Urothelial Carcinoma

34

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Abstract

Our understanding of urothelial carcinoma (UC) has advanced significantly over the past three decades to provide a better understanding of the molecular basis of these tumors and the different clinical behaviors of low- and high-grade urothelial carcinoma. Fluorescence in situ hybridization is currently used to monitor UC patients for recurrent tumor and to detect new bladder tumors in patients with hematuria. The detection of cells with FGFR3 mutations in urine shows promise as a way to detect low-grade UC. Assessing upper urinary tract UC for defective mismatch repair with microsatellite instability testing or immunostains for MLH1, PMS2, MSH2, and MSH6 helps identify patients that may have Lynch syndrome. While targeted therapies are being investigated for use in advanced bladder cancer, progress has been slow and molecular profiling of urothelial carcinoma for guiding targeted therapy of UC is not currently clinically indicated.

Keywords

Bladder cancer • Urothelial carcinoma • UroVysion • Fluorescence in situ hybridization • FGFR3 • P16 • Microsatellite instability • Lynch syndrome

Introduction

The two main types of urothelial carcinoma (UC) are papillary UC (pTa) and "flat" UC (pTis), also known as carcinoma in situ (CIS). Approximately 80 % of UC are papillary and approximately 20 % are CIS. Most UC arise from the bladder, but UC also originate from the ureters and renal pelvis, and patients sometimes have tumor involving both the lower and upper urinary tract. Papillary tumors tend to recur but not progress to invasive cancer. CIS is aggressive and tends to progress to muscle-invasive cancer. UC tumorigenesis is a multistep process. Papillary UC may arise from areas of urothelial hyperplasia or from urothelial papillomas. Although most papillary tumors are low-grade and have little tendency to progress to invasive tumors, a small proportion are high grade and have significant potential to progress to invasive UC. Most invasive UC arise through the following sequence of events: normal urothelium to dysplasia to CIS to invasive cancer (Fig. 34.1). The schema used for staging UC of the bladder is shown in Table 34.1.

Molecular Basis of Disease

At the chromosomal level, the majority of low-grade papillary tumors are diploid or near-diploid, while the majority of high-grade papillary UC, CIS, and invasive UC (pTa tumors) are aneuploid. Based on array-based comparative genomic hybridization and fluorescence in situ hybridization (FISH) studies, noninvasive, low-grade pTa papillary UC have relatively few chromosomal abnormalities except for loss of all or part of chromosome 9, while CIS, high-grade pTa, and invasive UC (pT1 tumors) have a high number

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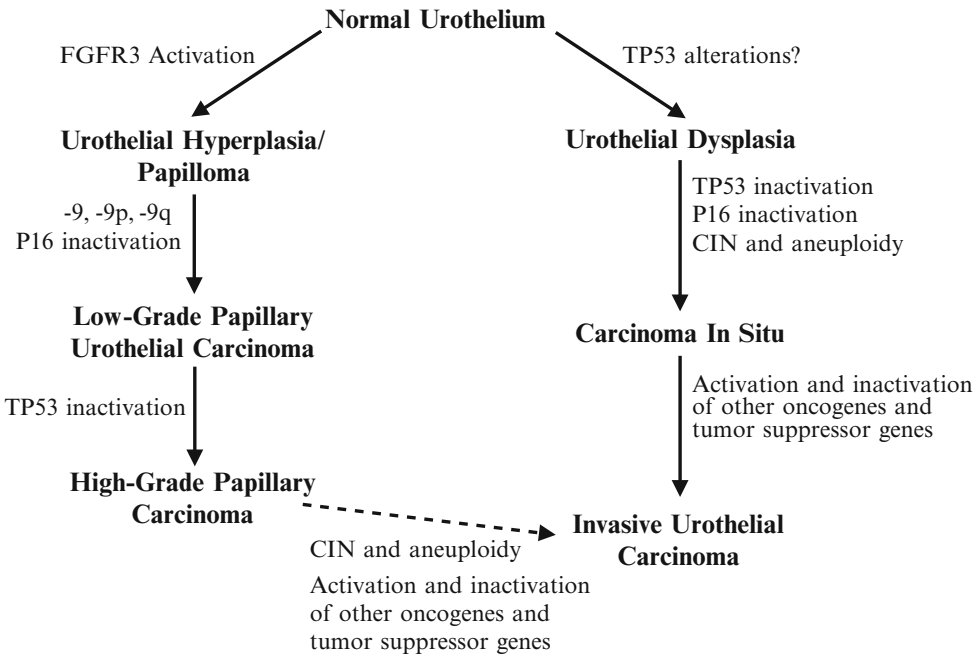

Figure 34.1 Genetic pathways for urothelial carcinoma tumorigenesis. Noninvasive papillary tumors are characterized by early activating mutations of the *FGFR3* gene, inactivating mutations or epigenetic alterations of the *P16* gene, and a diploid or near-diploid DNA content. Carcinoma in situ (CIS) and invasive tumors are characterized by early inactivating mutations within the *TP53* and *P16* genes, chromosomal instability (CIN), and an aneuploid DNA content. A small proportion of papillary tumors may acquire *TP53* alterations or alterations of other unknown genes that cause invasive potential of these tumors

Table 34.1 Pathologic staging of primary bladder urothelial carcinoma

Stage	Description
pTa	Noninvasive papillary
pTis	Carcinoma in situ
pT1	Invasion into lamina propria
pT2	Invasion into muscularis propria
pT3	Invasion through the muscularis propria and into bladder adventitia
pT4	Invasion into surrounding organs (e.g., colon)

of chromosomal gains and losses [1, 2]. The pT1 tumors also have loss of all or part of chromosome 9 but have numerous additional chromosomal abnormalities, which include whole or partial chromosomal losses and gains. Frequent sites of allelic imbalance (AI) in UC include 3p, 4p, 8p, 9p, 9q, 11p, 13q, 17p, and 18q based on microsatellite analysis (MA) [3–5]. Regions with high rates of AI are the sites of known or putative tumor suppressor genes. Many of the regions that show high rates of AI correspond to the areas of chromosomal gains and losses detected by aCGH.

Two important molecular genetic alterations that contribute to UC tumorigenesis are mutational and epigenetic alterations that inactivate the *P16* and *TP53* tumor suppressor genes. *P16* loss is one of the earliest events in the development of both papillary and flat/invasive UC [1, 5–7]. Mutations that inactivate *TP53* are found primarily in CIS and invasive UC and not low-grade papillary tumors and in part may be responsible for the aggressive behavior of these tumors [8, 9]. According to the Catalogue of Somatic Mutations in Cancer (COSMIC) database, other oncogenes and tumor suppressor genes mutated in decreasing order of frequency in UC include *FGFR3*, *PIK3CA*, *CDKN2A*, *HRAS*, *KRAS*, *PTEN*, *AKT1*, *APC*, *CTNB1*, and *NRAS* (http://www.sanger.ac.uk/genetics/CGP/cosmic/).

Defective DNA mismatch repair (MMR) is manifested as MSI at >30 % of microsatellite markers examined and in most cases is associated with a loss of expression of one of the DNA MMR proteins, hMSH2, hMLHl, hMSH6, or hPMS2. MMR is rarely observed in UC of the bladder but is found in approximately 20–30 % of upper urinary tract UC [10, 11]. The finding of defective MMR in an upper tract UC should prompt an investigation into the possibility that the patient may have hereditary nonpolyposis colorectal cancer (HNPCC) and a germline mutation of one of the DNA MMR genes.

Chromosomal instability (CIN) is present in invasive UC and CIS. It is likely that genes that maintain genomic stability are inactivated early during invasive UC tumorigenesis. CIN drives tumorigenesis and tumor progression by accelerating the mutation rate in tumor cells [12]. The genes responsible for CIN in invasive UC are not known, and the role of *TP53* inactivation in CIN has been a matter of debate. pTa tumors show little evidence of CIN but, as noted above, tend to be diploid or near-diploid tumors with relatively few chromosomal alterations. Chromosome 9 and *P16* alterations play a major role in the formation of low-grade pTa tumors.

In addition, most low-grade papillary UC and urothelial papillomas have missense mutations of the fibroblast growth factor 3 (*FGFR3*) gene, while mutations of this gene are less common in invasive UC and CIS [13].

Taken together, two genetic pathways lead to the development of UC [1, 7]. One pathway leads to the formation of noninvasive papillary UC and the other to the development of CIS/invasive UC (Fig. 34.1). The pathway for noninvasive papillary UC is characterized by the presence of *FGFR3* mutations and/or chromosome 9 alterations and *P16* inactivation. The pathway for invasive UC is characterized by early alterations in the *TP53* and *P16* genes, late alterations of other tumor suppressor genes and oncogenes, chromosomal instability, and aneuploidy. The genetic differences between noninvasive papillary and CIS/invasive tumors likely explain the markedly different behavior and prognosis of these tumors [14].

Clinical Utility of Testing

In general, clinical molecular tests for solid tumors can be categorized as being useful for predicting predisposition to developing tumor, aiding in a diagnosis of the tumor type, detecting the presence of tumor, predicting prognosis, or guiding therapy. Examples of assays that are currently being used or investigated for each of these indications for UC are presented below.

Available Assays

The most clinically useful clinical molecular tests, as described below, are the following:

- MSI analysis and DNA MMR protein immunohistochemistry (IHC) of upper urinary tract UC to assess for Lynch syndrome (LS)
- FISH for UC detection
- *FGFR3* mutation analysis for UC detection

Numerous assays with a variety of clinical purposes for UC have been investigated but have not yet transitioned into the clinical use. *TP53* mutations are common in UC, especially in high-grade UC [15]. Assays that assess for *TP53* status could potentially be used to assess prognosis and detect tumor recurrence. Some studies have shown that TP53 overexpression detected by IHC analysis of formalin-fixed, paraffin-embedded tumors is associated with worse prognosis and higher risk of muscle invasion [8, 16], while others have not [17]. IHC analysis of bladder tumors for TP53 expression has not been widely utilized by urologists or pathologists. A few studies have shown that the antiapoptotic protein survivin may be a sensitive and specific marker for the detection of recurrent UC [18], but blinded prospective studies are needed to further evaluate the clinical utility of this assay. Alterations in certain genes, such as glutathione S-transferase M1 and N-acetyltransferase that encode proteins that metabolize carcinogens, may increase an individual's risk of developing bladder cancer, especially among smokers, but assays for these alterations also have not been used clinically.

Interpretation of Results

MSI Analysis and DNA MMR Protein IHC of Upper Tract UC to Assess for LS

Defective DNA MMR is rarely observed in UC of the bladder but is found in approximately 20–30 % of upper tract UC [10, 11]. Patients with early onset upper tract UC or an upper tract UC and a family history of LS-related tumors should be evaluated for LS. This evaluation can consist of assessing the tumor for defective DNA MMR with MSI testing and/or DNA MMR IHC. Patients whose tumors exhibit high-level MSI (MSI-H phenotype) have defective DNA MMR and almost always show loss of expression of one or more of the DNA MMR proteins by IHC. Most histopathology laboratories perform immunostains for four DNA MMR proteins: MLH1, PMS2, MSH2, and MSH6. The most common pattern of protein expression loss in tumors that exhibit defective DNA MMR is loss of MLH1 and PMS2 with retention of staining for MSH2 and MSH6. This pattern of expression is most often due to epigenetic silencing of the *MLH1* gene through promoter hypermethylation. However, some patients with MLH1 and PMS2 loss have a germline mutation in the *MLH1* gene and consequently have HNPCC. Less common IHC staining patterns are loss of MSH2 and MSH6 with retention of MLH1 and PMS2, loss of MSH6 alone, or loss of PMS2 alone. These three patterns are strongly associated with the presence of a germline mutation in the *MSH2*, *MSH6*, and *PMS2* genes, respectively. Patients with genetically proven LS are at risk of developing various tumors such as colorectal cancer, endometrial cancer, upper tract UC, gastric cancer, and sebaceous skin tumors and should undergo regular surveillance for these tumors.

Fluorescence In Situ Hybridization for UC Detection

Urine cytology has been the primary laboratory method for diagnosing and monitoring UC for the past 50 years. Urine cytology has excellent specificity but poor sensitivity for the

detection of UC [19]. The problem with false-negative urine cytology test results, if combined with a negative cystoscopy, is that clinical surveillance regimens recommend rescreening in 3 months, allowing an undetected tumor to progress to a higher, potentially incurable state before it is detected. This is of particular concern for grade 3 UC, which routinely progress if not removed or treated. The suboptimal sensitivity of urine cytology has prompted the development of new tests with improved sensitivity for UC detection.

Most UC are characterized by numerical and structural chromosomal abnormalities and a marked degree of CIN with variation in the chromosomal abnormalities found from cell to cell. The finding of aneusomy (i.e., abnormal chromosome copy number) and CIN in a population of cells by FISH is strongly correlated with the presence of malignancy. UroVysion (Abbott Molecular, Des Plaines, IL) is a FISH assay that has been developed for the detection of UC in urine. This assay utilizes four FISH probes, CEP3, CEP7, CEP17, and LSI 9p21, that are labeled with red, green, aqua, and yellow fluorophores, respectively [2, 19]. UroVysion received FDA approval in 2001 for monitoring UC patients for tumor recurrence and FDA approval in 2005 for assessing patients with hematuria (gross or microscopic) for bladder cancer. Representative examples of patients with FISH-positive and FISH-negative findings are shown in Fig. 34.2.

Meta-analysis of the sensitivity and specificity of UroVysion was 72 % (69–75 %) and 83 % (82–85 %), respectively [20]. The sensitivity of UroVysion for the detection of CIS, invasive UC, and high-grade papillary tumors is > 95 % [21]. The sensitivity of UroVysion is lower for low-grade papillary tumors than other UC, but is still significantly better than cytology for low-grade tumors. Though further studies are needed, it is possible that the low-grade tumors not detected by FISH have lower invasive potential and the intervals between cystoscopy could be extended. Studies suggest that UroVysion can detect recurrent UC before it is clinically evident by cystoscopy [21–24]. In the trial that led to FDA approval [23], Sarosdy et al. reported that 36 patients had a negative cystoscopic examination but a positive FISH result. With continued follow-up, 15 (41.7 %) of these cases were found to have biopsy-proven tumor recurrence with time-to-tumor diagnosis of 3–16 months (mean 6.0 months). Conversely, among 68 patients who had a negative cystoscopy and a negative FISH result, only 13 (19.1 %) had a biopsy-proven recurrence at 3–19 months (mean 11.2 months). The time to recurrence was significantly less ($p = 0.014$) for the patients with a positive FISH result but a negative cystoscopy than for patients with a negative FISH result and a negative cystoscopy [23].

UroVysion FISH testing has several other clinical uses. The clinical management of patients with equivocal cytology results is challenging because fewer than half of these patients will have bladder cancer on clinical follow-up. Equivocal cytology results can lead to unnecessary and expensive clinical investigations. Patients with an equivocal cytology and positive FISH result are at higher risk for having bladder cancer and should be followed more aggressively [25, 26]. FISH is also useful for assessing noninvasive bladder cancer patients undergoing bacillus Calmette-Guerin (BCG) treatment for reduction of recurrence risk following therapy. A 2005 study by Kipp et al. found that patients with a positive FISH result following intravesical therapy were 4.6 times more likely to have recurrent bladder cancer and 9.4 times more likely to have follow-up muscle-invasive

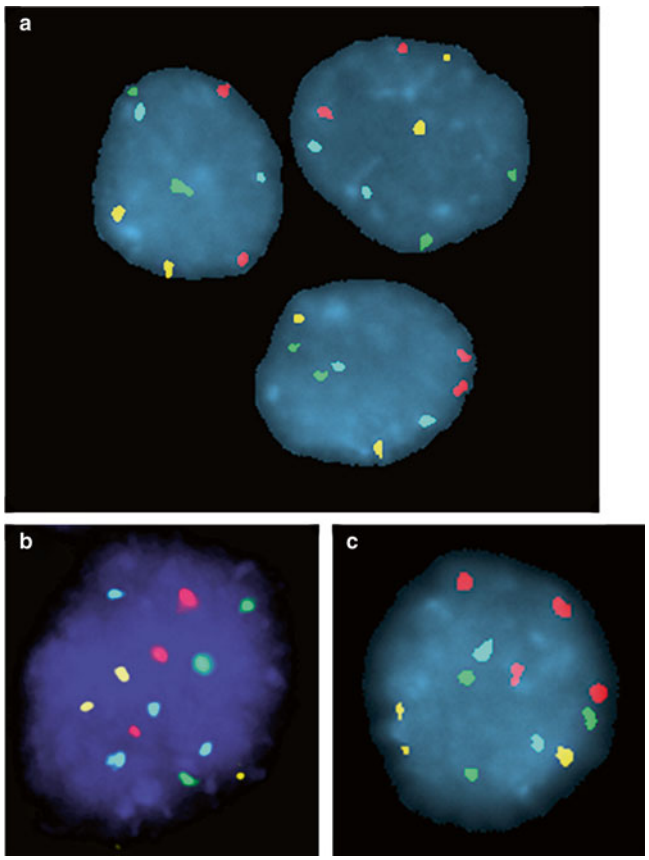

Figure 34.2 Representative examples of FISH results for nonneoplastic urothelial cells (*panel* **a**) and UC cells (*panels* **b** and **c**) using the UroVysion FISH assay (Abbott Molecular, Des Plaines, IL). Nonneoplastic cells generally have two signals for each of the four probes, though occasional nonneoplastic cells show only one signal for one or more of the probes due to random overlap of signals or imperfect hybridization efficiency. UC cells generally have gains for two or more of the probes (i.e., polysomy) of the UroVysion probe set. The finding of just a few cells with polysomy has high specificity for the presence of malignancy. *Panel* **b** shows UC cells with a gains of all four probe signals, CEP3(*red*), CEP7(*green*), CEP17 (*aqua*) and LSI 9p21 (*yellow*). *Panel* **c** shows UC cells with gain of CEP3 (*red*) and CEP7 (*green*) probe signals.

bladder cancer than patients with a negative FISH result [27]. Similar results were obtained by Mengual et al. [28] and Savic et al. [29], who found that patients with a positive post-BCG FISH result had 3.0 and 3.8 times higher risk of tumor recurrence, respectively.

Although other tumor markers are currently available for diagnosing bladder cancer (e.g. BTA stat [Polymedco, Inc., Cortlandt Manor, NY]; NMP22 [Alere, Orlando, FL]), the high sensitivity and specificity of the UroVysion FISH probe set makes this test one of the most commonly used molecular markers for detecting UC in urine cytology specimens. The primary disadvantage of the FISH assay is that it requires more effort than conventional cytology or point-of-care assays such as the BTA stat test. Typical turnaround time for the FISH assay is 1–2 days, though the test can be performed in a single day. Automated FISH enumeration instruments such as the Metasystems (Newton, MA), BioView (Billerica, MA), and Ikonisys (New Haven, CT) systems are used by some clinical laboratories. These systems may increase the ease of FISH test performance, reduce the cost of testing, and increase the throughput and sophistication of the data that can be obtained. Another shortcoming of the FISH test is its inability to detect some low-grade papillary tumors. An assay for UC cells that harbor *FGFR3* mutations (see *FGFR3* Mutation Analysis for UC Detection section below) may complement FISH and allow for the detection of virtually all UC.

FGFR3 Mutation Analysis for UC Detection

The detection of cells with *FGFR3* mutations in the urine is a promising way to detect the low-grade papillary tumors that are not detected by cystoscopy, cytology, or assays such as FISH [13, 30, 31]. FGFR3 is a tyrosine kinase receptor. Germline point mutations in various domains of *FGFR3* are associated with human skeletal disorders such as hypochondroplasia and achondroplasia, and somatic mutations of *FGFR3* have been identified in bladder cancer and myeloma. Interestingly, two groups have demonstrated a high frequency of somatic *FGFR3* point mutations in low-grade papillary UC and urothelial papilloma but not in high-grade papillary UC, CIS, or invasive UC [13, 30]. Billerey et al. found that the frequency of *FGFR3* mutations by stage was pTa 74 %, pTis 0 %, pT1 21 %, and pT2 to pT4 16 % [13]. UC of grade 1 showed 84 %, grade 2 showed 55 %, and grade 3 showed 7 %. The most common *FGFR3* mutation was an S249C mutation (33 of 48 tumors; 69 %), but R248C, G372C, Y375C, and K652E mutations also were identified. The difference in the frequency of *FGFR3* mutations between low-grade and high-grade tumors was highly significant ($p < 0.0001$) and is consistent with the current model of bladder tumor progression in which the most common precursor of invasive UC is CIS (Fig. 34.1). A commercial test kit for *FGFR3* mutation analysis is not available currently.

Laboratory Issues

Laboratory tests can be broadly divided into FDA-cleared/approved tests and laboratory-developed tests (LDT). Laboratories should verify that they can reproduce the performance characteristics that are published in the package inserts of FDA-approved tests. Laboratories that develop LDTs are responsible for establishing the performance characteristics of the LDT. Analytical validation of an LDT should include determining the accuracy, precision (reproducibility), reportable range, reference range, analytical sensitivity, and analytical specificity of the assay. In addition, the laboratory should conduct or be able to cite studies that demonstrate the clinical validity and utility of the LDT, including the positive and negative predictive values of the test. For a diagnostic assay, clinical validation would address the clinical sensitivity and specificity of the assay. For a prognostic assay, evidence of clinical validity would come from studies with Kaplan-Meier analyses and likelihood or hazard ratios.

As with all clinical tests, appropriate controls should be included with each run. For PCR-based tests, this would include positive, negative, and "no DNA" controls and analytical sensitivity and precision controls when a quantitative result is produced. Positive and negative controls for the UroVysion FISH test can be obtained from Abbott Molecular, Inc. These controls are non-hybridized slides prepared from cultured normal male lymphoblast cells and cultured bladder cancer cell lines. Each control slide consists of two separate target areas in which each of the different cell types has been applied. Clinical laboratories must enroll in proficiency testing when available and if not available establish internal methods to assess proficiency. Proficiency testing is available for UroVysion testing through the College of American Pathologists (CAP) Cytogenetics resource committee and for microsatellite instability (MSI) testing through the CAP Molecular Oncology resource committee.

Conclusions and Future Directions

Relatively few clinical molecular tests are used for the diagnosis and management of patients with UC. The main tests that are currently being used or developed are intended for bladder cancer detection in urine specimens. Messenger RNA expression profiling assays (e.g., Oncotype DX [Genomic Health, Redwood City, CA] and MammaPrint [Agendia, Inc., Irvine, CA]) have been used for prognosis and to guide therapy for patients for some tumor types such as breast cancer. Similar assays are not available for UC patients yet but could have clinical utility. Currently, no therapies are directed to specific molecular targets in UC. Therapies that target the FGFR3 tyrosine kinase receptor are being developed, and it is possible that the mutation status of the *FGFR3* gene may identify patients who are

most likely to respond to these therapies [32]. In the near future it is likely that next-generation sequencing of UC will guide targeted therapy.

Conflict of Interest Dr. Halling receives industry funding from Abbott Laboratories and royalties from the sale of the UroVysion probe set.

References

1. Richter J, Jiang F, Gorog JP, et al. Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. Cancer Res. 1997;57:2860.
2. Sokolova IA, Halling KC, Jenkins RB, et al. The development of a multitarget, multicolor fluorescence in situ hybridization assay for the detection of urothelial carcinoma in urine. J Mol Diagn. 2000;2:116.
3. Dalbagni G, Presti Jr JC, Reuter VE, et al. Molecular genetic alterations of chromosome 17 and p53 nuclear overexpression in human bladder cancer. Diagn Mol Pathol. 1993;2:4.
4. Knowles MA, Elder PA, Williamson M, et al. Allelotype of human bladder cancer. Cancer Res. 1994;54:531.
5. Rosin MP, Cairns P, Epstein JI, et al. Partial allelotype of carcinoma in situ of the human bladder. Cancer Res. 1995;55:5213.
6. Ruppert JM, Tokino K, Sidransky D. Evidence for two bladder cancer suppressor loci on human chromosome 9. Cancer Res. 1993;53:5093.
7. Cairns P, Shaw ME, Knowles MA. Initiation of bladder cancer may involve deletion of a tumour-suppressor gene on chromosome 9. Oncogene. 1993;8:1083.
8. Esrig D, Elmajian D, Groshen S, et al. Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med. 1994;331:1259.
9. Tsutsumi M, Sugano K, Yamaguchi K, et al. Correlation of allelic loss of the P53 gene and tumor grade, stage, and malignant progression in bladder cancer. Int J Urol. 1997;4:74.
10. Gonzalez-Zulueta M, Ruppert JM, Tokino K, et al. Microsatellite instability in bladder cancer. Cancer Res. 1993;53:5620.
11. Hartmann A, Zanardo L, Bocker-Edmonston T, et al. Frequent microsatellite instability in sporadic tumors of the upper urinary tract. Cancer Res. 2002;62:6796.
12. Jallepalli PV, Lengauer C. Chromosome segregation and cancer: cutting through the mystery. Nat Rev Cancer. 2001;1:109.
13. Billerey C, Chopin D, Aubriot-Lorton MH, et al. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am J Pathol. 2001;158:1955.
14. Sauter G, Mihatsch MJ. Pussycats and baby tigers: non-invasive (pTa) and minimally invasive (pT1) bladder carcinomas are not the same! J Pathol. 1998;185:339.
15. Uchida T, Wada C, Ishida H, et al. p53 mutations and prognosis in bladder tumors. J Urol. 1995;153:1097.
16. Sarkis AS, Dalbagni G, Cordon-Cardo C, et al. Nuclear overexpression of p53 protein in transitional cell bladder carcinoma: a marker for disease progression. J Natl Cancer Inst. 1993;85:53.
17. Shariat SF, Weizer AZ, Green A, et al. Prognostic value of P53 nuclear accumulation and histopathologic features in T1 transitional cell carcinoma of the urinary bladder. Urology. 2000;56:735.
18. Smith SD, Wheeler MA, Plescia J, et al. Urine detection of survivin and diagnosis of bladder cancer. J Am Med Assoc. 2001;285:324.
19. Halling KC, King W, Sokolova IA, et al. A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. J Urol. 2000;164:1768.
20. Hajdinjak T. UroVysion FISH test for detecting urothelial cancers: meta-analysis of diagnostic accuracy and comparison with urinary cytology testing. Urol Oncol. 2008;26:646.
21. Halling KC, Kipp BR. Bladder cancer detection using FISH (UroVysion assay). Adv Anat Pathol. 2008;15:279.
22. Bubendorf L, Grilli B, Sauter G, et al. Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. Am J Clin Pathol. 2001;116:79.
23. Sarosdy MF, Schellhammer P, Bokinsky G, et al. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. J Urol. 2002;168:1950.
24. Skacel M, Fahmy M, Brainard JA, et al. Multitarget fluorescence in situ hybridization assay detects transitional cell carcinoma in the majority of patients with bladder cancer and atypical or negative urine cytology. J Urol. 2003;169:2101.
25. Lotan Y, Bensalah K, Ruddell T, et al. Prospective evaluation of the clinical usefulness of reflex fluorescence in situ hybridization assay in patients with atypical cytology for the detection of urothelial carcinoma of the bladder. J Urol. 2008;179:2164.
26. Kipp BR, Halling KC, Campion MB, et al. Assessing the value of reflex fluorescence in situ hybridization testing in the diagnosis of bladder cancer when routine urine cytological examination is equivocal. J Urol. 2008;179:1296.
27. Kipp BR, Karnes RJ, Brankley SM, et al. Monitoring intravesical therapy for superficial bladder cancer using fluorescence in situ hybridization. J Urol. 2005;173:401.
28. Mengual L, Marin-Aguilera M, Ribal MJ, et al. Clinical utility of fluorescent in situ hybridization for the surveillance of bladder cancer patients treated with bacillus Calmette-Guerin therapy. Eur Urol. 2007;52:752.
29. Savic S, Zlobec I, Thalmann GN, et al. The prognostic value of cytology and fluorescence in situ hybridization in the follow-up of nonmuscle-invasive bladder cancer after intravesical Bacillus Calmette-Guerin therapy. Int J Cancer. 2009;124:2899.
30. van Rhijn BW, Montironi R, Zwarthoff EC, et al. Frequent FGFR3 mutations in urothelial papilloma. J Pathol. 2002;198:245.
31. van Rhijn BW, Lurkin I, Chopin DK, et al. Combined microsatellite and FGFR3 mutation analysis enables a highly sensitive detection of urothelial cell carcinoma in voided urine. Clin Cancer Res. 2003;9:257.
32. Guagnano V, Furet P, Spanka C, et al. Discovery of 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamin o]-pyrimidin-4-yl}-1-methyl-urea (NVP-BGJ398), a potent and selective inhibitor of the fibroblast growth factor receptor family of receptor tyrosine kinase. J Med Chem. 2011;54:7066.