Genitourinary Neoplasms

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Frequently Asked Questions

1. **What are the most common molecular assays currently available in the sporadic genitourinary (GU) system neoplasia?**
   - With increasing understanding of the genetic landscape of the tumors of the genitourinary system, it is proved that many types of GU tumors are associated with, and even defined by, recurrent genomic abnormalities.
   - Table 11.1 reviews the majority of the clinically relevant molecular assays currently available that may aid in the diagnosis, prognosis, and treatment of neoplasia in the genitourinary system.
   - It should be noted that there remain significant practice gaps for the implementation of this increasing knowledge into clinical practice.
2. **When should a molecular assay for *VHL* mutations or 3p deletions be considered in working up a renal cell carcinoma?**
   - More than 90% of sporadic clear cell renal cell carcinoma (CCRCC) harbors genomic alterations, most commonly copy number loss, on chromosome arm 3p, on which the tumor suppressor genes, such as *VHL*, *PBRM1*, *BAP1*, and *SETD2*, are located [1–3].
   - Most diagnostic pathology practice in a routine setting is based on histologic evaluation, possibly combined with immunohistochemistry (IHC). In rare challenging cases or in cases with a small biopsy or scant material, a molecular assay, including mutation analysis, FISH assay, and methylation studies, for 3p loss or *VHL* mutation can certainly provide supporting evidence for diagnosis [2].

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Y. Ding, L. Zhang (eds.), *Practical Oncologic Molecular Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-73227-1_11

Table 11.1 Main molecular assays in the genitourinary neoplasms

Organ	Target	Diagnosis	Method	Specimen
Kidney	*VHL* gene and chromosome 3	Clear cell RCC	FISH, sequencing	Fresh or FFPE tissue
	Chromosome 7 and 17 trisomy Chromosome Y deletion in male patients	Papillary RCC	FISH	Fresh or FFPE tissue
	TFE3 and *TFEB*	Translocation-associated RCC	IHC, FISH	Fresh or FFPE tissue
	ALK	ALK-rearranged RCC	Gene sequencing, FISH	Fresh or FFPE tissue
	Chromosome 7 and 17 trisomy Chromosome Y deletion in male patients *KRAS* mutation	Papillary renal neoplasm with reverse polarity	Gene sequencing, FISH	Fresh or FFPE tissue
	NTRK	Cellular congenital mesoblastic nephroma	IHC, FISH, sequencing	Fresh or FFPE tissue,
Bladder	Chromosome 3, 7, 9p, and 17; *TERT* promoter mutations; *FGFR* gene alterations	Urothelial carcinoma	FISH, molecular techniques	Urine, fresh or FFPE tissue, cytology smear, urine
Prostate	*ERG*	Prostatic adenocarcinoma and small cell carcinoma	IHC, FISH	Fresh or FFPE tissue
	TMPRSS2: *ERG* and *PCA3*	Screening for prostatic adenocarcinoma	TMA	Urine
	BRAF, *RAF1*	ETS-negative prostate cancer	FISH, sequencing	Fresh or FFPE tissue
	AR signaling status, AR-V7	Castration-resistant prostatic adenocarcinoma	Molecular techniques	Fresh or FFPE tissue, blood
	BRCA1, *BRCA2*, *HOXB13*	Pathogenic germline mutations increasing risk of prostate cancer	Molecular techniques	Fresh or FFPE tissue, blood
Testis	Isochromosome 12p	Germ cell tumor	FISH	FFPE tissue, semen

RCC renal cell carcinoma; *FISH* fluorescence in situ hybridization; *IHC* immunohistochemistry

- Notably, FISH analysis is unable to detect some cases of *VHL* loss, and other copy number assessment should be considered to identify copy number deletion, missense, and truncating mutations in *VHL*, which commonly occur in CCRCC.
- Emerging biomarkers, such as *VHL*, *PBRM1*, *BAP1*, and *SETD2*, although not being used routinely, may have increasing roles in renal cancer management. For example, it has been demonstrated that tumors harboring *PBRM1* mutation have more favorable behavior, whereas tumors with *BAP1* or *SETD2* mutations likely to have more aggressive behavior [4, 5].

3. **What are the commonly seen genetic alterations in chromophobe renal cell carcinoma?**
   - Like other RCC, chromophobe RCC can be usually diagnosed by typical histologic evaluation, with IHC in some cases.
   - Conventional cytogenetics can be used to detect commonly seen cytogenetic changes in chromophobe RCC including:
     - Hypodiploidy
     - Loss of chromosomes 1,2,6,10,13,17,21 and Y [6]
   - Commonly seen gene mutations include [7]:
     - Mutations in tumor suppressor genes: *TP53* (20–30%), *PTEN* (5–10%)
     - Rearrangements in *TERT* promoter region (~10%)
   - For tumors with hybrid chromophobe and oncocytic morphology and Birt-Hogg-Dube syndrome is suspected; genetic counseling and analysis of the *FLCN* (folliculin) gene should be considered [8, 9].
4. **What molecular assays can be used to aid the subtyping of papillary renal cell carcinoma (PRCC)?**
   - Papillary RCC (PRCC) is the second most common type of RCC and accounts for 15–20% of RCCs.
   - Papillary RCC is further subdivided into type 1 and type 2 PRCC, in which type 1 is more uniform on morphologic, immunophenotypic, and molecular features than type 2.
   - The difference between type 1 and type 2 PRCC is not only histologic but also prognostic and genomic, as patients with type 2 PRCC typically have worse outcomes than patients with type 1 PRCC.
   - Type 1 PRCC is frequently associated with trisomy of chromosomes 7 and 17 and loss of chromosome Y, whereas recent studies have shown that although these cytogenetic changes can also be observed in type 2 PRCC, it is more characterized by other genetic alterations. Indeed, type 2 PRCC is now considered likely more than one diagnostic entity (Table 11.2) [10, 11].
   - Nonetheless, there is currently no clinically available molecular assay to aid in the diagnosis of type 2 PRCC,

Table 11.2 Comparison of type 1 and type 2 PRCC

	Type 1 PRCC	Type 2 PRCC
Histology and immunophenotype	More uniform	Likely a mixture of multiple entities
cytogenetic changes	Trisomy or polysomic chromosomes 7 and 17 Loss of chromosome Y Gain of chromosomes 3,12,16, and 20	Gain of chromosomes 12,16, and 20
Gene mutations	*MET* mutations in the hereditary papillary RCC syndrome and sporadic type I PRCC [12–18]	*CDKN2A* silencing, *SETD2* mutations [10]

but FISH for trisomy 7 and/or 17 could be used in cases in which type 1 PRCC is a consideration. Notably, type 1 PRCC can have considerable morphologic overlap with mucinous tubular and spindle cell carcinoma and clear cell papillary RCC, both of which lack trisomies 7 and 17; thus, FISH for trisomy 7 and/or 17 may be especially useful in this differential diagnosis.

- Papillary renal neoplasm with reverse polarity is a newly proposed entity. It shows architectural and immunohistochemical overlap with PRCC. However, it has distinctively a single layer of apically located nuclei with positive GATA3 and LICAM and negative vimentin immunostaining. This sets it apart from PRCC, clear cell PRCC, and Xp11 translocation RCC. Recurrent *KRAS* point mutation identified in this entity is distinctly different from other renal cell neoplasms.

5. **What molecular assays should be considered to help differentiate translocation-associated TCC (tRCC) from other subtypes of RCC?**
   - Translocation-associated RCC (tRCC) is a subtype of RCC defined by a translocation involving the microphthalmia (MiT) subfamily of transcription factors includes the most common *TFE3* located at Xp11.2 and less common *TFEB*, *TFC*, and *MITF* [19].
   - The diagnosis of tRCC is mainly based on typical morphologic features such as nested and papillary growth pattern, mixture of clear and eosinophilic cells with unusually voluminous cytoplasm, psammomatous calcifications, and hyalinized stroma.
   - In the difficult cases with considerable morphologic overlap between tRCC and other RCC subtypes, such as CCRCC and PRCC.
     - Immunohistochemistry, such as melanocytic markers, TFE3 or TFEB, is often useful but not sensitive or specific for tRCC.
     - Break-apart FISH analysis for *TFE3* and *TFEB* gene rearrangements is highly sensitive and specific for tRCC and should be used to aid the diagnosis of tRCC [20, 21].
     - Other molecular techniques including sequencing can be used to detect gene rearrangement in cases with suspected false-negative FISH results and uncommon fusions not covered by FISH.
6. **What molecular assays are available when a hereditary RCC syndrome is considered?**
   - Hereditary RCC syndromes mainly include:
     - Hereditary leiomyomatosis and RCC (HLRCC) syndrome
     - Succinate dehydrogenase (SDH)-deficient RCC
     - von Hippel-Lindau syndrome
     - Hereditary papillary RCC
     - Birt-Hogg-Dube syndrome
     - Tuberous sclerosis
   - When encountering one or multiple renal tumors in a young patient, communication with clinicians, genetic counseling, and molecular assays for germline mutations should be considered to evaluate for a hereditary renal cancer syndrome (Table 11.3).
7. **What are the commonly seen genetic alterations in Wilms tumor?**
   - Wilms tumor is the most common childhood renal malignancy and could be associated with a variety of syndromes, which mainly include:
     - WAGR syndrome: **W**ilms tumor, **a**niridia, **g**enitourinary abnormalities, and mental **r**etardation
     - Denys-Drash and Frasier syndrome
     - Beckwith-Wiedemann syndrome
   - Genetic alteration has been identified in one-third of Wilms tumor-associated syndromes and is summarized in Table 11.4. In addition, mutations in *CTNNB1*, *WTX*, and *TP53* are identified in 10–15% of these syndromes [27].
8. **What is the most used molecular test in urothelial carcinoma (UC) screening?**
   - The most widely used molecular assay in UC screening is urine-based UroVysion (Vysis Inc) test.
   - UroVysion is a FISH assay performed on exfoliated cells in urine that assesses the aneuploidy of chromosomes 3, 7, and 17, as well as the loss of chromosome 9p21 locus, all of which are abnormalities characteristic of UC [28].
   - UroVysion test can be used as an aid for the initial diagnosis of bladder carcinoma in patients with hematuria. Because of its relatively high sensitivity and specificity for UC, this test has been implemented into many bladder cancer screening programs.
   - UroVysion can also be used to monitor tumor recurrence in patients with a history of UC or for stratification of patients with an abnormal cytology result and no clinical or cystoscopic evidence of a bladder tumor

Table 11.3 Major molecular genetic alterations in hereditary renal cell syndromes

Hereditary RCC syndromes	Associated neoplasms	Germline mutation	Mutation detection
Hereditary leiomyomatosis and RCC (HLRCC) syndrome [22, 23]	Leiomyomatosis of the skin and uterus **RCC** with type 2 papillary RCC-like morphology with prominent nucleoli Pheochromocytoma (rarely)	Fumarate hydratase (*FH*) gene with autosomal-dominant fashion	IHC for FH; IHC for 2SC (accumulates in the cytoplasm of HLRCC-associated RCC); Sequencing of *FH* gene
Succinate dehydrogenase (SDH)-deficient RCC [24, 25]	Paraganglioma/ pheochromocytoma Gastrointestinal stromal tumor **SDH-deficient RCC** Pituitary adenoma	One of the *SDH* genes (A–D), most commonly *SDHB* with autosomal-dominant fashion	SDHB IHC (loss of SDHB expression by IHC confirms inactivation of an *SDH* gene but is not necessarily diagnostic of inactivation of the *SDHB* gene) Sequencing of *SDH* genes
von Hippel-Lindau syndrome [26]	**Multiple CCRCC and renal cysts** Hemangioblastoma of the CNS and retina Pheochromocytoma Pancreatic cysts and neuroendocrine tumors Epididymal and broad ligament cystadenomas Endolymphatic sac tumors of the inner ear	*VHL* gene	Gene sequencing, FISH
Hereditary papillary RCC	Multiple, bilateral PRCCs	*MET* gene	Gene sequencing
Birt-Hogg-Dube syndrome	Multiple, bilateral kidney tumors, including a characteristic hybrid oncocytic tumor	*FCLN* gene	Gene sequencing
Tuberous sclerosis	A morphologically unique RCCs	*TSC1* and *TSC2* genes	Gene sequencing

CNS central nervous system; *2SC* 2-succinyl-cysteine

Table 11.4 Major genetic changes of Wilms tumor-associated syndromes

Congenital syndromes associated with Wilms tumor	Genetic changes
WAGR syndrome	Deletion mutations in *WT1* gene
Denys-Drash syndrome	Missense mutations in *WT1* gene
Beckwith-Wiedemann syndrome	Loss of imprinting or uniparental disomy of *IGF2* gene in chromosome 11p15.5

[29]. However, more recent studies demonstrate that the sensitivity and positive predictive value of UroVysion, particularly for low-grade urothelial carcinoma, may not be as optimal as initially thought [30].

9. **Besides UroVysion assay, what are other molecular assays available for UC and their indications?**
   - Besides cytogenetic alterations in UC, *TERT* promoter point mutations are commonly present in 60% to 80% of UC, and it appears to be early events in the oncogenesis of UC [31, 32].
   - Because *TERT* promoter mutations do not occur in reactive urothelial lesions [33], it has practical implications to test this mutation under at least three situations:
     1. In the differential diagnosis of UC versus non-neoplastic benign mimics (e.g., cystitis glandularis)
     2. In the differential diagnosis of UC versus other GU malignancy, such as prostatic cancer
     3. In urine cytology case suspicious for recurrent UC screening
   - Limitations of *TERT* promoter mutation test include the following:
     – It is not specific for UC. *TERT* promoter mutations are also present in benign urothelial neoplasms, such as urothelial papilloma, papillary urothelial neoplasm of low malignant potential, bladder squamous cell carcinoma, sarcomatoid carcinoma, and urachal carcinomas [34–37]. In addition, they are also reported to be present in neoplasms of other organs, such as glioblastoma and melanoma.
     – A negative *TERT* promoter mutation result does not exclude the possibility of a urothelial neoplasm.
   - *FGFR3* mutations, primarily point mutations and translocations, can be detected in nonmuscle-invasive bladder cancer as well as up to 10–15% of muscle-invasive bladder cancer cases [38, 39]. Because FGFR3 is a target of pan-FGFR inhibitors, the National Comprehensive Cancer Network (NCCN) has recently recommended that molecular tests for *FGFR3* mutations should be considered in patients with advanced stage bladder cancer.

10. **What is the clinical utility of ETS gene fusions in diagnosis of prostate adenocarcinoma?**
    - The ETS family of transcription factors is composed of approximately 27 members which are frequently involved in gene fusions. ETS fusion genes have been detected in a variety of malignancies such as *EWS* gene fusions in Ewing's sarcoma, *TEL*(*ETV6*) gene fusions in leukemia, and *ERG* gene fusions in prostate cancer [40–42].
    - More than 20 *ERG* fusion partners have been reported in prostate cancer, and about 50% is fusion of *TMPRSS2* to *ERG* [43, 44].
    - *ERG* fusion transcripts can be detected in up to 50% of prostate tumors. Although the diagnosis of most prostate tumors are based on histologic and immunohistochemistry evaluation, FISH or sequence for *ERG* may provide help in the difficult cases to aid the diagnosis of prostatic adenocarcinoma versus a benign process.
    - Most ETS rearrangements can be detected by FISH using a break-apart probe for *ERG* (chromosome 21q22). IHC using an anti-ERG antibody, which detects the *ERG* gene fusion product, can also detect *ERG* aberrations.
    - Limitations:
      – A negative *ERG* FISH or IHC result does not exclude the diagnosis of prostatic adenocarcinoma.
      – IHC for ERG may be positive in high-grade prostatic intraepithelial neoplasia (HGPIN).
      – Overexpression of *ERG* by itself is not a diagnostic criterion for malignancy.
11. **Besides the ETS gene family, what are other commonly observed molecular genetic abnormalities in prostatic cancer and their prognostic significance?**
    - *PTEN*, the tumor-suppressor gene, and proliferation index Ki67 are emerging biomarkers in localized prostate cancer and may be used to guide clinical management.
    - *PTEN* inactivation, either by gene deletion, rearrangement, or truncation mutations, have been described in about 20% of primary and up to 40% of metastatic prostate cancer. Depending on the mutation type, FISH or IHC assays are the commonly used methods to assess *PTEN* status [45–48].
    - Clinical significance of *PTEN* inactivation in prostate cancer include [46, 48–50]:
      – *PTEN* inactivation is associated with rising levels of prostate-specific antigen (PSA) in the serum.
      – Patients with heterogeneous or subclonal *PTEN* loss generally have worse outcomes than those with intact *PTEN* but better outcome than patients with homogenous or clonal *PTEN* loss.
      – Patients with compound *PTEN* inactivation and *ERG* rearrangement have better clinical outcomes compared with those with *PTEN* inactivation but wild-type *ERG* gene.
    - Because *PTEN* inactivation shows a strong positive correlation with pathologic stage in prostate cancer, the analysis of *PTEN* status and Ki67 level should be considered to facilitate the assessment of the pathologic grade of the tumor especially in the core biopsy settings [51].
12. **For prostatic cancer, what is the current utilization of gene expression profiling (GEP) assay?**
    - Gene expression profiling (GEP) is introduced to risk-stratify prostatic cancer patients and guide treatment decisions between therapeutic intervention and active surveillance.
    - Several commercially available clinical GEP assays have been developed, including Prolaris® assay (Myriad Genetics, Salt Lake City, UT), OncotypeDX® Prostate Cancer Assay (Genomic Health, Redwood City, CA), Decipher® Prostate Cancer Classifier (GenomeDx Biosciences, Vancouver, BC, Canada), and ProMark™ Protein Biomarker Test (Metamark Genetics, Cambridge, MA). The characteristic features of these assays are summarized in Table 11.5 [52–57].
    - Although with potential benefit in providing additional information in aiding treatment decisions and preventing unnecessary rebiopsy procedure and its related cost, GEP assay's clinical utility is still not well defined at current practice, and most of such tests are performed upon clinician's requests. Further evidence of GEP performance and patients' follow-up is desirable to evaluate its value to guide future utilization.
13. **Is there a prostate cancer screening algorithm available?**
    - A review of more than 60 studies of screening for prostate cancer including approximately 2 million people demonstrated that prostate-specific antigen (PSA) screening has been shown to substantially reduce prostate cancer mortality. It is also known to be associated with false-positive results, overdiagnosis, unnecessary biopsies with associated risks of morbidity, and increased risks associated with treatments that may not prolong life. A novel or modified screening algorithm is imperative to replace the PSA-alone prostate cancer screening practice.

Table 11.5 Representative gene expression profiling assays for prostate cancer

GEP	Sample type	Targets	Risk calculation
ConfirmMDx® Prostate Cancer Assay	Previously biopsied prostate cancer negative tissue	Detect the DNA methylation status of *GSTP1*, *APC*, and *RASSF1* genes using methylation-specific PCR (MSP)	The likelihood of GS ≤ 6 and GS ≥ 7 prostate cancer being detected on repeat biopsy is calculated by incorporating DNA methylation intensity with clinical risk factors, including PSA, DRE, age, and histopathology of the previous biopsy
Decipher® Prostate Cancer Classifier	Radical prostatectomy tissue in newly diagnosed patients with localized cancer	A GEP panel of 22 genes	A continuous risk score between 0 and 1 to predict the probability of clinical metastasis within 5 years of radical prostatectomy
OncotypeDX® Prostate Cancer Assay	Prostate biopsy	A GEP panel of 17 genes (12 cancer-related and 5 reference genes) to generate a GPS.	Combination of GPS (0–100) with PSA, Gleason score, and tumor stage
Prolaris® assay	Prostate biopsy or prostatectomy sample	A GEP panel of 46 genes (31 CCP genes and 15 housekeeper genes) to generate a CCP score	Combination of GS, PSA, clinical stage, and CCP score
ProMark™ Protein Biomarker Test	Prostate biopsy	A quantitative protein based multiplex immunofluorescence in situ imaging platform measuring eight protein biomarkers	An algorithmically derived risk score between 1 and 100

GS Gleason score; *PSA* prostate-specific antigen; *DRE* digital rectal exam; *CCP* cell cycle progression; *GPS* genomic prostate score [58]

- Several new screening tests, including serum or blood-based such as 4Kscore, prostate health index (PHI), and Stockholm3(STHLM3) test and urine-based such as prostate cancer antigen 3 (PCA3) and HOXC6/DLX1, have been shown to be more accurate and generally better than PSA-alone screening. Combinations of molecular tests with multiparametric magnetic resonance imaging (mpMRI) are also gaining popularity for its ability to determine clinically significant cancer.
- In summary, a shared decision-making approach is currently used for prostate cancer screening, and patients are encouraged to decide for themselves whether the benefits of screening outweigh the harms.

14. **What are the commonly seen molecular changes in testicular germ cell tumors (GCTs)?**
    - Germ cell tumors (GCTs) account for most testicular neoplasms, especially in young adult men.
    - Alteration of chromosome 12p is the hallmark biomarker of germ cell tumors. Isochromosome 12p is the most common alteration observed in about ~80% of cases seen in almost all invasive tumors, but not in isolated germ cell neoplasia in situ without an adjacent invasive component [58].
    - Most common genetic changes in GCTs are the copy number gain of chromosome 12p, which can be detected in ~80% of GCTs by FISH, microarray, or next-generation sequencing (NGS).
    - Driver mutations in *KIT*, *KRAS*, and *NRAS* genes have also been reported in 5–30% of seminoma and up to 15% of non-seminoma patients [59–62].

Case Presentations

Case 1

Learning Objective

Histological, immunophenotypic, and molecular features of papillary renal neoplasm with reverse polarity.

Case History

The patient is a 54-year-old female with a history of end-stage renal disease who was found to have an incidental 2-cm right renal mass during routine workup for consideration of renal transplantation. She underwent total nephrectomy.

Histologic Findings

Histologic examination showed an intracystic papillary tumor. The papillae were arborized and covered by a single layer of cuboidal cells with eosinophilic cytoplasm. The nuclei were monotonous and rounded and were characteristically apical in location. They had a low WHO/ISUP nuclear grade with no prominent nucleoli. The papillary cores were fibrotic and contained sparse inflammatory cells. No hemorrhage, necrosis, or mitotic figures were seen. The tumor was positive for GATA3 and L1CAM and negative for AMACR and vimentin by immunohistochemistry (Fig. 11.1a–d).

- *Question 1: After reviewing this preliminary information, what are the major differential diagnosis?*
- *Question 2: Which molecular studies could be ordered to help the diagnosis?*

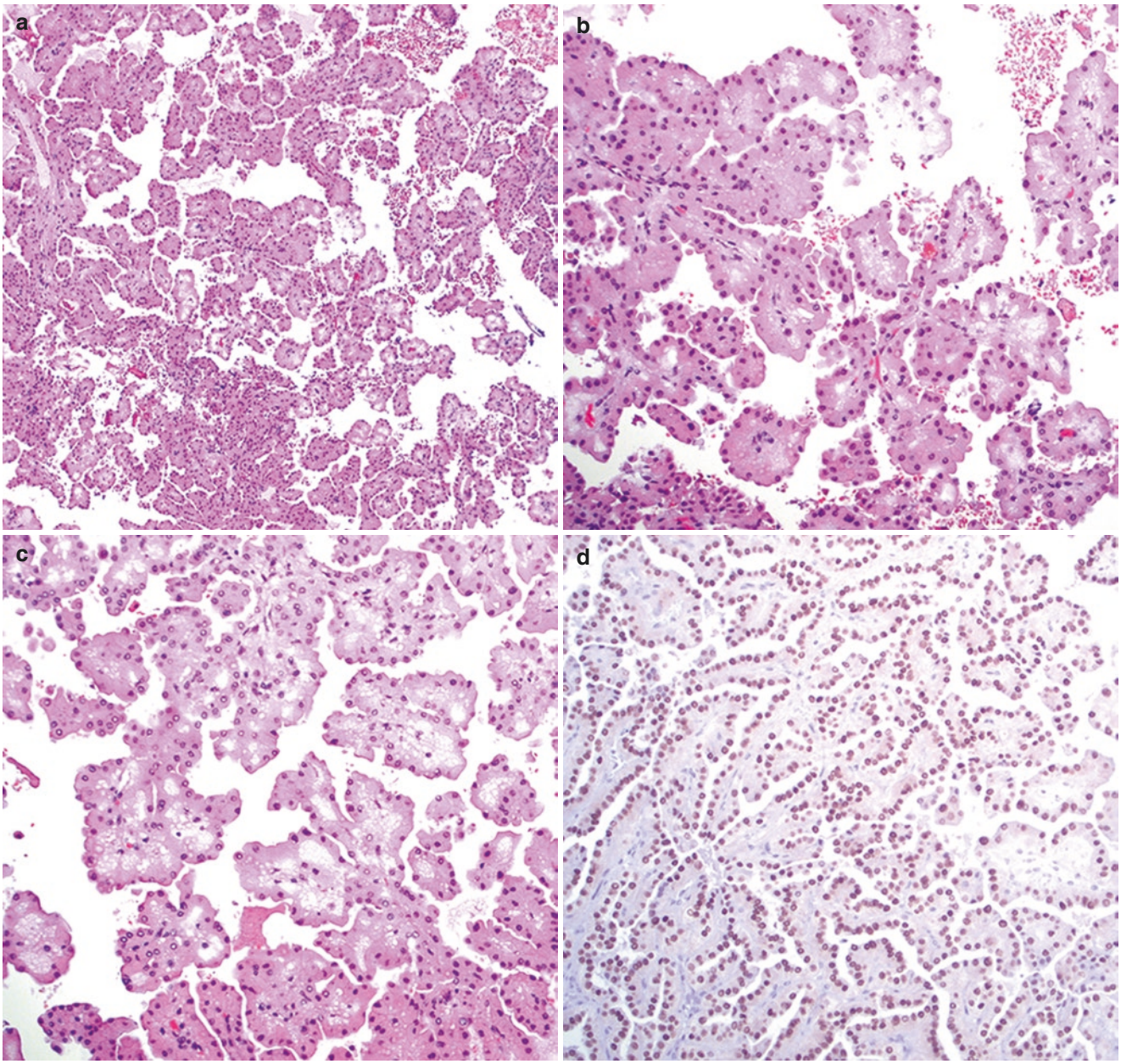

Fig. 11.1 Papillary renal neoplasm with reverse polarity. It is formed by arborizing papillary architecture with centrally hyalinized fibrovascular core (H&E stain, 100×) [53] (**a**). The papillae are covered by a single layer of cuboidal cells with eosinophilic cytoplasm and apically located nuclei (reversely polarized) (H&E stain, 200×) (**b**, **c**). Prominent intracytoplasmic vacuolization is present (**c**). GATA-3 immunostain is uniformly positive (H&E stain, 200×) (**d**)

Based on the described histologic findings, the major differential diagnosis includes papillary renal cell carcinoma, type 1 or type 2, and other papillary renal cell neoplasms. FISH analysis to identify the presence of chromosomal abnormalities including gains or losses of 3p, 7, 17, and Y would provide useful information to diagnose.

Molecular Genetic Study

FISH analysis showed the presence of trisomy of chromosome 7 and disomy of chromosome 17 (Fig. 11.2). Next-generation sequencing identified *KRAS* p.G12V (c.34G > T) mutation.

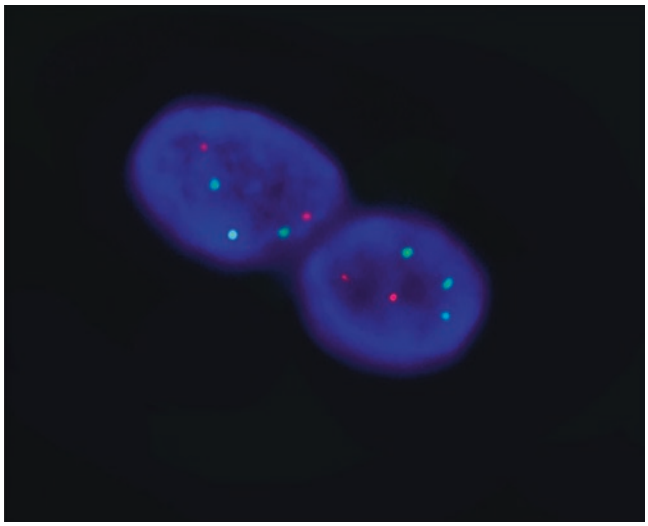

Fig. 11.2 FISH analysis showed the presence of trisomy 7 and disomy of chromosome 17

Final Diagnosis
Papillary renal neoplasm with reverse polarity

Follow-Up
The patient was followed up for 48 months and had no evidence of tumor recurrence or metastasis.

Discussion
Papillary renal neoplasm with reverse polarity is an epithelial renal tumor and a newly proposed entity [63]. Although papillary renal neoplasm with reverse polarity has papillary or tubulopapillary architecture, which overlaps with PRCC, it also has distinctively apically located nuclei away from the basement membrane. Together with other morphological findings, including the single layer of eosinophilic cells with finely granular cytoplasm, inconspicuous nucleoli, and lack of intracellular hemosiderin, mitotic figures or necrosis, these features set this entity apart from PRCC, clear cell PRCC, and Xp11 translocation RCC. Immunophenotypically, papillary renal neoplasm with reverse polarity are positive for GATA3 and LICAM and negative for vimentin and AMACR (except for blush-like positive in some cases).

Besides the well-known histologic heterogeneity in papillary renal cell carcinoma (PRCC), especially in type 2, type 1 PRCCs are associated with *MET* alterations, whereas a variety of gene alterations, such as *CDKN2A* inactivation and *SETD2* mutations, have been reported in type 2. *KRAS* point mutation is associated with papillary renal neoplasm with reverse polarity which is distinctly different from other renal cell neoplasms [64].

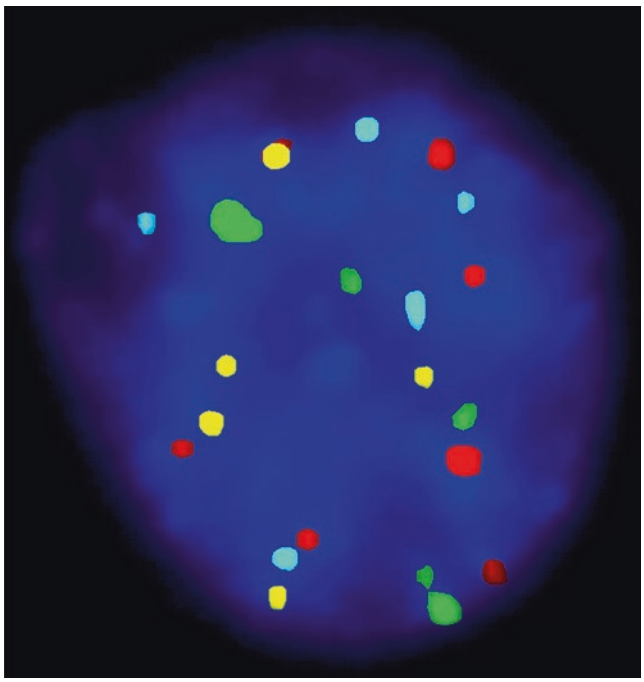

Fig. 11.3 UroVysion FISH analysis was positive for polysomy of chromosomes 3, 7, 17, and 9p21

Case 2

Learning Objective
Utilization of UroVysion FISH analysis for bladder cancer screening

Case History
The patient is an 84-year-old male with a history of prostatectomy for high-grade prostatic adenocarcinoma with salvage radiation and hormonal therapy. Fifteen years after his prostatectomy, he presented with gross hematuria, and cytology showed rare atypical urothelial cell suspicious for high-grade urothelial carcinoma. The urine specimen was sent for UroVysion analysis.

Molecular Genetic Study
UroVysion FISH analysis was positive for polysomy of chromosomes 3, 7, 17, and 9p21 (Fig. 11.3). The patient underwent a cystoscopy which was sent for pathology evaluation.

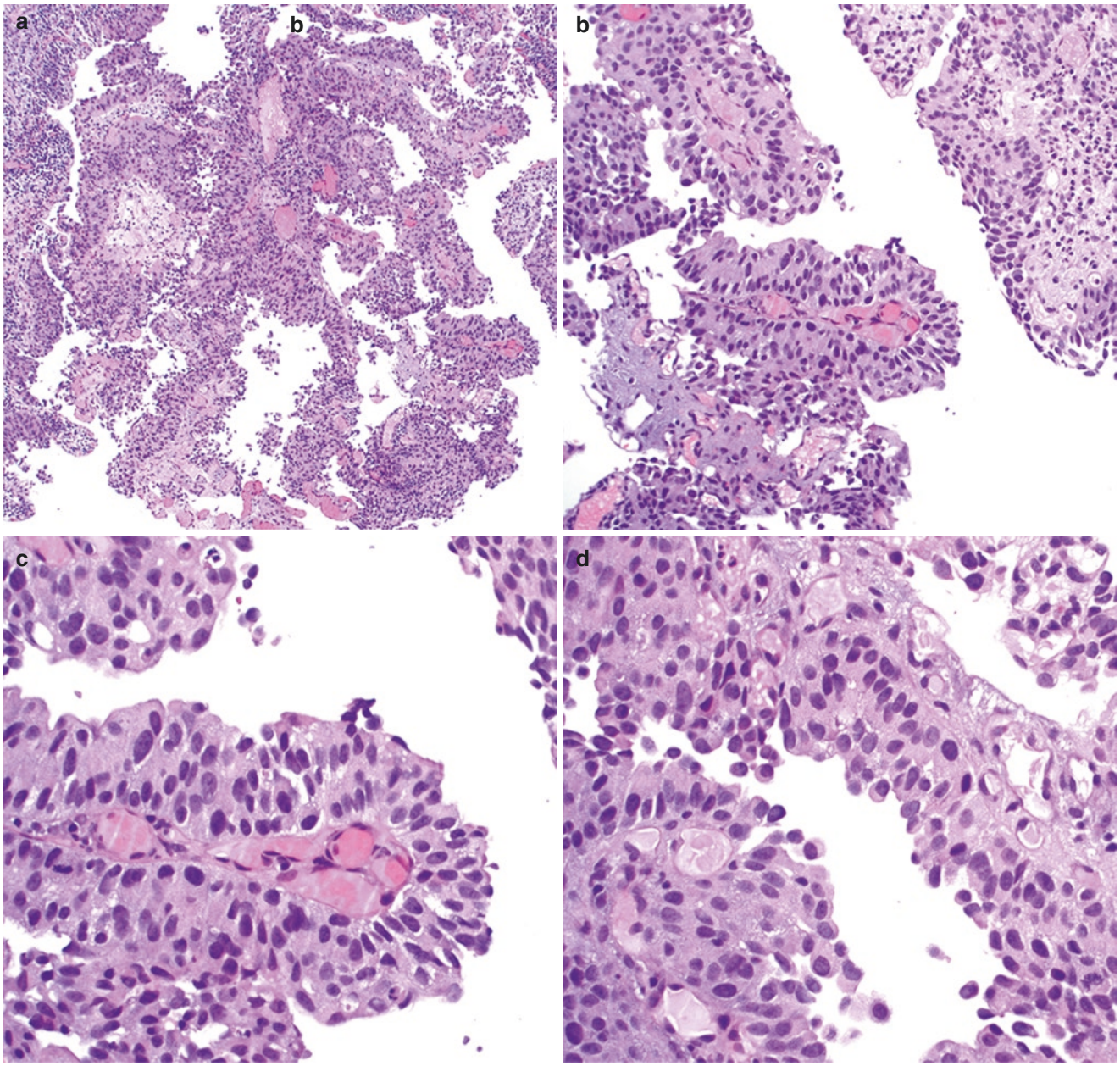

Fig. 11.4 High-grade papillary urothelial carcinoma. The papillae are covered by proliferating urothelial cells (H&E stain, 100×) (**a**), showing disorderly oriented nuclei, with marked cytologic atypia, nuclear hyperchromasia, and prominent nucleoli (H&E stain, 200×) (**b**–**d**)

Histologic Findings

Histologic examination shows neoplastic urothelial proliferation formed of multiple fibrovascular cores, covered by variably thickened urothelial cells. The cells show moderate to significant cytologic atypia, nuclear enlargement, and hyperchromasia. Loss of nuclear polarity and prominent nucleoli are also seen (Fig. 11.4a–d).

Final Diagnosis

Papillary urothelial carcinoma, noninvasion, high grade

Follow-Up

The patient had a tumor recurrence 2 years later which was cystoscopically resected and showed invasion into the lamina propria. He was followed up for 70 months with no evidence of recurrence.

Discussion

This case represents a typical situation when UroVysion FISH study is indicated for bladder cancer screening. Besides it can also be used to monitor tumor recurrence based on its high specificity for high-grade urothelial carcinoma.

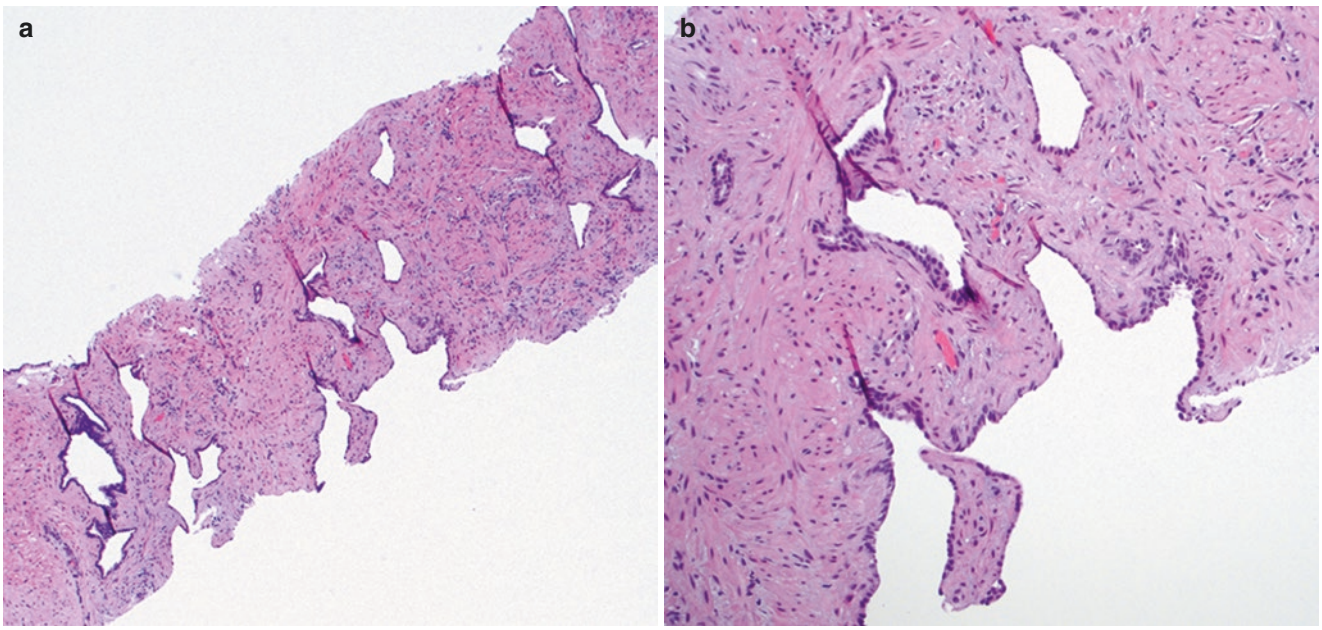

Fig. 11.5 Prostatic atrophy. The prostatic parenchyma shows widely spaced prostatic glands with dilated lumens and separated by prominent stromal elements (H&E stain, 40×) (**a**). The glands are lined by cells with minimal amount of cytoplasm and crowded nuclei (H&E stain, 200×) (**b**)

Case 3

Learning Objective

Emerging gene expression profiling (GEP) assay for risk-stratify patients with increased risk for prostate cancer

Case History

The patient is a 69-year-old male with no known significant medical history, who presented for evaluation of two episodes of elevated prostate specific antigen (PSA) of up to 6.25 ng/ml during surveillance examination within 6 months.

Histologic Findings

Histologic examination showed cores of prostatic tissue composed of glands with dilated lumens that are lined by cells with minimal amount of cytoplasm and crowded nuclei. Stromal fibrosis is also prominent (Fig. 11.5a–b).

Molecular Genetic Study

The case was sent to ConfirmMDx for prostate cancer DNA (*GSTP1*, *APC*, and *RASSF1*) methylation study and was negative.

Final Diagnosis

Benign prostatic tissue with atrophy

Follow-Up

Given the negative biopsy and ConfirmMDx findings, the patient elected to be followed up by serial PSA monitoring. The subsequent PSA levels fell below 4 ng/ml.

Discussion

The ConfirmMDx assay (MDxHealth, Irvine, CA) is a commercially available test designed to improve patient stratification, and it could be considered in men with an elevated PSA level (≥4.0 ng/ml) and/or abnormal digital rectal exam (DRE) but with cancer-negative prostate biopsy [65, 66]. The DNA methylation status of three genes, *GSTP1*, *APC*, and *RASSF1*, is evaluated on the core biopsy, and the result indicates the likelihood of Gleason score (GS) ≤ 6 and GS ≥7 prostate cancer being detected. Urologists should incorporate the ConfirmMDx result together with PSA, DRE, age, and histopathology findings to stratify patients for active monitoring or repeat biopsy/MRI examination.

References

1. Brugarolas J. Molecular genetics of clear-cell renal cell carcinoma. J Clin Oncol. 2014;32(18):1968–76.
2. Magers MJ, Cheng L. Practical molecular testing in a clinical genitourinary service. Arch Pathol Lab Med. 2020;144(3):277–89.
3. Klatte T, Said JW, Seligson DB, Rao PN, de Martino M, Shuch B, et al. Pathological, immunohistochemical and cytogenetic features of papillary renal cell carcinoma with clear cell features. J Urol. 2011;185(1):30–5.
4. Chen YB, Xu J, Skanderup AJ, Dong Y, Brannon AR, Wang L, et al. Molecular analysis of aggressive renal cell carcinoma with unclassified histology reveals distinct subsets. Nat Commun. 2016;7:13131.
5. Piva F, Santoni M, Matrana MR, Satti S, Giulietti M, Occhipinti G, et al. BAP1, PBRM1 and SETD2 in clear-cell renal cell carcinoma: molecular diagnostics and possible targets for personalized therapies. Expert Rev Mol Diagn. 2015;15(9):1201–10.

6. Speicher MR, Schoell B, du Manoir S, Schrock E, Ried T, Cremer T, et al. Specific loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 in chromophobe renal cell carcinomas revealed by comparative genomic hybridization. Am J Pathol. 1994;145(2):356–64.
7. Davis CF, Ricketts CJ, Wang M, Yang L, Cherniack AD, Shen H, et al. The somatic genomic landscape of chromophobe renal cell carcinoma. Cancer Cell. 2014;26(3):319–30.
8. Delongchamps NB, Galmiche L, Eiss D, Rouach Y, Vogt B, Timsit MO, et al. Hybrid tumour 'oncocytoma-chromophobe renal cell carcinoma' of the kidney: a report of seven sporadic cases. BJU Int. 2009;103(10):1381–4.
9. Petersson F, Gatalica Z, Grossmann P, Perez Montiel MD, Alvarado Cabrero I, Bulimbasic S, et al. Sporadic hybrid oncocytic/chromophobe tumor of the kidney: a clinicopathologic, histomorphologic, immunohistochemical, ultrastructural, and molecular cytogenetic study of 14 cases. Virchows Arch. 2010;456(4):355–65.
10. Pitra T, Pivovarcikova K, Alaghehbandan R, Hes O. Chromosomal numerical aberration pattern in papillary renal cell carcinoma: review article. Ann Diagn Pathol. 2019;40:189–99.
11. Saleeb RM, Brimo F, Farag M, Rompre-Brodeur A, Rotondo F, Beharry V, et al. Toward biological subtyping of papillary renal cell carcinoma with clinical implications through histologic, immunohistochemical, and molecular analysis. Am J Surg Pathol. 2017;41(12):1618–29.
12. Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet. 1997;16(1):68–73.
13. Zbar B, Tory K, Merino M, Schmidt L, Glenn G, Choyke P, et al. Hereditary papillary renal cell carcinoma. J Urol. 1994;151(3):561–6.
14. Dharmawardana PG, Giubellino A, Bottaro DP. Hereditary papillary renal carcinoma type I. Curr Mol Med. 2004;4(8):855–68.
15. Rhoades Smith KE, Bilen MA. A review of papillary renal cell carcinoma and MET inhibitors. Kidney Cancer. 2019;3(3):151–61.
16. Gilbert JA. Savolitinib for MET-driven papillary renal cell carcinoma. Lancet Oncol. 2017;18(8):e440.
17. Akhtar M, Al-Bozom IA, Al HT. Papillary Renal Cell Carcinoma (PRCC): an update. Adv Anat Pathol. 2019;26(2):124–32.
18. Lubensky IA, Schmidt L, Zhuang Z, Weirich G, Pack S, Zambrano N, et al. Hereditary and sporadic papillary renal carcinomas with c-met mutations share a distinct morphological phenotype. Am J Pathol. 1999;155(2):517–26.
19. Argani P. MiT family translocation renal cell carcinoma. Semin Diagn Pathol. 2015;32(2):103–13.
20. Green WM, Yonescu R, Morsberger L, Morris K, Netto GJ, Epstein JI, et al. Utilization of a TFE3 break-apart FISH assay in a renal tumor consultation service. Am J Surg Pathol. 2013;37(8):1150–63.
21. Rao Q, Williamson SR, Zhang S, Eble JN, Grignon DJ, Wang M, et al. TFE3 break-apart FISH has a higher sensitivity for Xp11.2 translocation-associated renal cell carcinoma compared with TFE3 or cathepsin K immunohistochemical staining alone: expanding the morphologic spectrum. Am J Surg Pathol. 2013;37(6):804–15.
22. Harrison WJ, Andrici J, Maclean F, Madadi-Ghahan R, Farzin M, Sioson L, et al. Fumarate hydratase-deficient uterine leiomyomas occur in both the syndromic and sporadic settings. Am J Surg Pathol. 2016;40(5):599–607.
23. Trpkov K, Hes O, Agaimy A, Bonert M, Martinek P, Magi-Galluzzi C, et al. Fumarate hydratase-deficient renal cell carcinoma is strongly correlated with fumarate hydratase mutation and hereditary leiomyomatosis and renal cell carcinoma syndrome. Am J Surg Pathol. 2016;40(7):865–75.
24. Gill AJ. Succinate dehydrogenase (SDH) and mitochondrial driven neoplasia. Pathology. 2012;44(4):285–92.
25. Gill AJ. Succinate dehydrogenase (SDH)-deficient neoplasia. Histopathology. 2018;72(1):106–16.
26. Cheng L, Williamson SR, Zhang S, Maclennan GT, Montironi R, Lopez-Beltran A. Understanding the molecular genetics of renal cell neoplasia: implications for diagnosis, prognosis and therapy. Expert Rev Anticancer Ther. 2010;10(6):843–64.
27. Huppmann AR. Educational case: Wilms tumor. Acad Pathol. 2018;5:2374289518781582.
28. Halling KC, Kipp BR. Bladder cancer detection using FISH (UroVysion assay). Adv Anat Pathol. 2008;15(5):279–86.
29. Moonen PM, Merkx GF, Peelen P, Karthaus HF, Smeets DF, Witjes JA. UroVysion compared with cytology and quantitative cytology in the surveillance of non-muscle-invasive bladder cancer. Eur Urol. 2007;51(5):1275–80. discussion 80
30. Dimashkieh H, Wolff DJ, Smith TM, Houser PM, Nietert PJ, Yang J. Evaluation of urovysion and cytology for bladder cancer detection: a study of 1835 paired urine samples with clinical and histologic correlation. Cancer Cytopathol. 2013;121(10):591–7.
31. Giedl J, Rogler A, Wild A, Riener MO, Filbeck T, Burger M, et al. TERT core promotor mutations in early-onset bladder cancer. J Cancer. 2016;7(8):915–20.
32. Rachakonda PS, Hosen I, de Verdier PJ, Fallah M, Heidenreich B, Ryk C, et al. TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. Proc Natl Acad Sci U S A. 2013;110(43):17426–31.
33. Zhong M, Tian W, Zhuge J, Zheng X, Huang T, Cai D, et al. Distinguishing nested variants of urothelial carcinoma from benign mimickers by TERT promoter mutation. Am J Surg Pathol. 2015;39(1):127–31.
34. Wang CC, Huang CY, Jhuang YL, Chen CC, Jeng YM. Biological significance of TERT promoter mutation in papillary urothelial neoplasm of low malignant potential. Histopathology. 2018;72(5):795–803.
35. Isharwal S, Hu W, Sarungbam J, Chen YB, Gopalan A, Fine SW, et al. Genomic landscape of inverted urothelial papilloma and urothelial papilloma of the bladder. J Pathol. 2019;248(3):260–5.
36. Kurtis B, Zhuge J, Ojaimi C, Ye F, Cai D, Zhang D, et al. Recurrent TERT promoter mutations in urothelial carcinoma and potential clinical applications. Ann Diagn Pathol. 2016;21:7–11.
37. Cheng L, Davidson DD, Wang M, Lopez-Beltran A, Montironi R, Wang L, et al. Telomerase reverse transcriptase (TERT) promoter mutation analysis of benign, malignant and reactive urothelial lesions reveals a subpopulation of inverted papilloma with immortalizing genetic change. Histopathology. 2016;69(1):107–13.
38. Sjodahl G, Lauss M, Lovgren K, Chebil G, Gudjonsson S, Veerla S, et al. A molecular taxonomy for urothelial carcinoma. Clin Cancer Res. 2012;18(12):3377–86.
39. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell. 2017;171(3):540–56. e25
40. Bohlander SK. ETV6: a versatile player in leukemogenesis. Semin Cancer Biol. 2005;15(3):162–74.
41. Khoury JD. Ewing sarcoma family of tumors. Adv Anat Pathol. 2005;12(4):212–20.
42. Hollenhorst PC, McIntosh LP, Graves BJ. Genomic and biochemical insights into the specificity of ETS transcription factors. Annu Rev Biochem. 2011;80:437–71.
43. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer. 2008;8(7):497–511.
44. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005;310(5748):644–8.
45. Jamaspishvili T, Berman DM, Ross AE, Scher HI, De Marzo AM, Squire JA, et al. Clinical implications of PTEN loss in prostate cancer. Nat Rev Urol. 2018;15(4):222–34.
46. Lotan TL, Wei W, Ludkovski O, Morais CL, Guedes LB, Jamaspishvili T, et al. Analytic validation of a clinical-grade PTEN

immunohistochemistry assay in prostate cancer by comparison with PTEN FISH. Mod Pathol. 2016;29(8):904–14.
47. Picanco-Albuquerque CG, Morais CL, Carvalho FL, Peskoe SB, Hicks JL, Ludkovski O, et al. In prostate cancer needle biopsies, detections of PTEN loss by fluorescence in situ hybridization (FISH) and by immunohistochemistry (IHC) are concordant and show consistent association with upgrading. Virchows Arch. 2016;468(5):607–17.
48. Lotan TL, Heumann A, Rico SD, Hicks J, Lecksell K, Koop C, et al. PTEN loss detection in prostate cancer: comparison of PTEN immunohistochemistry and PTEN FISH in a large retrospective prostatectomy cohort. Oncotarget. 2017;8(39):65566–76.
49. Bismar TA, Hegazy S, Feng Z, Yu D, Donnelly B, Palanisamy N, et al. Clinical utility of assessing PTEN and ERG protein expression in prostate cancer patients: a proposed method for risk stratification. J Cancer Res Clin Oncol. 2018;144(11):2117–25.
50. Reid AH, Attard G, Ambroisine L, Fisher G, Kovacs G, Brewer D, et al. Molecular characterisation of ERG, ETV1 and PTEN gene loci identifies patients at low and high risk of death from prostate cancer. Br J Cancer. 2010;102(4):678–84.
51. Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, et al. PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. Clin Cancer Res. 2011;17(20):6563–73.
52. Cuzick J, Berney DM, Fisher G, Mesher D, Moller H, Reid JE, et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. Br J Cancer. 2012;106(6):1095–9.
53. Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. Eur Urol. 2014;66(3):550–60.
54. Erho N, Crisan A, Vergara IA, Mitra AP, Ghadessi M, Buerki C, et al. Discovery and validation of a prostate cancer genomic classifier that predicts early metastasis following radical prostatectomy. PLoS One. 2013;8(6):e66855.
55. Kornberg Z, Cooperberg MR, Cowan JE, Chan JM, Shinohara K, Simko JP, et al. A 17-gene genomic prostate score as a predictor of adverse pathology in men on active surveillance. J Urol. 2019;202(4):702–9.
56. Freedland SJ, Gerber L, Reid J, Welbourn W, Tikishvili E, Park J, et al. Prognostic utility of cell cycle progression score in men with prostate cancer after primary external beam radiation therapy. Int J Radiat Oncol Biol Phys. 2013;86(5):848–53.
57. Ross AE, Johnson MH, Yousefi K, Davicioni E, Netto GJ, Marchionni L, et al. Tissue-based genomics augments post-prostatectomy risk stratification in a natural history cohort of intermediate- and high-risk men. Eur Urol. 2016;69(1):157–65.
58. Al-Obaidy KI, Chovanec M, Cheng L. Molecular characteristics of testicular germ cell tumors: pathogenesis and mechanisms of therapy resistance. Expert Rev Anticancer Ther. 2020;20(2):75–9.
59. McIntyre A, Summersgill B, Grygalewicz B, Gillis AJ, Stoop J, van Gurp RJ, et al. Amplification and overexpression of the KIT gene is associated with progression in the seminoma subtype of testicular germ cell tumors of adolescents and adults. Cancer Res. 2005;65(18):8085–9.
60. Kemmer K, Corless CL, Fletcher JA, McGreevey L, Haley A, Griffith D, et al. KIT mutations are common in testicular seminomas. Am J Pathol. 2004;164(1):305–13.
61. Litchfield K, Summersgill B, Yost S, Sultana R, Labreche K, Dudakia D, et al. Whole-exome sequencing reveals the mutational spectrum of testicular germ cell tumours. Nat Commun. 2015;6:5973.
62. Cheng L, Lyu B, Roth LM. Perspectives on testicular germ cell neoplasms. Hum Pathol. 2017;59:10–25.
63. Al-Obaidy KI, Eble JN, Cheng L, Williamson SR, Sakr WA, Gupta N, et al. Papillary renal neoplasm with reverse polarity: a morphologic, immunohistochemical, and molecular study. Am J Surg Pathol. 2019;43(8):1099–111.
64. Al-Obaidy KI, Eble JN, Nassiri M, Cheng L, Eldomery MK, Williamson SR, et al. Recurrent KRAS mutations in papillary renal neoplasm with reverse polarity. Mod Pathol. 2020;33(6):1157–64.
65. Waterhouse RL Jr, Van Neste L, Moses KA, Barnswell C, Silberstein JL, Jalkut M, et al. Evaluation of an epigenetic assay for predicting repeat prostate biopsy outcome in African American men. Urology. 2019;128:62–5.
66. Van Neste L, Groskopf J, Grizzle WE, Adams GW, DeGuenther MS, Kolettis PN, et al. Epigenetic risk score improves prostate cancer risk assessment. Prostate. 2017;77(12):1259–64.