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Omics sciences and precision medicine in Urothelial Carcinoma

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Abstract

This comprehensive review explores the potential of omics sciences— such as genomics, transcriptomics, proteomics, and metabolomics—in advancing the diagnosis and therapy of urothelial carcinoma (UC), a prevalent and heterogeneous cancer affecting the urinary tract. The article emphasizes the significant advancements in understanding the molecular mechanisms underlying UC development and progression, obtained through the application of omics approaches. Genomic studies have identified recurrent genetic alterations in UC, while transcriptomic analyses have revealed distinct gene expression profiles associated with different UC subtypes. Proteomic investigations have recognized protein biomarkers with diagnostic and prognostic potential, and metabolomic profiling has found metabolic alterations that are specific to UC. The integration of multi-omics data holds promises in refining UC subtyping, identifying therapeutic targets, and predicting treatment response. However, challenges like the standardization of omics technologies, validation of biomarkers, and ethical considerations need to be addressed to successfully translate these findings into clinical practice. Omics sciences offer tremendous potential in revolutionizing the diagnosis and therapy of UC, enabling more precise diagnostic methods, prognostic evaluations, and personalized treatment selection for UC patients. Future research efforts should focus on overcoming these challenges and translating omics discoveries into meaningful clinical applications to improve outcomes for UC patients. ***Clin Ter 2023; 174 Suppl. 2 (6):1-10 doi: 10.7417/CT.2023.2466***

Key words: *Urothelial Carcinoma, precision medicine, genomics, biomarkers, metabolomics*

Introduction

Urothelial carcinoma is a type of cancer that arises from the cells lining the urinary system, including the bladder, ureters, and renal pelvis (1). It is a significant public health concern, with an estimated 430,000 new cases and 165,000 deaths worldwide in 2020 (2). In the United States alone, approximately 83,000 new cases of bladder cancer are diagnosed annually, with over 17,000 deaths (3). It is more prevalent in men than women, and the risk of developing this disease increases with age (4).

Urothelial carcinoma is a complex and heterogeneous disease that can present in non-invasive or invasive forms, with varying clinical and molecular features (5). Non-invasive urothelial carcinoma, also known as papillary urothelial neoplasms of low malignant potential (PUNLMP) or non-invasive papillary urothelial carcinoma (NIPUC), accounts for approximately 70% of bladder cancers (6). It is typically slow-growing and has a good prognosis, with a low risk of progression to invasive disease. Invasive urothelial carcinoma, on the other hand, can spread to other organs and tissues, leading to metastasis and poor prognoses.

Despite advances in diagnosis and treatment, urothelial carcinoma, also known as transitional cell carcinoma, continues to have poor prognosis, especially in advanced stages (7). This type of cancer is the most common form of bladder cancer and arises from the urothelial cells lining the urinary tract. The current treatment options include surgery, chemotherapy, radiation therapy, and immunotherapy (8). However, the effectiveness of these therapies varies depending on the stage and molecular profile of the tumor (9). To improve patient outcomes, there is an urgent need

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to identify novel biomarkers and therapeutic targets specific to urothelial carcinoma (10). These markers and targets could potentially help in the development of personalized treatment strategies and improve response rates (11). This is particularly important considering the heterogeneity of urothelial carcinoma and the diverse molecular alterations observed in different patients (12).

Omics sciences, including genomics, proteomics, metabolomics, and microbiomics, have revolutionized our understanding of cancer biology and have opened new avenues for the diagnosis, prognosis, and treatment of various cancers, including urothelial carcinoma (13). These omics approaches involve comprehensive analysis of molecular data, encompassing DNA, RNA, proteins, metabolites, and their interactions with the environment and microbiome (14). Genomics, proteomics and metabolomics studies have identified recurrent genetic alterations that drive urothelial carcinoma growth and survival (15). By understanding the altered metabolism in cancer cells, researchers can develop targeted therapies to disrupt these pathways and inhibit tumor progression. In addition to the tumor cells themselves, the role of the microbiome in cancer development and treatment response is being increasingly recognized. Microbiomics has revealed associations between specific microbial compositions and urothelial carcinoma (16). Understanding the interactions between the microbiome and tumor cells can provide insights into the mechanisms underlying carcinogenesis and potentially lead to the development of microbiome-based interventions for urothelial carcinoma.

In this review, we have discussed the use of omics sciences in refining the diagnosis and therapy of urothelial carcinoma. Specifically, we focused on the application of genomics, proteomics, metabolomics, and microbiomics in the diagnosis, prognosis, and treatment of urothelial carcinoma. We propose future directions involving the integration of multi-omics data, the development of novel therapeutic strategies, and the implementation of personalized approaches to cancer management. Omics sciences offer potential in identifying biomarkers, therapeutic targets, and advancing our knowledge of cancer biology in urothelial carcinoma. However, to validate their clinical utility and overcome challenges for widespread adoption, it is crucial to conduct standardized assays, perform biomarker validation, and conduct large-scale studies in clinical practice.

Genetics of cancer

Cancer is a genetic disease that arises from the accumulation of genetic alterations in cells that disrupt normal cellular functions, including cell proliferation, differentiation, and apoptosis. Germline mutations that predispose to cancer, such as mutations in the BRCA1/2 genes, have been extensively studied in breast and ovarian cancer, but their role in urothelial carcinoma is less clear (17). Somatic mutations in oncogenes and tumor suppressor genes play a crucial role in the development and progression of urothelial carcinoma (18). These genetic alterations contribute to the dysregulation of key signaling pathways involved in cell growth, proliferation, and survival. Understanding the role of specific genes in urothelial carcinoma can provide insights into the underlying molecular mechanisms and potential therapeutic targets.

One of the most frequently mutated genes in urothelial carcinoma is fibroblast growth factor receptor 3 (or FGFR3): its mutations are predominantly observed in low-grade non-invasive urothelial carcinomas, including papillary urothelial neoplasms of low malignant potential (PUNLMP) and non-invasive papillary urothelial carcinoma (NIPUC). These mutations lead to constitutive activation of the FGFR3 signaling pathway, promoting cell proliferation and inhibiting differentiation (19, 20). Targeting FGFR3 signaling has emerged as a potential therapeutic strategy for urothelial carcinoma, with the development of FGFR inhibitors currently under investigation in clinical trials (19).

Another commonly mutated gene in urothelial carcinoma is TP53, which encodes the p53 tumor suppressor protein. TP53 mutations are more frequently observed in high-grade invasive urothelial carcinomas. The loss or dysfunction of p53 function leads to impaired DNA damage response and cell cycle control, resulting in genomic instability and increased tumor aggressiveness (21). In urothelial carcinoma, TP53 mutations are associated with poor prognosis and resistance to chemotherapy (22). Targeting mutant p53 or restoring wild-type p53 function represents a potential therapeutic approach for urothelial carcinoma treatment (23).

In addition to FGFR3 and TP53, other genes frequently implicated in urothelial carcinoma include ERBB2 (HER2), PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha), and KDM6A (lysine-specific demethylase 6A) (24, 25). ERBB2 amplifications and overexpression have been observed in a subset of urothelial carcinomas, particularly in muscle-invasive and metastatic cases (26). Targeting ERBB2 with anti-HER2 therapies, such as trastuzumab and pertuzumab, has shown promise in selected patients with ERBB2-positive urothelial carcinoma (27). PIK3CA mutations, which activate the PI3K/AKT/mTOR signaling pathway, are present in a subset of urothelial carcinomas and may predict response to targeted therapies (28). KDM6A is an X-linked histone demethylase that is frequently mutated or deleted in urothelial carcinoma, and its loss is associated with a more aggressive phenotype (29).

Qin et al. (2020) conducted a review focusing on the development of FGFR inhibitors in combination with immune checkpoint inhibitors for the treatment of urothelial carcinoma. The study discussed the rationale behind combining these two classes of drugs and highlighted the potential synergistic effects. It emphasized the potential of FGFR inhibitors as a therapeutic option to enhance the efficacy of immune checkpoint inhibitors in urothelial carcinoma (17). Baldia et al. (2016) investigated FGFR alterations in squamous differentiated bladder cancer and identified them as a potential therapeutic target in this specific subtype. The study suggested that FGFR-targeted therapies may benefit a subset of squamous differentiated bladder cancer patients, indicating the potential for personalized treatment approaches (31).

Wu et al. (2019) examined the significance of TP53 mutation in bladder cancer progression and its impact on treatment decisions. The study highlighted the role of TP53

mutations as a prognostic factor and emphasized their importance in guiding therapeutic strategies for bladder cancer patients (32). Borowczak et al. (2023) evaluated the prognostic role of p53 and its correlation with CDK9 in urothelial carcinoma. The study investigated the expression of p53 and CDK9 and their association with clinical outcomes. The findings suggested a potential prognostic value for p53 and its correlation with CDK9 expression in predicting disease progression and patient outcomes (33).

Jenkins et al. (2022) focused on HER2 overexpression and amplification in uterine carcinosarcomas with serous morphology. The study identified the presence of HER2 alterations in a subset of uterine carcinosarcomas with serous morphology, highlighting the potential for HER2-targeted treatment strategies in this specific subtype (34). Yorozu et al. (2020) assessed the HER2 status in molecular subtypes of urothelial carcinoma of the renal pelvis and ureter. The study investigated the prevalence of HER2 alterations in different molecular subtypes and their potential association with clinical characteristics, contributing to the understanding of the molecular heterogeneity of urothelial carcinoma (35).

Tharin et al. (2023) analyzed the PIK3CA and PIK3R1 tumor mutational landscape in a pan-cancer patient cohort, exploring the frequency and types of mutations and their relevance in guiding treatment decisions across various cancer types (36). Moreover, Ross et al. (2013) conducted a study focused on exploring the PIK3CA mutation spectrum in urothelial carcinoma. The researchers aimed to understand the different types of PIK3CA mutations present in this type of cancer and how these mutations contribute to signaling pathways and phenotypic outcomes (37).

Thus, somatic mutations in oncogenes and tumor suppressor genes, such as FGFR3, TP53, ERBB2, PIK3CA, and KDM6A, contribute to the pathogenesis of urothelial carcinoma by driving abnormal cell growth, survival, and invasion. These genetic alterations have important clinical implications, as they can serve as potential diagnostic and prognostic biomarkers, as well as therapeutic targets for precision medicine approaches in urothelial carcinoma treatment. Table 1 shows key genetic mutations in urothelial carcinoma.

Tumor genomics

Tumor genomics plays a crucial role in understanding the genetic landscape of urothelial carcinoma, encompassing various genetic alterations like somatic mutations, copy number alterations, and chromosomal rearrangements. The advent of next-generation sequencing (NGS) technologies has revolutionized the field by enabling comprehensive analysis of the tumor genome and transcriptome, leading to the discovery of novel driver mutations and potential therapeutic targets.

In urothelial carcinoma, the identification of actionable mutations has emerged as one of the most promising applications of tumor genomics. These actionable mutations are specific genetic alterations that can be targeted by drugs designed to inhibit or modulate the activity of the affected genes or pathways. For instance, the FGFR inhibitor erdafitinib has received approval for the treatment of advanced urothelial carcinoma with FGFR alterations (40). FGFR alterations, such as activating mutations or gene fusions, occur in a subset of urothelial carcinomas and can be identified through genomic profiling (41). Clinical trials have demonstrated the efficacy of erdafitinib in patients with FGFR-altered urothelial carcinoma, highlighting the importance of genomic profiling in guiding targeted therapy selection (42).

Another promising therapeutic approach in urothelial carcinoma is the use of poly(ADP-ribose) polymerase (PARP) inhibitors (43). PARP inhibitors exploit the concept of synthetic lethality, where cancer cells with defects in DNA repair pathways—such as those harboring mutations in DNA repair genes like BRCA1 and BRCA2—become highly dependent on PARP-mediated DNA repair mechanisms (44). Clinical trials have shown promising results for the PARP inhibitor olaparib in urothelial carcinoma patients with DNA repair gene mutations, providing a potential targeted treatment option for this subset of patients (45).

Another promising biomarker for monitoring tumor burden and treatment response in urothelial carcinoma has emerged: analyzing circulating tumor DNA (ctDNA) (46), which is composed by small fragments of DNA that are released into the bloodstream by tumor cells (47). By analy-

Table 1. Key Gene Mutations in Urothelial Carcinoma and their Clinical Implications.

Gene	Function/Pathway Involved	Frequency of Mutations in Urothelial Carcinoma	Clinical Implications	References
FGFR3	Activation of FGFR3 signaling pathway	Frequently mutated in low-grade non-invasive urothelial carcinoma	Potential therapeutic target; FGFR inhibitors under investigation	(16, 30, 31)
TP53	DNA damage response and cell cycle control	Commonly found with high frequency in high-grade invasive urothelial carcinoma	Associated with poor prognosis and resistance to chemotherapy	(32, 33)
ERBB2 (HER2)	Cell proliferation and survival	Amplification and overexpression in subset of urothelial carcinomas	Target of anti-HER2 therapies; promising in ERBB2-positive cases	(34, 35)
PIK3CA	Activation of PI3K/AKT/mTOR signaling	Mutations present in a subset of urothelial carcinomas	Predictive of response to targeted therapies	(36, 37)
KDM6A	X-linked histone demethylase	Frequent mutation or deletion in urothelial carcinoma	Associated with aggressive phenotype; potential therapeutic target	(38, 39)

zing ctDNA, real-time information on the genetic alterations present in the tumor can be obtained non-invasively (48). Several studies have demonstrated the potential of ctDNA analysis in predicting treatment response, monitoring disease progression, and detecting minimal residual disease in urothelial carcinoma patients (46). This approach holds promise for tailoring treatment strategies, assessing treatment efficacy, and detecting disease recurrence. For example, a study by Powles et al. (2021) investigated the utility of ctDNA analysis in predicting response to immune checkpoint inhibitors in metastatic urothelial carcinoma patients (49). The study found that patients with detectable ctDNA at baseline had worse outcomes compared to those with undetectable ctDNA. Moreover, changes in ctDNA levels during treatment were correlated with treatment response and survival outcomes. Similarly, another study demonstrated the potential of ctDNA analysis in detecting minimal residual disease after radical cystectomy in urothelial carcinoma patients, with ctDNA detection being associated with an increased risk of disease recurrence (46). Integration of genomic profiling, including ctDNA analysis, into routine clinical practice holds promise for improving patient outcomes and optimizing treatment strategies in urothelial carcinoma (50).

Lotan et al. compared urine markers and cytology for surveillance of patients with urothelial carcinoma (UC) using the Cxbladder Monitor test (51). This test exhibited superior sensitivity and negative predictive value compared to cytology, NMP22 ELISA, and NMP22 BladderChek tests, with consistent negative results for false negatives across all markers. In another study, Zeng et al. detected chromosomal aberrations in urothelial carcinoma using whole-genome sequencing technology and proved that the customized bioinformatics workflow had high performance in identifying chromosomal aberrations (52). Table 2 presents the findings derived from clinical trials, prospective studies, and retrospective studies utilized in the investigation of UC (urothelial carcinoma).

Loriot et al. (2019) aimed to assess the efficacy of erdafitinib, an FGFR inhibitor, in patients with locally advanced or metastatic urothelial carcinoma. The study demonstrated promising outcomes, particularly in terms of response rates and progression-free survival. Erdafitinib emerged as an effective targeted therapy for patients with FGFR-altered advanced urothelial carcinoma, highlighting its potential as a treatment option in this patient population (53). Sweis et al. (2018) conducted a clinical trial to investigate the use of olaparib, a PARP inhibitor, in urothelial bladder cancer patients with DNA damage response gene mutations. The study observed the clinical activity of olaparib in this specific subgroup of patients, suggesting its potential as a targeted therapy. The findings provided valuable insights into the personalized treatment approach for urothelial carcinoma based on the presence of DNA repair gene mutations (54).

Fenner (2021) performed a prospective study that focused on utilizing ctDNA (circulating tumor DNA) as a biomarker in urothelial cancer. The study revealed that detectable ctDNA at baseline was associated with worse outcomes, and changes in ctDNA levels correlated with treatment response and survival. This finding emphasized the potential utility of ctDNA as a valuable tool for monitoring disease progression and evaluating treatment response in urothelial carcinoma patients (55). Moreover, Das (2021) carried out another prospective study that explored the role of ctDNA detection as a predictor of disease recurrence after radical cystectomy in urothelial carcinoma patients. The study found an association between the presence of ctDNA and an increased risk of disease recurrence, suggesting its potential as a prognostic marker in this setting (56), while Faltas et al. (2017) conducted a retrospective study with the aim of identifying recurrent genetic alterations in urothelial carcinoma. The study revealed the presence of somatic mutations and copy number alterations, which were found to be associated with pathology outcomes. These findings suggested the existence of distinct molecular subtypes within urothelial carcinoma, providing valuable insights into the genetic landscape of the disease (57).

In addition, Al-Ahmadie and Iyer (2015) conducted a prospective study utilizing integrated genomic analysis to characterize subtypes of urothelial carcinoma based on their distinct molecular characteristics. The study highlighted the prognostic implications of genetic alterations and gene expression patterns in urothelial carcinoma. This comprehensive understanding of the genetic landscape and molecular heterogeneity of the disease has the potential to improve diagnosis and guide treatment decisions (58).

Liu et al. (2022) investigated the molecular mechanisms underlying urothelial carcinoma with FGFR3 mutations. The study aimed at understanding the specific genetic alterations and molecular pathways associated with FGFR3, providing valuable insights into potential targeted therapies for this particular subtype of urothelial carcinoma (59).

Al-Obaidy and Cheng (2021) explored the pathogenesis and treatment implications of fibroblast growth factor receptor (FGFR) gene alterations in urothelial carcinoma. The study shed light on the role of FGFR gene alterations in the development and progression of the disease, paving the way for potential targeted therapies aimed at this specific molecular target (60).

An interesting study by Al-Ahmadie and Iyer (2018) provided updates on the genetics and molecular subtypes of urothelial carcinoma, including select variants, through a comprehensive review. The review emphasized the importance of understanding the genetic landscape and molecular heterogeneity of urothelial carcinoma for improved diagnosis and personalized treatment strategies (61).

Pharmacogenomics

Pharmacogenomics focuses on how drug response and toxicity are impacted by genetic variations: the pharmacokinetics and pharmacodynamics of drugs can be influenced by genetic variations in drug-metabolizing enzymes, drug transporters, and drug targets, thus leading to variable drug efficacy and toxicity, which plays a crucial role in personalized medicine approaches for urothelial carcinoma.

The association between genetic variations and treatment outcomes has been extensively studied in urothelial carcinoma patients receiving chemotherapy and immunotherapy. In a Phase II study, cisplatin-gemcitabine regimen with bevacizumab showed promising overall radiographic response rates and improved median overall survival com-

Table 2 displays evidence from clinical trials and prospective and retrospective studies for UC.

Gene Mutation	Type of study	Target therapy	References
FGFR alterations	Clinical Trial	Erdafitinib, an FGFR inhibitor, showed efficacy in FGFR-altered advanced urothelial carcinoma.	(53)
DNA repair gene mutations	Clinical Trial	Olaparib, a PARP inhibitor, demonstrated promising results in urothelial carcinoma with DNA repair gene mutations.	(54)
ctDNA	Prospective Study	Detectable ctDNA at baseline correlated with worse outcomes, and changes in ctDNA levels correlated with treatment response and survival outcomes.	(55)
ctDNA	Prospective Study	ctDNA detection was associated with an increased risk of disease recurrence after radical cystectomy.	(56)
Somatic mutations, copy number alterations	Retrospective Study	Identified recurrent genetic alterations in urothelial carcinoma and their association with patient outcomes.	(57)
Genetic alterations, gene expression	Prospective Study	Integrated genomic analysis revealed subtypes of urothelial carcinoma with distinct molecular characteristics and prognostic implications.	(58)
FGFR3, TP53	Retrospective Study	Investigated the relationship between FGFR3 and TP53 mutations and their association with patient outcomes and response to therapy.	(59, 60)
Genomic alterations, molecular subtypes	Integrative Molecular Analysis	Identified molecular subtypes of urothelial carcinoma with distinct genomic alterations and clinical characteristics.	(61)
Somatic mutations, copy number alterations	Integrative Molecular Analysis	Identified recurrent genetic alterations in urothelial carcinoma and their association with patient outcomes.	(62, 63)
DNA repair gene alterations	Prospective Study	Detected DNA repair gene alterations in urothelial carcinoma and their association with prognosis and response to chemotherapy.	(64, 65)

pared to chemotherapy alone (66). Ongoing Phase III trials are further evaluating these regimens in advanced urothelial carcinoma patients (50). The prognostic significance of the VEGF axis, particularly VEGFA, has been supported in urothelial cancer, and understanding angiogenesis can aid in theragnostics and patient stratification (67).

In the realm of immunotherapy, immune checkpoint inhibitors have been approved for bladder cancer treatment, but response rates remain modest (65). PD-L1 expression in tumor samples has shown promise as a potential biomarker for immunotherapy response, although its use as a selection criterion is not widely implemented (68). Mutational load, reflecting the number of mutations in the tumor genome, has also emerged as a predictive biomarker for immunotherapy response, as higher mutational load has been correlated with increased likelihood of response to anti-PD-L1 antibodies (69). Other genetic variations—such as those in the CYP2D6 gene and drug transporter genes like ABC transporters—have been explored as potential predictive markers for chemotherapy outcomes (70). Genome-wide association studies (GWAS) have provided insights into the genetic basis of chemotherapy response in urothelial carcinoma, identifying genetic loci associated with treatment outcomes (71).

Biological therapies

Immunotherapy presents a promising approach for treating UC, using drugs that stimulate the immune system to target cancer cells. For example, the treatment of UC has been revolutionized by immune checkpoint inhibitors (ICIs), a type of immunotherapy that has gained approval (72). ICIs function by blocking proteins that hinder the immune system's ability to attack cancer cells, thereby enhancing immune recognition and response against cancer cells. Multiple clinical trials have demonstrated the efficacy of ICIs in UC treatment. In a study, patients with metastatic UC who received the ICI pembrolizumab exhibited higher response rates and longer progression-free survival compared to those who received chemotherapy (73). Another study highlighted that the ICI atezolizumab improved overall survival in patients with locally advanced or metastatic UC (72). ICIs generally exhibit better tolerability with fewer side effects when compared to chemotherapy.

Monoclonal antibodies (mAbs) also demonstrate promise in UC treatment. These targeted drugs are designed to bind to specific proteins on the surface of cancer cells, aiding in their destruction or impeding their growth and spread. Atezolizumab, an approved mAb for UC treatment, targets PD-L1, a protein expressed on some cancer cells. By blocking PD-L1, atezolizumab activates the immune system to attack cancer cells (74). Other mAbs-targeting proteins (such as Nectin-4, which is frequently overexpressed in UC cases), like enfortumab vedotin, have shown favorable results in clinical trials and have gained approval for locally advanced or metastatic UC treatment (75).

Emerging in UC treatment are biomarker-driven strategies that leverage biomarkers like genetic mutations or protein expression levels to identify patients likely to benefit from specific treatments. FGFR3 mutations have been studied as a biomarker in UC, present in approximately 20% of cases, and associated with a more favorable response to FGFR inhibitors (75). Several FGFR inhibitors are under development for UC treatment, with promising

results observed in clinical trials involving patients with FGFR3 mutations. Another biomarker of interest in UC is PD-L1 expression, which may indicate a higher likelihood of response to ICIs (72). However, PD-L1 expression alone does not guarantee response, prompting the exploration of other biomarkers to identify patients who will benefit most from ICIs.

Proteomic biomarkers

Proteomics is a rapidly evolving field that aims to identify and quantify the entire set of proteins expressed by a cell, tissue, or organism. Proteomic biomarkers have the potential to provide valuable information about the molecular mechanisms underlying UC development and progression, as well as to predict treatment response and clinical outcomes. In recent years, several studies have focused on identifying proteomic biomarkers in UC using various techniques, such as mass spectrometry, immunohistochemistry, and bioinformatics.

One of the most promising proteomic biomarkers in UC is fibroblast growth factor receptor 3 (FGFR3), a transmembrane receptor that regulates cell proliferation and differentiation. FGFR3 mutations are present in up to 80% of low-grade non-invasive UC cases and are associated with a favorable prognosis (76). Moreover, FGFR3 expression levels have been shown to correlate with tumor grade, stage, and recurrence in UC patients. Therefore, FGFR3 has been proposed as a potential diagnostic and prognostic biomarker in UC.

Another proteomic biomarker that has attracted attention in UC is aquaporin-1 (AQP1), a water channel protein that plays a role in cell migration and invasion (77). AQP1 overexpression has been observed in high-grade invasive UC and is associated with poor survival outcomes. Furthermore, AQP1 has been shown to enhance the sensitivity of UC cells to cisplatin, a commonly used chemotherapy drug. Therefore, AQP1 may serve as a predictive biomarker for chemotherapy response in UC patients.

Researchers designed another study to identify plasma protein biomarkers for early diagnosis of bladder carcinoma (78). They employed 2D-DIGE and mass spectrometry techniques, which led to the identification of fifteen differentially expressed proteins. Among them, haptoglobin exhibited high sensitivity and specificity in distinguishing between low-grade bladder cancer patients and controls. These findings indicate the potential of haptoglobin and other identified proteins as biomarkers for early detection of bladder cancer, emphasizing the need for further validation and investigation. Moreover, another study used plasma samples from bladder cancer patients and compared these to normal samples using 2-dimensional SDS-PAGE, image gel analysis, and MALDI-TOF mass spectrometry, resulting in the identification of three groups of proteins with altered abundance (11). The first group included modified forms of plasma transferrin, fibrinogen gamma, and complement C3b, absent in normal plasma, while the second group comprised proteins such as haptoglobin, alpha-2-macroglobulin, vitamin D-binding protein, and pigment epithelium-derived factor, found in higher quantities in cancerous samples. The third group consisted of three molecular forms of immunoglobulin M (IgM), significantly lower in relative abundance in cancerous plasma samples. Table 3 describes the role of protein biomarkers, metabolites, and microbes in urothelial cancers.

Metabolomic and microbiomic prognostic indicators

Metabolomic profiling of urine samples has demonstrated its potential in predicting the prognosis of urothelial carcinoma. Several studies have investigated the metabolomic profile of urine samples from urothelial carcinoma patients, aiming to identify metabolites that correlate with disease progression and patient outcomes. For example, in a study by Issaq et al (79), urine samples from 48 healthy individuals and 41 patients with transitional cell carcinoma (bladder cancer) were analyzed using a high-performance liquid chromatography-mass spectrometry approach.

The statistical analysis, using positive ionization mass spectrometry, accurately predicted the status of all 48 healthy urine samples and all 41 bladder cancer urine samples, demonstrating a sensitivity and specificity of 100% for bladder cancer detection. Moreover, their analysis also supported these results, correctly identifying 46 out of 48 healthy urine samples and 40 out of 41 bladder cancer urine samples. A study by Pasikanti et al. (2017) analyzed urine samples from urothelial carcinoma patients and identified alterations in metabolites associated with amino acid metabolism, lipid metabolism, and energy metabolism. Furthermore, they found that specific metabolites, including creatinine, taurine, and citrate, exhibited significant associations with patient prognosis, suggesting their potential as prognostic biomarkers. Pasikanti et al. conducted a study to investigate urinary metabotyping of bladder cancer using two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS) (80). They analyzed urine samples from bladder cancer patients and non-cancer controls. The OPLS-DA model demonstrated high specificity (100%) and sensitivity (71%) in detecting bladder cancer, outperforming cytology. The study also identified metabolites and perturbed metabolic pathways associated with bladder cancer, including alterations in the tryptophan-quinolinic metabolic axis. In another work, gas chromatography/time-of-flight mass spectrometry was used for urinary metabolic profiling of 24 BC patients and 51 non-BC controls (81). Multivariate analysis, including principal component analysis and OPLS-DA, demonstrated a clear differentiation between BC patients and non-BC subjects based on global urinary metabolic profiles. Urinary metabonomics achieved 100% sensitivity in detecting BC, outperforming urinary cytology which achieved only 33% sensitivity.

In addition to metabolomics, microbiomic analysis has shown promise as a prognostic indicator in urothelial carcinoma. Several studies have explored the association between microbial communities and clinical outcomes in urothelial carcinoma patients. Numerous studies have shown that microbial populations can exert an influence on urological conditions, indicating the potential involvement of microbes in the continuum of health and disease states (85). The precise nature and role of these microbes are still

Table 3. Role of Protein Biomarkers, Metabolomics, and Microbiomics in Urothelial Cancer: Key Findings from Selected Studies

Study	Focus	Key Findings	References
Lemaska-Perek et al. (2019)	Protein Biomarkers	Identified potential plasma biomarkers of bladder cancer through proteomic analysis, which could aid in diagnosing and monitoring the disease.	(11)
Blanca et al. (2016)	Protein Biomarkers	Identified FGFR3 and Cyclin D3 as urine biomarkers for bladder cancer recurrence.	(76)
Morrissey et al. (2016)	Protein Biomarkers	Examined urine aquaporin-1 and perilipin-2 concentrations as biomarkers for renal cell carcinoma screening.	(77)
Nedjad et al. (2015)	Protein Biomarkers	Discovered a circulating proteomic signature for detecting biomarkers in bladder cancer patients.	(78)
Amara et al. (2019)	Metabolomics	Discussed the use of metabolomics in bladder cancer research, highlighting its potential for identifying metabolic alterations and developing diagnostic and therapeutic strategies.	(14)
Issaq et al. (2008)	Metabolomics	Detected bladder cancer in human urine by metabolomic profiling.	(79)
Pasikanti et al. (2013)	Metabolomics	Urinary metabotyping of bladder cancer using metabolomic analysis.	(80)
Pasikanti et al. (2010)	Metabolomics	Developed a noninvasive urinary metabonomic diagnostic model for bladder cancer.	(81)
Alfano et al. (2016)	Microbiomics	Explored the interplay between the extracellular matrix and the microbiome in urothelial bladder cancer, emphasizing the complex interactions and their implications in cancer development and progression.	(16)
Buevi Popovi et al. (2018)	Microbiomics	Investigated the urinary microbiome associated with bladder cancer, providing insights into the potential role of microbiota in the disease.	(82)
Wu et al. (2018)	Microbiomics	Identified specific microbial species, such as Acinetobacter baumannii, in bladder cancer patients, suggesting their potential involvement in the disease.	(83)
McConnell et al. (2013)	Microbiomics	Discussed Gram-positive anaerobic cocci as opportunistic pathogens associated with urothelial cancer.	(84)

under investigation, but their impact on bladder cancer carcinogenesis has been evident in the long-standing observation of the association between squamous cell carcinoma of the bladder and urogenital schistosomiasis. S. haematobium, in particular, has consistently been reported to be associated with this type of bladder cancer, potentially contributing to pathogenesis through mechanisms such as epithelial damage, chronic inflammation, and oxidative stress (86).

Despite the significance of microbial involvement in bladder cancer, only a limited number of studies have reported detailed analyses of the urinary microenvironment in urothelial bladder cancer. In one study, Xu et al. compared the urine microbiota of healthy individuals and that of bladder cancer patients, and observed an enrichment of Streptococcus in the urine of patients with urothelial carcinoma (87). Streptococcus abundance was nearly absent in most healthy individuals, and, in cases where Streptococcus abundance was low in cancer samples, Pseudomonas or Anaerococcus were the most abundant genera. However, the study had limitations due to its small sample size. Another similar study compared bacterial communities in urine samples from healthy individuals and from cancer patients, revealing Firmicutes as the most abundant phylum in both groups, followed by Actinobacteria, Bacteroidetes, and Proteobacteria. Operational taxonomic units (OTUs) belonging to the genus Fusobacterium were found to be more abundant in the bladder cancer group (82). Confirming these findings, an independent analysis of 42 bladder cancer tissues detected Fusobacterium nucleatum sequences using protein chain reaction in 11 samples. Additionally, the genera Veillonella, Streptococcus, and Corynebacterium were found to be more abundant in healthy urine samples.

In recent investigations, patients with bladder cancer demonstrated an increase in bacterial richness, defined by the number of unique OTUs in a sample. This greater bacterial richness was also observed in urine from patients with non-muscle invasive bladder cancer (NMIBC) who had a high risk of recurrence or progression, based on the European Organization for Research and Treatment of Cancer (EORTC) scoring system (83). Therefore, higher bacterial richness may serve as a potential indicator of the high risk of recurrence and progression in NMIBC. Notably, Acinetobacter and Anaerococcus were found to be more abundant in bladder cancer patients compared to the non-cancer group (83). Acinetobacter baumannii, known for its virulence factors, can invade epithelial cells, degrade phospholipids, and form biofilms, enabling evasion from the host immune response (84). Anaerococcus, a member of the Gram-positive anaerobic cocci, has been reported to induce inflammation and remodeling of the extracellular matrix (ECM) (88). The research work proposes that the interplay between the ECM, microbiome, and inflammation plays a crucial role in the onset, progression, and relapse of bladder cancer (16).

Conclusions

The advent of omics sciences has ushered in a new era in our comprehension of urothelial carcinoma, offering

unprecedented opportunities for advancements in diagnosis, prognosis, and treatment. The comprehensive exploration of tumor genomics, pharmacogenomics, biological therapies, proteomic biomarkers, and metabolomic and microbiomic prognostic indicators has illuminated the path towards refined strategies for managing urothelial carcinoma. Nonetheless, the complexity inherent in the heterogeneity of urothelial carcinoma necessitates the establishment of standardized assays and rigorous biomarker validation protocols. Moving forward, it is crucial for future research endeavors to concentrate on forging personalized approaches to urothelial carcinoma by integrating multifaceted omics data with clinical parameters, while simultaneously identifying novel therapeutic targets and devising innovative strategies to overcome resistance mechanisms.

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Conflicts of interest statement

Authors declare no conflict of interest.

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