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REVIEW

Molecular Biomarkers of Bladder Cancer: A Mini-Review

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Summary

Cancers are quite common, but mostly very serious diseases and therefore belong to the most important areas of scientific research activity. Bladder cancer is one of the most common malignancies, it is a heterogeneous disease with significant diagnostic, therapeutic, and prognostic problems. It represents a disease with a variable course and a different response to therapy. The "conventional" prognostic markers used so far cannot reliably predict the natural course of the disease or estimate the tumor response to the chosen type of treatment. Molecular markers can provide us with the opportunity to diagnose a bladder tumor early, identify patients who are at risk of recurrence, or predict how tumors will respond to therapeutic approaches. As a result, diagnostics are found to help clinicians find the best therapeutic options for patients with bladder cancer. In this study, we focused on a brief description of potential molecular markers in bladder tumors in the context of precise diagnostics. Last but not least, we also focused on a new approach to the treatment of cancer using nanomaterials.

Key words

Apoptosis • Biomarker from urine • miRNA • Serum • GATA3 • FGFR3 • Sp1 • Bcl-2

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Introduction

Bladder cancer is the fifth most common malignancy in humans and the second most commonly diagnosed tumor after prostate cancer. Every year, there are approximately 400000 new patients with this disease in the world. In men, it occurs 4 times more often than in women. According to global morbidity and mortality data from GLOBOCAN, an estimated 573278 people were diagnosed worldwide in 2020 and 212546 patients died of bladder cancer. In recent years, the incidence of bladder cancer in women from developing countries is on the rise, which may be related to an increase in smoking rates among women [1]. Early and accurate diagnosis has an impact on the successful treatment of any cancer. The standard diagnostic methods currently used by doctors require invasive procedures such as cystoscopy and biopsy. These methods often lack the sensitivity to detect all cancers [2,3]. These inaccuracies in capturing the clinical and biological potential of the tumor lead to excessive and inadequate treatment and therapy effects. Recently, more and more teams are interested in the use of molecular-genetic methods in the early diagnosis of a bladder tumor. There are many studies that look at molecular markers. Molecular markers detected in urine, tissue, or blood offer promising opportunities to improve understanding of the biology of specific cancers and their influence on the micro- and macroenvironment. This could help identify the disease earlier, risk stratifies patients, improve prognosis and prediction, and aid in targeted therapy. Molecular biomarkers and pathways

that are involved in the development of bladder cancer are the main key to understanding and identifying, which we can subsequently use for use in clinical practice to create personalized therapy for treatment.

In this study, we focused on a brief description of potential molecular markers in bladder tumors in the context of precise diagnostics. Last but not least, we also focused on a new approach to the treatment of cancer using nanomaterials.

Pathology of bladder tumors

Tumor-mimicking processes include mainly granulomas, which are mostly after operations performed on the bladder. These changes may resemble sarcoma. They are formed by spindle cells, any manifestations of pleiomorphism of cells are absent here. Benign tumors include inverted papilloma, adenomatoid tumors, leiomyomas, hemangioma, and others. The historical term "superficial bladder cancer" is used to describe tumors that do not penetrate the muscles. We can also include papillae urothelial carcinoma called pTa, carcinoma in situ pTis. A muscle-penetrating tumor refers to pT1 [4]. Bladder tumors can also be classified according to low-grade and high-grade. Low-grade bladder tumor attacks the muscular lining of the bladder wall. In contrast, a high-grade bladder tumor attacks the muscle lining and metastasizes. Bladder tumors belong to a heterogeneous group, relapses and progression to a higher stage are typical for them. Tumors arise suddenly in different places of the bladder and metastasize [5]. Bladder cancers are usually identified as non-muscularly invasive, yet about a third of them develop aggressive recurrent bladder tumors. Even today, the diagnosis of the bladder is mainly established using cytology, which is characterized by insufficient sensitivity [6]. Bladder cancer is a heterogeneous disease, and the existence of finding a single biomarker is unlikely. It is therefore important to extend current knowledge about the mechanism of bladder tumor formation to the molecular level. There are several theories of the development of bladder cancer. The first of them was pronounced back in 1953. The development of bladder cancer has been found to have two distinct molecular pathways [7]. One pathway assumes the formation of a tumor from a single transformed cell. Molecular diagnostics of several samples confirmed the theory of tumor formation from a single cell since mutations on the TP53 and RB1 genes were detected, which confirmed the assumption of accumulation of genetic changes and the subsequent transformation of a healthy cell into a pathological cancer cell. This process is now called carcinogenesis. On the contrary, molecular analyses of recent decades show that there is another way. Recently, it has been considered that the tumor may also arise as a result of affecting the expression of FGFR3 (Fibroblast Growth Factor Receptor 3). This factor is one of the most commonly mutated factors in bladder cancer. Close associations were also found between its molecular subtypes and clinicopathological characteristics [7].

Molecular markers detected from tissue

Biomarkers detected from bladder tumor tissue will help us identify patients who are at risk of recurrence or predict how tumors will respond to therapeutic approaches. These potential biomarkers include, for example, FGFR3, VEGF-C, GATA3, FOXA1, P53, etc.

The FGFR3 gene belongs to the fibroblast growth factor receptor (FGFR) family, with its amino acid sequence highly conserved among members and among distinct species. Members of the FGFR family differ from each other in affinities for ligands and distribution in tissues. The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately affecting mitogenesis and differentiation [8]. It plays an important role in many important cellular processes, including regulation of proliferation, differentiation, apoptosis, angiogenesis, wound healing, and embryogenesis [9,10]. Genomic changes in the FGFR gene have been extensively studied, and it was found that changes in the FGFR gene were discovered in several types of human cancer. 7.1 % change in fibroblast factor receptors [11]. In bladder tumors, up to 15 % had a somatic change to FGFR3, 7 % FGFR1 amplification, and 6 % gene fusion. This factor has been found to play an important role mainly in the lower stage of bladder cancer [8,12]. FGFR3 stimulates SCD1 activity and thus promotes tumor growth in bladder tumor cells. The FGFR3 and RAS pathway can be activated in bladder tumors at any stage. In up to 80 %, this pathway is active in the lower stages of bladder cancer, where it is mainly associated with point mutations on the FGFR3 gene, which was found to pose a higher risk of recurrence [13,14].

VEGF-C (vascular endothelial growth factor C) is the first lymphangiogenesis factor discovered. VEGF-C may promote proliferation, invasion, metastasis,

and resistance to mitomycin C BCa cells. Mechanisms that increased the Bcl-2/Bax ratio, caspase-3 inactivation, and increased MMP-9 expression are thought to be associated with this [15,16]. Chen *et al.* linked the activity of the VEGF-C gene to tumor grade [17].

GATA3 is a member of the GATA family and is a transcription factor with a zinc finger originally identified as specific to the T cell line. It has an important role in the differentiation of breast epithelium, urothelium, and subgroups of T-lymphocytes [18]. GATA3 has been extensively studied in relation to mammary gland morphogenesis and breast cancer pathogenesis [19]. Using animal models, GATA3 has been shown to play a critical role in breast carcinogenesis [19,20]. GATA3 loss has also been linked to breast tumor progression and metastases. The functional role of GATA3 in the evolution and progression of BC remains largely unclear. However, it was shown that increased expression of GATA3 in a muscle-invasive BC tumor correlates with a higher risk of disease progression. A normal level of GATA3 poses a lower risk of progression. High expression of GATA3 is a predictor of poor prognosis for the patient. The complete loss represents a poor prognosis for patients [21-23].

Several recent studies have noted that a member of the FOX family has a role in urothelial differentiation (FOXA1). FOXA1 is expressed in normal cells and participates in normal urogenital development. Reduced expression of the FOXA1 gene was correlated with increasing tumor stage. Complete loss of expression of FOXA1 is associated with a high histological degree BC [24]. FOXA1, based on a study by Sikic *et al.*, seems to be the marker most suitable as a surrogate marker to distinguish non-basal subtypes from basal subtypes [25].

A bladder tumor can arise from a single mutated cell, accumulating genetic changes, the so-called “two hits” on two prototypical suppressor genes RB1 (retinoblastoma gene) and TP53. What forms the basis for the progression of a healthy cell into a cancer cell [7].

P53, a transcription factor, is also referred to as a genome guard. TP53 gene is found in several cells, where it protects cellular integrity, and has many other functions such as induction of apoptosis, inhibition of cell proliferation, or cell cycle arrest (Fig. 1) [26]. Loss of function of the TP53 gene may lead to reduced regulation of the cell cycle and thus inhibition of apoptosis [27]. p53 protein has the ability to activate the transcription of pro-apoptotic genes [28]. The accumulation of TP53 is a predictor factor of poor prognosis in advanced-stage bladder tumors. TP53 expression has also been found to be associated with disease recurrence [29].

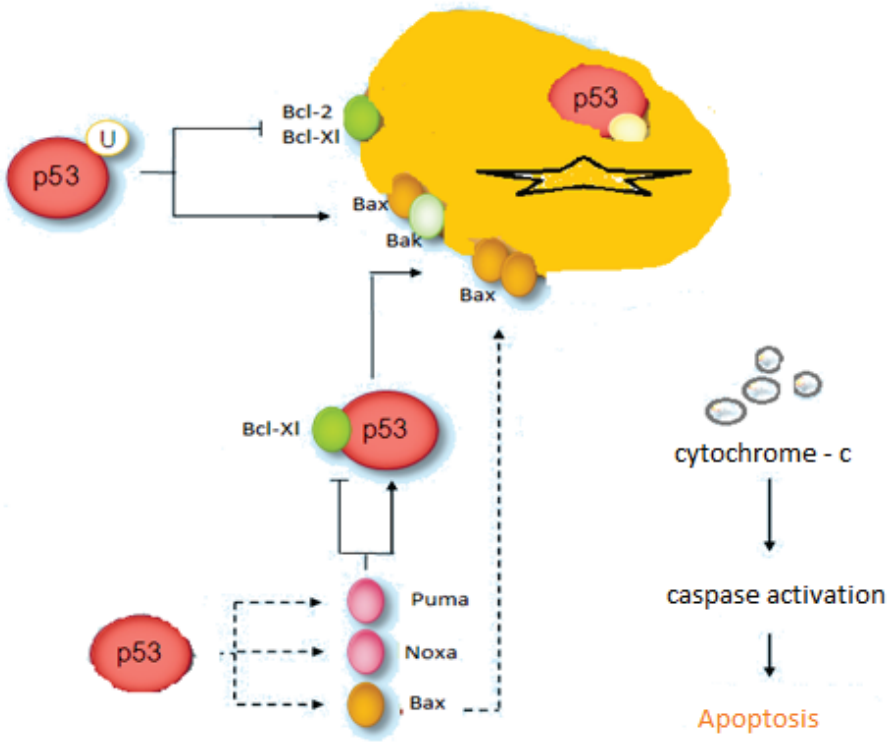

Fig. 1. The mitochondrial pathway of p53 apoptosis. Nuclear p53 induces expression of Puma protein, which releases p53z binding to Bcl-X1. Free p53 causes oligonucleosis of Bax and translocation of mitochondria. In mitochondria, p53 acts to oligomerize Bax and Bak, which act against Bcl-2, these changes lead to membrane disruption.

Another unfavorable marker is the loss of expression of RB1, which is a tumor suppressor gene. RB1 is one of the major regulators of the cell cycle and its changes are related to carcinogenesis in several types of cancer [30]. Molecular analyses have found that bladder tumors with mutations on the RB1 gene show low levels of expression of the FGFR3 gene at the same time. That may be related to poor survival in a bladder tumor.

Another promising specific marker that plays key functions in the process of carcinogenesis and cancer progression is the specific protein Sp1. The specific Sp1 protein is a member of the Sp family of Kruppel factors and acts as a transcription factor by binding to specific promoter elements of its target genes. Sp1 regulates genes that are mainly responsible for cell growth, apoptosis, or the cell cycle [31]. These genes are believed to be involved in cellular functions that form the basis of tumor initiation and progression. In recent years, studies have shown that the overexpressed Sp1 gene is involved in the formation of various tumors such as stomach cancer, and pancreatic adenocarcinoma [32,33]. A study by Zhu *et al.* looked at the Sp1 gene as a prognostic marker in urothelial cancer. They managed to associate high expression with poor patient survival and observed high expression of this gene in tumors with higher histological grades and in samples with metastases [34]. They hypothesize that Sp1 could be a potential independent prognostic biomarker, especially for patients after bladder tumor surgery. It would serve to identify patients with an aggressive type of tumor and consequently poor clinical outcomes [35].

One of the most common antiapoptotic genes studied in bladder tumors is survivin, which we consider to be a marker of prediction of recurrence. However, new and more reliable markers are also being sought. A promising apoptotic biomarker could be genes belonging to the Bcl family (Table 1) of apoptosis promoting such as Bad, Bak, and Bid, but also apoptosis-inhibiting genes such as Bcl-2. The labile balance between these Bcl proteins shifts towards apoptosis under stress. Also, the cleavage of Bid by the action of caspase 8 activates Bcl-2, thereby connecting the internal signaling pathway to induce apoptosis. Abnormalities in these molecular processes play an important role in the development of various malignancies, the progression of the disease. One mechanism to avoid controlled cell death is the overexpression of anti-apoptotic genes such as Bcl-2. In different tumors, the Bcl-2 gene has different effects on tumorigenesis and tumor progression. Increased expression of this gene reduced overall survival in prostate and breast tumors [35]. Conversely, in renal cells, increased expression was significantly correlated with better patient survival [36]. In bladder tumors, Bcl-2 expression has been associated with a higher tumor grade [37]. These genes belonging to the Bcl family in relation to bladder tumors have not yet been adequately described, therefore they deserve attention in the future, and they can offer us a better understanding of the process of bladder cancer.

Table 1. Members of the Bcl family.

Anti-apoptotic members	Pro-apoptotic members	Pro-apoptotic-BH3-only members
Bcl-2	Bax	Bad
Bcl-x_l	Bok/Mtd	Bik/nbk/Blk
Bcl-w	Bcl-xs	Bid
A1	Bak	Noxa
Mcl-1	Bcl-gl	Puma
Boo		Bmf

Molecular markers detected from serum miRNAs

A very promising alternative to finding markers from tissue biopsies is a fluid biopsy. Compared to tissue biopsy, we can talk about a non-invasive procedure that represents minimal intervention for the patient. In this way, we can get real-time information about the disease and provide clinicians with fast, accurate diagnoses in a short time to help them find the best therapeutic options. Samples of liquid biopsy can be considered serum, blood, or urine. Since miRNAs are found in body fluids such as serum and urine, they are less susceptible to degradation, so they have huge potential in treating

bladder tumors than for their non-invasive approach. Promising possibilities are provided by obtaining miRNA from serum from patients with a bladder tumor.

MicroRNAs (miRNAs) are small approximately 22 nucleotides endogenously non-encoding RNA molecules negatively regulating gene expression levels through binding to microRNA-binding elements in 3'UTR sequences of target mRNAs [38]. Probably more than half of all genes coding for proteins are regulated by miRNAs, making them unique candidates for biomarkers [39]. While in normal cells the expression of miRNAs is strictly regulated, tumorigenesis is characterized by deregulation of their expression, which can affect the risk of cancer, the effectiveness of treatment, and the prognosis for the patient. It follows that one miRNA can regulate the expression of multiple genes. Alterations in some microRNAs may be related to tumor formation, and these miRNAs may be perceived as tumor-suppressor or oncogenic [40,41]. Since their discovery in 1993, more than 2000 have been experimentally verified in humans [42]. Therefore, miRNAs are considered promising novel diagnostic markers (Table 2). Studies show that miRNAs are involved in the appearance and inhibition of various tumors. Thus, their overexpression or excessive suppression of their expression could predict the emergence of various types of cancer. We already know today that miRNAs are predictors, for example, of pulmonary adenocarcinoma [43]. Recent studies have also shown the association of miRNA with bladder tumors (miR-141, miR-34a) [44].

Table 2. The potential biomarkers of miRNA.

miRNA	Expression	Target Pathways
miR-34a	Downregulated	Cell cycle control
miR-143	Downregulated	PI3K/ACP MAPK signaling
miR-145	Downregulated	Apoptosis
miR-203	Downregulated	Apoptosis/PI3K-Akt signaling
miR-200	Downregulated	Inhibit EMT
miR-129	Upregulated	Signal transduction

MiR-145 and miR-143 are tumor suppressors that show reduced expression in bladder tumor tissue and can suppress cell proliferation and migration as well as promote apoptosis. MiR-20 and miR-183, on the other hand, have an increased level of expression in BC tissue. High expression of miR-143 is also associated with poor survival, since at the same time the tumor suppressor SOX4 and GALNT are downregulated [45]. MiR-145 is one of the most frequently reduced miRNA regulators in BC. MiR-141 and miR-205 are among the poor indicators of prognostic biomarkers of overall survival in BC [7,46]. MiR-146-5p appears to be a suitable adept for a marker related to higher-grade of bladder cancer [6]. However, low miR-200c values have also been found to be associated with the progression of non-invasive bladder cancer [47]. Chen *et al.* discovered a panel of 33 upregulated miRNAs and 41 downregulated miRNAs in a bladder tumor [48]. MiR-34a belongs to tumor suppressors and expression causes inhibition of cellular migration. If anti-apoptotic genes such as Bcl-w are suppressed, then increased levels of miR-203a-3p expression are observed [49].

Molecular markers detected from urine

Urine biopsy is one of the promising alternatives in the search for new biomarkers in bladder tumors. Urine biopsy from patients can provide us with important information about the development of the disease, and help in the diagnosis and prognosis of the disease.

Urine is an easily accessible biological fluid. Urine examination is one of the basic clinical-biochemical procedures that significantly contribute to diagnosing and monitoring the course of the disease. Urine analysis uses a wide range of methods from the simplest to demanding and fully automated methods. Urine contains several different cell types such as epithelial cells, white blood cells, red blood cells, urothelial cells, proteins, immune cells, and EBCC cells (cells released from a tumor that have been found in urine). And also thanks to EBCC cells, scientists' interest in finding markers in urine has increased [50]. EBCC

cells can be obtained from urine by filtration based on membrane size. Filtration has been found to improve the sensitivity of obtaining EBCC cells. With a bladder tumor, it was found that filtration improves sensitivity in detecting the recurrence of the disease. EBCC cells were detected in up to 87 % of filtered samples. When using antibodies, selectivity rises to 99 %. [50-52]. We already know that these cells are a suitable indicator for prognosis in breast cancer or colorectal cancer [53,54]. Currently, EBCC cells are not used for BC screening, but they have great potential, mainly due to their high specificity [55]. EBCC-type cells should be in the interest of scientific inquiry. The focus of scientists should be on morphology, the amount of cell concentration obtained, viability, and subsequent use in the diagnosis of diseases. These cells may contain information about the emerging tumor, which can help clinicians catch the tumor at an early stage.

Among the substances that we can obtain in a non-invasive way from urine in a bladder tumor are cytokeratins. Cytokeratin (CK-20) is expressed in BC cells, but not in normal healthy cells in urine. A large study of potential markers over 3000 in urine was conducted, CK-20 in this study came out as the best marker with a sensitivity of 78-87 % using RT-PCR methods. Other studies have shown that with the help of immunostaining, we can achieve a sensitivity of 82 % [55].

Potential markers that are excreted into the urine by tumor cells are already mentioned in markers obtained from tissue, the VEGF marker. Elevated levels of VEGF in urine detected by ELISA tests are associated with disease recurrence [55].

In the urine, we can find different extracellular vesicles. Extracellular vesicles include exosomes, microvesicles, and apoptotic bodies, which are relatively stable in urine. A gene has been detected in extracellular vesicles obtained from urine that can be linked to bladder cancer KRT17. Keratin, cytoskeletal type I 17, is a protein that is encoded in humans by the KRT17 gene. Loss or mutation can promote apoptosis or reduce immunity. Recent studies have shown that knockdown KRT17 could be an effective avenue to treat osteosarcoma [56]. Studies of bladder tumors have revealed that overexpressed KRT17 occurs in the pT1 stage BC. Other studies have linked reduced expression of KRT17 to poor prognosis for BC patients. Although the function and impact of the KRT17 gene are unclear in a bladder tumor, it is a possible predictive biomarker.

The use of markers in urine is of importance mainly from the point of view of patient stratification. For example, patients with asymptomatic microhematuria (AMH) have a higher risk of BC. In patients with AMH, urinary markers should be used as another option for a diagnostic strategy. The prevalence of AMH in the adult population is 18 %, only a low percentage of patients are recommended for examination of a bladder tumor, which leads to late diagnosis. It is in this disease that urinary markers would help to identify risk groups [57,58].

Targeted drug transport in the treatment of bladder tumor

Research in the field of cancer in recent years has brought a great deal of knowledge that can help in targeted and personalized treatment in the future. For clinical practice, it is important not only to understand the pathophysiology of processes in the development of tumor diseases but also to the progress and improvement of drug transport and effective targeted influence at the tumor site are crucial. Intensive biomedical research also focuses on the development of so-called nanocarriers for targeted drug transport. Currently, we know more than 40 products based on nanocarriers (nano drugs) in clinical practice [59]. The first generation of nano drugs focused on drug transport and drug accumulation in the tumor. The new generation focuses mainly on active targeting, that is, so that the drug accumulates only at the tumor site, thereby increasing its effectiveness and reducing undesirable effects. The drug is bound to specific molecules, these systems can be liposome-based, polymeric, magnetic, or silica-based, carbon. These materials have a wide range of applications not only in the diagnosis but also in the treatment of cancer [60]. Manju *et al.* have been able to demonstrate the effectiveness of gold nanoparticles. The association of the curcumin-hyaluronic acid complex with gold nanoparticles promoted tumor cell targeting and dose reduction in colon tumors [61]. An integral part of this research is also the assessment of the safety of nanosystems for the human body, as well as a more detailed understanding of the nanosystem-cell interaction. The requirements for carriers used for targeted drug transport can be described in two simple words: biocompatibility and functionality. Hou *et al.* led research on a nanosystem based on silicon deposits with bound doxorubicin in mice with bladder tumors. In doing so, they managed to design a sustained drug release system,

which resulted in overwhelmingly effective suppression of the bladder tumor. They also demonstrated that treatment with this nanosystem prolonged the survival of mice with bladder tumors, as opposed to mice treated with conventional methods using Doxorubicin [62].

Conclusions

The use of molecular markers in urine, tissue, or blood offers potential opportunities to improve understanding of bladder cancer biology, which can help identify disease earlier, stratify patient risk, improve outcome prediction, or aid in targeted therapy. Based on them, we can identify those patients who are most at risk of recurrence. We can also predict how tumors will respond to different therapeutic approaches. Bladder tumors today are diagnosed by a combination of cytology and histology. Although these methods are quite expensive, they reveal little of the molecular side of the tumor, and the results are often subjective. Recent advances in our understanding of the molecular characteristics of a bladder tumor potentially can help correlate molecular outcomes with clinical outcomes. The result of this effort should be to provide diagnostics that would help clinicians find the best therapeutic options for patients suffering from bladder tumors. The use of nanoparticles in the diagnosis and treatment of oncological diseases undoubtedly brings great benefits. The aim of using nanoparticles in the treatment of oncological diseases is to significantly increase the effectiveness of the drug, reduce its toxicity, increase the benefit of treatment and comfort of patients, and, last but not least, the economic efficiency of treatment.

Conflict of Interest

There is no conflict of interest.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-249. https://doi.org/10.3322/caac.21660
2. Ng K, Stenzl A, Sharma A, Vasdev N. Urinary biomarkers in bladder cancer: A review of the current landscape and future directions. Urol Oncol 2021;39:41-51. https://doi.org/10.1016/j.urolonc.2020.08.016
3. Repiska V, Radzo E, Biro C, Bevizova K, Bohmer D, Galbavy S. Endometrial cancer--prospective potential to make diagnostic process more specific. Neuro Endocrinol Lett 2010;31:474-476.
4. Nieder AM, Soloway MS. Eliminate the term "superficial" bladder cancer. J Urol 2006;175:417-418. https://doi.org/10.1016/S0022-5347(05)00290-9
5. Bryan RT, Tselepis C. Cadherin switching and bladder cancer. J Urol 2010;184:423-431. https://doi.org/10.1016/j.juro.2010.04.016
6. Bratu O, Marcu D, Anghel R, Spinu D, Iorga L, Balescu I, Bacalbasa N, ET AL. Tumoral markers in bladder cancer (Review). Exp Ther Med 2021;22:773. https://doi.org/10.3892/etm.2021.10205
7. Nagata M, Muto S, Horie S. Molecular Biomarkers in Bladder Cancer: Novel Potential Indicators of Prognosis and Treatment Outcomes. Dis Markers 2016;2016:8205836. https://doi.org/10.1155/2016/8205836
8. Ascione CM, Napolitano F, Esposito D, Servetto A, Belli S, Santaniello A, Scagliarini S, ET AL. Role of FGFR3 in bladder cancer: Treatment landscape and future challenges. Cancer Treat Rev 2023;115:102530. https://doi.org/10.1016/j.ctrv.2023.102530
9. Keegan K, Johnson DE, Williams LT, Hayman MJ. Isolation of an additional member of the fibroblast growth factor receptor family, FGFR-3. Proc Natl Acad Sci U S A 1991;88:1095-1099. https://doi.org/10.1073/pnas.88.4.1095
10. Harsanyi S, Novakova ZV, Bevizova K, Danisovic L, Ziaran S. Biomarkers of Bladder Cancer: Cell-Free DNA, Epigenetic Modifications and Non-Coding RNAs. Int J Mol Sci 2022;23:13206. https://doi.org/10.3390/ijms232113206
11. Helsten T, Schwaederle M, Kurzrock R. Fibroblast growth factor receptor signaling in hereditary and neoplastic disease: biologic and clinical implications. Cancer Metastasis Rev 2015;34:479-496. https://doi.org/10.1007/s10555-015-9579-8

12. AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine through an International Consortium. Cancer Discov 2017;7:818-831. https://doi.org/10.1158/2159-8290.CD-17-0151
13. Moch H, Humphrey PA, Ulbright TM, Reuter VE. *WHO Classification of Tumours of the Urinary System and Male Genital Organs, 4th ed.*; IARC Press, Lyon, France, 2016. https://doi.org/10.1016/j.eururo.2016.02.029
14. Inamura K. Bladder Cancer: New Insights into Its Molecular Pathology. Cancers (Basel) 2018;10:100. https://doi.org/10.3390/cancers10040100
15. Zhang HH, Qi F, Shi YR, Miao JG, Zhou M, He W, Chen MF, ET AL. RNA interference-mediated vascular endothelial growth factor-C reduction suppresses malignant progression and enhances mitomycin C sensitivity of bladder cancer T24 cells. Cancer Biother Radiopharm 2012;27:291-298. https://doi.org/10.1089/cbr.2010.0919
16. Zhang C, Hu J, Li H, Ma H, Othmane B, Ren W, Yi Z, ET AL. Emerging biomarkers for predicting bladder cancer lymph node metastasis. Front Oncol 2021;11:648968. https://doi.org/10.46903/gjms/19.01.943
17. Chen JX, Deng N, Chen X, Chen LW, Qiu SP, Li XF, Li JP. A novel molecular grading model: combination of Ki67 and VEGF in predicting tumor recurrence and progression in non-invasive urothelial bladder cancer. Asian Pac J Cancer Prev 2012;13:2229-2234. https://doi.org/10.7314/APJCP.2012.13.5.2229
18. Miettinen M, McCue PA, Sarlomo-Rikala M, Rys J, Czapiewski P, Wazny K, Langfort R, ET AL. GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. Am J Surg Pathol 2014;38:13-22. https://doi.org/10.1097/PAS.0b013e3182a0218f
19. Li Y, Ishiguro H, Kawahara T, Kashiwagi E, Izumi K, Miyamoto H. Loss of GATA3 in bladder cancer promotes cell migration and invasion. Cancer Biol Ther 2014;15:428-435. https://doi.org/10.4161/cbt.27631
20. Asselin-Labat ML, Sutherland KD, Vaillant F, Gyorki DE, Wu D, Holroyd S, Breslin K, ET AL. Gata-3 negatively regulates the tumor-initiating capacity of mammary luminal progenitor cells and targets the putative tumor suppressor caspase-14. Mol Cell Biol 2011;31:4609-4622. https://doi.org/10.1128/MCB.05766-11
21. Morris G, Stoychev S, Naicker P, Dirr HW, Fanucchi S. The forkhead domain hinge-loop plays a pivotal role in DNA binding and transcriptional activity of FOXP2. Biol Chem 2018;399:881-893. https://doi.org/10.1515/hsz-2018-0185
22. Inoue S, Mizushima T, Fujita K, Meliti A, Ide H, Yamaguchi S, Fushimi H, ET AL. GATA3 immunohistochemistry in urothelial carcinoma of the upper urinary tract as a urothelial marker and a prognosticator. Hum Pathol 2017;64:83-90. https://doi.org/10.1016/j.humpath.2017.04.003
23. Miyamoto H, Yao JL, Chaux A, Zheng Y, Hsu I, Izumi K, Chang C, ET AL. Expression of androgen and oestrogen receptors and its prognostic significance in urothelial neoplasm of the urinary bladder. BJU Int 2012;109:1716-1726. https://doi.org/10.1111/j.1464-410X.2011.10706.x
24. DeGraff DJ, Clark PE, Cates JM, Yamashita H, Robinson VL, Yu X, Smolkin ME, ET AL. Loss of the urothelial differentiation marker FOXA1 is associated with high grade, late stage bladder cancer and increased tumor proliferation. PLoS One 2012;7:e36669. https://doi.org/10.1371/journal.pone.0036669
25. Sikic D, Eckstein M, Wirtz RM, Jarczyk J, Worst TS, Porubsky S, Keck B, ET AL. FOXA1 Gene expression for defining molecular subtypes of muscle-invasive bladder cancer after radical cystectomy. J Clin Med 2020;9:994. https://doi.org/10.3390/jcm9040994
26. Esrig D, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen SC, Nichols PW, ET AL. Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med 1994;331:1259-1264. https://doi.org/10.1056/NEJM199411103311903
27. Amaral JD, Xavier JM, Steer CJ, Rodrigues CM. The role of p53 in apoptosis. Discov Med 2010;9:145-152.
28. Jeffers JR, Parganas E, Lee Y, Yang C, Wang J, Brennan J, MacLean KH, ET AL. Puma is an essential mediator of p53-dependent and -independent apoptotic pathways. Cancer Cell 2003;4:321-328. https://doi.org/10.1016/S1535-6108(03)00244-7
29. Shariat SF, Bolenz C, Karakiewicz PI, Fradet Y, Ashfaq R, Bastian PJ, Nielsen ME, ET AL. p53 expression in patients with advanced urothelial cancer of the urinary bladder. BJU Int 2010;105:489-495. https://doi.org/10.1111/j.1464-410X.2009.08742.x
30. Shariat SF, Tokunaga H, Zhou J, Kim J, Ayala GE, Benedict WF, Lerner SP. p53, p21, pRB, and p16 expression predict clinical outcome in cystectomy with bladder cancer. J Clin Oncol 2004;22:1014-1024. https://doi.org/10.1200/JCO.2004.03.118

31. Safe S, Imanirad P, Sreevalsan S, Nair V, Jutooru I. Transcription factor Sp1, also known as specificity protein 1 as a therapeutic target. Expert Opin Ther Targets 2014;18:759-769. https://doi.org/10.1517/14728222.2014.914173
32. Jiang NY, Woda BA, Banner BF, Whalen GF, Dresser KA, Lu D. Sp1, a new biomarker that identifies a subset of aggressive pancreatic ductal adenocarcinoma. Cancer Epidemiol Biomarkers Prev 2008;17:1648-1652. https://doi.org/10.1158/1055-9965.EPI-07-2791
33. Jiang W, Jin Z, Zhou F, Cui J, Wang L, Wang L. High co-expression of Sp1 and HER-2 is correlated with poor prognosis of gastric cancer patients. Surg Oncol 2015;24:220-225. https://doi.org/10.1016/j.suronc.2015.05.004
34. Zhu J, Lu Z, Ke M, Cai X. Sp1 is overexpressed and associated with progression and poor prognosis in bladder urothelial carcinoma patients. Int Urol Nephrol 2022;54:1505-1512. https://doi.org/10.1007/s11255-022-03212-6
35. Hess J, Stelmach P, Eisenhardt A, Rübben H, Reis H, Schmid KW, Bachmann HS. Impact of BCL2 polymorphisms on survival in transitional cell carcinoma of the bladder. J Cancer Res Clin Oncol 2017;143:1659-1670. https://doi.org/10.1007/s00432-017-2404-8
36. Itoi T, Yamana K, Bilim V, Takahashi K, Tomita F. Impact of frequent Bcl-2 expression on better prognosis in renal cell carcinoma patients. Br J Cancer 2004;90:200-205. https://doi.org/10.1038/sj.bjc.6601454
37. Pollack A, Wu CS, Czerniak B, Zagars GK, Benedict WF, McDonnell TJ. Abnormal bcl-2 and pRb expression are independent correlates of radiation response in muscle-invasive bladder cancer. Clin Cancer Res 1997;3:1823-1829. https://doi.org/10.1016/S0360-3016(97)00147-8
38. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 2010;466:835-840. https://doi.org/10.1038/nature09267
39. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;19:92-105. https://doi.org/10.1101/gr.082701.108
40. Medina PP, Slack FJ. microRNAs and cancer: an overview. Cell Cycle 2008;7:2485-2492. https://doi.org/10.4161/cc.7.16.6453
41. Medina PP, Nolde M, Slack FJ. OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. Nature 2010;467:86-90. https://doi.org/10.1038/nature09284
42. Pasquinelli AE, Hunter S, Bracht J. MicroRNAs: a developing story. Curr Opin Genet Dev 2005;15:200-205. https://doi.org/10.1016/j.gde.2005.01.002
43. Wang SS, Fang YY, Huang JC, Liang YY, Guo YN, Pan LJ, Chen G. Clinical value of microRNA-198-5p downregulation in lung adenocarcinoma and its potential pathways. Oncol Lett 2019;18:2939-2954. https://doi.org/10.3892/ol.2019.10610
44. Li X, Chen W, Li R, Chen X, Huang G, Lu C, Wen Z, ET AL. Bladder cancer diagnosis with a four-miRNA panel in serum. Future Oncol 2022;18:3311-3322. https://doi.org/10.2217/fon-2022-0448
45. Dyrskjøt L, Ostenfeld MS, Bramsen JB, Silahtaroglu AN, Lamy P, Ramanathan R, Fristrup N, ET AL. Genomic profiling of microRNAs in bladder cancer: miR-129 is associated with poor outcome and promotes cell death in vitro. Cancer Res 2009;69:4851-4860. https://doi.org/10.1158/0008-5472.CAN-08-4043
46. Wang H, Li Q, Niu X, Wang G, Zheng S, Fu G, Wang Z. miR-143 inhibits bladder cancer cell proliferation and enhances their sensitivity to gemcitabine by repressing IGF-1R signaling. Oncol Lett 2017;13:435-440. https://doi.org/10.3892/ol.2016.5388
47. Wiklund ED, Gao S, Hulf T, Sibbritt T, Nair S, Costea DE, Villadsen SB, ET AL. MicroRNA alterations and associated aberrant DNA methylation patterns across multiple sample types in oral squamous cell carcinoma. PLoS One 2011;6:e27840. https://doi.org/10.1371/journal.pone.0027840
48. Chen YH, Wang SQ, Wu XL, Shen M, Chen ZG, Chen XG, Liu YX, ET AL. Characterization of microRNAs expression profiling in one group of Chinese urothelial cell carcinoma identified by Solexa sequencing. Urol Oncol 2013;31:219-227. https://doi.org/10.1016/j.urolonc.2010.11.007
49. Lenherr SM, Tsai S, Silva Neto B, Sullivan TB, Cimmino CB, Logvinenko T, Gee J, ET AL. MicroRNA expression profile identifies high grade, non-muscle-invasive bladder tumors at elevated risk to progress to an invasive phenotype. Genes (Basel) 2017;8:77. https://doi.org/10.3390/genes8020077
50. Chen CK, Liao J, Li MS, Khoo BL. Urine biopsy technologies: Cancer and beyond. Theranostics 2020;10:7872-7888. https://doi.org/10.7150/thno.44634

51. Andersson E, Dahmcke CM, Steven K, Larsen LK, Guldberg P. Filtration device for on-site collection, storage and shipment of cells from urine and its application to DNA-based detection of bladder cancer. PLoS One 2015;10:e0131889. https://doi.org/10.1371/journal.pone.0131889
52. Macgregor-Ramiasa M, McNicholas K, Ostrikov K, Li J, Michael M, Gleadle JM, Vasilev K. A platform for selective immuno-capture of cancer cells from urine. Biosens Bioelectron 2017;96:373-380. https://doi.org/10.1016/j.bios.2017.02.011
53. Lv Q, Gong L, Zhang T, Ye J, Chai L, Ni C, Mao Y. Prognostic value of circulating tumor cells in metastatic breast cancer: a systemic review and meta-analysis. Clin Transl Oncol 2016;18:322-330. https://doi.org/10.1007/s12094-015-1372-1
54. Rahbari NN, Aigner M, Thorlund K, Mollberg N, Motschall E, Jensen K, Diener MK, ET AL. Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. Gastroenterology 2010;138:1714-1726. https://doi.org/10.1053/j.gastro.2010.01.008
55. Hong M, He G, Goh S, Low AWX, Tay KJ, Lim TKH, Yeong J, Khor LY, Lim TS. Biomarkers for Precision Urothelial Carcinoma Diagnosis: Current Approaches and the Application of Single-Cell Technologies. Cancers (Basel) 2021;13:260. https://doi.org/10.3390/cancers13020260
56. Wu J, Xu H, Ji H, Zhai B, Zhu J, Gao M, Zhu H, Wang X. Low Expression of Keratin17 is Related to Poor Prognosis in Bladder Cancer. Onco Targets Ther 2021;14:577-587. https://doi.org/10.2147/OTT.S287891
57. Mariani AJ, Mariani MC, Macchioni C, Stams UK, Hariharan A, Moriera A. The significance of adult hematuria: 1,000 hematuria evaluations including a risk-benefit and cost-effectiveness analysis. J Urol 1989;141:350-355. https://doi.org/10.1016/S0022-5347(17)40763-4
58. Fajkovic H, Halpern JA, Cha EK, Bahadori A, Chromecki TF, Karakiewicz PI, Breinl E, Merseburger AS, Shariat SF. Impact of gender on bladder cancer incidence, staging, and prognosis. World J Urol 2011;29:457-463. https://doi.org/10.1007/s00345-011-0709-9
59. Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. Nanomedicine 2013;9:1-14. https://doi.org/10.1016/j.nano.2012.05.013
60. Muthu MS, Leong DT, Mei L, Feng SS. Nanotheranostics - application and further development of nanomedicine strategies for advanced theranostics. Theranostics 2014;4:660-677. https://doi.org/10.7150/thno.8698
61. Manju S, Sreenivasan K. Gold nanoparticles generated and stabilized by water soluble curcumin-polymer conjugate: blood compatibility evaluation and targeted drug delivery onto cancer cells. J Colloid Interface Sci 2012;368:144-151. https://doi.org/10.1016/j.jcis.2011.11.024
62. Hou DY, Zhang NY, Wang MD, Xu SX, Wang ZJ, Hu XJ, Lv GT, ET AL. In situ constructed nano-drug depots through intracellular hydrolytic condensation for chemotherapy of bladder cancer. Angew Chem Int Ed Engl 2022;61:e202116893. https://doi.org/10.1002/anie.202116893