



REVIEW OF THE BLADDER CANCER MOLECULAR CLASSIFICATION PROPOSED: A NEW ERA – NEW TAXONOMY

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SUMMARY – The management of bladder cancer patients largely depends on pathologic staging and grading, and current morphological classification does not always show the individual patient's risk. Despite modern surgical techniques, pre- and postoperative therapies, clinical outcomes of these patients have not changed over decades. Today, there are new biomarkers for bladder cancer showing changes in tumor biology and progression, as a result of changes in the pathways affecting cell signaling, proliferation, apoptosis, epigenetic changes, angiogenesis, and modulation of host immune response. Assessment of multiple biomarkers associated with those pathways offers new understanding of tumor behavior while identifying important panels of predicting patient management and outcomes. In this review, the most important molecules and basics of the novel molecular classification of bladder cancer are presented.

Key words: *Bladder cancer; Molecular classification*

Introduction

Bladder cancer is the most common malignancy involving the urinary system. Different predictive calculations evaluating cancer risk progression include tumor morphological subtype, grade and stage, presence of carcinoma *in situ*, vascular invasion, previous recurrence rate, tumor diameter, and number of tumors¹. These are important for therapy and prognosis, as well as for active patient follow up²⁻⁴. In routine, these findings are subjective and depend on a good trained pathologist, which can largely vary in small

centers where pathologist cannot subspecialize in one field⁵. There is also the requirement of high-quality material necessary for histopathologic evaluation, as well as good sampling due to huge heterogeneity of bladder cancer⁶.

The currently valid and still worldwide used morphological classification of urothelial cancer, according to the World Health Organization urogenital tumor classification¹, is loudly suggested to be replaced with new molecular taxonomy. Bladder cancer harbors chromosomal aberrations such as aneusomies, amplifications and deletions, which affect almost all chromosomes (Figs. 1 and 2)⁷. Important and intermittent genetic alterations have been identified in the TP53, FGFR3, PIK3CA and RB1 genes. Bladder cancer also frequently harbors somatic TERT promoter mutations, which occur early in the process of bladder carcinogenesis. Nevertheless, few pathways have

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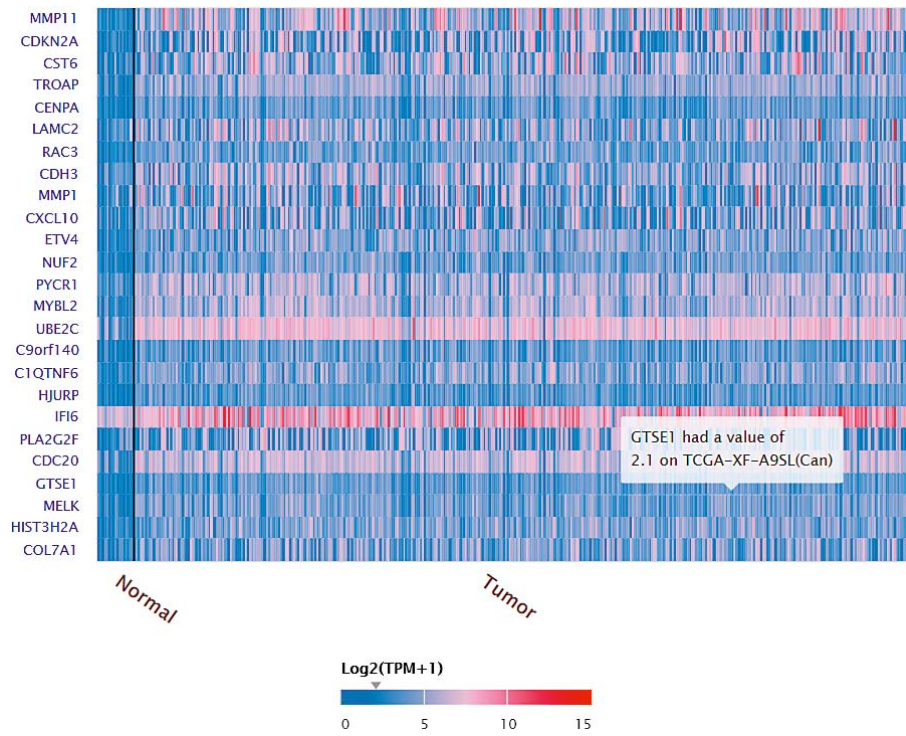


Fig. 1. The most common overexpressed genes in bladder urothelial carcinoma.

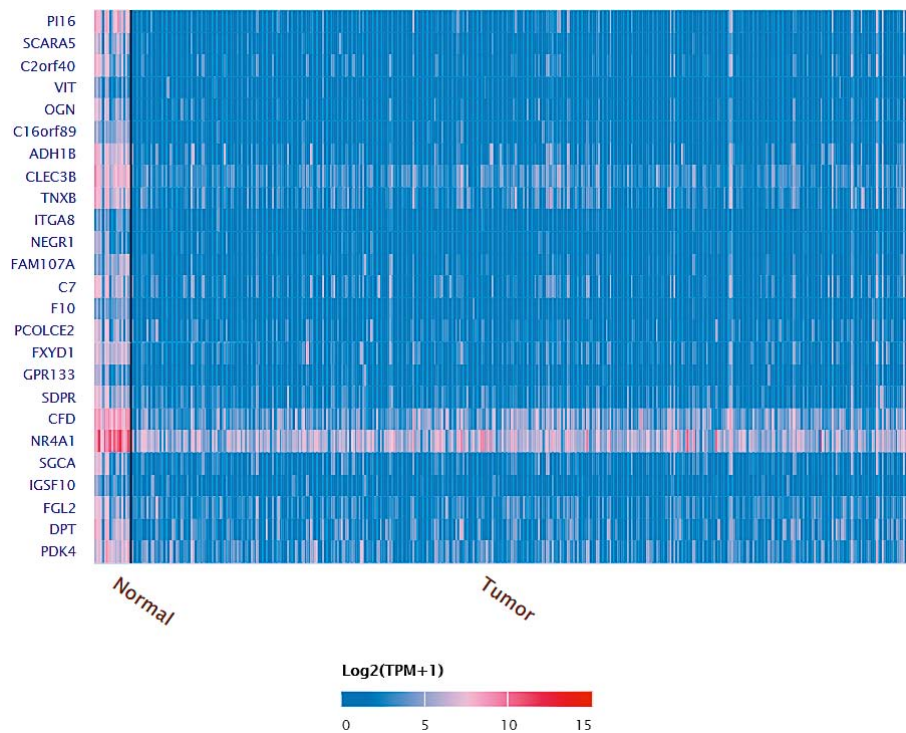


Fig. 2. The most common underexpressed genes in bladder urothelial carcinoma.

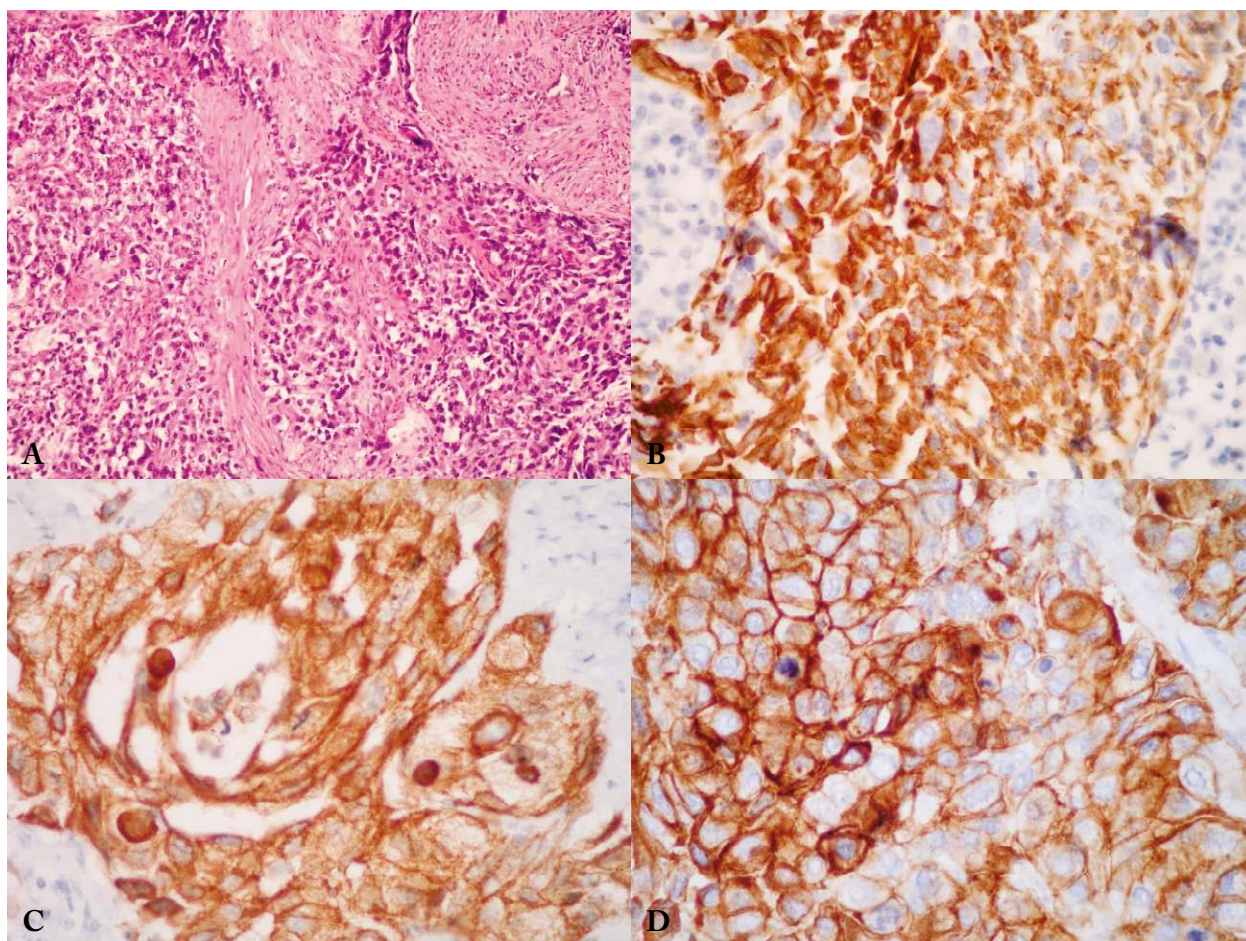


Fig. 3. High grade invasive urothelial cancer with 'basal-like' immunohistochemistry phenotype (HE, $\times 100$) (A); CK7 ($\times 400$) (B); CK5/6 ($\times 400$) (C); CD44 ($\times 400$) (D).

been identified as the leading courses of bladder carcinogenesis and simple introduction into routine work is possible while it can be determined by the use of immunohistochemistry, which is nowadays an integral part of most pathologic laboratories².

Discussion

During the last decade, many facts have emerged in the field of genetic and epigenetic bladder carcinogenesis. Those data support the existence of two main pathways for bladder cancer, and roughly, it can be translated into non-muscle-invasive and muscle-invasive cancer¹. Most patients with newly diagnosed bladder cancer present with a non-muscle-invasive bladder cancer (NMIBC); they comprise up to 80% of urothelial cancer cases, include low- and high-grade papillary

noninvasive and superficially invasive tumors (pTa and pT1), and they mostly reveal modifications in fibroblast growth factor receptor 3 (FGFR3), PI3-kinase catalytic subunit α (PIK3CA) and GTPase HRAS (HRAS). They show mutations in the genes for chromatin-modifying enzymes (H3K27, KDM6A) as well. Those tumors usually show low mitotic and proliferation activity (low Ki-67/MIB-1 index). NMIBC tumors have frequent recurrences and very low mortality, with up to 20% of tumors progressing into muscle-invasive tumors (MI)^{1,2,8}.

Muscle-invasive tumors (pT2, pT3) mostly develop from flat urothelial lesions, i.e. carcinoma *in situ* (CIS) and may express papillary or non-papillary phenotype. They reveal p53, RB1 and gene cyclin-dependent kinase inhibitor 2A (CDKN2A) modifications and genomic instability, as well as high mitotic and

proliferation activity (high Ki-67/MIB-1 index). They show mutations in the genes for chromatin-modifying enzymes as well, but mostly for H3K4 and KMT2D. MI tumors are aggressive tumors with a high mortality rate^{7,8}.

Van Rhijn *et al.*⁹ describe so called 'molecular grade' for NMI tumors, a prognostic variable based on FGFR3 mutation status and proliferation index due to favorable outcome of tumors with FGFR3 overexpression. Mutated FGF3 activates RAS pathway, but a small percentage of NMI bladder cancers harbor RAS mutation without FGFR mutation.

One of the first prognostic markers important in the prognosis of MI and NMI tumors was TP53, which is mutated in most of the MI bladder cancers and can coexist with RB1 inactivation.

The PIK3/AKT/MTOR pathway mutations are described in the subset of MI cancers, and epigenetic alterations were found to be good predictive markers for progression and survival^{7,9,10}.

Gene expression profiling showed different molecular pathways, as well as genetic mutations and epigenetic modifications at a high extent, and according to different groups of authors, it is shown that urothelial carcinoma may be divided into two major molecular groups using simple immunohistochemistry staining, luminal and basal^{2,11,12}.

Basal types (or squamous-like subgroup) (Fig. 3) show p63, CK5/6, EGFR and CD44 expression and are negative for CK20 immunostaining (expression of basal markers and downregulation of urothelial differentiation markers; KRT5/6+ KRT14+ FOXA1-GATA3- phenotype). This group is associated with worse prognosis but better response to neoadjuvant chemotherapy. The third of the MI and a small subset of NMI bladder cancers are classified in the basal subtype group. Luminal type is related to FGFR3 mutation and expression of estrogen receptor, ERBB2 and PPAR γ . It can be divided in two subgroups: urothelial-like, with urothelial differentiation, harboring alterations in the FGFR3-pathway; most of the NMI bladder tumors are part of this group. Second is the luminal subtype: genomic unstable/infiltrated/p53-like has expression of urothelial differentiation markers but not related to the FGFR3-pathway and is found to be sensitive to PD-L1 inhibitor therapy and resistant to neoadjuvant therapy. A type (claudin low) which is described in relation to epithelial-mesenchymal transi-

tion (EMT) with rich lymphocytic reaction, as well as a neuronal subtype expressing neuroendocrine genes and mutation of TP53 and RB-1 are described in MI bladder cancer and comprise a small subset of these tumors^{2,10-14}. In some types, the immune-related subgroups were described, with high expression of immune genes and variable expression of EMT genes¹⁴⁻¹⁶.

Some of the subtypes described are related to characteristic morphological and immunohistochemical phenotype, although additional studies are required.

Due to the huge mutation burden and heterogeneity of bladder cancer, therapy of MI tumors has remained the same for decades; platinum-based combination chemotherapy is the first-line treatment for patients with metastatic MI urothelial cancer. In the previously described molecular classification, the basal-like subtype is a candidate for neoadjuvant chemotherapy. For long time, second-line therapy was not standardized and in recent years several agents and strategies, immune checkpoint inhibitors targeting PD-1/PDL-1 have been developed and showed significant therapeutic response in metastatic urothelial cancer^{17,18}.

Conclusion

Increasing knowledge on cancer genomics is changing the diagnostic and therapeutic approach to genitourinary (GU) cancers. Immunotherapy is gradually becoming a key factor in the therapeutic algorithm for patients with GU cancers at different stages of the disease. Current knowledge about genomic GU cancer supports creating patient groups for targeted therapies. Robust and reliable biomarkers are crucial in this process, but recent insights indicate that a single biomarker for patient selection may not be feasible, given that tumor progression, as well as immune responses are dynamic processes and evolve over time.

Patients need unique treatment and controlled modalities, however, histopathologic finding determined by morphological features is still necessary for clinical intervention and therapy decisions. Molecular classification shows some overlap with pathologic grade and stage, and the molecular taxonomy proposed is related to different treatment possibilities, but additional studies are needed to propose definitive classification criteria.

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Sažetak

PREGLED NOVOPREDLOŽENE MOLEKULARNE KLASIFIKACIJE KARCINOMA
MOKRAĆNOG MJEHURA: NOVA ERA – NOVA TAKSONOMIJA*Š. Šoipi, M. Vučić, B. Spajić, B. Krušlin, M. Tomić i M. Ulamec*

Liječenje karcinoma mokraćnog mjehura uvelike ovisi o patohistološkom tipu, gradusu i stadiju tumora, a današnja morfološka klasifikacija ne može u potpunosti odrediti individualne potrebe i procjenu rizika za pojedinačne bolesnike. Unatoč modernim kirurškim tehnikama i novijim terapijskim mogućnostima klinički ishod bolesnika s tumorom mokraćnog mjehura u posljednjih nekoliko desetljeća nije se promijenio. Danas su poznati brojni biološki biljezi koji se odnose na karcinom mokraćnog mjehura, a pokazuju promjene u biologiji i progresiji tumora te su rezultat različitih promjena u signalnim putevima stanice, proliferaciji, apoptozi, epigenetskim promjenama, angiogenezi ili modulaciji imunog odgovora domaćina. Procjena više bioloških biljega povezanih s navedenim putevima omogućuje prikaz ponašanja tumora, jer identificira važne panele predviđanja terapije i ishoda. U ovom prikazu stavlja se naglasak na najvažnije molekule koje čine osnovu novopredložene molekularne klasifikacije karcinoma mokraćnog mjehura

Ključne riječi: *Karcinom mokraćnog mjehura; Molekularna klasifikacija*