needs to be increased in future studies to improve the accuracy of the study results. It is hoped that the analysis of this article can provide a theoretical basis for clinical analysis, from the perspective of hospital, hope that doctors develop rapidly, from the perspective of patients, early diagnosis and treatment of patients, improve the clinical symptoms of patients, give scientific treatment for patients, reduce the pain of patients.

In conclusion, this study was analyzed from the data level and urine level, and found that TMEFF2 gene methylation changes in bladder cancer, which has an impact on biological behavior, indicating that bladder cancer and genetics have an impact. There are some shortcomings in this study, no verified urine samples and no expression in bladder cancer tissues. Without follow-up of the collected samples, the study time is short, so in future studies, the sample content will be increased to serve as a screening test for bladder cancer. I hope that the article analysis can provide a little advice for the diagnosis of bladder cancer.

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ISSN 2618-1584 Vol. 5, Issue 4: 62-66, DOI: 10.25236/FMSR.2023.050411

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