The Molecular Pathology of Bladder Carcinoma and Future Perspectives.

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ABSTRACT:

Molecular diagnostics applications are now an integral part of the management algorithms of several solid tumors such as breast, colon, and lung. In stark contrast, the current clinical management of urologic malignancies is lagging behind. Clinically robust molecular tests that can identify patients that are more likely to respond to a given targeted agent or even those in need of a more aggressive treatment based on well-validated molecular prognosticators are still lacking. Several promising biomarkers for detection, prognosis, and targeted therapeutics are now under evaluation. The following review discusses some of the candidate biomarkers that may soon make their transition to clinical assays in bladder cancer patients.

Superficial and muscle invasive urothelial carcinoma (URCa) of the bladder display two distinct clinical phenotypes in regards to biologic behavior and prognosis. Increasingly, molecular evidence supporting two divergent pathways of pathogenesis for superficial and invasive disease is accumulating. Superficial URCa is thought to originate from benign urothelium through hyperplasia with only a small contribution (10-15%) to the pool of high grade non-invasive and subsequently invasive URCa. The majority of invasive tumors appear to originate through progression from dysplasia to flat CIS and high grade non invasive URCa where genetic instability lead to accumulation of genetic alterations promoting progression to invasive lesions.

Clinically, a significant proportion of superficial tumors (pTa and pT1) are deemed to recur following TURB with only a minority of cases enduring progression to higher-grade, deeply invasive aggressive tumors.

The superficial URCa pathogenesis pathway is primarily based on alterations in the tyrosine kinase receptor FGFR-3 and H-RAS oncogene. The pathogenic pathway for muscle invasive URCa primarily involves alterations in tumor suppressor genes p53, p16 and Rb.
Figure 1: Divergent molecular pathways of oncogenesis in superficial and muscle invasive urothelial carcinoma of urinary bladder. Genetic alterations are depicted in key stages of disease progression.
As illustrated in figure 1, p53 and Rb alterations are also needed for the progression of a subset of superficial lesions that lead to higher grade muscle invasive URCa.

The currently established clinicopathologic prognostic parameters in superficial lesions include: pTNM stage, WHO/ISUP grade, size of tumor, disease multifocality, presence of CIS and frequency and rate of prior recurrences. Prognostic parameters that can accurately predict the subset of superficial tumors that will progress are actively sought in order to identify patients in need for vigilant surveillance and aggressive treatment plan. Equally needed are markers that will improve prognostication in muscle invasive disease given the current poor outcome (60% 10 year overall survival) in this group of patients.

Molecular Pathways of Oncogenesis in Urothelial Carcinoma:

Chromosome 9 alterations are the earliest genetic alterations in both pathways of URCa development. These changes are thought to impart the necessary milieu of genetic instability that in turn allows for the accumulation of subsequent genetic defects. Among other common chromosomal gains and deletions, gains of chromosomes 3q, 7p, and 17q and 9p21 deletions (p16 locus) are of special interest given their potential diagnostic value. A multitarget interphase FISH based urine cytogenetic assay was developed based on the above numerical chromosomal alterations. Such test is now commercially available. Initially FDA approved for surveillance of recurrence in previously diagnosed URCa patients, the test subsequently was also approved for screening in high risk patients with hematuria. The multicolor FISH assay appears to enhance the sensitivity of routine urine cytology analysis and can be used in combination with routine cytology as a reflex testing in cases with atypical cytology. A sensitivity range of 69-87% and a specificity range of 89-96% have been reported with the multitarget interphase FISH assay. With
the exception of one study, the multitarget FISH urine assay has been shown to be more sensitive than routine cytology. An additional advantage of urine based FISH testing could be the anticipatory positive category of patients identified by such assay. The latter category refers to patients where FISH assay detects molecular alteration of URCa in urine cells several months prior to cancer detection by cystoscopy or routine cytology. In the study by Yoder et al., two thirds of the 27% of patients categorized as “anticipatory positive” developed URCa that was detected by cystoscopy up to 29 months later. Such encouraging results point to the great potential of molecular testing in early detection and allocation of vigorous/frequent follow-up cystoscopy in at risk patients.

The current detailed understanding of the molecular pathways involved in URCa development and progression has fueled the field of molecular prognostication, theranostics and targeted therapy in bladder cancer. Several recent gene expression studies have highlighted differentially expressed genes that may play a role in predicting recurrence and progression in URCa. Table 1 summarizes the established clinicopathologic and potential molecular prognostic parameters in superficial and muscle invasive urothelial carcinoma of bladder. A series of recent studies have pointed to the potential prognostic value of receptor tyrosine kinase (RTK) markers such as FGFR-3, EGFR and other ERB family members (ERBB2/HER2 and ERBB3) in superficial and invasive bladder cancer disease. Early studies by Sarkis et al. revealed p53 alterations to be a strong independent predictor of disease progression in URCa (superficial, muscle invasive as well as CIS). P53 has also been shown to be predictive of increased sensitivity to chemotherapeutic agents that damage DNA. More recent studies have demonstrated a synergistic role for combining p53 evaluation with other cell cycle control elements such as pRb, p21 and p27, demonstrating the superiority of multi-markers approach.
compared to prior single marker approach for prognostication \cite{31-33, 36}. In a recent study by Shariat et al. \cite{22}, superficial URCa patients with TURB demonstrating synchronous immunohistochemical alterations in all four tested p53, p21, pRb and p27 markers were at significantly lower likelihood of sustained disease free survival (DFS) compared to patients with only three markers altered. In turn, those with three altered markers did worse than patients with only two altered markers which in turn had a lower DFS than those with only one alteration as shown in figure 2.

**Figure 2: Synergistic prognostic role of immunohistochemical analysis of four markers (p53, p21, pRb and p27) in superficial URCa. adapted from Shariat et al. \cite{22}.**

![Figure 2: Synergistic prognostic role of immunohistochemical analysis of four markers (p53, p21, pRb and p27) in superficial URCa.](image)

A similar synergistic prognostic role of multiple molecular markers (p53, pRb and p21) was demonstrated by Chatterjee et al. \cite{20} in patients undergoing cystectomy for invasive URCa using IHC technique. Such multimarker approach of prognostication could soon result in a new standard of care in URCa management once additional multi-institutional, preferably
prospective, trials confirm the above findings. An encouraging such development is the recent report of the bladder consortium multi-institutional trial confirming the role of proliferation index (as measured by Ki67 on IHC) as a prognosticator in URCa. The current clinicopathologic based prognostic approach to predicting progression in superficial URCa is soon to be supplemented by a molecular guided approach based on markers among those listed in table 1.
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<thead>
<tr>
<th>Clinicopathologic Prognostic Parameters</th>
<th>Muscular invasive urothelial carcinoma</th>
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<tbody>
<tr>
<td><strong>WHO/ISUP Grade</strong></td>
<td>pTNM</td>
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<tr>
<td><strong>pT stage</strong></td>
<td>LVI</td>
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<tr>
<td><strong>Presence of associated CIS/Dysplasia</strong></td>
<td>Resistance to neoadjuvant chemotherapy</td>
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<td><strong>Disease duration</strong></td>
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<td><strong>Time to and frequency of recurrences</strong></td>
<td><strong>Divergent Histology:</strong></td>
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<tr>
<td><strong>Multifocality</strong></td>
<td>Micropapillary</td>
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<td><strong>Tumor Size (&gt;3 cm)</strong></td>
<td>Osteoclast rich</td>
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<td><strong>Failure of prior BCG Rx</strong></td>
<td>Undifferentiated/Giant cell</td>
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<tr>
<td><strong>Presence of LVI</strong></td>
<td>Plasmacytoid</td>
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<td><strong>Depth of Lamina propria Invasion</strong></td>
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<tr>
<th>Emerging Potential Molecular Prognostic Parameters</th>
<th>Muscular invasive URCa</th>
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<tr>
<td>Superficial URCa</td>
<td>Muscle Invasive URCa</td>
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<tr>
<td>Ki 67 proliferation index</td>
<td>Loss of E Cadherin</td>
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<tr>
<td>Ploidy Status</td>
<td>p53 inactivation</td>
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<tr>
<td>Loss of E Cadherin</td>
<td>Alteration of Rb expression</td>
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<tr>
<td>p53 inactivation</td>
<td>Loss of p21 expression</td>
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<tr>
<td>Loss of Rb expression</td>
<td>Alteration of p16 expression</td>
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<tr>
<td>Loss of p21 expression</td>
<td>EGFR overexpression</td>
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<td>Loss of p27 expression</td>
<td>HER2 overexpression/amplification</td>
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<td>VEGF mRNA overexpression</td>
<td>TSP1 overexpression</td>
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<td>HIF 1A overexpression</td>
<td>mTOR</td>
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<td>ERBB3, ERBB4 overexpression</td>
<td>Phos S6 expression (protective)</td>
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<td>FGFR3 Mutation overexpression</td>
<td>Epigenetic Alterations:</td>
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<tr>
<td>TSP1 Overexpression</td>
<td>RASSF1 promotor hypermethylation</td>
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<td></td>
<td>E Cad promotor hypermethylation</td>
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<td>EDNRB promotor hypermethylation</td>
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Table 1: Established clinicopathologic and potential molecular prognostic parameters in superficial and muscle invasive urothelial carcinoma of bladder.

Finally, from a targeted therapy perspective, RTK-HRAS-MAPK (superficial disease) and p53-pRb (muscle-invasive disease) pathogenic pathways as well as angiogenesis pathway of the tumor microenvironment offer tremendous new opportunities for future management of URCa. Phase II trials evaluating the role of tyrosine receptor kinase inhibitors (TKI) targeting EGFR with small molecules such as Gefitinib and Sorafinib or monoclonal antibodies (MoAb) such as Cetuximab are underway. Other Phase II trials are addressing the role of Herceptin (anti HER2 MoAb) and Bevacizumab (anti VEGF MoAb) in URCa. Phase I trials testing the safety of p53 or
Rb intravesical gene therapy are being evaluated. One strategy example is the intravesical introduction of a wild type p53 loaded replication deficient Adenovirus (Ad5CMV-TP53) in an ambitious attempt to compensate for the loss of p53 function in invasive URCa.\textsuperscript{50-54}

In summary, our recent understanding of the complex molecular alterations involved in the development and progression of urologic malignancies is yielding novel diagnostic and prognostic molecular tools and opening the doors for experimental targeted therapies in these prevalent, frequently lethal solid tumors.

REFERENCES


