Gene mutation detection of urinary sediment cells for NMIBC early diagnose and prediction of NMIBC relapse after surgery

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Abstract

Early diagnose of bladder cancer could lead to good prognosis and high 5-year-survival rate. Among bladder cancer, about 75% patients with were nonmuscle-invasive bladder cancer (NMIBC). Patients were painful and easily get infected during bladder cancer diagnosis, which mainly depends on invasive cystoscopy and low-sensitivity urine exfoliation cytology. Meanwhile, relapse after surgery was also becoming the major problem for patients. Exploring noninvasive, high-sensitivity, and painless method is very important and meaningful for NMIBC treatment.

Firstly, we found potential related gene mutation sites for NMIBC by searching COSMIC database and related study. Urinary sediment cells of patients both in normal group (patients with benign) and NMIMC group were collected before and after operation for potential gene mutation site detecting. Meanwhile, the urinary sediment cells of relapse patients and good prognosis people in NMIBC group after surgery were also collected for further Gene mutation detection and NMIBC relapse after surgery prediction.

Fourteen genes (152 mutation sites) were selected between 95 NMIBC patients and 67 control patients, which were FGFR3, TP53, PIK3CA, and others. Compared with control group, mutation ratio of above 14 genes was higher in NMIBC group. NMIBC diagnose model was established by 5 times cross-validation and had a good effects, which included the all mutation site in FGFR3, TP53, PIK3CA, ARID1A, STAG2, and KTM2D. On the contrary, the relapse rate was 30.5% among 95 patients for about 1.5-year follow-up time. Compared with control group, smoking rate and tumor grade were higher in relapse group. Meanwhile, mutation rate of FGFR3, TP53, PIK3CA, ERBB3, and TSC1 in relapse group were higher than that in normal group. According to the mutation sites of FGFR3, TP53, PIK3CA, and ERBB3 and the combination of urinary sediment cells genetic mutation and relapse status, a predicted model for NMIBC relapse was also established, which had 90% accuracy.

The diagnosed NMIBC model (based on FGFR3, TP53, PIK3CA, ARID1A, STAG2, and KTM2D gene mutation) and predicted relapse model (based on FGFR3, TP53, PIK3CA, and ERBB3 gene mutation) possess high accuracy and would be applied in early diagnose and early predicting relapse of patients.

Abbreviations: NMIBC = nonmuscle-invasive bladder cancer, NPV = negative predictive value, PPV = positive predictive value.

Keywords: gene mutation, liquid biopsy, nonmuscle-invasive bladder cancer, urinary sediment cells

1. Introduction

In developed countries, the incidence of bladder cancer ranks 4th among male malignant tumors, which causing a serious disease burden.[1,2] Nonmuscle-invasive bladder cancer (NMIBC) accounts for 70% to 80% in bladder cancer, which 70% patients are Ta, 25% patients are T1, and 5% patients are CIS. For NMIBC patients, surgery is the main therapy for the treatment. At present, early diagnosis of bladder cancer can lead to a good prognosis and a 5-year survival rate of 94%. The diagnosis of bladder cancer nowadays mainly depends on cystoscopy and urine exfoliation cytology. The characteristic of cystoscopy are mainly invasive, which always resulted in pain, infection and misdiagnosis (62–84% sensitivity and 43–98% specificity) for patients.[2] Meanwhile, urine exfoliation cytology has media sensitivity. Therefore, it is of great significance to develop a noninvasive, sensitive and painful diagnostic method for bladder cancer. On the contrary, compared with normal cells, gene mutations were always detected in tumor cells. Therefore, with the development of liquid biopsy and 2nd generation sequencing technology, noninvasive and early diagnosis of bladder cancer can be achieved by detecting biomarkers in

urine, such as DNA methylation and mutant genes. Urinary sediment cells were cell mix in urine, which included many kinds of cells, such as red blood cells, white blood cells, renal tubular epithelial cells, and tumor cells in NMIBC patients.

Bladder cancer has a high relapse rate, which could be up to 60% to 70%.[3] What is more, the malignance also was increased after surgery. Meanwhile, millions of patients undergo examinations for preventing relapse.[4] Therefore, early and simple prediction before relapse has great significance to the patient prognosis. Owing to its simple operation and sustainable monitoring characteristics, genetic detection of urinary sediment cells can be well applied to monitor relapse after surgery.

The interaction among gene regulation, epigenetic regulation, and environmental factors leads to the occurrence of cancer. Epigenetic regulations, such as DNA methylation, play important roles in the development of cancer. Many studies have noninvasively diagnosed bladder cancer[5] and predicted relapse of bladder cancer[6] by detecting DNA methylation information in urine sediment cells of bladder cancer patients.

Researchers found that the accuracy of predicting relapse of bladder cancer by panel composed of methylation levels of SOX1, IRAK3, and L1-MET genes was 35% higher than that by cystoscopy and 15% by cytologic examination. There results demonstrated that the combination of multiple markers in multiple urine sediment cells has a high accuracy in detecting relapse of bladder cancer.[6] In addition to DNA methylation, gene mutation also plays an important role in the progress of bladder cancer. According to COSMIC database statistics, the percentage of TERT mutation in bladder cancer patients is 52%, that of FGFR3 mutation is 35%, and that of TP53 mutation is 28%. Furthermore, these mutant genes should be involved in the development of bladder cancer.

The most suitable specimen for detecting the molecular characteristics of bladder cancer is still tissue. However, in the process of cancer formation and progression, some cancer cells will detach from the primary lesion and metastasize to local or distant areas, and enter the body fluid (blood, urine, pleural, ascites, etc). The cancer cells entering the body fluid carry the genetic information of the primary tumor. Therefore, the detection of DNA mutation in urine sediment cells can also reflect the molecular characteristics of tumor tissue to a certain extent. The key is to find suitable molecular markers and use high-sensitivity detection technology. In this study, we will examine the consistency of tissue biopsy and urine examination to guarantee the accuracy of the results.

Therefore, the aim of our study is to detect hotspot gene mutations in urinary sediment cells (including TERT, FGFR3, TP53, etc) for bladder cancer diagnose with noninvasive and convenient and predict the relapse of bladder cancer patients after operation.

2. Materials and methods

2.1. Patients and prognosis follow-up

Patients were from the 1st affiliated hospital and the 3rd affiliated hospital from May 2017 to November 2018. The patient was suspected of having bladder cancer. Meanwhile, detailed clinicopathologic information of patients was also collected. After cystoscopy and pathologic diagnosis, patients were divided into 2 groups, control patients (n=67) who has hematuria and no macroscopic tumor at cystoscopy/CTM, and the other group was

NMIBC patients (n=95) who were diagnosed as malignant tumor. About 95 patients with NMIBC were followed up every 3 months. Relapse is defined as the occurrence of bladder cancer symptoms 3 months after treatment. The study was approved by the ethics Committee of Zhengzhou University.

2.2. Gene mutation information screening

In the COSMIC database (https://cancer.sanger.ac.uk/cosmic), we get the mutation information of bladder cancer through searching for the keyword "Bladder cancer" and selecting the corresponding tissue. In addition to referring to the COSMIC database, we search for bladder cancer-related gene mutation information by using the keywords "bladder cancer," "molecular characterization," "sequencing," and "gene mutation" in PubMed (www.pubmed.com). Finally, combined with COSMIC database and relevant literature, the potential gene mutation information in present study was determined.

2.3. Sample collection and mutation information detection

Urine samples (80–100 mL), sediment cells, and tumor tissues after surgery were collected, which were used to detect mutation information by next generation sequencing. Urine is collected and stored in a refrigerator at 4°C or sent for inspection immediately. Urine sediment cells are isolated from urine with DNA kit (QIAGEN DNeasy kit, German). Tumor tissue was immersed in normal saline after operation and sent for inspection immediately or embedded in paraffin to make paraffin section. The DNA libraries were prepared by kit (Illumina NGS Fast Library Prep Set) and interrupted by ultrasound for 10 cycles at 30 seconds ultrasound, 30 seconds interval per cycle.

2.4. Gene mutation consistency analysis between urine sediment cells and tumor tissues

In this study, 32 pairs of urine sediment cell and tissue samples were collected, including 22 pairs in NMIBC group and 10 pairs in control group. If the mutation information detected in a patient's tissue is identical to that detected in urine sediment cells at one or more loci, the mutation consistency is considered. If none of them were detected, they were wild-type consistency. The overall consistency was the sum of wild type consistency and mutation consistency. If there is no consistent site, the patient's tissue and urine sediment cell mutation information is inconsistent.

2.5. Diagnose and early prediction models establishment

According to the results of sequencing, the genes were sequenced by the random forest model. Then 50 NMIBC patients and 40 control patients were randomly selected as training sets to construct a diagnose model. The diagnose model was then tested in the remaining 45 NMIBC and 27 control samples. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were used to evaluate the accuracy of this model by 5-fold cross-validation. To further study the relapse issues, the patients in NMIBC group were followed up for about 1.5 years. Kept record of relapse and related clinicopathologic features both in relapsed and nonrelapsed patients. Predicted model was also established according to genetic mutation and relapse. Sensitivity, specificity, PPV, and NPV were used to evaluate the accuracy of this model.
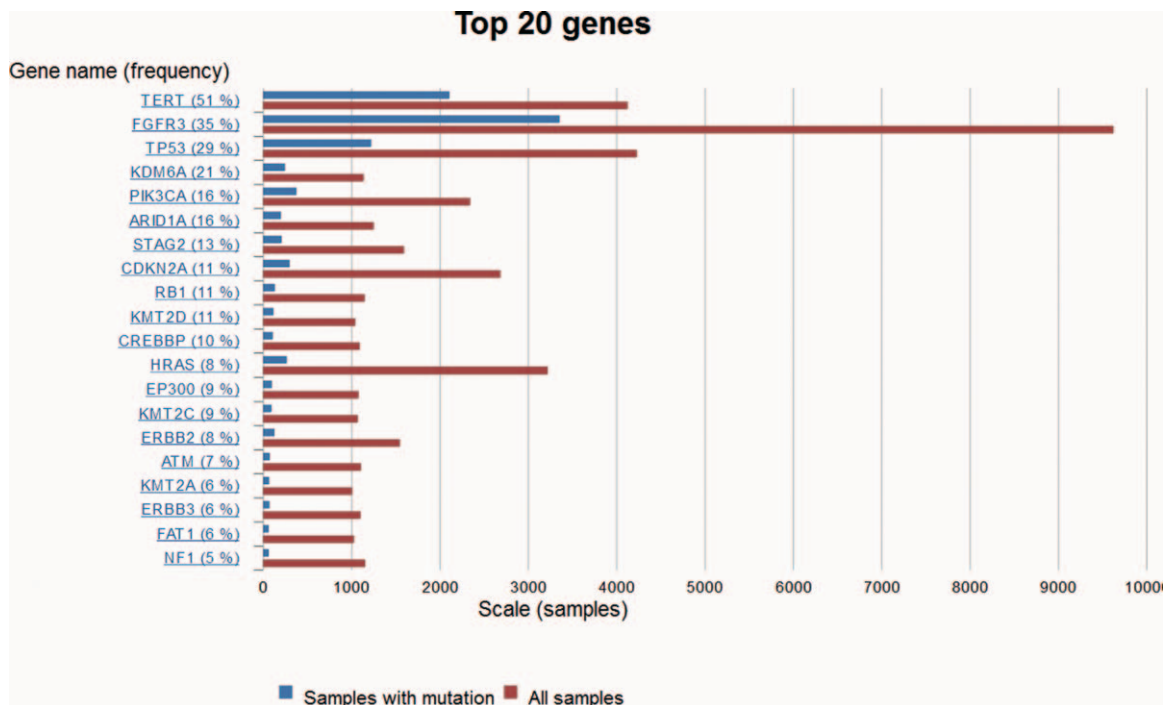
Figure 1. Bladder cancer-related top 20 genes in COSMIC database.

2.6. Statistics analysis

Data are expressed as mean ± standard error mean, and the differences between groups were evaluated using SPSS 16.0 software. The data between the 2 groups were compared by 2 independent samples t test. Chi-squared test was used to compare the counting data. The model was constructed by 5-fold cross-validation (random sampling of some samples as training sets, the remaining samples as test sets). $P < .05$ showed that the difference was statistically significant.

Figure 2. Bladder cancer-related gene mutation result from citation summary. The citations were Eur Urol 2016; 70(6): 916–919; Nature 2014; 507(7492): 315–322; Nat Genet 2011; 43(9): 875–878; and Nat Genet 2013; 45(12): 1459–1463.

3. Results

3.1. Information of gene mutation and patients

By searching COSMIC database (top 20 genes were found) and related literature, 14 important mutation genes related to bladder cancer were selected in this study and the total number of mutation sites was 152. The mutation genes included FGFR3, TP53, PIK3CA, ERBB3, TSC1, NF1, ERBB2, FGFR1, CDKN2A, ARID1A, STAG2, KTM2D, CREBBP, and HRAS (Fig. 1) and their specific gene mutations were also provided (Table S1, http://links.lww.com/MD/D154). Corresponding 14 bladder cancer-related gene mutation proportion from previous study was also provided in pie chart (Fig. 2).

About 95 NMIBC patients (65.4 ± 12.5 years average age) and 67 control patients (54.4 ± 6.3 years average age) were selected for further analysis. The male rate of patients in NMIBC group was up to 80%, which is higher than that in control group. Meanwhile, the number of smoke in NMIBC group was also higher than that in control group (Table 1).

In present study, mutations at any site of the gene were considered gene mutation positive. The positive rate of gene mutation sites in urinary sediment cells in control group was 56.7% (38/67) and the positive rate in NMIBC was 93.7% (89/95) (Table 2). It was found that the mutation frequency of some sites in NMIBC was significantly higher than that in control group, while most of the mutation sites had no significant difference between the NMIBC group and control groups (Fig. 3). The range of mutation sites detected in the 2 groups was 13 and 16, respectively. The range of mutation genes in the 2 groups was 6 and 9, respectively (Table 2). Among potential 14 genes, FGFR3, TP53, and STAG2 have extremely significant difference ($P < .01$). PIK3CA, ERBB2, ARID1A, KTM2D, and HRAS have significant difference ($P < .05$). The other genes (ERBB3, TSC1, NF1, FGFR1, CDKN2A, and CREBBP) have no statistical differences (Table 3).
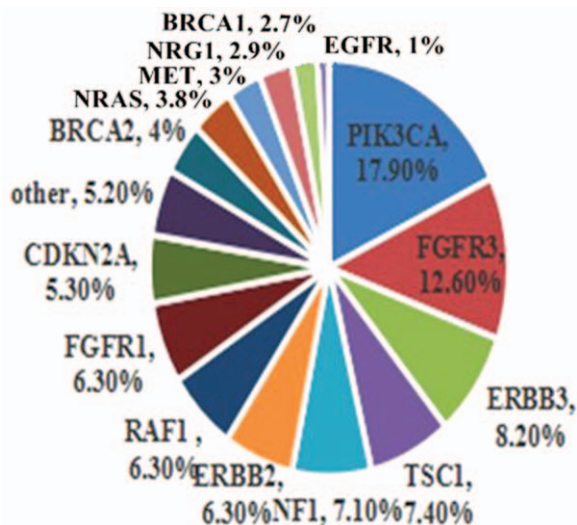
Table 1

Clinicopathologic characteristics of research subjects.

Features	NMIBC (n=95)	Control (n=67)	P
Age, yr			
Average age (χ±s)	65.4±12.5	54.4±6.3	.013
Range	29–84	23–80	
Gender			
Male	76 (80.0%)	33 (49.3%)	.00
Female	19 (20.0%)	34 (50.7%)	
Number of smokers			
Smokes	55 (57.9%)	17 (25.4%)	.00
Nonsmokers	40 (42.1%)	50 (74.6%)	
Tumor staging			
pTa/pT1/CIS	71 (74.7%)/14 (14.7%)/10 (10.6%)	NA	NA
Grade			
Low	57 (60.0%)	NA	NA
High	38 (40.0%)	NA	

NMIBC = nonmuscle-invasive bladder cancer.

To ensure the reliability of the results, consistency analysis of gene mutations in urine and tissue samples were done. We selected 22 samples from NMIBC and 10 samples from control group. We found 14 gene mutations consistency between urine and tissue samples both in NMIBC and control group were above than 0.6 (Table S2). Among them, consistency of ARID1A, KTM2D, and CREBBP mutations between urine and tissue samples in NMIBC and control group was above 0.8 (Fig. 4).

3.2. Comparison of clinicopathology and gene mutation between relapsed and nonrelapsed patients

To further study relapse, 95 patients with NMIBC were followed up for an average of 1.5 years. The relapse rate of bladder cancer was 30.5% (29 recurred in 95 cases). Comparing the clinicopathologic characteristics of the 2 groups, it was found that there were differences in smoking rate and tumor grade between the 2 groups, but there was no statistical difference in age, sex, and tumor stage (Table 4).

For gene mutation rate in 2 groups, TP53 has extremely significant difference ($P < .01$) and FGFR3, PIK3CA, ERBB3, and TSC1 have significant difference ($P < .05$), while other gene has no statistical differences (Table 5).

3.3. Diagnosis and prediction model establishment

To make better use of the earlier results, we established diagnosis and prediction model for NMIBC early diagnose and NMIBC relapse prediction after surgery.

Table 2

Number of mutation sites and mutation genes in NMIBC and control groups.

	Control	NMIBC
Mutation sites		
≥1 site	56.7% (38/67)	93.7% (89/95)
Number of mutation sites	0–13	0–16
Mutated genes		
≥1 gene	56.7% (38/67)	93.7% (89/95)
Number of mutated genes	0–6	0–9

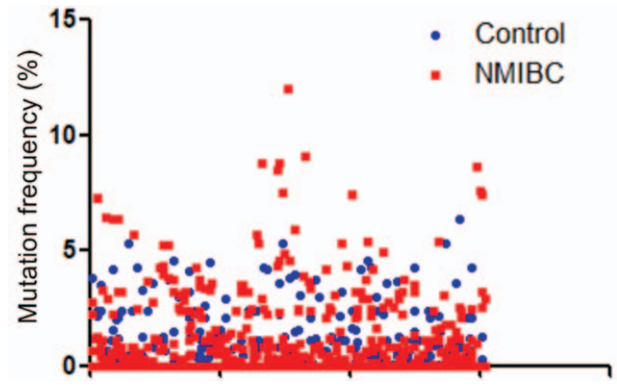NMIBC = nonmuscle-invasive bladder cancer.

Figure 3. Gene mutation frequency richness of 152 mutation sites.

A 5-fold cross-validation method was used to construct a diagnostic model for NMIBC. The model incorporates all the loci of 6 genes: FGFR3, TP53, PIK3CA, ARID1A, STAG2, and KTM2D. The sensitivity, specificity, PPV, and NPV of training set model for diagnosis of NMIBC are between 0.83 to 0.92, 0.79 to 0.94, 0.86 to 0.90, and 0.80 to 0.89 respectively. The sensitivity, specificity, PPV, and NPV of validation set model for diagnosis of NMIBC are between 0.80 to 0.91, 0.80 to 0.89, 0.80 to 0.86, and 0.79 to 0.89, respectively (Table 6). At the same time, the sensitivity, specificity, PPV, and NPV of the model were 0.93, 0.80, 0.94, and 0.75 respectively, and the area of AUC curve was 0.93 (Fig. 5).

The predictive model is a multifactor model which contains all mutation sites of FGFR3, TP53, PIK3CA, and ERBB3. The 5-fold cross-validation shows that the sensitivity, specificity, PPV, and NPV of the model are 0.87 to 0.95, 0.80 to 0.90, 0.88 to 0.93, and 0.79 to 0.91. The sensitivity, specificity, PPV, and NPV of all samples were 0.95, 0.85, 0.92, and 0.82, respectively, after receiver-operating characteristic curve analysis (Table 7). We further calculated the respective proportions of 4 genes and their combinations in the relapsed and nonrelapsed groups. The results showed that the proportion of FGFR3, TP53, PIK3CA, and ERBB3 gene mutations (at any site) was 20.7% in the relapsed group (Fig. 6A), and the rate in the nonrelapsed group was 4.5% (Fig. 6B).

Table 3

Mutation ratio of 14 genes in patient of 2 groups.

Gene	Control (n=67)	NMIBC (n=95)	P
FGFR3	10.4% (7/67)	27.4% (26/95)	.008
TP53	11.9% (8/67)	29.5% (28/95)	.008
PIK3CA	4.5% (3/67)	15.8% (15/95)	.024
ERBB3	3.0% (2/67)	9.5% (9/95)	.169
TSC1	1.5% (1/67)	7.4% (7/95)	.089
NF1	1.5% (1/67)	6.3% (6/95)	.137
ERBB2	0% (0/67)	9.5% (9/95)	.011
FGFR1	1.5% (1/67)	5.3% (5/95)	.402
CDKN2A	0% (0/67)	6.3% (6/95)	.137
ARID1A	4.5% (3/67)	16.8% (16/95)	.024
STAG2	3.0% (2/67)	15.8% (15/95)	.009
KTM2D	1.5% (1/67)	10.5% (10/95)	.027
CREBBP	3.0% (2/67)	9.5% (9/95)	.106
HRAS	0% (0/67)	7.4% (7/95)	.023

Figure 4. (A) The consistency of urine sediment cells and tissues in NMIBC group. (B) The consistency of urine sediment cells and tissues in control group. The black band represents a positive mutation in the corresponding sample. The white band represents the negative mutation of the corresponding sample.

4. Discussion

Bladder cancer is the most common malignant tumor of genitourinary system, and its incidence is still on the rise in recent years. At present, the diagnosis of bladder cancer mainly depends on cystoscopy and cytologic examination of urine exfoliation. In recent years, cystoscopy technology has also been improved, such as fluorescent cystoscopy, narrowband light cystoscopy, and so on. However, the accuracy of cystoscopy in the early diagnosis and prognosis of bladder cancer (such as relapse and metastasis) is limited. Therefore, researchers have

Table 4

Clinicopathologic comparison between relapsed and nonrelapsed groups.

Features	Relapsed group (n = 29)	Nonrelapsed group (n = 66)	P
Age, yr			
Average age ($\chi \pm s$)	65.9 ± 9.5	64.8 ± 7.4	.655
Range	29-80	31-84	
Gender			
Male	18 (62.1%)	34 (51.5%)	.378
Female	11 (37.9%)	32 (48.5%)	
Number of smokers			
Smokes	20 (69.0%)	28 (44.4%)	.000
Nonsmokers	9 (31.0%)	38 (55.6%)	
Tumor staging			.374
pTa/pT1/CIS	23 (79.3%)/5 (17.2%)/ 1 (3.5%)	48 (72.8%)/9 (13.6%)/ 9 (13.6%)	
Grade			.002
Low	5 (10.3%)	31 (47.0%)	
High	26 (89.7%)	33 (53.0%)	

Table 5

Genetic mutation between relapsed and nonrelapsed groups.

Gene	Relapsed group (n = 29)	Nonrelapsed group (n = 66)	P
FGFR3	44.8% (13/29)	19.7% (13/66)	.026
TP53	51.7% (15/29)	19.7% (13/66)	.003
PIK3CA	27.6% (8/29)	10.6% (7/66)	.028
ERBB3	20.7% (6/29)	4.5% (3/66)	.013
TSC1	17.2% (5/29)	3.3% (2/66)	.015
NF1	10.3% (3/29)	4.5% (3/66)	.285
ERBB2	13.8% (4/29)	7.6% (5/66)	.341
FGFR1	10.3% (3/29)	3.0% (2/66)	.141
CDKN2A	10.3% (3/29)	4.5% (3/66)	.285
ARID1A	24.0% (7/29)	13.6% (9/66)	.208
STAG2	24.0% (7/29)	12.1% (8/66)	.139
KTM2D	17.2% (5/29)	7.6% (5/66)	.157
CREBBP	13.8% (4/29)	7.6% (5/66)	.341
HRAS	13.8% (4/29)	4.5% (3/66)	.112

shifted their attention to the development of noninvasive, accurate, simple, and inexpensive detection technology.

With the development of liquid biopsy and 2nd-generation sequencing technology, the gene loci with low mutation abundances closely related to bladder cancer can be detected directly from urine sediment cells or supernatants of bladder cancer patients. In present study, urine and tissue consistency of 14 genes were analyzed, and the consistency of each gene was found to be different, ranging from 0.6 to 0.82. Previous findings have proved that urine DNA detection can well reflect mutation information in tissues, and the consistency of mutation information of FGFR3 in urine DNA with that in tissues is 91%.[7]

It is reliable and promising to use these loci to achieve early diagnosis and prognosis judgment. On the contrary, the occurrence and development of bladder cancer is accompanied by gene changes, including gene mutation, DNA methylation, and gene copy number variation. Gene mutation may be the most potential mutation type for diagnosis, which can be used as a diagnostic marker for NMIBC and detection relapse.

Urinary sediment cells of NMIBC patients contain lots of mutation information, whose mutation ratio and abundance were all higher than that in control patients. In this study, 152 mutation sites of 14 genes closely related to bladder cancer were selected as candidate markers based on COSMIC oncology database and related study. The male proportion in the NMIBC group is as high as 80%, which indicates that NMIBC mainly occurs in male patients. Six of them (FGFR3, TP53, PIK3CA, ARID1A, STAG2, and KTM2D) were selected as diagnostic markers and 4 (FGFR3, TP53, PIK3CA, and ERBB3) as relapse markers.

Overall, FGFR3 mutation is strongly associated with PIK3CA mutation. The mutation of PIK3CA was found in 26% of the tumors with mutation of FGFR3, while only 6.9% of the wild-type tumors with mutation of PIK3CA.[8] Studies have shown that the activation of FGFR3 promotes the growth of tumors through lipid metabolism associated with PI3K signal.[9] In present study, the mutation rate of PIK3CA in NMIBC group was significantly higher than that in control group. Sun et al have also demonstrated that PI3K pathway in bladder cancer is related to the clinicopathologic characteristics and disease progression of patients.[10]

It was found that there were FGFR3 overexpression in 85% of mutant tumors, but there were FGFR3 overexpression in 42% of wild-type tumors. This suggests that the mutation of FGFR3 is most closely related to bladder cancer than overexpression. Activation mutations of FGFR3 are present in almost 50% of bladder cancer, and are higher in low grade and low stage tumors, ranging from 60% to 70%.[11] Therefore, the mutation of FGFR3 in urinary sediment cells may be more suitable for the detection of low-grade and low-stage bladder cancer. In addition, we also found that the proportion of FGFR3 in relapsed patients was significantly higher than that in nonrelapsed patients. Furthermore, the predicting relapse model of bladder cancer patients after surgery has high accuracy through detecting FGFR3 combined with other gene mutation in urinary sediment cell. This is consistent with previous studies, which 70% patients with relapse of NMIBC detected FGFR3mutations in urine

Table 6

Accuracy of diagnosis model after 5-fold cross-validation.

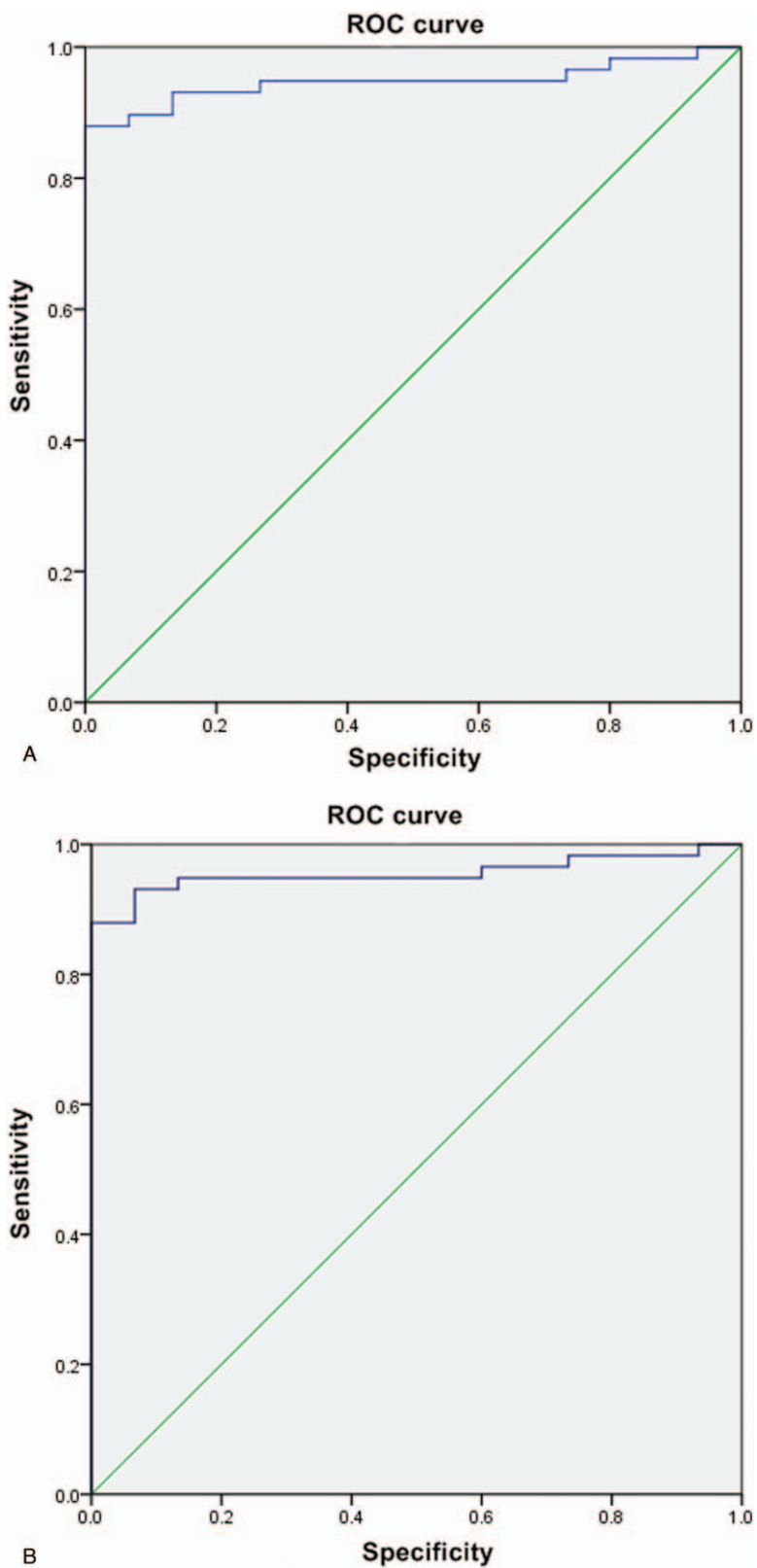| Model	Training set				Validation set			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Model 1	0.83	0.94	0.90	0.82	0.80	0.89	0.84	0.85
Model 2	0.90	0.87	0.87	0.86	0.91	0.80	0.86	0.79
Model 3	0.87	0.90	0.88	0.89	0.81	0.88	0.80	0.85
Model 4	0.92	0.79	0.86	0.80	0.89	0.83	0.83	0.78
Model 5	0.84	0.88	0.89	0.86	0.85	0.86	0.82	0.81

Figure 5. (A) Receiver-operating characteristic (ROC) curve of diagnosis model. (B) Analysis of ROC curve on predictive model.

Table 7

Accuracy of predictive model after 5-fold cross-validation.

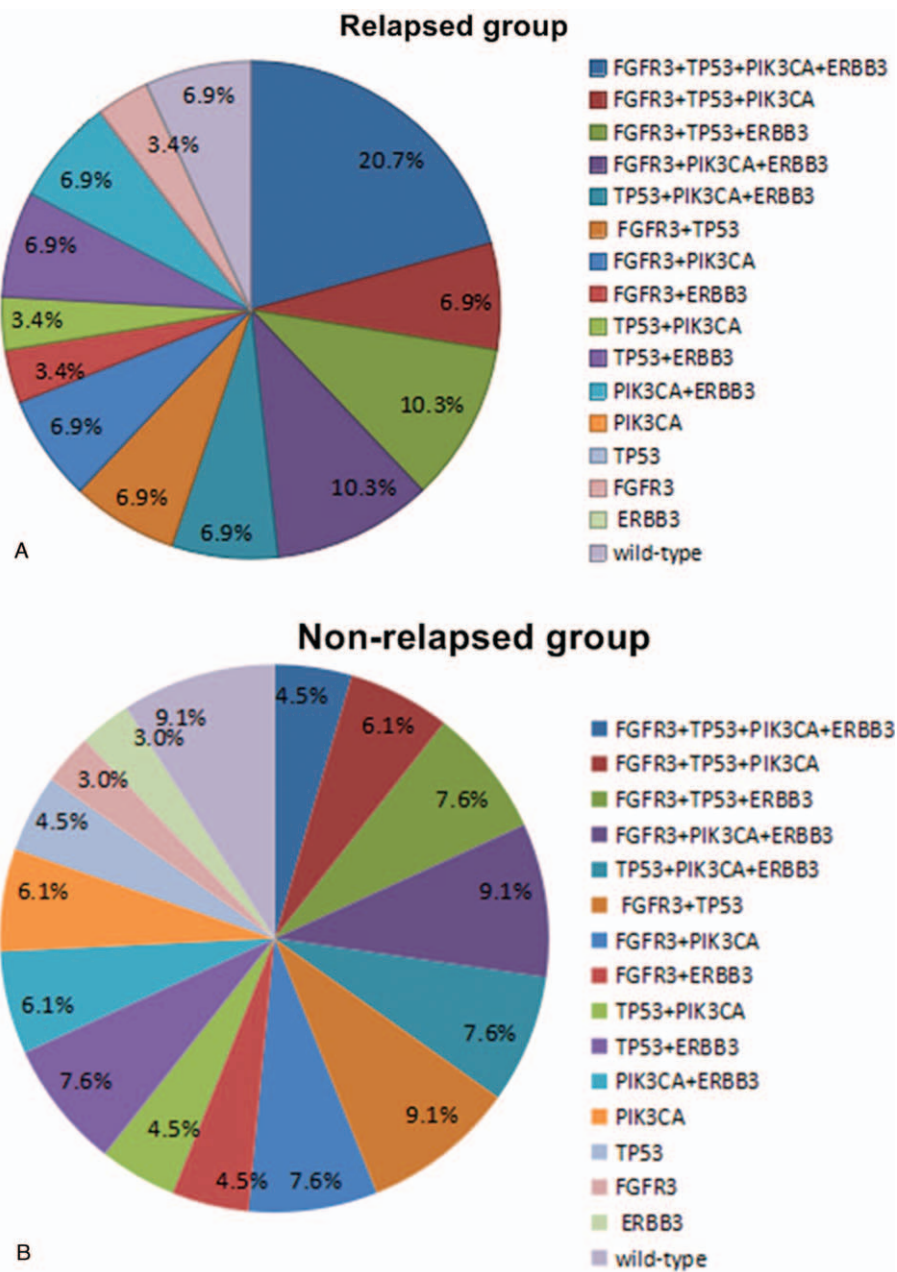| Model	Training set				Validation set			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Model 1	0.87	0.90	0.91	0.80	0.90	0.84	0.90	0.85
Model 2	0.90	0.88	0.89	0.85	0.91	0.82	0.89	0.90
Model 3	0.92	0.87	0.93	0.85	0.93	0.83	0.90	0.86
Model 4	0.95	0.85	0.92	0.82	0.94	0.80	0.90	0.79
Model 5	0.89	0.90	0.88	0.91	0.92	0.83	0.93	0.81

Figure 6. Constituent ratio of gene mutation between recurrent group (A) and nonrecurrent group (B).

sediment before operation, but no mutations in FGFR3 were found in patients without relapse.[12]

Tumor suppressor gene p53 can inhibit the growth of malignant tumor cells through cycle arrest and promoting apoptosis. The mutation of p53 is closely related to the progress of bladder cancer and the increase of mortality.[13] However, the relationship between p53 mutation and overall survival and disease-free progression survival remains unclear. This study found that the proportion of p53 in relapse group was significantly higher than that in nonrelapse group, and p53 could be combined with other genes to predict the relapse of bladder cancer.

So far, many markers related to the prognosis of bladder cancer have been found. Because of the heterogeneity of bladder cancer, single marker has limited value in predicting the prognosis of bladder cancer. Combining multiple markers can improve the accuracy of prediction. But this does not mean that the more gene mutation information is included, the accuracy of prediction is better. Increasing the number of gene mutations in the model may improve the sensitivity, but at the same time, the specificity and the rate of missed diagnosis will increase. Therefore, the ideal model is relatively high in both sensitivity and specificity, which the area under the AUC curve is the largest. The sensitivity and specificity of two models in our manuscript was higher than that previous study.[14]

In conclusion, the present study aims to detecting mutated genes of sediment cells for NMIBC early diagnose and prediction of NMIBC relapse after surgery. We constructed diagnostic and predictive models containing mutation information of FGFR3, TP53 and PIK3CA. These two models have high accuracy and high application value in the diagnosis and predicting relapse of bladder cancer patients.

Author contributions

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Writing – review & editing: Feng Zhu.

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