

REVIEW

The Era of Molecular Taxonomy of Neoplasms of the Urinary Tract

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ABSTRACT

Urothelial carcinoma of the bladder is a common malignancy causing an estimated 150,000 deaths per year. Treatment for muscle-invasive bladder cancer has not advanced beyond cisplatin-based combination chemotherapy and surgery in the past 30 years and no new drugs for the disease have been approved during that time. In contrast to other malignancies, molecular diagnostics are not established yet for routine clinical management for patients with urothelial cancer of the bladder. Studies have identified gene expression patterns that classify tumors into clinically relevant molecular subtypes. These distinct molecular profiles may help define subsets of patients who are expected to respond positively to chemotherapy and to molecularly targeted therapy. In the current review, two molecular taxonomies of bladder cancer are investigated.

INTRODUCTION

Urothelial carcinoma of the bladder is a major cause of morbidity and mortality worldwide. It is the seventh most common cancer with an estimated global incidence of 330,380 new cases in 2012 causing an estimated 150,000 deaths per year.^{1,2} Bladder cancer is 3 to 4 times more common in males than females. The median patient age at diagnosis is 65-70 years. Approximately 70-80% of patients with newly diagnosed bladder cancer present with nonmuscle invasive or early invasive disease. With these types of tumors, recurrence is common (50-70% of cases), but progression occurs only in 15-25% of the cases.³ Treatment for muscle-invasive bladder cancer has not advanced beyond cisplatin-based combination chemotherapy and surgery in the past 30 years and no new drugs for the disease have been approved during that time.⁴ A new molecular approach to the diagnosis and treatment of bladder cancer is hence required. Molecular taxonomy of bladder cancer is a step forward to better managing patients with this disease. In the current review, two molecular taxonomies of bladder cancer are investigated.

MOLECULAR PATHWAYS

Of all the adult malignancies studied, urothelial bladder carcinoma displays the

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third largest number of DNA alterations, slightly fewer than lung cancer and melanoma.⁵ Molecular genetic evidence supports the existence of two distinct pathogenetic pathways for bladder cancer development, corresponding to two distinct biological and clinical phenotypes; nonmuscle invasive and muscle-invasive urothelial carcinoma. Disruption of the P13K-AKT-mTOR pathway and alterations in the tyrosine kinase receptor gene *FGFR3* and the oncogene *HRAS* are associated with non-muscle-invasive bladder cancer. The main genetic alterations underlying muscle-invasive bladder cancer involve tumor suppressor genes which encode proteins that regulate cell cycle and apoptosis pathways, including *TP53*, *CDKN2A*, *CCND1*, *CDKN1B* and *RB1*.⁶

MOLECULAR TAXONOMY

Studies have identified gene expression patterns that classify tumors according to major pathological characteristics that have defined clinically relevant molecular subtypes of bladder cancer. These subtypes show distinct molecular profiles that differ in survival rates and may help to define subsets of patients who are expected to respond positively to chemotherapy, while sparing others from unnecessary therapy-related toxicity.

The Sjobahl et al study from Lund University has defined 5 major molecular subtypes of urothelial cell carcinoma: Urobasal A, Genomically Unstable, Urobasal B, SCC-like and Infiltrated.⁷ These five molecular subtypes were defined by distinct gene expression signatures specific for cell cycle, cytokeratins, cell adhesion, receptor tyrosine kinases and immune response. Tissue microarrays were constructed and stained with 13 antibodies to detect these subtypes also on the protein level.

The majority of Urobasal A tumors was nonmuscle invasive of low pathological grade and showed very good prognosis. They were characterized by elevated gene expression of *FGFR3*, *CCND1*, and *TP63*, as well as cytokeratin-5. This subtype showed activity of cell-cycle genes operating before the cell-cycle restriction point, indicating a phenotype reminiscent of undifferentiated urothelial cells. These tumors have also retained most cell adhesion genes. Most Genomically Unstable molecular subtype tumors were of high grade and as close as 40% were muscle-invasive. They were characterized by frequent *TP53* mutations, *CCNE* and *ERBB2* expression. In contrast to the Urobasal A tumors, Genomically Unstable tumors showed increased activity of late cell-cycle genes, for example *CCNA*, *CCNB* and *CDC20*. A major difference between the Urobasal A and the Genomically Unstable subtype was that the latter did not show expression of the basal/intermediate cytokeratins but rather of cytokeratin-20 associated with umbrella cells. The SCC-like subtype was characterized by high expression of basal cytokeratins normally not expressed in the urothelium, *CK4*, *CK6A*, *CK6B*, *CK6C*,

CK14 and *CK16*, as well as by bad prognosis. The Urobasal B tumors showed several similarities to the Urobasal A tumors, such as high *FGFR3* mutation frequency, elevated *FGFR3*, *CCND1*, *TP63* levels and expression of the *FGFR3* signature. This group however showed frequent *TP53* mutations and expression of several cytokeratins, specific for the SCC-like subtype. In addition, 50% of these cases were muscle-invasive. The Infiltrated subtype most likely represents a heterogeneous class of tumors. This subtype's special characteristic was a very strong immunologic and extracellular-matrix (ECM) signal, indicating the presence of tumor-infiltrating cells such as T-lymphocytes and myofibroblast cells.

This study has also investigated the relation between pathologic stage (Tis, Ta, T1, T2, T3 and T4), pathologic grade (G1, G2, G3) and molecular classification with interesting results. The molecular phenotype was irrelevant to pathologic stage and grade, emphasizing the molecular subtypes as intrinsic properties of the tumor. This proves that the molecular subtyping can be implemented synergistically with pathologic classification, providing additional information and differentiating the tumors even further.

Another major study of Choi et al has discovered three molecular subtypes of muscle-invasive bladder cancer that resembled established molecular subtypes of breast cancer: Basal, Luminal and p53-like.⁸ The Basal-like subtype is characterized by p63 activation, squamous differentiation, *CK5/6* positivity, *EGFR* and *CD44* expression as well as lack of *CK20*. This subtype is clinically aggressive but potentially sensitive to neoadjuvant chemotherapy. The Luminal-like subtype was typically enriched for activating *FGFR3* mutations, active estrogen receptor pathway and an *ERBB2* and *PPARγ* expression profile (all potential targets of therapy). The third molecular subtype discovered by Choi et al was characterized by a wildtype *TP53* gene expression signature. These p53-like muscle-invasive bladder tumors were consistently resistant to neoadjuvant methotrexate, vinblastine, doxorubicin and cisplatin chemotherapy. Interestingly, tumors of the luminal and basal-like subtypes also displayed a wildtype *TP53* expression signature upon resistance to chemotherapy.

Similarities can be extracted by comparing the two molecular classifications mentioned above. All the SCC-like subtype tumors described by Sjobahl et al corresponded to the Basal-like subtype described by Choi et al.^{7,8} The p53-like subtype described by Choi et al and the Infiltrated subtype described by Sjobahl et al were enriched with extracellular matrix biomarkers and all Urobasal A tumors were confined to Luminal-like subtype.^{7,8}

Lately there has been huge research and clinical interest in immunotherapy. Cancers with a high rate of somatic mutations such as urothelial bladder cancer appear to respond well to anti-PDL1 therapy. Further work will help to clarify the relationship between mutational frequency and response to anti-PDL1 immunotherapy.⁹

**CONCLUSION - THE ROLE
OF MOLECULAR TAXONOMY**

As our understanding of the complex molecular mechanisms involved in bladder cancer development deepens, our approach to the diagnosis and management of this disease continues to evolve. Molecular subtyping of bladder cancer adds valuable additional information to current pathologic staging and grading providing also many novel potential targets of therapy. Next-generation sequencing technology will make molecular taxonomy of bladder cancer even more accessible in everyday practice providing the much-needed progress in the management of patients with this disease.

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