Molecular Pathology

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Mutational Frame

Development and progression of bladder UC occur mainly through genomic modifications affecting almost all chromosomes. All types of genetic changes that include aneusomies, epigenetic alterations, activating or silencing mutations, amplifications, and deletions are commonly seen in this disease [1, 2].

Numerical Chromosomal Alterations

The most frequently detected copy number aberrations in UC are on chromosomes 1, 8, 9, 10, 11, 13, and 14 [3]. These changes offer the necessary setting of genetic instability that in turn allows for the accumulation of succeeding genetic defects. Although most non-muscle invasive bladder cancer (NMIBC) are diploid or near diploid, loss of specific regions is common and associated with higher recurrence [4–6]. A study comparing genetic deviations between Ta and T1 tumors has found that losses of 9q (54%), 9p (39%), and Y (28%) and gain of 1q (14%) were more prevalent in Ta tumors, whereas deletions at 2q (36%), 8p (32%), and 11p (21%) and gains at 1q (54%), 8q (32%), 3p, 3q, 5p, 6p, and 10p (18% each) were more common in T1 neoplasia [6]. Notably, loss of 9q has also been shown in normal surface epithelium adjacent to tumor. Loss of 9q appears to be an early marker of local genomic instability and may act in the initiation of bladder cancer [5, 7]. NOTCH1 and TSC1 are the candidate tumor suppressor genes on chromosome 9q that may factor in the cancer pathogenesis. Gains of chromosomes 3q, 7p, and 17q and 9p21 deletions (p16 locus) are of special note which give them potential diagnostic and prognostic significance [8] (see Urovysion below).

Mutations

Mutations in bladder cancer (BC) mainly involve the genes responsible for neoplastic transformation, signal transduction, cell cycle regulation, DNA damage repair, transcription, and chromatin remodeling. Overall mutation rates in muscle invasive bladder carcinoma (MIBC) are very high (mean 8.2 and median 5.8 per megabase in coding regions according to The Cancer Genome Atlas (TCGA) data, only slightly fewer than lung cancer and melanoma [9, 10]). Recurrent genetic alterations include mutations in the coding region of many genes such as *FGFR3*, *PIK3CA*, *KDM6A*, *STAG2*, and *TP53* [10, 11] as well as in numerous non-coding regions such as *TERT*, *PLEKHS1*, *WDR74*, *TBC1D1*, *LEPROTL1*, and *GPR126* [12, 13].

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H. Zhou et al. (eds.), *Urinary Bladder Pathology*, https://doi.org/10.1007/978-3-030-71509-0_14

High mutation load in invasive UC is mainly thought to be driven by the APOBEC (apolipoprotein B mRNA editing enzyme catalytic polypeptide-like) mutagenesis. APOBEC is a member of the evolutionary conserved family of cytidine deaminases that are involved in the intrinsic response to infection, modification, and clearance of viral DNA. TCGA Bladder Cancer Group has shown that the somatic mutations in UC are dominated by a C:G → T:A [9]. This is characteristic of mutations caused by the APOBEC family [14]. APOBEC-a and APOBEC-b mutation signatures account for 67% of all single nucleotide variants (SNVs) within MIBC. A second frequent mutational signature is associated with ERCC2 mutations and thought to be responsible for ~20% of all SNVs. ERCC2 encodes a DNA helicase that has a central role in the nucleotide excision DNA repair pathway. A third signature in the TCGA analysis is likely related to 5-methylcytosine deamination and has been associated with 8% of SNVs.

The most frequently mutated gene in the bladder cancer is the *TERT* (telomerase reverse transcriptase) promoter [13, 15, 16]. *TERT* encodes the catalytic subunit of the telomerase complex which is upregulated in the majority of cancers and is essential for vanquishing senescence and apoptosis by maintaining the 3′ telomere length at the ends of chromosomes [17]. Somatic *TERT* promoter mutations occur early in the process of bladder carcinogenesis [16, 18, 19]. Mutations generate consensus binding motifs for ETS transcription factors, increasing TERT expression and activity. Given that telomere shortening acts as a mitotic clock, the activation of telomerase elongates telomeres at the ends of chromosomes, which is essential for the continued growth of cancer cells [20].

Activating mutations of *FGFR3*, a gene located at chromosome 4p16.3, are common in bladder UC, particularly in the subset of low-grade and low-stage tumors, where their frequency reaches up to 70–80% [18]. They map to three mutation hotspots in exons 7 (codons 248 and 249), 10 (codons 372, 373, 375, 382, and 393), and 15 (codon 653) [21].

One of the most frequently mutated gene is *TP53* in muscle-invasive UCs and has been detected in nearly 50% of the cases [9]. Mouse double minute 2 homolog (*MDM2*) is another gene functioning in cell cycle regulation. *MDM2* amplification and overexpression are seen in 7% of UCs and mutually exclusive with *TP53* mutation. *RB1* mutation is a frequent accompaniment of *TP53* mutation, is observed in 17% of cases, and is mutually exclusive with *CDKN2A* deletion.

Mixed-lineage leukemia 2 (*MLL2*) gene belongs to the group of chromatin remodeling genes involved in epigenetic regulation. It is another frequently mutated gene and found in around 28% of UCs. Other frequently mutated genes include lysine (K)-specific methyltransferase 2C (*KMT2C*), ataxia telangiectasia mutation (*ATM*), FAT atypical cadherin 1 (*FAT1*), CREB-binding protein (*CREBBP*), *ERBB2*, spectrin alpha non-erythrocytic 1 (*SPTAN1*), and lysine (K)-specific methyltransferase 2A (*KMT2A*).

The recurrent gene fusions are rarely observed in UC [10]. Less than 5% of bladder cancers harbor *FGFR3-TACC3* (transforming acidic coiled-coil containing protein 3) fusions and even less frequently *TSEN2* (tRNA splicing endonuclease subunit 2)-*PPARG* (peroxisome proliferator-activated receptor gamma) and *MKRN2* (makorin ring finger protein 2)-*PPARG* translocations [22].

Epigenetic Alterations

Aberrant DNA methylation and histone modification play a role in regulating gene expression and may contribute to carcinogenesis. Several groups have documented that hypermethylation of *RARB*, *RASSF1*, and *DAPK* is linked to aggressiveness in UC [23].

Chromatin-modifying genes (CMGs) are the regulators of gene expression and commonly mutated in the malignancies [10, 24]. It was found that the two most commonly mutated CMGs in NMIBC were *KDM6A* (38%) and *ARID1A* (28%) [25]. *KDM6A* mutation frequency is 52% in low grade (LG) Ta, 38% in high grade (HG) Ta, 32% in HGT1, and 24% in

MIBC, whereas *ARID1A* mutation frequency is 9% in LGTa, 28% in HGTa, 18% in HGT1, and 24% in MIBC cases. Frequency of *KDM6A* mutations was found elevated in the female patients with Ta tumors (72%) compared to men (42%). *ARID1A* has been associated with increased risk of recurrence, which may be linked to increased aggressiveness or BCG resistance [25].

Molecular Pathways

Bladder UC is believed to develop via a field effect that involves multiple sites in the mucosa, leading to multifocal and metachronous tumorigenesis [18, 26]. Urothelial cells in the affected field gain additional genetic alterations and become malignant by clonal evolution.

UC develops along two oncogenic tracks: papillary (~80% of bladder cancers) and nonpapillary (~20% of bladder cancers), with some overlapping molecular profile (Fig. 14.1). Low-grade (LG) papillary tumors are superficial, and they arise from premalignant lesions referred to as urothelial dysplasia (low-grade intraurothelial neoplasia), whereas nonpapillary lesions are generally high grade (HG) and develop from urothelial dysplasia that progresses to carcinoma in situ (high-grade intraurothelial neoplasia). Low-grade papillary UCs have high propensity for recurrence after transurethral resections, but they usually do not penetrate the basement membrane of surface epithelium to invade the bladder wall. On the other hand, urothelial CIS is notorious for frequent transformation to invasive and metastatic cancer. It is also known that some of the low-grade papillary tumors (~10 to 15%) may progress to the noninvasive high-grade papillary UC and subsequently invasive UC. The MIBC cohort in The Cancer Genome Atlas (TCGA) study has demonstrated the mutual exclusiveness of alterations between *CDKN2A* and *TP53*, *CDKN2A* and *RB1*, *TP53* and *MDM2*, and *FGFR3* and *RB1* gene pairs. Similar analyses showed the co-occurrence of mutations in the *TP53* and *RB1* genes and in the *FGFR3* and *CDKN2A* genes [10]. It has now been widely accepted that the Ras pathway is a major driver of the papillary track, whereas the p53/RB1 and PTEN-related pathways contribute to the aggressive and invasive phenotype [2, 18, 27, 28]. Most CIS lesions gain TP53 mutations early in evolution and do not acquire FGFR3 mutations [29]. On the other hand, some low-grade papillary

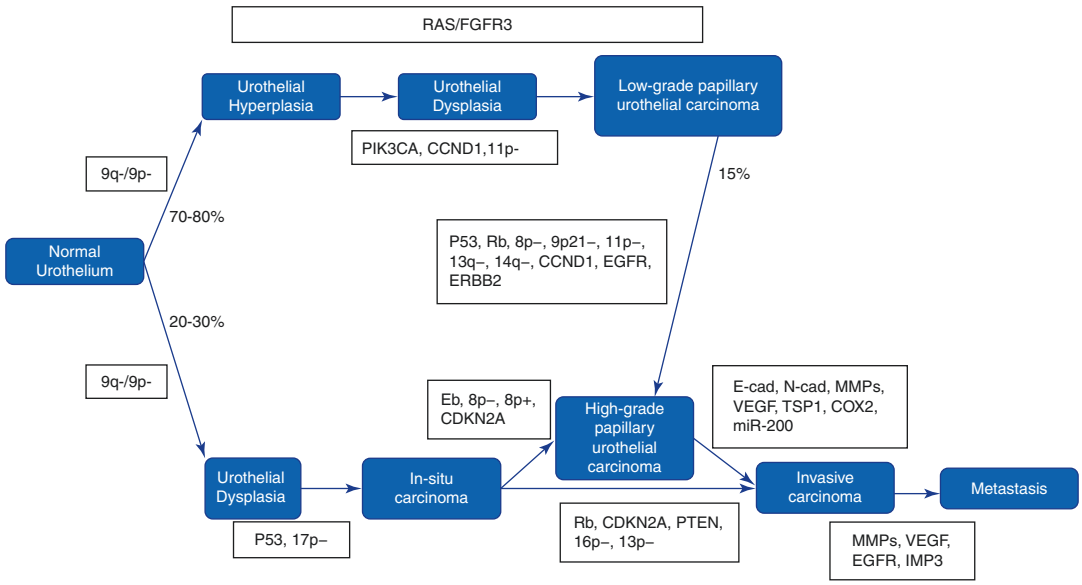

Fig. 14.1 Molecular pathways of urothelial carcinoma

tumors with *FGFR3* mutation may acquire additional mutations of the *TP53* gene and chromosomal losses of 9p21 (the locus that includes *CDKN2A*) and may progress to high-grade and invasive carcinoma [29–31].

Low-Grade Tumors

FGFR3/RAS Pathway: The FGFR3/RAS pathway is active mainly in low-grade noninvasive papillary UC. FGFR3 signals through Ras (RAS-MAPK-ERK pathway) and regulates cell cycle entry and proliferation. The most common *FGFR3* mutations facilitate ligand-independent receptor dimerization, leading to transphosphorylation and downstream signaling. Activating point mutation in *FGFR3* is most common in Ta tumors (~80%), with decreased frequency in high-grade Ta (59%), T1 (10–34%), and MIBC (10–20%) [25, 32, 33]. *FGFR3* mutations have been associated with a higher risk of recurrence in noninvasive papillary bladder cancer and favorable clinical outcomes in pT1 tumors [18, 27, 34, 35]. Approximately 10% of low-grade bladder carcinomas harbor mutations in *RAS* genes (*HRAS*, *KRAS*, or *NRAS)* [36] which do not co-occur with *FGFR3* mutations [37]. *FGFR3* fusion proteins are also implicated in bladder cancer pathogenesis, with in-frame *FGFR3-TACC3* fusions being the most common [10]. TACC3 is upstream of FGFR3 signaling, and fusion protein causes constitutive activation of the MAPK-ERK pathway [38]. *FGFR3-TACC3* fusions appear more commonly associated with MIBC.

High-Grade Tumors

TP53/RB1 Pathway: The TP53/RB1 pathway is an important regulator of cell cycle progression and plays an important role in the development of aggressive UCs [18, 39]. The mutation or deletion of *TP53* has been observed predominantly in CIS and MIBC. According to TCGA cohort data [10], 89% of MIBCs have an inactivated TP53 cell cycle pathway, with *TP53* mutations in 48%, *MDM2* amplification in 6%, and *MDM2* overexpression in 19% of cases. Seventeen percent of MIBCs harbor *RB1* mutations often with concurrent *TP53* mutations [40]. *CDKN2A* (*p16*), which functions as a negative regulator of the RB1 pathway, is found to be mutated (7%) or deleted (22%).

Evidence suggests LG noninvasive papillary UC, which classically has a high frequency of *FGFR3* mutation, progresses to high-grade and invasive carcinoma through mutations in *TP53* and chromosomal losses of 9p21, the locus that includes *CDKN2A* [30, 31]. In contrast, most CIS lesions develop *TP53* mutations early and do not acquire *FGFR3* mutations [29].

PIK3/AKT/MTOR Pathway: In vitro studies show that the ablation of p53 in a background of mutant Ras induces superficial papillary tumors but is insufficient to trigger cancer invasion, suggesting that additional complex genetic events are needed to induce a thoroughly aggressive and invasive phenotype [41]. Up to 40% of bladder UCs show the activation of the phosphoinositide 3-kinase/protein kinase B (or AKT)/mechanistic target of rapamycin (PI3K/AKT/mTOR) pathway. The PIK3/AKT/MTOR pathway regulates important steps in tumorigenesis and tumor progression. This pathway is activated by receptor tyrosine kinases including ERBB2, ERBB3, and FGFR3. The upstream pathway activator *ERBB2* encodes human epidermal growth factor receptor 2 (Her2), which is mitogenic for cell growth. It is amplified, mutated, or overexpressed in 12% of MIBCs or a subset of high-grade NMIBC cases [9, 25, 28]. When present in NMIBC, *ERBB2* amplification is associated with high risk of progression and concomitant CIS [42–44]. *ERBB2* mutations are commonly found in the extracellular domain and are likely reflect APOBEC mutational signature [10]. *PIK3CA* (cancer-associated phosphatidylinositol 3-kinase) encodes the catalytic subunit of PI3K, and its mutations are seen more frequently in NMIBC than in MIBC (Ta, 40–50%; T1, 6–20%; MIBC, 22%) [10, 13, 45]. They are more commonly located in the helical domain than in the kinase domain, likely due to the mutagenic activity of APOBEC. *PIK3CA* mutations appear to be associated with a favorable

outcome in patients who undergo radical cystectomy [46]. The reduced expression of phosphatase and tensin homolog (PTEN) is a negative regulator of the PIK3/AKT/MTOR pathway. Inactivating deletions or mutations of the *PTEN* gene has been observed in many MIBC cases. Loss of PTEN was significantly associated with non-papillary, high-grade and invasive tumors, supporting that the involvement of the PI3K/AKT/mTOR pathway might be a potential driver of an invasive phenotype. *AKT1* and *TSC1* are other tumor suppressor genes and negative regulators of this pathway. However, they are not as frequently mutated [18].

Urothelial Proliferation of Unknown Malignant Potential (Urothelial Hyperplasia) and Dysplasia

The deletion of chromosome 9 is prevalent in urothelial hyperplasia and dysplasia [40, 47, 48], suggesting that this deletion occurs in the early stage of bladder cancer. In one study, chromosome 9 deletions were detected in 37% of cases of flat urothelial hyperplasia with or without associated papillary lesions, in addition to chromosome 8 deletions in 10% and *FGFR3* mutations in 23% of the cases [49]. The *FGFR3*/*HRAS* mutations are frequently found during the development of urothelial hyperplasia [2, 27, 28, 50, 51]. FGFR3/RAS pathway enables tumors to progress from urothelial hyperplasia to noninvasive papillary tumors with high recurrence rates. Expression of ectopic mutant FGFR3 in normal urothelial cells has been shown to induce aberrant activation of the MAPK and PLCg1 signaling pathways and increase cell proliferation [21]. In animal models the activating mutations of the Ras gene caused the development of urothelial dysplasia and low-grade superficial papillary UC [50, 52]. The dose of activated Ras was related to phenotypic change. A low copy number of mutant Ras induced urothelial dysplasia, whereas a high copy number led to the development of low-grade superficial papillary tumors.

Tumor Progression

Approximately, 15–20% of patients with NMIBC progress to muscle invasive disease [53] which is referred to as secondary MIBC. Two of the candidate genes proposed in tumor progression are E2F1 and CDKN2A. E2F1 is a regulator of cellular apoptosis that has been linked to tumor invasion and metastasis in various cancer types [53, 54]. Upregulation of E2F1 and its downstream targets, EZH2 and SUZ12, have been shown in patients with NMIBC progressing to muscle-invasive disease [55]. CDK2NA is a cell cycle regulator involved in G1-S arrest. CDKN2A is lost in the invasive portion of NMIBCs, and only tumors with progression lose both TP53 and CDK2NA [56].

Urothelial Papilloma (UP)

Results of molecular studies in UPs are variable. Rates of reported TERT promoter mutations vary from 46% to 0% [57, 58]. Similarly range of FGFR3 mutations varies from 75% [59] to 0% [58]. In a recent study, 10 of 11 UPs had oncogenic mutations in the RAS/ERK signaling pathway (seven *KRAS*, one *HRAS*, one *KRAS* plus *HRAS* and one *BRAF* mutations) [58]. Only one case harbored oncogenic *FGFR3* or *TERT* promoter mutations. This lesion was likely a recurrent carcinoma despite papilloma histology as the tumor also had oncogenic *PIK3CA*, *KMT2D*, and *CDKN1A* mutations and arose in a patient who had history of several low-grade noninvasive papillary urothelial carcinomas, prior and subsequent to UP.

Inverted Urothelial Papilloma (IUP)

There is variability in the reported results of molecular studies in inverted papillomas. Some groups report *FGFR3* mutations in 9.8–45% (a mean of 18%) of inverted papillomas [60, 61], but others have found no change in *FGFR3* gene [62]. Similarly, some tumors have been reported to harbor 9p deletions (in 3.9% of cases), 9q

deletions (in 13.2%), and 17p deletions (in 51%) [60]. The most common molecular alterations in IUP appear in the MAP kinase/ERK pathway, *HRAS* and *KRAS* mutations being predominant. Recurrent *HRAS* mutations (Q61R) have been reported in 60% to over 90% of cases [57, 62].

TERT promoter mutations are rare in inverted urothelial papilloma, with most studies showing inverted papillomas lack these mutations [57, 60–63]. This information and the benign behavior and frequent mutations in the MAP kinase/ERK pathway in these lesions have been taken as evidence that IUPs are a distinct type of indolent low-grade urothelial neoplasia that does not progress to carcinoma [64].

Urothelial Carcinoma with Variant Histology

Urothelial Carcinoma with Divergent Differentiation

The literature on the molecular characteristics of divergent (glandular and/or squamous) differentiation in UC is scant, but it is very likely that there is overlap with those of UC, particularly in the presence of high rates of TERT promoter mutations [65, 66].

Plasmacytoid Urothelial Carcinoma (PUC)

PUCs are characterized by loss of E-cadherin expression similar to lobular or diffuse carcinomas of the breast and stomach. Somatic *CDH1* truncating mutations are mostly responsible from E-cadherin loss as they have been identified in 84% of PUC; *CDH1* promoter hypermethylation occurs less frequently [67]. Aside from *CDH1* alterations, the genomic landscape of PUC is generally similar to that of coexistent conventional UC, suggesting that both histologic subtypes potentially evolve from a common cell of origin [67, 68]. No germline *CDH1* mutations have been reported in PUC.

Micropapillary Urothelial Carcinoma (MPUC)

Genomic expression profile of micropapillary cancer reveals that more than 6000 genes are aberrantly expressed when compared to conventional UC [69]. The micropapillary expression signature is also present in conventional UC component accompanying MPUC, suggesting that micropapillary variant arises from a unique subset of conventional UCs.

Consistently higher rates of *ERBB2* amplification have been reported in MPUC than in conventional UC [70]. *ERBB2* amplification is associated with a worse outcome following radical cystectomy in some series [71]. A study has shown that *ERBB2* amplification is more commonly identified in the micropapillary variant than conventional UC when both components are present [72] although the rate of *ERBB2* amplification in the conventional urothelial component in these mixed (micropapillary + conventional urothelial) tumors is much higher than the reported rates in UC not containing micropapillary component [10, 73, 74].

It has been reported that in MPUC there is common downregulation of miR-296 and activation of chromatin-remodeling complex RUVBL1, with overexpression of its downstream target genes such as lysine-specific demethylase 4B (*KDM4B*), insulin-like growth factor-binding protein 3 (*IGFBP3*), and disintegrin and metalloproteinase domain-containing protein 15 (*ADAM15*) [75, 76]. These are known to be involved in cell growth, DNA damage repair, and metastasis.

Sarcomatoid Urothelial Carcinoma

The sarcomatous and urothelial components within the same tumor share common clonal origin. More recently, it has been shown that sarcomatoid UC is enriched with mutations *in TP53*, *RB1*, and *PIK3CA* and is associated with overexpression of epithelial-mesenchymal transition markers [77–80].

Nested Variant of Urothelial Carcinoma

Up to now, only a few molecular findings have been reported related to this tumor type, the most common being the high rate of *TERT* promoter mutations as well as occasional mutations in *TP53*, *JAK3*, and *CTNNB1*. These findings suggest that this UC subtype harbors molecular alterations similar to those of UC in general [81, 82]. Documentation of *TERT* promoter mutation can be beneficial in difficult cases such as small biopsies as it is not found in benign mimickers of nested UC.

Small Cell/Neuroendocrine Carcinoma of the Bladder (SmCC)

One of the most common findings in SmCC is the near ubiquitous presence of loss-of-function co-alterations of *TP53* and *RB1*. One study reported mutations of *TP53* and *RB1* in 90% and 87% of cases, respectively (80% of tumors displaying co-alterations of both) [83]. Even in tumors with no loss-of-function mutations in *RB1* gene, RB protein expression was lost immunohistochemically, suggesting an alternative mechanism for RB suppression, such as epigenetic silencing.

Small cell carcinoma has a high somatic mutational burden driven predominantly by an APOBEC-mediated mutational process [84]. Genes that are commonly mutated in UC are also found mutated in bladder SmCC, including *TERT* promoter mutations (95%) and truncating alterations in genes involved in chromatin modification such as *CREBBP*, *EP300*, *ARID1A*, and *KMT2D* in ~75% of cases [83, 84]. Unlike UC, there is near absence of *KDM6A* truncating mutations, *CDKN2A* deletion, and *CCND1* amplifications in SmCC [83]. SmCC is associated with a high level of chromosomal instability, and whole genome duplication is seen in 72% of tumors. RNA sequencing reveals novel fusion transcripts, including an in-frame Pvt1 oncogene (*PVT1*)-*ERBB2* fusion, which is associated with aberrant *ERBB2* expression.

Studies investigating the clonal connection between the small cell and urothelial components within the same tumor have shown that there are shared changes between the two components as well as different alterations in each component. These findings further support the common clonal origin for SmCC and coexisting conventional UC [83].

Micro-RNA (miRNA)

Over 200 miRNAs or miRNA families/clusters are aberrantly expressed in UC [85]. The downregulated miRNAs may serve as tumor suppressors. miR-145 appears to be the most frequently downregulated miRNA in bladder cancer. The upregulated miRNAs may contribute to tumor progression. miR-21 has been shown to be upregulated in the tissues, plasma, and urinary exosomes of patients with bladder carcinoma, but its role in UC still needs further investigation. Circulating miRNAs in body fluids, especially in urine, constitute an important cancer signature and carry the potential to be the useful molecular markers for diagnosis, prognosis, classification, and recurrence of UC. miR-146a-5p is frequently overexpressed in the urine of UC patients, which indicates its potential as a novel biomarker for the rapid and early diagnosis.

Inheritance

Upper tract UC is a characteristic tumor of Lynch syndrome (an autosomal dominant disorder caused by a defect in a DNA mismatch repair (MMR) gene). Invasive upper tract UCs are MSI-high/MMR-deficient in ~20% of cases [86]. Emerging evidence suggests an increased (but smaller) risk of urothelial neoplasia in the bladder as well [87]. The 10-year risk for urothelial cancer in patients already diagnosed with Lynch syndrome is 2%. The patients with Lynch syndrome seem to develop urothelial tumors mainly when MSH2 is affected by a germline mutation [88].

Bladder cancer has been reported in patients with hereditary retinoblastoma, possibly related to radiation and/or cyclophosphamide therapy. Bladder cancer can be a component of Costello syndrome. Patients with this syndrome have been reported to develop papillary UC during childhood [87].

Molecular Biomarkers for Tumor Detection and Surveillance

Analysis of desquamated urothelial cells in urine is a valuable source for noninvasive detection of bladder cancer. Urine cytology is an important tool in this respect for both diagnosis and follow-up of UC. However, its overall low sensitivity, especially in low-grade tumors, limits its utility. By the help of accumulating data about pathogenesis and molecular background of urothelial neoplasia, several urine-based noninvasive assays have now become available for early detection and surveillance of the disease with higher sensitivity and specificity.

- The Urovysion assay: This test is multitarget, multicolor fluorescence in situ hybridization (FISH) assay and explores four common chromosomal alterations (aneuploidy of chromosomes 3, 7, and 17 and losses in 9p21) in high-grade UC cells shed to the urine [89]. It was reported that almost all invasive tumors including pT1 as well as a large fraction of the noninvasive bladder tumors were identified by this assay. Most studies also claim that adding this test to standard urine cytology increases sensitivity for detecting recurrence [90].

- Mutation detection assays: Urine-based mutational tests performed on cellular DNA have higher sensitivity than urine cytology and can detect low-grade tumor, an advantage over FISH. They mainly evaluate the genes altered in bladder cancer, such as *TERT* promoter and *FGFR3*, with focus on mutational hotspots [91, 92]. The noninvasive test may be useful for monitoring patients and triage cystoscopy. A positive result may serve as a warning of future recurrence if the subsequent cystoscopy is unable to show a tumor. Mutations in the *TERT* promoter occur early and are very common in UC regardless of grade, stage, and morphologic variants including papillary urothelial neoplasm of low malignant potential [93, 94]. *TERT* promotor mutations do not occur in reactive urothelial proliferations. Thus, they also have great diagnostic utility in distinguishing UC from its benign mimics. *FGFR3* mutations in the cell-free DNA obtained from blood were identified in 68% of patients with advanced or metastatic UC in one study [95].

- UroSEEK: This is a urine-based molecular assay recently developed for the detection and surveillance of urothelial neoplasms [96]. It is designed to detect alterations in 11 genes (*TERT*, *FGFR3*, *PIK3CA*, *TP53*, *HRAS*, *KRAS*, *ERBB2*, *CDKN2A*, *MET*, *MLL*, and *VHL*) commonly mutated in bladder cancer and copy number changes on 39 chromosome arms. Combined with cytology, the test detects 95% of bladder UC, 75% of upper tract UC, and 68% of recurrent bladder carcinoma. The advantage of the assay over cytology is more evident in low-grade tumors as UroSEEK detects 67% of these cases whereas cytology does none.

Molecular Markers for Treatment

The potential therapeutic molecular targets have been identified overall in 70% of the bladder cancers; however, none of them has been integrated into clinical practice, waiting for the results of ongoing studies and clinical trials.

***FGFR Inhibitors*:** A very high proportion of bladder tumors are characterized by *FGFR3* dysregulation. Activating point mutations of *FGFR3* are found in up to 80% of low-grade and low-stage UC of the bladder. Upregulated expression of FGFR3 protein is also found in a significant number of tumors which lack point mutations and are predominantly muscle invasive [21]. Thus, *FGFR3* may be an important therapeutic target in both noninvasive and invasive UC. Several studies have shown in preclinical models that silencing or inhibition of *FGFR3* has a profound inhibitory effect on some UC cells leading to decreased proliferation, reduced

anchorage-independent growth, and enhanced apoptosis [97–99]. Therefore, FGFR inhibitors have been proposed as novel therapeutic agents in the treatment of bladder tumors [100], and clinical trials of such agents have been initiated. In a phase II trial of erdafitinib (an FGFR inhibitor) for metastatic UC with *FGFR3* alterations, the overall response rate was 40% [101]. The study of BGJ398 and erdafitinib showed significant clinical activity in patients with refractory metastatic cancers whose tumors contained activating *FGFR3* mutations or fusions, which led to the recent US Food and Drug Administration (FDA) approval of erdafitinib. The US FDA also approved a companion diagnostic test for FGFR3 mutations and fusions. Given the high frequency of *FGFR3* mutation in NMIBC, *FGFR3* may be a rational target in NMIBC as well.

DNA Damage Response (DDR) Gene Alterations and Treatment: *ERCC2* is among the DDR-related genes, and its alterations are detected in 10–15% of MIBCs. Mutations in *ERCC2* and other genes involved in DNA damage response and repair have recently been shown to be associated with improved response not only to cisplatin-based chemotherapy but also to immune checkpoint blockade and radiation therapy for advanced UC [102–104]. Forty percent of responders to neoadjuvant chemotherapy (NAC) have been seen to have nonsynonymous *ERCC2* gene alteration versus 7% in non-responders [105]. Other DDR genes such as *ATM*, *RB1*, and *FANCC* appear as potential biomarkers for response to NAC as well [106, 107].

Mammalian Target of Rapamycin (mTOR) Inhibitors: The potential therapeutic vulnerabilities also include the targets in the PI-3 kinase/AKT/mTOR and in the receptor tyrosine kinase (RTK)/mitogen-activated protein kinase (MAPK) pathways. Patients with mutations that activate mTOR pathway may benefit from mTOR inhibitors. *TSC1* is the negative regulator of mTOR, and its loss may be associated with increased cell growth and survival in high-risk NMIBC [4]. mTOR inhibitors may be an effective therapy to prevent recurrence of tumors with *TSC1* loss.

***Other Potential Targets*:** Urothelial carcinoma with carcinogenesis by *EGFR*, *ERBB2*, *ERBB3*, *PIK3CA*, or *RAS* alterations may benefit from targeted therapy. Chromatin regulatory genes have been found more frequently mutated in UC than other common cancers, further supporting additional therapeutic options [10]. Long non-coding RNAs (lncRNAs) are long RNA transcripts greater than 200 nucleotides in length that do not code for any proteins. The lncRNA urothelial cancer-associated 1 (UCA1) has been associated with cisplatin chemotherapy resistance through activation of Wnt signaling [108].

Bacillus Calmette-Guérin (BCG) Responsiveness

Certain glutathione pathway genomic variations and immune system gene single nucleotide polymorphisms reveal potential to predict recurrence and progression-free survival after BCG therapy [109–111]. *IL-8* (−251 T > A) polymorphism has been associated with an increased recurrence-free survival (RFS) in BCG-treated patients [112]. Gene polymorphisms in *XPA*, *XPC*, *XPD*, *XPG*, *XPF*, *ERCC1*, *ERCC2*, *XRCC1*, *XRCC4*, *APEX1*, *GSTM1*, *CCNB1*, *PON1*, and *SLCO1B* have been linked to reduced RFS or increased recurrence risk after BCG treatment. High tumor mutation burden and loss of *CDK2NA* may predict progression to MIBC in high-risk NMIBC treated with BCG [56]. *ARID1A* mutation has been associated with increasing stage and aggressiveness and may serve as a predictive biomarker of resistance in patients undergoing BCG therapy or a potential therapeutic target to enhance BCG response [25].

Conclusions

The discovery of the molecular changes and pathways involved in bladder cancer is fundamental to understand its biological heterogeneity. Analysis of specific alterations can be used to plan targeted therapies, and predict clinical outcomes and responsiveness to personalized therapies.

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