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Review Article

Molecular Biology of Urothelial Carcinoma

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Abstract

Objective: Urothelial carcinoma (UC) is a common malignant tumor worldwide. Extensive genomic data analysis revealed that UC has a complex molecular character. From the perspective of cancer hallmarks reviewed the molecular biology participated in the tumorigenesis of UC. **Data Sources:** We inspected the results of multiple studies of UC focusing on the hallmarks of cancer. **Results:** UC has distinctive molecular pathways involved in sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, promotion of replicative immortality, induction of angiogenesis, activation of invasion and metastasis, genome instability and mutation, tumor-promoting inflammation, reprogramming of energy metabolism, and evasion of immune destruction. **Conclusion:** From the perspective of the hallmark of cancer, we revealed the many-sided biological behavior of UC.

Keywords: Hallmarks of cancer, molecular characterization, urothelial carcinoma

Introduction

Urothelial carcinoma (UC) is the most common tumor type arising from the urinary tract.[1] The tumor can derive from the urothelium of the upper urinary tract (renal pelvis and ureter) or the urothelium of the lower urinary tract (urinary bladder and urethra). Previous studies have shown that the biological behavior and the gene expression profiles of UCs arising from both locations are highly similar.[2,3] This finding indicates that tumorigenesis of UC from anywhere in the urinary tract employs common pathways. In developed countries, the incidence of upper urinary tract UCs (UTUCs) is less frequent than that of urinary bladder UCs (UTUBs), and the ratio of the incidence of UC in the renal pelvis, ureter, and urinary bladder is approximately 3:1:51. However, the prevalence of UTUCs is high in certain populations such as those with Balkan endemic nephropathy, Chinese herb nephropathy, or phenacetin abuse.[2,4] In Taiwan, the ratio of the incidence of UC in the renal pelvis, ureter, and urinary bladder is approximately 1:2.08:6.72. The higher incidence of UTUC in Taiwan may partly be explained by the presence of environmental pollution

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and the increase in herbal consumption.[3] Currently, the carcinogenesis of UC is still poorly understood, despite the increasing number of studies identifying the prognostic value of biomarkers.

Histologically, UC was graded as low grade or high grade according to nuclear features, pleomorphism, mitosis, and cell arrangement. Low-grade UC (LGUC) is characterized by enlarged but monotonous nuclear features, loss of polarity, and rare mitosis [Figure 1]. These cases frequently show papillary configurations and rarely develop stromal invasion. In contrast, high-grade UC (HGUC) has moderate-to-marked nuclear pleomorphism, prominent nucleoli, and frequent mitosis. The papillary structure is frequently fused, and it tends to develop into an invasive tumor. Occasionally, carcinoma *in situ* (CIS) is associated with HGUC, which shows high-grade tumor cell pagetoid spreading along the surrounding urothelium.[5] In the urinary bladder, LGUC is frequently associated with activating point mutations in the gene that encodes fibroblast growth factor receptor 3 (FGFR3).[6] For UC of the urinary bladder (UBUC), tumors that invade the proper muscle layer have a worse outcome than those that do not and thus need more aggressive treatment. Currently, many classification systems have been published and are available for molecular subtyping of UBUC. Some of them focus mainly on either nonmuscle-invasive bladder cancer (NMIBC), such as the UROLMOL[7] system, or muscle-invasive bladder cancer (MIBC), such as the Cartes d'Identité des Tumeurs-Curie,[8] University of North Carolina,[9] MD Anderson Cancer Center,[10] and Cancer Genome Atlas (TCGA)[11] systems. Other systems, such as the LUND[12] and Baylor[13] systems, can be applied to both NMIBC and MIBC. Although the complexity and taxonomy are different between each system, there are similar clusters across the different systems. For example, in MIBC, a group of cancers rich in PPARG and estrogen receptor transcription can be identified across the systems. They share similar biomarkers with luminal subtypes of breast cancer and preserved uroplakins and KRT20.[10] FGFR3 mutations can be detected in more than half of this subgroup. Histologically, tumors carrying these two molecular changes usually show papillary configurations. In addition, patients with this subtype of MIBC, the luminal-papillary (LumP) type, have a relatively good outcome.[14,15] The UROLMOL system classified NMIBC into three subclasses. Class 1 consists mostly of low-grade tumors with mutations in early cell cycle genes. Class 2 NMIBC shares similar luminal cluster genes with Class 1 tumors but has mutations in late cell cycle genes and regularly progresses to MIBC over time in high-grade tumors. Class 3 tumors show basal-like gene expression.[7] The well-characterized molecular features not only provide information on UBUC behavior but also offer crucial guidance for their treatment.

Similar to those in UBUC, FGFR3 and TP53/MDM2 gene mutations are frequent genetic events in UTUC. Low-grade tumors regularly have activating FGFR3 mutations and lack TP53 mutations. However, the TP53 mutation is more prevalent in high-grade UBUC. One-third of high-grade UBUCs have FGFR3 mutations instead of TP53 alterations and

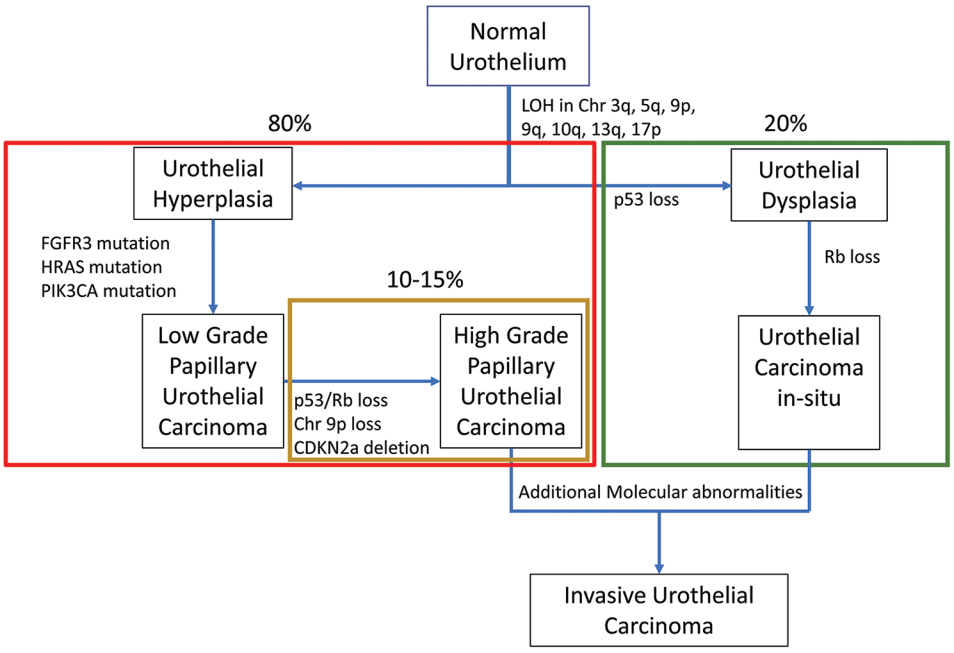

Figure 1: A brief review of bladder carcinogenesis. The process starts with loss of heterozygosity of related chromosomes in the normal bladder epithelium and gives rise to flat atypia or papillary hyperplasia. Around 80% of the lesion developed into low-grade papillary urothelial carcinoma after associated molecular aberrations occurred. A small portion (~15%) of these lesions developed into high-grade papillary urothelial carcinoma after disruption of the tumor suppressor genes. On the other hand, 20% of cases may acquire the loss of function of the p53 and developed urothelial dysplasia. The lesions may progress into urothelial carcinoma *in situ* which frequently associated with loss of expression of RB protein. High-grade papillary urothelial carcinoma and urothelial carcinoma *in situ* have a high propensity to develop into invasive urothelial carcinoma

have a relatively promising clinical outcome.[16] UTUC also has a higher prevalence of HRAS mutations and a lower prevalence of RB1 and ERBB2 mutations.[17] Somatic microsatellite instability (MSI) has been reported in UTUC. A previous study showed that DNA mismatch repair (MMR) deficiency is observed in 7% of UTUC cases.[18] The transcriptome of UTUC is less characterized, in contrast to that of UBUC, probably due to the rarity of this tumor worldwide. The first comprehensive transcriptomic analysis of UTUC was conducted by Moss *et al*. and involved 31 untreated snap-frozen tumor samples. Four clusters of UTUC were identified: cluster 1 resembled the UBUC luminal subtype; cluster 2 resembled the UBUC basal subtype, had 100% FGFR3 mutations, and low bladder recurrence; cluster 3 had 100% FGFR3 and 71% PIK3CA mutations, no TP53 alterations, and high bladder recurrence; and cluster 4 had 50% TP53 mutations, frequent high-grade, advanced-stage disease, and shorter survival. UTUC of cluster 3 and cluster 4 showed a trend of poorer overall survival.

Loss of heterozygosity is considered an early genetic event of UC development and can be found in premalignant lesions of UC, which are also known as low-grade intraurothelial neoplasia.[19] From here, the tumors progressed along one of the two different tracks of UC development. Most of them developed noninvasive papillary LGUC, which has a characteristic exophytic papillary configuration. Approximately 10%–20% of these LGUCs will transform into more aggressive HGUCs, which tend to invade the underlying stroma. A small portion of low-grade intraurothelial neoplasia progresses to high-grade intraurothelial neoplasia/CIS, which also frequently develops into invasive UC.[20,21] Recently, an analysis of high-throughput data revealed that UCs have complex genomic alterations and thus confer different biological behaviors.

The concept of hallmarks of cancer (HOCs) was introduced by Hanahan and Weinberg to provide a logical framework for understanding the remarkable diversity of neoplastic diseases.[22,23] The number of HOCs has been expanded and refined during the past two decades. To better illustrate the many facets of UCs, we will discuss the molecular biology behind the development of UCs from the perspective of the ten HOC.

Sustained Proliferative Signaling

Sustained proliferation of neoplastic cells is the fundamental feature of cancer. Increased mitogenic signaling is the key to maintaining the growth of cancer cells. Cancer can increase the signal through increased growth factor ligand production, elevating the growth receptor levels on cancer cells, altering the structure of the receptor, or modifying elements of the downstream cascade.[22,23] The past two molecular changes, considered growth factor-independent pathways, are important for the development of UC. Activating point mutations of the gene that encodes FGFR3, a tyrosine kinase receptor, are consistently observed in LGUCs. The persistent activation of FGFR3 activates the downstream mitogen-activated protein kinase and phosphoinositide 3-kinase (PI3K) pathways, which are important for the regulation of cell growth and proliferation.[24] HRAS is a member of the GTPase family and is a downstream element of the FGFR3 receptor. Mutation of the HRAS oncogene is another important genetic alteration in UCs and has a similar effect on downstream pathways as FGFR3. Their mutations are mutually exclusive.[25] Another frequent genetic alteration associated with LGUCs is mutations in the PIK3CA gene, which consistently activate the PI3K/AKT/mTOR pathway.[26] Unlike HRAS alterations, PIK3CA is associated with concurrent FGFR3 mutations. LGUC harboring PIK3CA/STAG2 mutations has a higher propensity to develop into noninvasive HGUC.[27] This finding highlighted the critical role of the PI3K/AKT/mTOR pathway in the development of HGUC. These molecular alterations are considered important signals that promote urothelial hyperplasia to LGUC.[6]

Evasion of Growth Suppressors

To benefit from the sustained growth signals, tumor cells must bypass the sturdy cell cycle to proliferate. This process is tightly regulated by the products of tumor suppressor genes.[22,23] The TP53 and RB proteins play a central role in regulating the process. In HGUC, inactivation of TP53 and RB genes is key molecular features. Zhang *et al*. showed that transgenic mice harboring the SV40T transgene developed bladder tumors mimicking human urothelial CIS and invasive UCs.[21] TP53 regulates the cell cycle by activating the transcription of the CDKN1a gene to generate the p21 protein. In HGUCs, TP53 is commonly affected by missense or loss-of-function mutations. Such mutations can be easily displayed using immunohistochemistry, as HGUC normally shows diffusely positive or negative staining results. Upon phosphorylation, RB releases E2F and promotes the expression of genes needed for the cell cycle to progress. Concurrent defects of these suppressor genes are important in promoting, but not initiating, UC invasiveness.[28] The *CDKN2A* mutation is also a frequent genetic event in UCs. Through different types of splicing, *CDKN2A* encodes the p14 and p16 proteins. The p14 protein inhibits the transcription of the MDM2 gene, which in turn prevents the degradation of TP53.[29] A previous study revealed that homozygous deletion of CDKN2A is frequently associated with FGFR3-mutated UCs and contributes to tumor aggressiveness and invasiveness.[30] The phosphatase and tensin homolog gene is another tumor suppressor gene that is also found to promote UC invasiveness.[31] We can conclude that Ras pathways are primarily involved in papillary tumor formation pathways, and genes associated with tumor suppression are more likely to confer increased aggressiveness.

Resistance to Cell Death

Apoptosis is one of the defense mechanisms to prevent the body from developing cancer.[32-34] The increased cancer-promoting signaling and DNA damage associated with hyperproliferation are stresses that trigger apoptosis. Tumor cells usually develop

strategies to bypass apoptosis throughout the tumorigenic process.[22,23] The loss of TP53 function hinders the response of apoptosis to stress.[35] Previous results showed that the Fas receptor is located on the cell surface and activates the extrinsic apoptotic pathway when binding with FasL. Invasive UCs tend to decrease Fas expression on the surface to avoid apoptosis and may release soluble Fas to neutralize FasL. UCs with these alterations usually behave more aggressively clinically.[36,37] Other apoptosis-related markers, including caspase-3, BCL-2, and survivin, also have prognostic impacts on surviving patients.[38] Autophagy is a physiological process that enables cells to break down cellular organelles and recycle them for further biosynthesis and energy metabolism.[39,40] This process is kept in a basal state for normal cells and can be activated upon exposure to stress. Cancer cells can utilize this process to survive through stresses induced during treatment.[41] Autophagic activity is upregulated in UCs. The activity is AMPK/mTOR dependent and is associated with tumor progression. Inhibiting autophagy upon cell starvation activates intrinsic apoptotic pathways.[42-44] The crosstalk between autophagy and apoptosis can be an important target for the treatment of UCs.

Promotion of Replicative Immortality

Most normal cells go through a limited number of cell growth and division cycles that are tightly regulated by telomeres.[45,46] Telomeres are composed of multiple tandem repeats of short GT-rich sequences and protect the end of chromosomes from loss of genetic materials and formation of unstable chromosome structures. The telomeres become shorter as DNA replicates and eventually lose their capping function. The shortened telomere activates DNA damage responses and triggers cell cycle arrest, and the cells enter an irreversible nonproliferative but viable senescence state. Telomerase, composed of telomerase reverse transcriptase (TERT) and a telomerase RNA component, is a specialized DNA polymerase that adds telomere repeat segments to the ends of telomeric DNA to counteract the progressive loss of telomeres. In normal nongermline tissue, telomerase activity is suppressed through the repression of TERT gene transcription.[23,47] However, telomerase activity can be detected in almost all cancers, including UCs, reflecting the fundamental requirements for cancer cells to replicate repetitively. TERT promoter mutation is an early event in UC tumorigenesis and promotes the upregulation of telomerase activity in UCs.[48,49] TERT promoter mutations can be detected in 60%–80% of UCs and do not occur in benign urothelial proliferation; thus, they can be useful diagnostic tools for the detection of UCs.[48,50] In addition to telomere lengthening, telomerase and TERT participate in various biological processes, including cell survival, apoptosis, and DNA repair.[47]

Induction of Angiogenesis

Vascular networks are crucial to maintain the delivery of nutrients and oxygen and remove waste products in tumors.[22,23] During tumorigenesis, increased metabolic activity and energy consumption create a hypoxic state within the tumor and activate the "angiogenic switch."[51] Hypoxia inducible factor 1 (HIF-1) production is increased, resulting in the activation of vascular endothelial growth factor receptor-1 (VEGF1) and VEGFR-1 transcription.[52] In UC, HIF-1a is overexpressed and is correlated with a high proliferation index, increased VEGF expression, increased microvessel density, and poor survival.[53,54] VEGF-A plays a central role in angiogenesis. This molecule is overexpressed in UBCs and likely confers tumor aggressiveness.[55,56] Interestingly, the mRNA levels of VEGF and angiopoietins are significantly higher in low-grade and low-stage UC than in their high-grade and high-stage counterparts. VEGF immunostaining also revealed a lack of "hot spot" expression in HGUCs. This evidence suggests that VEGF activity is high during the early tumorigenesis of UCs and decreases as vascular remodeling is less pronounced during the late stage.[57]

Activation of Invasion and Metastasis

The invasion and metastasis of cancer involve multistep processes.[22,23] Although many studies have disclosed regulatory molecules associated with tumor metastasis, the complex mechanism of invasion and metastasis is still unclear. Epithelial–mesenchymal transition is an important step in the beginning of this process. In UCs, loss of cell adhesion markers and an increase in mesenchymal markers are associated with invasive tumors and higher tumor grades.[58] E-cadherin is a cell–cell adhesion glycoprotein, and loss of function contributes to cancer progression, invasion, and metastasis. In UTUBs, loss of E-cadherin expression is associated with tumor recurrence and tumor progression.[59,60] Integrins are transmembrane receptors involved in cell-extracellular matrix (ECM) adhesion. The crosstalk between E-cadherin and integrin plays a role in tumor invasion and metastasis.[61] When *ITGA5*, encoded by integrin alpha 5, was knocked down, UCs showed increased E-cadherin expression, a downregulated "stemness" phenotype, and impeded tumor metastasis.[62] The "stemness" phenotype refers to the expression of stem cell markers that are normally observed in embryonic and adult stem cells. Tumor cells with this phenotype usually show self-renewal and multilineage differentiation potential and are thus cancer stem cells. UCs with "stemness" phenotypes have a higher propensity to metastasize.[63] Several stemness-related pathways have been shown to be involved in the UC metastatic process including the Hedgehog, Notch, Wnt/beta-catenin, and PI3k/Akt pathways.[64] The interaction between tumor cells and their surrounding microenvironment also determines their metastatic propensity.[65] This microniche is orchestrated by inflammatory cells, stromal cells, ECMs, and soluble components. Although many problems have been addressed, many questions still need to be answered.

Genome Instability and Mutation

The frequency of mutation in the genome is determined by the sensitivity to mutagenic agents and the integrity of

the genomic maintenance machinery. Disruption of the maintenance machinery causes widespread genome instability in human cancer. This phenomenon is instrumental for tumor progression, as evolving tumor cells can accumulate favorable genotypes for tumor survival.[23] Deficiencies in MMR systems can cause MSI, resulting in the accumulation of mutations in the short tandem repeats of DNA and tending to cause DNA mismatch errors. Inactivation of the MMR gene (MLH1, PMS2, MSH2, or MSH6) is either caused by an inherited mutation (Lynch syndrome) or sporadic events. MMR deficiency/MSI-high events are found in <1% of UCs of the urinary bladder but can occur in up to 20% of UCs of the upper urinary tract.[66,67] With advances in molecular techniques, the evaluation of tumor mutational status is currently possible. Tumors with a high tumor mutational burden (TMB) are associated with a good response to immune checkpoint inhibitor therapies.[68] This finding may be due to tumors with high TMB generating more neoantigens and thus readily being recognized by the immune system. UC has relatively high somatic mutation frequencies, along with lung cancer and melanoma.[69] The mutation frequency shows a pattern of TCW > T/C mutations (where "W" corresponds to either A or T, with C to T transitions or C to G transversions), which is consistent with the characteristic mutational signature caused by the "apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like" (APOBEC) family of cytidine deaminases.[70] The APOBEC family has 11 members and is involved in C > U deamination in single-stranded DNA. TCGA and BGI datasets showed that UCs enriched with the APOBEC mutational signature (APOBEC-high) had better overall survival than those with low or no enrichment (APOBC-low). In addition, APOBEC-low UCs tend to be seen in LGUC and are associated with Asian ethnicity. Of all members of the APOBEC family, only APOBEC3A and APOBEC3B expression is associated with UC total mutational burden. APOBEC-high UCs tend to have mutations in DNA repair genes and chromatin regulatory genes such as *TP53, NCOR1, KMT2C, KMT2A, ATR, BRCA2,* and *ARID1A*. However, APOBEC-low UCs have a more frequent mutations in FGFR3 and HRAS.[71] It is likely that increased replicative stress and disruption of DNA repair genes can promote APOBEC mutagenesis.[72-74]

Tumor-Promoting Inflammation

The infiltration of inflammatory cells in cancer is considered a sign of the immune response to eradicate the tumor.[75] Later, it was found that this tumor-associated inflammation can exert a protumoral effect on cancer. Similar to wound healing, inflammatory cells release growth signals, proangiogenic factors, and ECM-modifying enzymes into the tumor microenvironment, facilitating tumor growth.[76-79] Tumor-associated macrophages (TAMs) have been widely studied in numerous cancers. Increased TAMs have been associated with poor outcome in some cancer types (breast, head and neck, melanoma, etc.) but not the others (colorectal and stomach).[80] There are two different phenotypes of macrophages: the antitumoral activated phenotype (M1) and the protumoral immunosuppressive phenotype (M2).[81] Increased M2-like CD163+ TAMs in UCs are associated with tumor recurrence and failure of Bacillus Calmette–Guerin therapy.[82,83] *In vitro* studies showed that bladder tumor cells were able to secrete M2 polarizing cytokines and transformed macrophages.[84] Notably, interleukin (IL)-10 can induce immunosuppressive macrophages and suppress activated T-cells. In addition, IL-10 induced the expression of programmed death ligand 1 (PD-L1) on macrophages.[85]

Based on solid evidence of a tumor-promoting role for TAMs, it is worth identifying treatment strategies for TAMs.[80] Multiple studies have evaluated the value of the neutrophil-lymphocyte ratio (NLR) in predicting survival or treatment outcome in UC. A systematic review revealed that a high NLR is correlated with worse overall recurrence-free and cancer-specific survival.[86] High tumor-associated neutrophils (TANs) can be seen in UCs and are associated with advanced tumor stage. Higher levels of IL-8 and transforming growth factor (TGF)-beta are released by UCs than by normal urothelium.[87,88] IL-8 is a potent chemoattractant for neutrophils, and its circulating level correlates with the NLR in UCs.[88,89] TGF-beta is responsible for transforming neutrophils into N2 phenotypes. N2 neutrophils release high levels of arginase and matrix metalloproteinase-9, which is known to confer tumor aggressiveness.[90] Based on solid evidence that the innate immune system influences downstream adaptive immunity, building a therapeutic strategy to manipulate intratumoral innate immunity is important.

Reprogramming of Energy Metabolism

To overcome deregulated cell proliferation, tumor cells will reprogram their energy metabolism for more efficient use of fuel and oxygen. Otto Heinrich Warburg demonstrated in 1924 that cancer cells are prone to glycolysis in glucose metabolism even with the support of adequate oxygen.[91-93] To overcome the less efficient ATP production, tumor cells upregulated glucose transport GLUT1 expression to facilitate the transport of glucose into the tumor cells. In urothelial neoplasms, GLUT-1 is mostly expressed in UCs but not in benign neoplasms. In addition, the upregulation of GLUT1 positively correlated with tumor grading and staging.[94,95] The upregulation of glycolysis in UCs likely participates in the early phase of tumorigenesis. As the tumor progresses, pyruvate metabolism increases and eventually becomes the main source of energy for the tumor.[96] Lactate dehydrogenase isoform A plays a major role in maintaining glycolysis in tumor cells and converts glucose storage into lactate. The increase in lactate production in UC results in indirect modification of the tumor microenvironment and leads to tumor progression.[97] In addition to aerobic glycolysis, other metabolic pathways were also likely reprogrammed in UCs including the lipid metabolic pathway. Previous studies showed that fatty acid oxidation and fatty acid synthesis were upregulated in UCs.[97] Fatty acid synthase (FASN) is a single multienzyme complex that

catalyzes fatty acid synthesis. Previous studies have shown that FASN overexpression participates in UC tumorigenesis and triggers apoptosis when exposed to a FASN inhibitor; thus, FASN is a novel therapeutic target.[98,99] In addition, various metabolites have been identified in the urine or serum of patients and show correlations with tumor involvement and/or grading.[97,100,101] These molecules can be useful biomarkers to identify a patient with UCs or for the detection of tumor recurrence.

Evasion of Immune Destruction

Our long-standing belief is that the human body is under constant immune surveillance and that incipient cancer cells are readily removed from the system.[23] Dendritic cells (DCs) mainly present antigens to T-cells and activate them. In UCs, there is an increased number of immature DCs expressing low HLA-DR, CD80, and CD86.[102] Immature DCs fail to activate T-cells and may shift T-cells toward a regulatory (Treg) phenotype.[103] Patients with UCs also have higher levels of Th2 cytokines (IL4, Il5, and IL10) and lower levels of Th1 cytokines (IL2 and IFN-gamma) in their serum.[104] This evidence indicates that UCs evade immune destruction by shifting away from the cell-mediated response. CD8+ T-cells are important for immune-mediated tumor destruction and have been at the center of immune-oncology during the past decade. A previous study observed a favorable outcome in a subset of UCs with increased intratumoral CD8+ T-cells; these cells can be used as a predictive marker for treatment response when coupled with FoxP3+ Treg cells. Exhaustion and functional impairment of T-cells can be seen in many cancers. The dysfunctional CD8+ T-cells expressed high levels of inhibitory receptors including PD-1, TIM-3, and Lag-3. The overexpression of PD-L1 on TAMs and tumor cells induced CD8+ T-cell exhaustion when binding to PD-1, thus impairing the CD8+ T-cell antitumor response. The PD-1/PD-L1 axis is the major target of many checkpoint inhibitor anticancer drugs. Nevertheless, activated and functional CD8+ T-cells can also express high levels of PD-1, which may hamper the treatment effect of anti-PD-1.[105]

Limitations

Most of the experiments mentioned above were conducted using UBUCs. The debate of whether UBUCs and UTUCs share similar biological behavior and molecular alterations is beyond the scope of this paper. However, we would like to note that the frequency of the involved molecules or pathways can be different in these two tumors. For example, RB1 gene mutations are common in UBUCs (12.9%) but are not detected in UTUCs.[106] MSI-high is seen in <1% of UBUCs but is detected in up to 20% of UTUCs.[49] Thus, although UBUC and UTUC share many similarities, careful consideration should be given when applied to UTUC. More studies are warranted to understand the biology of UTUC.

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Conflicts of interest

There are no conflicts of interest.

References

1. Lopez-Beltran A, Sauter G, Gasser T. Infiltrating urothelial carcinoma. In: WHO Classification of Tumours of the Urinary System and Male Genital Organs. Vol. 8. Lyon, France: International Agency for Research on Cancer (IARC) Press; 2016. p. 81-98.
2. Catto JW, Yates DR, Rehman I, Azzouzi AR, Patterson J, Sibony M, *et al*. Behavior of urothelial carcinoma with respect to anatomical location. J Urol 2007;177:1715-20.
3. Zhang Z, Furge KA, Yang XJ, Teh BT, Hansel DE. Comparative gene expression profiling analysis of urothelial carcinoma of the renal pelvis and bladder. BMC Med Genomics 2010;3:58.
4. Oosterlinck W. Ureteral tumour: A specific upper urinary tract transitional cell carcinoma. Eur Urol 2007;51:1164-5.
5. Compérat EM, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, *et al*. Grading of Urothelial Carcinoma and The New "World Health Organisation Classification of Tumours of the Urinary System and Male Genital Organs 2016". Eur Urol Focus 2019;5:457-66.
6. Akhtar M, Al-Bozom IA, Ben Gashir M, Taha NM. Intrinsic molecular subclassification of urothelial carcinoma of the bladder: Are we finally there? Adv Anat Pathol 2019;26:251-6.
7. Hedegaard J, Lamy P, Nordentoft I, Algaba F, Høyer S, Ulhøi BP, *et al*. Comprehensive transcriptional analysis of early-stage urothelial carcinoma. Cancer Cell 2016;30:27-42.
8. Rebouissou S, Bernard-Pierrot I, de Reyniès A, Lepage ML, Krucker C, Chapeaublanc E, *et al*. EGFR as a potential therapeutic target for a subset of muscle-invasive bladder cancers presenting a basal-like phenotype. Sci Transl Med 2014;6:244ra91.
9. Damrauer JS, Hoadley KA, Chism DD, Fan C, Tiganelli CJ, Wobker SE, *et al*. Intrinsic subtypes of high-grade bladder cancer reflect the hallmarks of breast cancer biology. Proc Natl Acad Sci U S A 2014;111:3110-5.
10. Choi W, Porten S, Kim S, Willis D, Plimack ER, Hoffman-Censits J, *et al*. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 2014;25:152-65.
11. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, *et al*. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. Cell 2017;171:540-.56E+27.
12. Marzouka NA, Eriksson P, Rovira C, Liedberg F, Sjödahl G, Höglund M. A validation and extended description of the Lund taxonomy for urothelial carcinoma using the TCGA cohort. Sci Rep 2018;8:3737.
13. Mo Q, Nikolos F, Chen F, Tramel Z, Lee YC, Hayashi K, *et al*. Prognostic power of a tumor differentiation gene signature for bladder urothelial carcinomas. J Natl Cancer Inst 2018;110:448-59.
14. Kamoun A, de Reyniès A, Allory Y, Sjödahl G, Robertson AG, Seiler R, *et al*. A consensus molecular classification of muscle-invasive bladder cancer. Eur Urol 2020;77:420-33.
15. Smelser WW, Woolbright BL, Taylor JA 3rd. Molecular subtyping of bladder cancer: Current trends and future directions in 2019. Curr Opin Urol 2019;29:198-202.
16. Sfakianos JP, Cha EK, Iyer G, Scott SN, Zabor EC, Shah RH, *et al*. Genomic characterization of upper tract urothelial carcinoma. Eur Urol 2015;68:970-7.
17. Hassler MR, Bray F, Catto JW, Grollman AP, Hartmann A, Margulis V, *et al*. Molecular characterization of upper tract urothelial carcinoma in the era of next-generation sequencing: A systematic review of the current literature. Eur Urol 2020;78:209-20.
18. Harper HL, McKenney JK, Heald B, Stephenson A, Campbell SC, Plesec T, *et al*. Upper tract urothelial carcinomas: Frequency of association with mismatch repair protein loss and lynch syndrome. Mod Pathol 2017;30:146-56.
19. Kamat AM, Hahn NM, Efstathiou JA, Lerner SP, Malmström PU, Choi W, *et al*. Bladder cancer. Lancet 2016;388:2796-810.
20. Cheng J, Huang H, Pak J, Shapiro E, Sun TT, Cordon-Cardo C, *et al*. Allelic loss of p53 gene is associated with genesis and maintenance,

but not invasion, of mouse carcinoma *in situ* of the bladder. Cancer Res 2003;63:179-85.
21. Zhang ZT, Pak J, Shapiro E, Sun TT, Wu XR. Urothelium-specific expression of an oncogene in transgenic mice induced the formation of carcinoma *in situ* and invasive transitional cell carcinoma. Cancer Res 1999;59:3512-7.
22. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.
23. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011;144:646-74.
24. Jebar AH, Hurst CD, Tomlinson DC, Johnston C, Taylor CF, Knowles MA. FGFR3 and Ras gene mutations are mutually exclusive genetic events in urothelial cell carcinoma. Oncogene 2005;24:5218-25.
25. Boulalas I, Zaravinos A, Karyotis I, Delakas D, Spandidos DA. Activation of RAS family genes in urothelial carcinoma. J Urol 2009;181:2312-9.
26. Dueñas M, Martínez-Fernández M, García-Escudero R, Villacampa F, Marqués M, Saiz-Ladera C, *et al*. PIK3CA gene alterations in bladder cancer are frequent and associate with reduced recurrence in non-muscle invasive tumors. Mol Carcinog 2015;54:566-76.
27. Sanli O, Dobruch J, Knowles MA, Burger M, Alemozaffar M, Nielsen ME, *et al*. Bladder cancer. Nat Rev Dis Primers 2017;3:17022.
28. He F, Mo L, Zheng XY, Hu C, Lepor H, Lee EY, *et al*. Deficiency of pRb family proteins and p53 in invasive urothelial tumorigenesis. Cancer Res 2009;69:9413-21.
29. Mitra AP, Hansel DE, Cote RJ. Prognostic value of cell-cycle regulation biomarkers in bladder cancer. Semin Oncol 2012;39:524-33.
30. Rebouissou S, Hérault A, Letouzé E, Neuzillet Y, Laplanche A, Ofualuka K, *et al*. CDKN2A homozygous deletion is associated with muscle invasion in FGFR3-mutated urothelial bladder carcinoma. J Pathol 2012;227:315-24.
31. Puzio-Kuter AM, Castillo-Martin M, Kinkade CW, Wang X, Shen TH, Matos T, *et al*. Inactivation of p53 and Pten promotes invasive bladder cancer. Genes Dev 2009;23:675-80.
32. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 2007;26:1324-37.
33. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. Nature 2004;432:307-15.
34. Evan G, Littlewood T. A matter of life and cell death. Science 1998;281:1317-22.
35. Control of Apoptosis by p53 | Oncogene. Available from: https://www.nature.com/articles/1207116. [Last accessed on 2020 Nov 17].
36. Svatek RS, Herman MP, Lotan Y, Casella R, Hsieh JT, Sagalowsky AI, *et al*. Soluble Fas – A promising novel urinary marker for the detection of recurrent superficial bladder cancer. Cancer 2006;106:1701-7.
37. Yamana K, Bilim V, Hara N, Kasahara T, Itoi T, Maruyama R, *et al*. Prognostic impact of FAS/CD95/APO-1 in urothelial cancers: Decreased expression of Fas is associated with disease progression. Br J Cancer 2005;93:544-51.
38. Karam JA, Lotan Y, Karakiewicz PI, Ashfaq R, Sagalowsky AI, Roehrborn CG, *et al*. Use of combined apoptosis biomarkers for prediction of bladder cancer recurrence and mortality after radical cystectomy. Lancet Oncol 2007;8:128-36.
39. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132:27-42.
40. Mizushima N. Autophagy: Process and function. Genes Dev 2007;21:2861-73.
41. Chandrasekar T, Evans CP. Autophagy and urothelial carcinoma of the bladder: A review. Investig Clin Urol 2016;57 Suppl 1:S89-97.
42. Ojha R, Singh SK, Bhattacharyya S, Dhanda RS, Rakha A, Mandal AK, *et al*. Inhibition of grade dependent autophagy in urothelial carcinoma increases cell death under nutritional limiting condition and potentiates the cytotoxicity of chemotherapeutic agent. J Urol 2014;191:1889-98.
43. Lin YC, Lin JF, Wen SI, Yang SC, Tsai TF, Chen HE, *et al*. Inhibition of high basal level of autophagy induces apoptosis in human bladder cancer cells. J Urol 2016;195:1126-35.
44. Sivridis E, Koukourakis MI, Mendrinos SE, Touloupidis S, Giatromanolaki A. Patterns of autophagy in urothelial cell carcinomas – The significance of "stone-like" structures (SLS) in transurethral resection biopsies. Urol Oncol 2013;31:1254-60.
45. Blasco MA. Telomeres and human disease: Ageing, cancer and beyond. Nat Rev Genet 2005;6:611-22.
46. Shay JW, Wright WE. Hayflick, his limit, and cellular ageing. Nat Rev Mol Cell Biol 2000;1:72-6.
47. Günes C, Wezel F, Southgate J, Bolenz C. Implications of TERT promoter mutations and telomerase activity in urothelial carcinogenesis. Nat Rev Urol 2018;15:386-93.
48. Kinde I, Munari E, Faraj SF, Hruban RH, Schoenberg M, Bivalacqua T, *et al*. TERT promoter mutations occur early in urothelial neoplasia and are biomarkers of early disease and disease recurrence in urine. Cancer Res 2013;73:7162-7.
49. Warrick JI, Knowles MA, Yves A, van der Kwast T, Grignon DJ, Kristiansen G, *et al*. Report from the International Society of Urological Pathology (ISUP) Consultation Conference on Molecular Pathology of Urogenital Cancers. II. Molecular Pathology of Bladder Cancer: Progress and Challenges. Am J Surg Pathol 2020;44:e30-46.
50. Amin MB, Trpkov K, Lopez-Beltran A, Grignon D, Members of the ISUP Immunohistochemistry in Diagnostic Urologic Pathology Group. Best practices recommendations in the application of immunohistochemistry in the bladder lesions: Report from the International Society of Urologic Pathology consensus conference. Am J Surg Pathol 2014;38:e20-34.
51. Liao D, Johnson RS. Hypoxia: A key regulator of angiogenesis in cancer. Cancer Metastasis Rev 2007;26:281-90.
52. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling-In control of vascular function. Nat Rev Mol Cell Biol 2006;7:359-71.
53. Chai CY, Chen WT, Hung WC, Kang WY, Huang YC, Su YC, *et al*. Hypoxia-inducible factor-1α expression correlates with focal macrophage infiltration, angiogenesis and unfavourable prognosis in urothelial carcinoma. J Clin Pathol 2008;61:658-64.
54. Deniz H, Karakök M, Yagci F, Güldür ME. Evaluation of relationship between HIF-1alpha immunoreactivity and stage, grade, angiogenic profile and proliferative index in bladder urothelial carcinomas. Int Urol Nephrol 2010;42:103-7.
55. Donmez G, Sullu Y, Baris S, Yildiz L, Aydin O, Karagoz F, *et al*. Vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and thrombospondin-1 (TSP-1) expression in urothelial carcinomas. Pathol Res Pract 2009;205:854-7.
56. Fauconnet S, Bernardini S, Lascombe I, Boiteux G, Clairotte A, Monnien F, *et al*. Expression analysis of VEGF-A and VEGF-B: Relationship with clinicopathological parameters in bladder cancer. Oncol Rep 2009;21:1495-504.
57. Quentin T, Schlott T, Korabiowska M, Käthei N, Zöller G, Glaser F, *et al*. Alteration of the vascular endothelial growth factor and angiopoietins-1 and -2 pathways in transitional cell carcinomas of the urinary bladder associated with tumor progression. Anticancer Res 2004;24:2745-56.
58. Baumgart E, Cohen MS, Silva Neto B, Jacobs MA, Wotkowicz C, Rieger-Christ KM, *et al*. Identification and prognostic significance of an epithelial-mesenchymal transition expression profile in human bladder tumors. Clin Cancer Res 2007;13:1685-94.
59. Breyer J, Gierth M, Shalekenov S, Aziz A, Schäfer J, Burger M, *et al*. Epithelial-mesenchymal transformation markers E-cadherin and survivin predict progression of stage pTa urothelial bladder carcinoma. World J Urol 2016;34:709-16.
60. Muramaki M, Miyake H, Terakawa T, Kumano M, Sakai I, Fujisawa M. Expression profile of E-cadherin and N-cadherin in non-muscle-invasive bladder cancer as a novel predictor of intravesical recurrence following transurethral resection. Urol Oncol 2012;30:161-6.
61. Canel M, Serrels A, Frame MC, Brunton VG. E-cadherin-integrin crosstalk in cancer invasion and metastasis. J Cell Sci 2013;126:393-401.
62. van der Horst G, Bos L, van der Mark M, Cheung H, Heckmann B, Clément-Lacroix P, *et al*. Targeting of alpha-v integrins reduces malignancy of bladder carcinoma. PLoS One 2014;9:e108464.
63. Kobayashi T. Understanding the biology of urothelial cancer metastasis. Asian J Urol 2016;3:211-22.
64. Zhao W, Li Y, Zhang X. Stemness-related markers in cancer. Cancer Transl Med 2017;3:87-95.
65. Lorusso G, Rüegg C. The tumor microenvironment and its contribution to tumor evolution toward metastasis. Histochem Cell Biol 2008;130:1091-103.
66. Kang TW, Lee JG, Jung SI, Choi YD, Choi C, Kwon DD, *et al*. A study

of microsatellite instability of upper urinary tract transitional cell carcinoma. Korean J Urol 2006;47:1269-77.
67. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ, *et al*. Landscape of microsatellite instability across 39 cancer types. JCO Precis Oncol 2017;2017:PO.17.00073.
68. Steuer CE, Ramalingam SS. Tumor mutation burden: Leading immunotherapy to the era of precision medicine? J Clin Oncol 2018;36:631-2.
69. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, *et al*. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013;499:214-8.
70. Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, *et al*. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. Nat Genet 2013;45:970-6.
71. Glaser AP, Fantini D, Wang Y, Yu Y, Rimar KJ, Podojil JR, *et al*. APOBEC-mediated mutagenesis in urothelial carcinoma is associated with improved survival, mutations in DNA damage response genes, and immune response. Oncotarget 2018;9:4537-48.
72. Hoopes JI, Cortez LM, Mertz TM, Malc EP, Mieczkowski PA, Roberts SA. APOBEC3A and APOBEC3B preferentially deaminate the lagging strand template during DNA replication. Cell Rep 2016;14:1273-82.
73. Middlebrooks CD, Banday AR, Matsuda K, Udquim KI, Onabajo OO, Paquin A, *et al*. Association of germline variants in the APOBEC3 region with cancer risk and enrichment with APOBEC-signature mutations in tumors. Nat Genet 2016;48:1330-8.
74. Cescon DW, Haibe-Kains B. DNA replication stress: A source of APOBEC3B expression in breast cancer. Genome Biol 2016;17:202.
75. Dvorak HF. Tumors: Wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650-9.
76. DeNardo DG, Andreu P, Coussens LM. Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. Cancer Metastasis Rev 2010;29:309-16.
77. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010;140:883-99.
78. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell 2010;141:39-51.
79. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: Links to genetic instability. Carcinogenesis 2009;30:1073-81.
80. Cortese N, Carriero R, Laghi L, Mantovani A, Marchesi F. Prognostic significance of tumor-associated macrophages: Past, present and future. Semin Immunol 2020;48:101408.
81. Sica A, Mantovani A. Macrophage plasticity and polarization: *In vivo* veritas. J Clin Invest 2012;122:787-95.
82. Suriano F, Santini D, Perrone G, Amato M, Vincenzi B, Tonini G, *et al*. Tumor associated macrophages polarization dictates the efficacy of BCG instillation in non-muscle invasive urothelial bladder cancer. J Exp Clin Cancer Res 2013;32:87.
83. Lima L, Oliveira D, Tavares A, Amaro T, Cruz R, Oliveira MJ, *et al*. The predominance of M2-polarized macrophages in the stroma of low-hypoxic bladder tumors is associated with BCG immunotherapy failure. Urol Oncol 2014;32:449-57.
84. Joseph M, Enting D. Immune responses in bladder cancer-role of immune cell populations, prognostic factors and therapeutic implications. Front Oncol 2019;9:1270.
85. Wang X, Ni S, Chen Q, Ma L, Jiao Z, Wang C, *et al*. Bladder cancer cells induce immunosuppression of T cells by supporting PD-L1 expression in tumour macrophages partially through interleukin 10. Cell Biol Int 2017;41:177-86.
86. Marchioni M, Primiceri G, Ingrosso M, Filograna R, Castellan P, De Francesco P, *et al*. The clinical use of the neutrophil to lymphocyte ratio (NLR) in urothelial cancer: A systematic review. Clin Genitourin Cancer 2016;14:473-84.
87. Miyamoto H, Kubota Y, Shuin T, Torigoe S, Dobashi Y, Hosaka M. Expression of transforming growth factor-beta 1 in human bladder cancer. Cancer 1995;75:2565-70.
88. Inoue K, Slaton JW, Kim SJ, Perrotte P, Eve BY, Bar-Eli M, *et al*. Interleukin 8 expression regulates tumorigenicity and metastasis in human bladder cancer. Cancer Res 2000;60:2290-9.
89. Morizawa Y, Miyake M, Shimada K, Hori S, Tatsumi Y, Nakai Y, *et al*. Correlation of immune cells and cytokines in the tumor microenvironment with elevated neutrophil-to-lymphocyte ratio in blood: An analysis of muscle-invasive bladder cancer. Cancer Invest 2018;36:395-405.
90. Shaul ME, Fridlender ZG. Cancer-related circulating and tumor-associated neutrophils - subtypes, sources and function. FEBS J 2018;285:4316-42.
91. Warburg O. On the origin of cancer cells. Science 1956;123:309-14.
92. Weinhouse S. On respiratory impairment in cancer cells. Science 1956;124:267-9.
93. Warburg OH. The metabolism of tumours: Investigations from the Kaiser Wilhelm Institute for Biology, Berlin-Dahlem. JAMA 1931;96:1982.
94. Chang S, Lee S, Lee C, Kim JI, Kim Y. Expression of the human erythrocyte glucose transporter in transitional cell carcinoma of the bladder. Urology 2000;55:448-52.
95. Lee JH, Kim YW, Chang SG. Glucose transporter-1 expression in urothelial papilloma of the bladder. Urol Int 2005;74:268-71.
96. Conde VR, Oliveira PF, Nunes AR, Rocha CS, Ramalhosa E, Pereira JA, *et al*. The progression from a lower to a higher invasive stage of bladder cancer is associated with severe alterations in glucose and pyruvate metabolism. Exp Cell Res 2015;335:91-8.
97. Massari F, Ciccarese C, Santoni M, Iacovelli R, Mazzucchelli R, Piva F, *et al*. Metabolic phenotype of bladder cancer. Cancer Treat Rev 2016;45:46-57.
98. Jiang B, Li EH, Lu YY, Jiang Q, Cui D, Jing YF, *et al*. Inhibition of fatty-acid synthase suppresses P-AKT and induces apoptosis in bladder cancer. Urology 2012;80:484.e9-15.
99. Zheng SS, Gao JG, Liu ZJ, Zhang XH, Wu S, Weng BW, *et al*. Downregulation of fatty acid synthase complex suppresses cell migration by targeting phosphor-AKT in bladder cancer. Mol Med Rep 2016;13:1845-50.
100. Wittmann BM, Stirdivant SM, Mitchell MW, Wulff JE, McDunn JE, Li Z, *et al*. Bladder cancer biomarker discovery using global metabolomic profiling of urine. PLoS One 2014;9:e115870.
101. Bansal N, Gupta A, Sankhwar SN, Mahdi AA. Low- and high-grade bladder cancer appraisal via serum-based proteomics approach. Clin Chim Acta 2014;436:97-103.
102. Xiu W, Ma J, Lei T, Zhang M. AG490 reverses phenotypic alteration of dendritic cells by bladder cancer cells. Oncol Lett 2018;16:2851-6.
103. Carrascal MA, Severino PF, Guadalupe Cabral M, Silva M, Ferreira JA, Calais F, *et al*. Sialyl Tn-expressing bladder cancer cells induce a tolerogenic phenotype in innate and adaptive immune cells. Mol Oncol 2014;8:753-65.
104. Satyam A, Singh P, Badjatia N, Seth A, Sharma A. A disproportion of TH1/TH2 cytokines with predominance of TH2, in urothelial carcinoma of bladder. Urol Oncol 2011;29:58-65.
105. Zhang Z, Liu S, Zhang B, Qiao L, Zhang Y, Zhang Y. T Cell Dysfunction and Exhaustion in Cancer. Front Cell Dev Biol 2020;8:17.
106. Lee JY, Kim K, Sung HH, Jeon HG, Jeong BC, Seo SI, *et al*. Molecular Characterization of Urothelial Carcinoma of the Bladder and Upper Urinary Tract. Transl Oncol 2018;11:37-42.