Bladder Cancer in the Genomic Era

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- **Context.**—Bladder cancer is a heterogeneous disease that exhibits a wide spectrum of clinical and pathologic features. The classification of bladder cancer has been traditionally based on morphologic assessment with the aid of immunohistochemistry. However, recent genomic studies have revealed that distinct alterations of DNA and RNA in bladder cancer may underlie its diverse clinicopathologic features, leading to a novel molecular classification of this common human cancer.

- **Objective.**—To update recent developments in genomic characterization of bladder cancer, which may shed insights on the molecular mechanisms underlying the origin of bladder cancer, dual-track oncogenic pathways, intrinsic molecular subtyping, and development of histologic variants.

Bladder cancer is a common malignancy in the United States and is estimated to affect 81,900 new patients and to cause 17,240 deaths in 2018. Among a wide variety of neoplasms that affects the bladder, urothelial carcinoma (UC) is the most common disease, accounting for approximately 90% of all bladder neoplastic disorders. Urothelial carcinoma develops in the stratified epithelial layer called urothelium of the urinary system along 2 tracks referred to as papillary and nonpapillary, leading to different but somewhat overlapping subsets of the disease with distinct molecular profile and challenges for clinical management. During the past decade several groups including The Cancer Genome Atlas (TCGA) Network performed genomic studies of bladder cancer by using a multiparameter approach, which provided detailed characterization of genetic and epigenetic alterations of the disease. These breakthroughs have transformed our view of bladder cancer biology, generated new hypotheses for therapy, provided the first opportunity to understand chemoresistance, and identified new targets for chemoresistant tumors. The identification of mutational landscape and intrinsic molecular subtypes that can be used for prognostication and therapy response prediction necessitates new diagnostic approaches and implementations of molecular profiles into pathologic diagnosis.

- **Data Sources.**—Peer-reviewed literature retrieved from PubMed search and authors’ own research.

- **Conclusions.**—Bladder cancer is likely to arise from different uroprogenitor cells through papillary/luminal and nonpapillary/basal tracks. The intrinsic molecular subtypes of bladder cancer referred to as luminal and basal exhibit distinct expression signatures, clinicopathologic features, and sensitivities to standard chemotherapy. Genomic characterization of bladder cancer provides new insights to understanding the biological nature of this complex disease, which may lead to more effective treatment.

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DUAL-TRACK CONCEPT OF BLADDER CARCINOGENESIS

The whole-organ histologic analyses of cystectomy specimens in correlation with clinicopathologic features have provided strong evidence that the papillary and nonpapillary forms of bladder cancers develop from distinct diffuse mucosal precursor conditions (Figure 1, A through F). Approximately 80% of bladder cancers are superficial papillary tumors that arise from diffuse in situ premalignant lesions referred to as low-grade intraurothelial neoplasia and also sometimes referred to as urothelial dysplasia. Papillary UCs are characterized by exophytic, fingerlike protrusions on the mucosal surface. These tumors often recur after local excision, but they usually do not invade the bladder wall or metastasize. The remaining bladder cancers (about 20%) present as invasive UCs, which develop from flat in situ precursor lesions, referred to as high-grade intraurothelial neoplasia or UC in situ. These tumors have a high propensity to invade the bladder wall and frequently give rise to distant metastasis. Approximately 10% to 15% of low-grade superficial papillary tumors eventually progress to high-grade invasive carcinomas. Interestingly, the progression is often preceded by the development of UC in situ within a papillary lesion or in the adjacent urothelial mucosa.

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The dual-track pathogenetic concept of bladder cancer is supported by molecular studies and animal models.11–14 In animal models the activating mutations of the Ras gene caused the development of urothelial dysplasia and low-grade superficial papillary urothelial carcinoma (UC). In the nonpapillary pathway, clonal evolution results in the establishment of a successor clone with microscopic features of high-grade intraurothelial neoplasia (HGJIN), which often shows a loss of major tumor suppressors such as retinoblastoma gene (RB) and has a high propensity for progression to an invasive high-grade nonpapillary UC. A, Normal urothelium expresses Ki-67 in proliferating basal cells and RB protein in parabasal cells. B, LGIN expresses Ki-67 and RB protein in the entire thickness. C, Low-grade papillary UC retains normal expression of the RB protein; insets to (C) show low- and high-power photomicrographs illustrating the expression of normal RB protein. D, HGJIN or UC in situ shows loss of RB protein expression. E, High-grade invasive UC shows loss of RB protein expression. Arrow shows expression of RB protein in endothelial cells adjacent to tumor, which serves as an internal positive control. F, HGJIN develops in bladder mucosa adjacent to a low-grade papillary UC. It is responsible for switching the pathway and progression of some low-grade papillary UCs to high-grade invasive UCs. Abbreviations: CIS, carcinoma in situ; FR, forerunner gene; LOH, loss of heterozygosity (hematoxylin-eosin, original magnifications ×100 [A, B, D, E, and F] and ×40 [C]; immunohistochemical stains, original magnifications ×100 [A, B, D, E, and F], ×40 [inset C, left], and ×200 [inset C, right]). Modified and reprinted with permission from Majewski et al.40 Lab Invest. 2008;88(7):694–721.
mutations of tumor suppressor genes such as phosphatase and tensin homolog (PTEN). Loss of PTEN expression was significantly associated with nonpapillary, invasive and high-grade tumors, suggesting that the involvement of the PI3K/AKT/mTOR pathway might be a potential driver of an invasive phenotype.15,16

Although in vitro studies document an important role of the Ras pathway in bladder cancer development, human bladder cancer samples have a surprisingly low rate of H-Ras mutations, suggesting the presence of an alternative mechanism activating the Ras pathway.17,18 A number of studies found that mutations of fibroblast growth factor receptor 3 (FGFR3) were present in more than 50% of papillary UCs and approximately 20% of invasive UCs, which provided an explanation for the upstream activation of the Ras pathway in a large proportion of bladder cancers.19–21 The high frequency of mutations and deletions of the TP53 gene in invasive UC indicates an important role of TP53 in the development of the clinically aggressive variant of the disease.6,7 Loss of TP53 function may also be caused by alterations in the “upstream” or “downstream” regulators of the pathway.22,23 Such alterations may not have as severe effects as the loss of TP53 itself, but may be oncogenically sufficient. It appears that the loss of the RB1 function may also be involved in the development of clinically aggressive forms of bladder cancer, although inactivating mutations of the RB1 gene are relatively uncommon and seen in only 17% of cases.6,7 Furthermore, both animal models and human tumor observations point toward the activation of the PTEN/PI3 kinase/AKT/mTOR pathway as a mechanism driving the development of an invasive phenotype of bladder cancer.15,24,25 It is, however, still unclear how the alterations in these pathways contribute to the development of the 2 distinct bladder cancer types. Nonetheless, these studies have provided strong evidence that the Ras pathway is a major driver of the papillary and invasive nonpapillary UC, suggesting that they may share a common origin.26,27

## Cellular Origin of Bladder Cancer

The exact origin of bladder cancer remains uncertain; however, the histologic and molecular analyses of the urothelial structure provide important clues. The bladder urothelium is composed of 3 different cell types based on their level of differentiation. The basal cells adjacent to the basement membrane are the least differentiated and express the basal cytokeratin (CK) 5/6 and CK14 but not CK18 and CK20, which are characteristic of more differentiated intermediate urothelial cell layers.26 The basal cells also express the surface-anchoring molecules, such as laminin receptor and β4-integrins.26,27 Another characteristic marker of basal cells is hyaluronic acid receptor (CD44).28 It is generally believed that stem cells or uroprogenitor cells with self-renewal capacity reside in the basal layer.29 Several groups have successfully isolated stem cells from the urothelium and reported that they exhibited a phenotype recapitulating that of the basal urothelial cells.27,28 The intermediate cells between the basal and luminal layers show moderate differentiation and express CK18, CD44, and CK5/6 at reduced levels.29 The proliferating potential of intermediate cells is limited and similar to the so-called partially committed cells described in other tissues.30,31 The surface or luminal cells are terminally differentiated umbrella cells that express uroplakins and CK20.30 Several studies have provided strong evidence that stem or uroprogenitor cells are the likely source of bladder cancer.26–28 In experimental systems, they are identified on the basis of their distinct surface markers.27,28 Cancer stem cells, similar to their benign counterparts, are also regulated through interactions with stromal cells and matrix components. Paracrine signaling is a key regulator of cancer stem cells and involves several important oncogenic pathways, such as Notch, wingless-type MMTV integration site family (Wnt), hedgehog, bone morphogenetic proteins and transforming growth factor β (BMPs/TGF-β), and integrin–matrix interactions.32

It is generally believed that field effects play an essential role in bladder carcinogenesis.33,34 Several studies have reported that the earliest oncogenic effects could be observed not only in areas of the bladder mucosa containing microscopically recognizable alterations such as dysplasia or carcinoma in situ but also in areas of the urothelium that might still be morphologically normal.35–37 Our whole-organ mapping analysis identified that the initial clonal expansion of preneoplastic urothelial cells was characterized by the deletion of 1 megabase DNA near the RB1 gene on the chromosome 13q14 region.38–40 The deletion involved large areas of the neoplastic urothelium as well as areas of the urothelium with minimal or no deviation from normal morphology. Surprisingly, the major tumor suppressor, RB1, was not mutated or hypermethylated in normal-appearing mucosa, suggesting that other alternative genes in this region, termed forerunner (FR) genes, were the drivers of this expansion (Figure 2). Five candidate FR genes, including integral membrane protein 2B (ITM2B), lysosphosphatidic acid receptor 6 (LPAR6), motilin receptor (MLNR), calcium-binding protein 39–like (CAB39L), and ADP ribosylation factor–like GTPase 11 (ART11), were identified as potential initiating drivers of bladder carcinogenesis.39 The FR genes are silenced primarily by hypermethylation and less frequently by mutation, leading to significant downregulation at the mRNA level in more than 50% of bladder cancer cell lines and human bladder cancer samples. Our studies suggest that dysregulation of the FR genes may be an initial molecular alteration associated with the field effect, whereas dysfunction of the RB1 gene may be a subsequent event associated with the development of bladder UC.38,39

Similar genetic alterations were detected in papillary and nonpapillary bladder UC, suggesting that they may share a common origin (Figure 2). Early whole-organ mapping studies identified 6 critical regions mapping to 3q22, 5q22–23, 9q21, 10p26, 13q14, and 17q13 that cooperated to drive the development of bladder cancer.40,41 The loss of genetic material in these regions appears to be similar in superficial papillary and invasive nonpapillary UC, and their synchronous involvement, ranging from 2 to 5 regions, can be documented in approximately 80% of bladder tumors. It also implies that secondary events are responsible for the divergence along the papillary and nonpapillary tracks. The original studies have shown that large deletions of genetic material were similar in papillary and nonpapillary tracks. On the other hand, more recent studies have demonstrated that the downstream target genes within these regions were different in the 2 forms of bladder carcinogenesis.34,42 The animal models tracking down the cellular origin of bladder cancer are in keeping with these observations and strongly suggest that the papillary and nonpapillary tracks are different from the outset and originate from distinct uroprogenitor cells.
INTRINSIC MOLECULAR SUBTYPES OF BLADDER CANCER

Molecular diversity underlies a spectrum of clinical behaviors of bladder cancer as their different responses to conventional and targeted therapies. Genomic expression analyses have shown that distinct expression signatures are associated with cancer progression, metastasis, and survival. Sjödahl et al. analyzed gene expression profiles of nonmuscle invasive UC, which led to the identification of 3 major molecular subtypes, including urothelial-like (Uro), genically unstable, and basal/squamous cell carcinoma-like (SCCL). These molecular subtypes showed different gene expressions; the Uro subtype expressed FGFR3 and cyclin D1 (CCND1), with the frequent loss of 9p21 (cyclin-dependent kinase inhibitor 2A [CDKN2A]); the genically unstable subtype expressed Forkhead box M1 (FOXM1) but not CK5, with the loss of RB1; and the basal/SCCL subtype expressed CK5 and CK14, but not FOXA1 and GATA-binding protein 3 (GATA3). The molecular subtypes of nonmuscle invasive UC also exhibited significant prognostic difference: the Uro group was associated with a good prognosis, the genically unstable group with an intermediate outcome, and the SCCL group with the worst prognosis. Furthermore, immunohistochemical analysis revealed marked differences of protein expressions in the molecular subtypes of nonmuscle invasive UC.

By performing whole-genome mRNA expression profiling, we found that muscle-invasive UC (MIUC) could be divided into 3 distinct intrinsic subtypes: basal, luminal, and p53-like (Figure 3, A). The molecular markers used in our profiling analysis generally reflected the gene expression signatures of normal basal (such as CK5/6, CK14, p63, and others) and luminal (such as uroplakins, CK18, CK20, GATA3, and others) urothelial layers. In addition to the distinct signatures of gene expressions, these molecular subtypes also demonstrated different clinicopathologic features. Basal UC was typically enriched with squamous and sarcomatoid features, and patients often presented with advanced or metastatic diseases. Although it was intrinsically aggressive, basal UC was highly sensitive to cisplatin-based chemotherapy. Luminal UC was enriched with...
papillary morphology, FGFR3 mutations, and erb-b2 receptor tyrosine kinase 2 (ERBB2) amplifications, and their gene expression profiles were controlled by peroxisome proliferator activator receptor γ (PPARγ). Luminal type could be further subdivided into 2 subtypes, p53-like and luminal, which were distinguished from one another by different levels of biomarkers that are characteristic of stromal infiltration and cell proliferation. Although the luminal subtypes were not as aggressive as basal UC, p53-like tumors were resistant to chemotherapy and might represent a therapeutic challenge.

To identify immunohistochemical markers that can help in the molecular classification of bladder cancers, we selected and evaluated a panel of immunohistochemical markers based on the genomic expression profiles. These markers included GATA3, CK18, uroplakin II, cyclin D1, and ERBB2 for the luminal markers; CK5/6, CK14, and p63 for the basal markers; and p16, B-cell lymphoma 2 (BCL2),...
smooth muscle actin (SMA), myosin, calponin, and desmin for the p53-like subtype markers. Parallel analyses of genomic expression profiles and immunohistochemical expression patterns found that a small set of immunohistochemical biomarkers, including CK5/6, CK14, CK20, GATA-3, and uroplakin, could be used to classify bladder cancer into different molecular subtypes. Interestingly, the analysis of the immunohistochemical stains of the p53-like tumors provided evidence that most markers were not distinctly positive in tumor cells and the p53-like signature profile likely resulted from stromal contamination. Furthermore, we found that the immunohistochemical expression levels of just 2 signature markers, GATA3 and CK5/6, were sufficient to classify bladder cancers into luminal and basal categories with greater than 90% accuracy, although the performance of this classifier should be further validated on larger independent cohorts.

The TCGA research group recently analyzed the RNA sequence data of 412 cases of bladder MIUC, which revealed 5 distinct molecular subtypes, including luminal-papillary (35%), luminal-infiltrated (19%), luminal (6%), basal-squamous (35%), and neuronal (5%). These molecular subtypes not only demonstrated different signatures of mRNA expressions but also exhibited distinct clinicopathologic features, which might lead to novel therapeutic approaches to this complex disease. The luminal-papillary subtype showed a prominent role of FGFR3 with a prevalence of FGFR3 gene mutations, amplification, and overexpression. The tumors were characterized by papillary morphology and associated with a low risk for cancer progression. Although the luminal papillary UC responded poorly to cisplatin-based chemotherapy, FGFR3 tyrosine kinase inhibitors may be effective for this subtype owing to the prevalence of FGFR3 alterations. The luminal-infiltrated subtype showed strong stromal reaction mixed with lymphocytes, smooth muscle, and myofibroblastic proliferation. Interestingly, the expression of immune checkpoint markers, including programmed death ligand-1 (PD-L1), programmed death receptor-1 (PD-1), and cytotoxic T-lymphocyte-associated protein (CTLA-4), were markedly elevated in this subtype. Although this subtype showed resistance to cisplatin-based chemotherapy, it might respond favorably to immunotherapy with immune checkpoint inhibitors. The luminal subtype expressed high levels of uroplakin genes as well as other genes associated with the terminally differentiated umbrella cells, suggesting that this subtype might derive from the intermediate cells with a transcriptional program leading to expression of markers characteristic of the normal umbrella cells. The basal-squamous subtype was more common in women and associated with squamous differentiation. It showed a high expression of basal markers (CD44, CK5, CK6A, CK14) as well as squamous differentiation markers, including transglutaminase 1 (TGM1), desmocollin 3 (DSC3), and PI3. This subtype was likely to respond to both cisplatin-based chemotherapy and immune checkpoint therapy, owing to the strong expression of PD-L1 and CTLA-4 immune markers. The neuronal subtype was characterized by expression of both neuroendocrine and neuronal genes. It also showed a high cell-cycle signature, indicating an active cell proliferation. Identifying this subtype largely depended on detecting expression of neuroendocrine/neuronal markers by either mRNA-Seq or immunohistochemistry, as the tumors did not exhibit the typical morphologic features associated with small cell or high-grade neuroendocrine carcinoma. Nonetheless, it had high levels of TP53 and RB1 mutations, like small cell carcinomas in other organs. Neuronal type was associated with the worst survival, making it important to recognize clinically. Like neuroendocrine neoplasms arising in other sites, this subtype might respond to etoposide-cisplatin chemotherapy, although this remains to be tested in prospective clinical trials.

It is evident from these investigations that bladder cancer is a molecularly heterogeneous disease. Although the names and numbers of subtypes by these groups were somewhat different, they showed striking similarities to the intrinsic basal and luminal subtypes identified in human breast cancers. There is a general consensus that the top-level separation occurs at the basal and luminal differentiation checkpoint. The luminal UC appears to evolve through the papillary track, while the basal UC develops via the nonpapillary track. The superficial papillary tumors are almost exclusively luminal, while the invasive bladder cancers are equally divided into luminal and basal types. The invasive tumors that show luminal expression signatures likely evolve from the preexisting papillary disease and represent a progression of superficial papillary tumors. Recently, we performed meta-analysis of the genomic expression profiles on several large cohorts, comprising a total of 937 bladder cancer samples with annotated clinical data. We found that bladder cancers can be consistently classified into 2 intrinsic molecular types, luminal and basal. The classification could be successfully performed, not only on fresh frozen tumor specimens but also on formalin-fixed and paraffin-embedded archival samples.

**MUTATIONAL LANDSCAPE**

Molecular alterations at the DNA level are a major driving force of the development and progression of bladder UC. Recently, several genome-wide analyses have attempted to dissect DNA alterations of urothelial cancer with a focus on those that originate in the bladder.

The most significant among these reports are comprehensive multiplatform genomic analyses of muscle invasive bladder UC by TCGA Research Network. Initially, TCGA performed a genomic analysis of 131 MIUCs and found that these tumors harbored a large number of DNA alterations, only slightly fewer than lung cancer and melanoma, but more than any other adult malignancies. On average, there were approximately 300 exonic mutations, 200 segmental alterations of genomic copy number, and 20 genomic rearrangements per sample. Subsequently, TCGA analyzed a larger cohort of 412 MIUCs and found recurrent mutations in 58 genes, including multiple genes involved in cell-cycle regulation, chromatin remodeling, and kinase signaling (Figure 3, B). The most frequently mutated gene was TP53, which was present in nearly 50% of MIUCs and mutually exclusive with the amplification and overexpression of mouse double minute 2 homolog (MDM2). The second most frequently mutated gene was mixed-lineage leukemia 2 (MLL2) (28%), which belongs to the group of chromatin-remodeling genes involved in epigenetic regulation. Another frequently mutated gene was RB1 (17%), which was mutually exclusive with CDKN2A deletion. Other significantly mutated genes included 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), which were involved in the regulation of cell proliferation, differentiation, and genetic stability. Less than 5% of bladder cancers harbored the recurrent gene fusions predominantly involving intrachromosomal FGFR3–TACC3 (transforming acidic coiled-coil containing protein 3) and less frequently, TSEN2 (tRNA splicing endonuclease subunit 2)–PPARG and MKRN2 (makorin ring finger protein 2)–PPARG.

High mutation load in MIUC was mainly driven by the apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) mutagenesis. APOBEC is a member of the evolutionary conserved family of cytidine deaminases that are involved in genomic clearance of foreign DNA. In bladder cancer, the APOBEC-a and APOBEC-b mutagenesis signatures accounted for 67% of all single nucleotide variants. Clustering analysis identified 4 distinct mutational signature groups, which were associated with distinct survival. Cluster 1 cancers (high APOBEC-signature mutagenesis and high mutation burden) showed an excellent 5-year survival probability (75%), as the high mutation burden might lead to a robust host immune response, inhibiting further tumor growth and metastasis. Cluster 2 cancers had the lowest mutation rate and the poorest 5-year survival (22%). Cluster 4 samples were enriched in ERCC2 mutations and were most common in smokers.

Overall, the potential therapeutic vulnerabilities were identified in 70% of the tumors, predominantly including targets in the PI-3 kinase/AKT/mTOR and in the receptor tyrosine kinase (RTK)/mitogen-activated protein kinase (MAPK) pathways. Chromatin regulatory genes were more frequently mutated in UC than in any other common cancer studied, further expanding the therapeutic options. Although these studies were mainly focused on the molecular landscape of MIUC, they dramatically improved our understanding of this aggressive disease.

The overall mutational landscape of luminal and basal bladder cancers was similar but several genes were distinctly enriched for their mutations in specific molecular subtypes. The mutations of 4 genes, including FGFR3, E74-like ETS transcription factor 3 (ELF3), cyclin-dependent kinase inhibitor (CDKN1A), and TSC complex subunit 1 (TSC1), were enriched in luminal tumors, while the basal tumors were enriched for the mutations of TP53, RB1, and nuclear factor erythroid-derived 2–like 2 (NFE2L2) genes. For some of these genes, their mutational enrichments in specific molecular subtypes were particularly evident when the mutational patterns of the functional domains of their encoded protein were analyzed.

**BLADDER UROTHELIAL CARCINOMA VARIANTS**

In addition to conventional UC, there are more than a dozen microscopically unique forms of bladder cancer described in the recent World Health Organization classification. Squamous and glandular differentiations are 2 common findings in otherwise conventional UC and have an uncertain clinical impact. On the other hand, the development of sarcomatoid, small cell, micropapillary, and plasmacytoid variants represents a progression of conventional disease that is associated with highly aggressive clinical behavior. Urothelial carcinomas with variant features pose a diagnostic and therapeutic dilemma and are associated with a disproportionate number of deaths. Unlike conventional UC, bladder cancers with variant histologic profiles have not been yet subjected to the same comprehensive genomic classification. It has been hypothesized that enrichment of specific mutations and activation of distinct molecular pathways in these tumors may open new avenues for diagnosis and therapeutic manipulation. The new genomic data concerning several most common forms of bladder cancer with variant histologic profiles, including micropapillary, plasmacytoid, and small cell carcinoma variants, are included in this review.

Micropapillary variant of UC is characterized by small morula-like tumor nests surrounded by empty spaces. It has a high propensity for metastasis to regional lymph nodes and distant organs, resulting in poor clinical outcome. Our studies of genomic expression profile of micropapillary cancer revealed that more than 6000 genes were aberrantly expressed when compared to conventional UC. The expression profile was characterized by dysregulation of multiple oncogenic pathways converging on transformation, cell cycle regulation, DNA damage repair, and signal transduction. Interestingly, the micropapillary expression signature was also present in conventional UC that contained foci of micropapillary features, suggesting that micropapillary variant arises from a unique subset of conventional UCs. The survival analyses using either the microscopically identified micropapillary features or the expression signature with hierarchical clustering showed that micropapillary UC was associated with more aggressive clinical behavior than conventional UC.

Micropapillary UCs were almost exclusively of luminal type and approximately half of them showed the expression signature of the p53-like subtype. In survival analysis, the p53-like subset of micropapillary UC appeared to be the most aggressive among the molecular subtypes. By immunohistochemistry micropapillary UCs were consistently positive for the luminal markers, such as GATA3 and uroplakin 2. In contrast, they were negative for basal markers, such as CD44 and CK14. The striking features of micropapillary UC were downregulation of miR-29b, with overexpression of its nearly 300 target genes and activation of chromatin-remodeling complex RuvB-like 1 (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) involved in cell growth, DNA damage repair, and metastasis.

Plasmacytoid UC variant is a distinct disease that is characterized by discohesive malignant cells with eccentric nuclei in a loose stroma with minimal fibrotic and inflammatory reaction. Plasmacytoid UC typically displays a diffuse growth pattern with a high tendency for peritoneal spread, leading to the positive margin at cystectomy. Recently, Al-Ahmadi et al showed that plasmacytoid UC is characterized by the presence of truncating mutations of cadherin 1 (CDH1) encoding E-cadherin in a large proportion of plasmacytoid UC. Furthermore, hypermethylation of the CDH1 promoter, an alternative mechanism of E-cadherin loss, was present in a small subset of plasmacytoid UCs with the wild-type CDH1. The CDH1 alterations resulted in loss of E-cadherin protein expression with negative immunohistochemical staining representing a good surrogate marker for this variant of bladder cancer. Although inactivating mutations in CDH1 were found exclusively in plasmacytoid variant, the patterns of other altered genes were similar in plasmacytoid variant and coexistent conventional UC, suggesting that both histologic
subtypes potentially evolve from a common cell of origin.61 Tumors with similar plasmacytoid morphology develop in breast and stomach and are referred to as lobular or diffuse carcinomas, respectively. Similar to bladder cancer both of these tumors frequently harbor point mutations of the CDH1 gene and are immunohistochemically negative for E-cadherin expression.62,63 Small cell carcinoma is a rare neuroendocrine variant of bladder cancer that is associated with particularly dismal prognosis.64 It frequently coexists with conventional UC.
representing a progression of preexisting tumor to a poorly differentiated small cell malignancy. Recent studies comparing small cell carcinoma with conventional UC pointed to divergent differentiation from a single-clone precursor, as determined by similar patterns of loss of heterozygosity and telomerase reverse transcriptase (TERT) promoter mutations in the 2 components. A comprehensive whole-genome analysis found that small cell carcinoma had a high somatic mutational burden driven predominantly by an APOBEC-mediated mutational process. RNA sequencing revealed dysregulated tumorigenic pathways and novel fusion transcripts, including an in-frame Pt1 oncogene (PVT1)-ERBB2 fusion, which was associated with aberrant expression of the ERBB2 gene. Furthermore, combined suppression of the TP53 and RB1 genes led to lineage switching from oncogene-addicted UC cells to neuroendocrine-like tumor cells. As bladder small cell carcinoma is histologically similar to small cell carcinoma of the lung, it is not surprising that these 2 tumors have overlapping molecular profiles and develop through a similar sequence of genomic alterations.

CONCLUSIONS

Recent studies based on genomic characterization of bladder UC have provided fundamental insights into the molecular biology of this complex disease. These studies have provided strong evidence that bladder UC is likely to develop from distinct uroepithelial cells and widespread mucosal field effects along the dual-track oncogenic pathways. A new class of genes, referred to as FR genes, is a driving force in the incipient phases of carcinogenesis, which sensitizes the field effects to the development of papillary and nonpapillary UC. The molecular characterization of bladder UC revealed different molecular subtypes that recapitulate the differentiation patterns of normal urothelium referred to as luminal and basal. These intrinsic molecular subtypes have distinct clinical behavior and sensitivities to frontline chemotherapy. Bladder cancer with variant histologic profiles such as micropapillary, plasmacytoid, and small cell show distinct mutational landscapes and expression signatures, which underlie their aggressive behaviors and may represent novel therapeutic targets. This new molecular paradigm of the disease opens new opportunities for correlative predictive and therapy sensitivity studies. These advances fundamentally change our understanding of the disease and expand the diagnostic and therapeutic options for patients affected by bladder cancer.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7–30.
2. Grignon DJ, Al-Amadie H, Alghaba F, et al. Tumors of the urinary tract. In: Moch H, Humphrey PA, Ulbright TM, Retuerto V, eds. WHO Classification of Tumours of the Urinary System and Male Genital Organs. 4th ed. Lyon, France: IARC Press; 2016:77–133. World Health Organization Classification of Tumours, vol 8.
3. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. Nature. 2007;446:7132:153–158.
4. Dambrau J, Hoadley KA, Chism DD, et al. Intrinsinc subtypes of high-grade bladder cancer reflect the hallmarks of breast cancer biology. Proc Natl Acad Sci U S A. 2014;111(8):3110–3115.
5. Chai W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell. 2014;25(2):152–162.
6. Cancer Genome Atlas Research Network. Distinct molecular characterization of urothelial bladder carcinoma. Nature. 2014;507:7492:315–325.
7. Robertson AG, Kim J, Al-Amadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell. 2017;171(3):540–556.e25.
38. Kim MS, Jeong J, Majewski T, et al. Evidence for alternative candidate genes near RB1 involved in Clonal expansion of in situ urothelial neoplasia. Lab Invest. 2006;86(2):175–190.

39. Lee S, Jeong J, Majewski T, et al. Forerunner genes contiguous to RB1 contribute to the development of in situ neoplasia. Proc Natl Acad Sci U S A. 2007;104(13):5372–5377.

40. Majewski T, Lee S, Jeong J, et al. Understanding the development of human bladder cancer by using a whole-organ genomic mapping strategy. Lab Invest. 2008;88(7):694–721.

41. Kram A, Li L, Zhang RD, et al. Mapping and genome sequence analysis of chromosome 5 regions involved in bladder cancer progression. Lab Invest. 2001;81(7):1039–1048.

42. Kim JH, Tuziak T, Hu L, et al. Alterations in transcription clusters underlie development of bladder cancer along papillary and nonpapillary pathways. Lab Invest. 2005;85(4):532–549.

43. Spiess PE, Czerniak B. Dual-track pathway of bladder carcinogenesis: practical implications. Arch Pathol Lab Med. 2006;130(6):844–852.

44. Sjodahl G, Lauss M, Lovgren K, et al. A molecular taxonomy for urothelial carcinoma. Clin Cancer Res. 2012;18(12):3377–3386.

45. Sjodahl G, Lovgren K, Lauss M, et al. Toward a molecular pathologic classification of urothelial carcinoma. Am J Pathol. 2013;183(3):681–691.

46. McConkey DJ, Choi W, Shen Y, et al. A prognostic gene expression signature in the molecular classification of chemotherapy-naïve urothelial cancer is predictive of clinical outcomes from neoadjuvant chemotherapy: a phase 2 trial of dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin with bevacizumab in urothelial cancer. Eur Urol. 2016;69(5):855–862.

47. Dadhania V, Zhang M, Zhang L, et al. Meta-analysis of the luminal and basal subtypes of bladder cancer and the identification of signature immunohistochemical markers for clinical use. EBiomedicine. 2016;12:105–117.

48. Lindgren D, Frigyesi A, Gudjonsson S, et al. Combined gene expression and genomic profiling define two intrinsic molecular subtypes of urothelial carcinoma and gene signatures for molecular grading and outcome. Cancer Res. 2010;70(9):3463–3472.

49. Gui Y, Guo G, Huang Y, et al. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. Nat Genet. 2011;43(9):875–878.

50. Iyer G, Al-Ahmadie H, Schultz N, et al. Prevalence and co-occurrence of actionable genomic alterations in high-grade bladder cancer. J Clin Oncol. 2013;31(25):3133–3140.

51. Chen F, Zhang Y, Bosse D, et al. Pan-urologic cancer genomic subtypes that transcend tissue of origin. Nat Commun. 2017;8(1):199.

52. Roberts SA, Lawrence MS, Klimczak LJ, et al. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. Nat Genet. 2013;45(9):970–976.

53. Amin MB. Histological variants of urothelial carcinoma: diagnostic, therapeutic and prognostic implications. Mod Pathol. 2009;22(suppl 2):S96–S118.

54. Paner GP, Annaiah C, Gulmann C, et al. Immunohistochemical evaluation of novel and traditional markers associated with urothelial differentiation in a spectrum of variants of urothelial carcinoma of the urinary bladder. Hum Pathol. 2014;45(7):1473–1482.

55. Liang Y, Heitzman J, Kamat AM, Dinney CP, Czerniak B, Guo CC. Differential expression of GATA-3 in urothelial carcinoma variants. Hum Pathol. 2014;45(7):1466–1472.

56. Willis DL, Porten SP, Kamat AM. Should histologic variants alter definitive treatment of bladder cancer? Eur Urol. 2013;63(3):435–443.

57. Guo CC, Dadhania V, Zhang L, et al. Gene expression profile of the clinically aggressive micropapillary variant of bladder cancer. Eur Urol. 2016;70(4):611–620.

58. Vaira V, Faversani A, Dobi T, et al. miR-296 regulation of a cell polarity-cell plasticity module controls tumor progression. Oncogene. 2012;31(21):7760–7768.

59. Gentili C, Castor D, Kaden S, et al. Chromosome missegregation associated with RUVBL1 deficiency. PloS One. 2015;10(7):e0133576.

60. Fox MD, Xiao L, Zhang M, et al. Plasmacytoid urothelial carcinoma of the urinary bladder: a clinicopathologic and immunohistochemical analysis of 49 cases. Am J Clin Pathol. 2017;147(5):500–506.

61. Al-Ahmadie HA, Iyer G, Lee BH, et al. Frequent somatic CDH1 loss-of-function mutations in plasmacytoid variant bladder cancer. Nat Genet. 2016;48(4):356–358.

62. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513(7517):202–209.

63. Cancer Genome Atlas Research Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61–70.

64. Wang G, Xiao L, Zhang M, et al. Small cell carcinoma of the urinary bladder: a clinicopathologic and immunohistochemical analysis of 81 cases [published online ahead of print May 12, 2018]. Hum Pathol. doi:10.1016/j.humpath.2018.05.003.

65. Kouba EJ, Cheng L. Understanding the genetic landscape of small cell carcinoma of the urinary bladder and implications for diagnosis, prognosis, and treatment: a review. JAMA Oncol. 2017;3(11):1570–1578.

66. Rickman DS, Beltran H, Demichelis F, Rubin MA. Biology and evolution of poorly differentiated neuroendocrine tumors. Nat Med. 2017;23(6):1–10.

67. Cheng L, Jones TD, McCarthy RP, et al. Molecular genetic evidence for a common clonal origin of urinary bladder small cell carcinoma and coexisting urothelial carcinoma. Am J Pathol. 2005;166(5):1533–1539.

68. Priemer DS, Wang M, Zhang S, et al. Small-cell carcinomas of the urinary bladder and prostate: TERT promoter mutation status differentiates sites of malignancy and provides evidence of common clonality between small-cell carcinomas of the urinary bladder and urothelial carcinoma [published ahead of print March 31, 2017]. Eur Urol Focus. doi:10.1016/j.euf.2017.03.007.

69. Shen P, Jing Y, Zhang R, et al. Comprehensive genomic profiling of neuroendocrine bladder cancer pinpoints molecular origin and potential therapeutics. Oncogene. 2018;37(22):3039–3044.

70. Chang MT, Penson A, Desai NB, et al. Small-cell carcinomas of the bladder and lung are characterized by a convergent but distinct pathogenesis. Clin Cancer Res. 2018;24(8):1965–1973.