

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

Hairong Wei, Weiming Wan, Hui Zhan, Jiansong Wang, Jian Chen*

Department of Urology, Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101, China

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

Received: January 8, 2021
Accepted: February 12, 2021
Published Online: February 21, 2021

***CORRESPONDING AUTHOR**

Jian Chen
E-mail: chenjian2016km@126.com

CITATION

Wei H, Wan W, Zhan H, et al., 2021, The Role of FGFR3 in the Diagnosis and Treatment of Bladder Cancer: A Review. *Cancer+*, 3(1):28–34.
DOI: [10.18063/cp.v3i1.302](https://doi.org/10.18063/cp.v3i1.302)

Copyright: © 2021. Wei et al.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), permitting all noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

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]. 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^[10]. 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^[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^[2].

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*

Table 1. Staging and description of the primary tumor of bladder cancer

Staging	Description
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma in situ: "flat tumor"
T1	Tumor invades subepithelial connective tissue
T2	Tumor invades muscle
T3	Tumor invades extravesical tissue
T4	Tumor invades any of the following: prostate stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall

expression and activate the STAT3 signaling pathway^[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^[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^[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^[17].

Roperch *et al.* had determined *FGFR3* mutation status (R248C, S249C, G372C, and Y375C) and tumor DNA methylation (HS3ST2, SLIT2, and SEPTIN9) in urine^[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^[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^[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^[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^[24]. 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^[25]. 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^[26]. 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^[27]. 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

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.	<ul style="list-style-type: none"> • High specificity and sensitivity • Less time-consuming • Low cost • Simple operation 	<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • Able to determine the absolute number of target molecules as low as a single copy to achieve absolute quantification of nucleic acid samples • Higher sensitivity and tolerance than ARMS-PCR 	<ul style="list-style-type: none"> • Only known mutations can be detected • Only one mutation can be detected at a time • 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.	<ul style="list-style-type: none"> • High sensitivity. • Able to achieve absolute quantification of single-molecule DNA 	<ul style="list-style-type: none"> • Only known mutations can be detected • Higher cost • More complicated operation • Prone to test errors

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^[28]. 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^[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^[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^[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^[32]. Similarly, all patients have *FGFR3* mutations (*R248C*, *S249C*, *G370C*, and *Y373C*) or *FGFR* gene fusions (*FGFR 3-TACC3*, *FGFR 3-BALAP2L1*, *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^[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.

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^[16], 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^[37]. 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^[38]. 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^[39]. 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^[40]. 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.

Acknowledgments

This study was supported by the research grant from Science and Technology Project of the Second Affiliated Hospital of Kunming Medical University (Grant no. 2019YK003).

Conflict of interest

The authors declared that they have no conflict of interest.

References

1. Helsten T, Elkin S, Arthur E, *et al.*, 2016, The FGFR Landscape in Cancer: Analysis of 4,853 Tumors by Next-Generation Sequencing. *Clin Cancer Res*, 22:259–67. DOI:

- 10.1158/1078-0432.ccr-14-3212.
2. Di Martino E, Tomlinson DC, Williams SV, et al., 2016, A Place for Precision Medicine in Bladder Cancer: Targeting the FGFRs. *Future Oncol*, 12:2243–63. DOI: 10.2217/fon-2016-0042.
3. Homami A, Kachoei ZA, Asgarie M, et al., 2020, Analysis of FGFR3 and HRAS Genes in Patients with Bladder Cancer. *Med J Islam Repub Iran*, 34:108.
4. Neuzillet Y, Paoletti X, Ouerhani S, et al., 2012, A Meta-analysis of the Relationship between FGFR3 and TP53 Mutations in Bladder Cancer. *PLoS One*, 7:e48993. DOI: 10.1371/journal.pone.0048993.
5. Garcia-Perdomo HA, Usubillaga-Velasquez JP, Zapata-Copete JA, et al. 2019, Mutations in CDKN2A and the FGFR3 Genes on Bladder Cancer Diagnosis: A Systematic Review and Meta-analysis. *World J Urol*, 37:2001–7. DOI: 10.1007/s00345-019-02779-7.
6. Nakanishi Y, Akiyama N, Tsukaguchi T, et al., 2015, Mechanism of Oncogenic Signal Activation by the Novel Fusion Kinase FGFR3-BAIAP2L1. *Mol Cancer Ther*, 14:704–12. DOI: 10.1158/1535-7163.mct-14-0927-t.
7. Costa R, Carneiro BA, Taxter T, et al., 2016, FGFR3-TACC3 Fusion in Solid Tumors: Mini Review. *Oncotarget*, 7:55924–38. DOI: 10.18632/oncotarget.10482.
8. Kim YS, Kim K, Kwon GY, et al., 2018, Fibroblast Growth Factor Receptor 3 (FGFR3) Aberrations in Muscle-invasive Urothelial Carcinoma. *BMC Urol*, 18:68. DOI: 10.1186/s12894-018-0380-1
9. Babina IS, Turner NC, 2017, Advances and Challenges in Targeting FGFR Signalling in Cancer. *Nat Rev Cancer*, 17:318–32. DOI: 10.1038/nrc.2017.8.
10. Sarkar S, Ryan EL, Royle SJ, 2017, FGFR3-TACC3 Cancer Gene Fusions Cause Mitotic Defects by Removal of Endogenous TACC3 from the Mitotic Spindle. *Open Biol*, 7:170080. DOI: 10.1098/rsob.170080.
11. Amaral AF, Méndez-Pertuz M, Muñoz A, et al., 2012, Plasma 25-Hydroxyvitamin D(3) and Bladder Cancer Risk According to Tumor Stage and FGFR3 Status: A Mechanism-based Epidemiological Study. *J Natl Cancer Inst*, 104:1897–904. DOI: 10.1093/jnci/djs444.
12. Neuzillet Y, Van Rhijn BW, Prigoda NL, et al., 2014, FGFR3 Mutations, but not FGFR3 Expression and FGFR3 Copy-Number Variations, are Associated with Favourable Non-Muscle Invasive Bladder Cancer. *Virchows Arch*, 465:207–13. DOI: 10.1007/s00428-014-1596-4.
13. Mao W, Huang X, Wang L, et al., 2019, Circular RNA hsa_circ_0068871 Regulates FGFR3 Expression and Activates STAT3 by Targeting miR-181a-5p to Promote Bladder Cancer Progression. *J Exp Clin Cancer Res*, 38:169. DOI: 10.1186/s13046-019-1136-9.
14. Sethakorn N, O'donnell PH, 2016, Spectrum of Genomic Alterations in FGFR3: Current Appraisal of the Potential Role of FGFR3 in Advanced Urothelial Carcinoma. *BJU Int*, 118:681–91. DOI: 10.1111/bju.13552.
15. Critelli R, Fasanelli F, Oderda M, et al., 2016, Detection of Multiple Mutations in Urinary Exfoliated Cells from Male Bladder Cancer Patients at Diagnosis and During Follow-up. *Oncotarget*, 7:67435–48. DOI: 10.18632/oncotarget.11883.
16. Borkowska EM, Traczyk-Borszyńska M, Kutwin P, et al., 2019, Usefulness of Droplet Digital PCR and Sanger Sequencing for Detection of FGFR3 Mutation in Bladder Cancer. *Urol Oncol*, 37:907–15. DOI: 10.1016/j.urolonc.2019.06.010.
17. Roperch J P, Hennion C, 2020, A Novel Ultra-sensitive Method for the Detection of FGFR3 Mutations in Urine of Bladder Cancer Patients Design of the Urodiag® PCR Kit for Surveillance of Patients with Non-muscle-Invasive Bladder Cancer (NMIBC). *BMC Med Genet*, 21:112. DOI: 10.1186/s12881-020-01050-w.
18. Roperch JP, Grandchamp B, Desgrandchamps F, et al., 2016, Promoter Hypermethylation of HS3ST2, SEPTIN9 and SLIT2 Combined with FGFR3 Mutations as a Sensitive/ Specific Urinary Assay for Diagnosis and Surveillance in Patients with Low or High-risk Non-muscle-invasive Bladder Cancer. *BMC Cancer*, 16:704. DOI: 10.1186/s12885-016-2748-5.
19. Blanca A, Requena MJ, Alvarez J, et al., 2016, FGFR3 and Cyclin D3 as Urine Biomarkers of Bladder Cancer Recurrence. *Biomark Med*, 10:243–53. DOI: 10.2217/bmm.15.120.
20. Cambier S, Sylvester RJ, Collette L, et al., 2016, EORTC Nomograms and Risk Groups for Predicting Recurrence, Progression, and Disease-specific and Overall Survival in Non-Muscle-invasive Stage Ta-T1 Urothelial Bladder Cancer Patients Treated with 1-3 Years of Maintenance *Bacillus Calmette-Guérin*. *Eur Urol*, 69:60–9. DOI: 10.1016/j.eururo.2016.01.055.
21. Larsen ES, Joensen UN, Poulsen AM, et al., 2020, *Bacillus Calmette-Guérin* Immunotherapy for Bladder Cancer: A Review of Immunological Aspects, Clinical Effects and BCG Infections. *Apmis*, 128:92–103. DOI: 10.1111/apm.13011.
22. Zhang N, Jiang G, Liu X, et al., 2016, Prediction of *Bacillus Calmette-Guerin* Response in Patients with Bladder Cancer after Transurethral Resection of Bladder Tumor by Using vGenetic Variation Based on Genomic Studies. *Biomed Res*

- Int*, 2016;9859021. DOI: 10.1155/2016/9859021.
23. Babjuk M, Burger M, Compérat EM, *et al.*, 2019, European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma *In Situ*)-2019 Update. *Eur Urol*, 76:639–57. DOI: 10.1016/j.eururo.2019.08.016.
 24. Langle YV, Belgorosky D, McCormick BP, *et al.*, 2016, FGFR3 Down-Regulation is Involved in *Bacillus Calmette-Guerin* Induced Bladder Tumor Growth Inhibition. *J Urol*, 195:188–97. DOI: 10.1016/j.juro.2015.06.093.
 25. Xie X, Lin J, Zhong Y, *et al.*, 2019, FGFR(3S249C) Mutation Promotes Chemoresistance by Activating Akt Signaling in Bladder Cancer Cells. *Exp Ther Med*, 18:1226–34. DOI: 10.3892/etm.2019.7672.
 26. Yang Z, Zhang R, Ge Y, *et al.*, 2018, Somatic FGFR3 Mutations Distinguish a Subgroup of Muscle-Invasive Bladder Cancers with Response to Neoadjuvant Chemotherapy. *EBioMedicine*, 35:198–203. DOI: 10.1016/j.ebiom.2018.06.011.
 27. Sung JY, Sun JM, Jeong BC, *et al.*, 2014, FGFR3 Overexpression is Prognostic of Adverse Outcome for Muscle-invasive Bladder Carcinoma Treated with Adjuvant Chemotherapy. *Urol Oncol*, 32:49.e23–31.
 28. Sharma P, Retz M, Siefker-Radtke A, *et al.*, 2017, Nivolumab in Metastatic Urothelial Carcinoma after Platinum Therapy (CheckMate 275): A Multicentre, Single-arm, Phase 2 Trial. *Lancet Oncol*, 18:312–22. DOI: 10.1016/s1470-2045(17)30065-7.
 29. Sweis RF, Spranger S, Bao R, *et al.*, 2016, Molecular Drivers of the Non-T-cell-Inflamed Tumor Microenvironment in Urothelial Bladder Cancer. *Cancer Immunol Res*, 4:563–8. DOI: 10.1158/2326-6066.cir-15-0274.
 30. Wang L, Gong Y, Saci A, *et al.*, 2019, Fibroblast Growth Factor Receptor 3 Alterations and Response to PD-1/PD-L1 Blockade in Patients with Metastatic Urothelial Cancer. *Eur Urol*, 76:599–603. DOI: 10.1016/j.eururo.2019.06.025.
 31. Bahleda R, Italiano A, Hierro C, *et al.*, 2019, Multicenter Phase I Study of Erdafitinib (JNJ-42756493), Oral Pan-Fibroblast Growth Factor Receptor Inhibitor, in Patients with Advanced or Refractory Solid Tumors. *Clin Cancer Res*, 25:4888–97. DOI: 10.1158/1078-0432.ccr-18-3334.
 32. Nadal R, Bellmunt J, 2019, Management of metastatic bladder cancer. *Cancer Treat Rev*, 76:10–21.
 33. Hahn NM, Bivalacqua TJ, Ross AE, *et al.*, 2017, A Phase II Trial of Dovitinib in BCG-Unresponsive Urothelial Carcinoma with FGFR3 Mutations or Overexpression: Hoosier Cancer Research Network Trial HCRN 12-157. *Clin Cancer Res*, 23:3003–11. DOI: 10.1158/1078-0432.ccr-16-2267.
 34. Milowsky MI, Ditttrich C, Duran I, *et al.*, 2014, Phase 2 Trial of Dovitinib in Patients with Progressive FGFR3-mutated or FGFR3 Wild-type Advanced Urothelial Carcinoma. *Eur J Cancer*, 50:3145–52. DOI: 10.1016/j.ejca.2014.10.013.
 35. Nogova L, Sequist LV, Garcia JM, *et al.*, 2017, Evaluation of BGJ398, a Fibroblast Growth Factor Receptor 1-3 Kinase Inhibitor, in Patients With Advanced Solid Tumors Harboring Genetic Alterations in Fibroblast Growth Factor Receptors: Results of a Global Phase I, Dose-Escalation and Dose-Expansion Study. *J Clin Oncol*, 35:157–65. DOI: 10.1200/jco.2016.67.2048.
 36. Van Rhijn BW, Mertens LS, Mayr R, *et al.*, 2020, FGFR3 Mutation Status and FGFR3 Expression in a Large Bladder Cancer Cohort Treated by Radical Cystectomy: Implications for Anti-FGFR3 Treatment?. *Eur Urol*, 78:682–7. DOI: 10.1158/1557-3265.bladder19-b23.
 37. Han Y, Liu X, Ye H, *et al.*, 2020, Lower Mutant-allele Tumor Heterogeneity is a Biomarker in FGFR3-mutant Bladder Cancer for Better Prognosis. *World J Surg Oncol*, 18:310. DOI: 10.21203/rs.3.rs-62350/v1.
 38. Breyer J, Wirtz RM, Erben P, *et al.*, 2018, High CDKN2A/p16 and Low FGFR3 Expression Predict Progressive Potential of Stage pT1 Urothelial Bladder Carcinoma. *Clin Genitourin Cancer*, 16:248–56.e2. DOI: 10.1016/j.clgc.2018.01.009.
 39. Kang HW, Kim YH, Jeong P, *et al.*, 2017, Expression Levels of FGFR3 as a Prognostic Marker for the Progression of Primary pT1 Bladder Cancer and its Association with Mutation Status. *Oncol Lett*, 14:3817–24. DOI: 10.3892/ol.2017.6621.
 40. Akanksha M, Sandhya S, 2019, Role of FGFR3 in Urothelial Carcinoma. *Iran J Pathol*, 14:148–55.