The Role of FGFR3 in the Diagnosis and Treatment of Bladder Cancer: A Review

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Abstract: Bladder cancer is the most common malignant tumor of the urinary system. The muscle-invasive bladder cancer (MIBC) is associated with poor prognosis; therefore, new systemic treatment is urgently needed. Although the prognosis of non-muscle-invasive bladder cancer (NMIBC) is relatively good, it is highly recurrent and requires lifelong monitoring that brings huge burden to patients and medical services. Thus, improving the diagnosis and treatment of bladder cancer is still a very important milestone to achieve. Fibroblast growth factor receptor 3 (FGFR3) gene mutations frequently occur in bladder cancer. The mutations are related to the development, progression, and prognosis of bladder cancer and may serve as effective biomarkers and therapeutic targets. An increase in the understanding of FGFR3 in recent years is expected to lead to new insights into the diagnosis and treatment of bladder cancer, thereby prolonging the survival of patients. Combined with relevant clinical research and basic research, this article reviews the application of FGFR3 in the diagnosis and treatment of bladder cancer.

Keywords: FGFR3, Bladder cancer, Biomarkers, Targeted therapy, Diagnosis

1. Introduction

Bladder cancer is the most common malignant tumor in the urinary system. Although the diagnosis and treatment techniques have been continuously improved, the exact pathogenesis of bladder cancer still remains to be elucidated and there are still rooms for improvement with regard to its treatment efficacy. The advancement of genetics and other disciplines has continued to deepen our understanding of bladder cancer biology. It is possible that a deep understanding of the genetic changes related to the occurrence and development of bladder cancer may facilitate the diagnosis and treatment of bladder cancer, thereby increasing the efficacy of individualized treatment and improving survival rate. FGFR3 gene alteration is one of the most common genetic events in bladder cancer. A large number of studies in recent years have revealed its important role in the diagnosis and treatment of bladder cancer. FGFR3 may be a potential gene marker for bladder cancer.

2. FGFR3 genetic alterations in bladder cancer

FGFR3 is a coding gene for tyrosine kinase receptor, and its encoded product is fibroblast growth factor receptor 3, which is involved in regulating various physiological processes including proliferation, differentiation, migration, and apoptosis. FGFR3 is also an important carcinogenic driver of bladder cancer, and the most common types of aberrations in bladder cancer include activating mutation, gene fusion, and upregulated expression[1].
2.1. FGFR3 mutations

So far, more than 10 different FGFR3 missense mutations have been reported in bladder cancer, of which R248C, S249C, and Y375C account for more than 85% of the mutations[2-3]. Mutations of FGFR3 activate and induce a variety of oncogenic signaling pathways, including RAS/mitogen-activated protein kinase (MAPK), phospholipase Cc1 (PLCc 1), phosphoinositide kinase 3 (PI3K), and signal transducers and activators of transcription (STAT) signal pathway. The frequency of FGFR3 mutations varies in different stages and grades of bladder cancer; for instance, the frequency decreases with an increase of cancer severity which is gauged by stages and grades. Bladder cancer can generally be categorized into Grade 1 (well differentiated), Grade 2 (moderately differentiated), and Grade 3 (poorly differentiated). The staging of primary tumor is shown in Table 1.

Neuzillet et al. reported that FGFR3 mutation frequency is 65%, 30.2%, and 11.5% in pTa, pT1, and pT2-4, respectively, while 69.8%, 68%, and 18.6% in G1, G2, and G3 grades, respectively[4]. The currently available studies merely report the mutation frequency of FGFR3 in different stages and grades of bladder cancer, but the specific mechanisms orchestrated by these mutations in the development of bladder cancer remain to be elucidated. FGFR3 mutations occur frequently in the early stage of bladder cancer, which makes it a potential tool for early diagnosis of bladder cancer. A recent meta-analysis conducted by Garcia-Perdomo et al. further emphasized that FGFR3 mutation has a strong correlation with the diagnosis of bladder cancer, supporting its use as a biomarker for specific screening and diagnosis of bladder cancer[5].

2.2. FGFR3 gene fusion

In bladder cancer, FGFR3 gene translocation and recombination lead to the formation of FGFR3 fusion gene. At present, the more common fusion genes include FGFR3-TACC3 fusion gene and FGFR3-BAIAP2L fusion gene. Aside from possessing high carcinogenic effect on cells, the products of these fusion genes can promote cell proliferation and transformation, as well as induce cell morphological transformation, anchorage-independent growth, and tumorigenicity[6,9]. The incidence of FGFR3-TACC3 and FGFR3-BAIAP2L in bladder cancer is about 2-6%[7,8]. Since most of the research results come from MIBC samples, the relationship with tumor grade or stage is still uncertain, but the incidence of point mutations in low-stage tumors is high, suggesting that gene fusion may be more common in T1 tumors and below. Current studies have found that the gene fusion products of FGFR3-TACC3 and FGFR3-BAIAP2L can activate RAS/MAPK, MAPK/ERK, and JAK/STAT signaling pathways, and FGFR3-BAIAP2L gene fusion product can also promote STAT1 phosphorylation, which, in turn, drives the occurrence of bladder cancer[6,9]. In addition, FGFR3-TACC3 fusion causes defects in cell mitosis and chromosome mis-segregation, which further leads to the formation of aneuploidy, a condition favorable for the progression of cancer[6,9]. Compared with the high frequency of FGFR3 gene mutations, further studies are needed to clarify the signaling pathways and carcinogenic mechanisms orchestrated by the products of the two fusion genes.

2.3. Upregulated expression of FGFR3

Normal physiological processes require precise fine-tuning of FGFR3 activity, including multi-level regulation of expression, activity, and downstream signals; the dysregulation in these aspects are related to bladder cancer. The upregulated expression of FGFR3 can be detected in all grades and stages of bladder cancer (including NMIBC and MIBC), which may be significantly related to FGFR3 mutations[10,11,12]. Of note, upregulated expression may occur in a higher proportion of NMIBC cases.

The exact mechanism of FGFR3 overexpression in bladder cancer has not been fully elucidated but it is important to note that upregulated expression of oncogenic proteins is often caused by gene amplification. However, high-level amplification of FGFR3 has not been reported, whereas low-level copy gain of the 4p16.3 region is only at low frequency. In a study concerning T1 tumors, upregulated expression of FGFR3 was detected in 63% of NMIBC cases, but three or more copies of FGFR3 were detected in six cases[12]. Similarly, in MIBC and metastatic bladder cancer, low-frequency copy number of FGFR3 has been observed, which is not sufficient to explain the relatively high frequency of the upregulation of wild-type FGFR3 expression in these tumors[12].

Mao et al. recently discovered that a circular RNA containing exons 4 and 8 produced at the FGFR3 gene locus, called has-circ-0068871, can upregulate FGFR3
expression and activate the STAT3 signaling pathway\cite{13}. The study found that hsa_circ_0068871, which is carcinogenic, is highly expressed in bladder cancer. While miR-181a-5p, a type of microRNA, is expressed at a low level in bladder cancer and acts as a tumor suppressor gene. The direct target of miR-181a-5p is FGFR3, and its expression is negatively correlated with FGFR3. Hsa_circ_0068871 acts as a sponge for miR-181a-5p to reduce the inhibition of FGFR3, contributing to the upregulated expression of FGFR3. Therefore, these RNAs may help clarify the specific mechanism of FGFR3 upregulation.

3. Detection of FGFR3 mutations in urine

The detection range of FGFR3 mutations in DNA isolated from patients’ urine is about 7-70%, and the huge disparity in detection may be due to factors such as detection methods and sample sizes\cite{14,15}. The commonly used detection methods are mainly polymerase chain reaction (PCR)-based, such as BEAMing, amplification refractory mutation system-polymerase chain (ARMS-PCR), and droplet digital PCR (ddPCR). The comparison of several detection methods is shown in Table 2.

ddPCR technology has a higher sensitivity for detecting FGFR3 gene mutations in urine, particularly in the detection of low levels of tumor DNA among a large excess of non-tumor DNA\cite{16}. Recently, the detection results of an ultra-sensitive multiplex PCR detection method called mutated allele specific oligonucleotide-PCR (MASO-PCR) that was developed by Roperch et al. are highly consistent with those of allele-specific PCR; therefore, a detection kit based on this new technology has been developed\cite{17}.

Roperch et al. had determined FGFR3 mutation status (R248C, S249C, G372C, and Y375C) and tumor DNA methylation (HS3ST2, SLIT2, and SEPTIN9) in urine\cite{18}. The results showed that the sensitivity and specificity for the diagnosis of bladder cancer were 97.6% and 84.8%, respectively, and the negative predictive value was 99.6%. In the same study, the researchers found that the use of FGFR3 mutations has sensitivity, specificity, and negative predictive value of 90.3%, 65.1%, and 97.0%, respectively, for detecting patient recurrence. The results also showed that the combined detection of FGFR3 mutation and DNA methylation in urine can be a useful strategy for the diagnosis and monitoring of bladder cancer. In another study, Blanca et al. evaluated the value of combined expression of FGFR3/cyclin D3 in urine to detect the recurrence of bladder cancer. The sensitivity and specificity of urinary expression of FGFR3/cyclin D3 and cystoscopy are equivalent, that is, 73% versus 80%, and 90% versus 84%, respectively\cite{19}. Therefore, determination of the urinary expression of FGFR3/cyclin D3 is recommended as a non-invasive biomarker for bladder cancer recurrence.

4. FGFR3 and drug resistance of bladder cancer

Transurethral resection of bladder tumor is the principal treatment of NMIBC, whereas post-operative intravesical infusion chemotherapy or immunotherapy is a very important auxiliary method.

Bacillus Calmette-Guérin (BCG) is currently recognized as the auxiliary treatment with the best curative effect. Since about 30-50% of patients still remained unresponsive to treatment or relapsed within 5 years\cite{20-23}, it is important to accurately identify the patients who would benefit from BCG treatment. At present, many biomarkers have been employed to predict the response to BCG treatment, including p53, cell cycle regulators, apoptosis inhibitors, cell adhesion molecules, and proliferation markers. However, the application of these biomarkers is limited due to some shortcomings, such as the lack of uniform diagnostic criteria and small sample size\cite{22}. FGFR3 has also shown potential role in predicting response to BCG treatment in some studies. Langle et al. reported that 41% of bladder tumors had lower FGFR3 expression after BCG treatment. Furthermore, it was also observed that BCG treatment could downregulate the expression of FGFR3 in murine tumor models, supporting the premise that downregulation of FGFR3 is associated with a good BCG response\cite{19}. However, large-scale clinical research is still warranted to validate the application of FGFR3 as a biomarker of BCG response.

Radical cystectomy (RC) is the principal mode of the treatment for MIBC and high-risk NMIBC, supplemented by cisplatin-based adjuvant/neoadjuvant chemotherapy. The previous studies have shown that FGFR3 mutation may promote the chemotherapy resistance of bladder cancer cells by activating the Akt signaling pathway and other pathways; therefore, FGFR3 mutation can be used as a predictive factor of chemotherapy sensitivity in patients\cite{23}. To study the predictive effect of FGFR3 on cisplatin chemotherapy response, Yang et al. found that 49% of the cisplatin-based neoadjuvant chemotherapy responders have activating mutations in FGFR3, corroborating that the FGFR3 mutation in MIBC is a potential predictive biomarker of cisplatin chemotherapy response\cite{20}. In addition, Sung et al. reported that FGFR3 overexpression occurred in 52.4% of the patients receiving cisplatin-based adjuvant chemotherapy and found that FGFR3 overexpression was associated with shorter disease-free survival and overall survival\cite{21}. The average disease-free survival (22.2 months vs. 50.1 months, P<0.05) and average overall survival (28.2 months vs. 63.6 months, P<0.05) of these patients were significantly lower. In a multivariate analysis, FGFR3 overexpression is still an important independent prognostic factor for disease-free survival and overall survival. In contrast, in patients without adjuvant chemotherapy, FGFR3 mutation or overexpression has no prognostic significance based on the multivariate analysis, indicating that FGFR3
overexpression may be related to cisplatin chemotherapy resistance. These findings suggest that FGFR3 may be used as a detection tool for cisplatin chemotherapy response.

In recent years, PD1/PDL1 immune checkpoint inhibitors, such as atezolizumab and pembrolizumab, have also been recommended for the treatment of MIBC. The luminal I or luminal papillary of bladder cancer has lower T cell infiltration and is associated with a lower response rate to immune checkpoint inhibitors\(^\text{[29]}\). There is a correlation between FGFR3 mutations and decreased T cell infiltration. The enrichment of FGFR3 mutations in luminal bladder cancer may lead to poor T cell infiltration, thereby affecting treatment response\(^\text{[29]}\). However, a recent study found that although FGFR3 gene mutation has a negative correlation with T cell infiltration, it has no direct correlation with the response of immune checkpoint inhibitors\(^\text{[30]}\). Regardless of FGFR3 status, the response rate and survival rate of all patients are similar. The possible explanation is that the low T cell infiltration caused by the FGFR3 mutation is offset by the TGF-β-related interstitial inflammatory response. Immune checkpoint inhibitors are second-line drugs for MIBC. There are currently no reliable biomarkers to predict treatment response. Thus, more research is needed to explore the mechanisms underlying chemotherapy resistance and potential methods to overcome the resistance.

### 5. FGFR3-targeted inhibitors

There are many types of FGFR family-targeted inhibitors. Erdafitinib (JNJ42756493) is an oral small-molecule FGFR inhibitor. As the first FGFR-targeted inhibitor for metastatic bladder cancer approved by the FDA, erdafitinib has a strong inhibitory effect on the activity of FGFR1-4 and is selective for other highly related kinases. In a Phase I clinical trial conducted by Bahleda et al., erdafitinib showed good tolerability and certain drug activity in advanced solid tumors, and had a good response rate (40%) especially in bladder cancer; all patients who respond to erdafitinib carried FGFR mutations or fusions\(^\text{[31]}\). In Phase II clinical trials, erdafitinib had an overall response rate of 42% (3% complete response and 39% partial response) and 80% disease control rate for metastatic bladder cancer\(^\text{[22]}\). Similarly, all patients have FGFR3 mutations (R248C, S249C, G370C, and Y373C) or FGFR gene fusions (FGFR 3-TACC3, FGFR 3-BAIAP2L1, FGFR2-BICC1, and FGFR2-CASP7). The patient’s median progression-free survival was 5.5 months, and the median overall survival was 13.8 months. For patients who had previously received immune checkpoint inhibitor therapy, the overall clinical response rate was as high as 70%.

Dovitinib (TKI258) is a non-selective tyrosine kinase inhibitor that targets FGFR1-3 at nanomolar concentrations. The kinase domains of FGFR1-3 show a high degree of structural similarity, and most selective inhibitors inhibit all three FGFRs to varying degrees\(^\text{[2,9]}\). According to a Phase II clinical trial conducted by Hahn et al., dovitinib always reached a bioactive concentration in the urothelium and showed an inhibitory effect on FGFR3 phosphorylation in NMIBC that does not respond to BCG treatment and has increased FGFR3 phosphorylation.

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**Table 2. Comparison of ARMS-PCR, ddPCR, and BEAMing**

| Names     | Principles and methods                                                                 | Advantages                                                                 | Limitations                                                                 |
|-----------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| ARMS-PCR  | Design primers with known mutation sites so that the 3'-terminal bases of the primer are complementary to those of the template to achieve amplification, thereby detecting mutations. | - High specificity and sensitivity <br>- Less time-consuming <br>- Low cost <br>- Simple operation | - Only known mutations can be detected |
| ddPCR     | The reaction system containing nucleic acid molecules is divided into tens of thousands to hundreds of thousands of nanoscale droplets, and the fluorescent signal of each droplet is analyzed one by one after PCR amplification. According to the principle of Poisson distribution and the number and proportion of positive droplets, the initial copy number or concentration of the target molecule is obtained. | - Able to determine the absolute number of target molecules as low as a single copy to achieve absolute quantification of nucleic acid samples <br>- Higher sensitivity and tolerance than ARMS-PCR | - Only known mutations can be detected <br>- Only one mutation can be detected at a time <br>- High cost |
| BEAMing   | Use specific PCR primers to combine with corresponding magnetic beads and perform amplification, and then use flow cytometry to detect fluorescent labels to determine mutations. | - High sensitivity. <br>- Able to achieve absolute quantification of single-molecule DNA | - Only known mutations can be detected <br>- Higher cost <br>- More complicated operation <br>- Prone to test errors |
Due to frequent toxicity, long-term administration is not feasible[33]. However, in a Phase II clinical trial, Milowsky et al. found that although dovitinib was well tolerated by patients, it has very limited single-agent activity in patients with previously treated advanced bladder cancer, regardless of FGFR3 mutation status[34].

BGJ398 is a selective inhibitor of FGFR1-3. The Phase I clinical trial of BGJ398 by Nogova et al. showed that after the failure of cisplatin-based chemotherapy, BGJ398 is safe and has good anti-tumor activity against advanced bladder cancer with FGFR3 mutation. Using different doses of BGJ398 treatment, 32% of the patients were under control, and the response rate and disease control rate were 38% and 75%, respectively, among patients with FGFR3 mutations, indicating a favorable therapeutic effect of BGJ398[35]. However, the results of Phase II clinical trial of BGJ398 in bladder cancer have not been reported. Other FGFR3-targeted inhibitors reported in recent years include AZD4547, Debio 1347, rogaratinib (BAY1163877) and TAS-120, but most of them are still in or have just passed the evaluation in Phase I clinical trials.

In addition to FGFR3-targeted inhibitors, other types of FGFR3-targeted drugs for bladder cancer are being developed. Some categories of FGFR3-targeted drugs are as follows: PI3K-beta inhibitors such as GSK2636771, and anti-epidermal growth factor receptor inhibitors such as afatinib, and monoclonal antibodies such as MFGR1877S and B-701.

6. FGFR3 and the prognosis of bladder cancer

The role of FGFR3 in predicting the prognosis and progression of bladder cancer is still controversial. A recent study confirmed that FGFR3 mutations are significantly associated with lower pT stage, tumor grade, absence of carcinoma in situ, pN0, low levels of p53, and longer disease-specific survival, but FGFR3 overexpression is only related to lower pT stage and tumor grade[36]. FGFR3 mutation and FGFR3 overexpression are related to different characteristics of bladder cancer that are often indicative of good prognosis. However, it is unclear why FGFR3 overexpression is related to lower pT stage and tumor grade.

A meta-analysis by Borkowska et al. also confirmed the correlation between the presence of FGFR3 mutations and better survival[37], indicating that FGFR3 gene alterations, especially FGFR3 gene mutations, imply better prognosis. Similarly, the study of Han et al. further clarified that in patients with FGFR3 mutations, lower mutant-allele tumor heterogeneity is an independent predictor of better prognosis[38]. From another perspective, however, FGFR3 gene alterations are related to the patient’s treatment responses that could influence patient’s prognosis in a different manner.

The study of Breyer et al. also clarified that the low expression of FGFR3 was significantly associated with worse progression-free survival, and multivariate Cox regression analysis pointed out that low FGFR3 expression was an independent predictor of progression-free survival[39]. On the contrary, the study by Kang et al. showed that FGFR3 mutation has no obvious prognostic significance for tumor recurrence or progression, but low FGFR3 expression is an independent predictor of cancer progression[40]. On the other hand, Akanksha et al. reported from an immunohistochemistry study that FGFR3 expression was positive in 80% of recurring high-grade non-invasive tumors, 72.7% in low-grade non-invasive tumors, and 14.3% in aggressive tumors[41]. Taken together, the expression of FGFR3 can be used as an indicator to judge the risk of recurrence, especially in non-invasive tumors. Despite the concerns raised by some studies, many findings point to the relationship of FGFR3 gene alterations to the prognosis of patients with bladder cancer.

7. Summary and outlook

FGFR3 is a promising biomarker in the diagnosis and treatment of bladder cancer. FGFR3 gene alterations such as gene mutations and gene fusion, as well as abnormal expression caused by amplification or translocation are common in bladder cancer and are related to the development and drug resistance of bladder cancer. However, its exact mechanism remains to be studied. In particular, the relationship between FGFR3 and the prognosis of bladder cancer is still not very clear. In recent years, a variety of biomarkers and indicators, such as neutrophil-to-lymphocyte ratio, gene markers in DNA damage repair pathway (such as ATM, ERCC2, BRCA1, and BRCA2) and markers involved in cell apoptosis regulation (such as Bcl-2), have been investigated for their application in the diagnosis and treatment of bladder cancer. The combination of multiple biomarkers including FGFR3 is anticipated to give rise to new strategies for the diagnosis and treatment of bladder cancer. In addition, the development of FGFR3-targeted drugs may also revolutionize the treatment of bladder cancer.

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Conflict of interest

The authors declared that they have no conflict of interest.

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