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REVIEW

Molecular biomarkers for predicting outcomes in urothelial carcinoma of the bladder

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Summary

Molecular biomarkers are used routinely in the clinical management of several tumours such as prostate, colon, ovarian and pancreatic cancer but management decisions in bladder cancer remain dependent on clinical and pathological criteria, which are limited in their ability to predict outcomes. Molecular markers are urgently needed in detection, surveillance and prognostication of bladder cancer as well as to predict treatment response to intravesical and systemic therapies. Advances in cancer genomics and platforms for biomarker profiling have led to a plethora of biomarkers, which must now be rigorously validated in the clinical setting. Pre-clinical and clinical studies exploring the role of emerging targeted therapies to risk stratify and reduce cancer progression are also needed.

Key words: Biomarkers, bladder cancer, epigenetics, genomics, methylation, microRNA, outcome.

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INTRODUCTION

There are two main clinical phenotypes of bladder cancer: non-muscle invasive bladder cancer (NMIBC), which accounts for the majority of cases and muscle invasive bladder cancer (MIBC). These two entities have distinct treatment paradigms and prognoses. Recent surveillance epidemiology and end results (SEER) data have shown that there has been minimal if any improvement in the outcomes of any stage of bladder cancer over the last three decades.[1] Coupled with this, the reliance on cystoscopy and imaging for surveillance has made bladder cancer the single most expensive solid tumour to treat from diagnosis to death.[2] For this reason, early detection, appropriate risk-stratification and targeted systemic therapy are urgently needed. Currently a set of clinical and pathological parameters (Table 1) are used to risk stratify bladder cancer but are limited in their ability to predict clinical outcomes. They are inherently biased by their retrospective nature and failure to account for variation in tumour biology. In sharp contrast to other tumour types such as breast, lung, renal cell, melanoma and colorectal cancers where insights gained from the molecular analysis has facilitated the development of targeted therapy, no such marker has been translated to the clinical management of bladder cancer. Biomarkers to accurately identify tumour subtype, risk of recurrence and progression, response to intravesical and systemic therapy and detect micrometastatic disease are needed to improve management of this cancer where the burden at both spectrums of the disease remains large. In this review we discuss the molecular pathogenesis and the current state of tissue-based biomarkers to predict outcomes in the disease.

DUAL PATHWAY OF BLADDER CARCINOGENESIS

Unlike many other epithelial cancers, current evidence supports the hypothesis that the development of low grade bladder cancer and high grade/invasive bladder cancer is driven by largely distinct molecular pathways[3] (Fig. 1).

Low grade non-muscle invasive tumours are genetically stable and are thought to develop from normal urothelium through hyperplasia. Such tumours are characterised by alterations in the *RAS-MAPK*, and *PI3K-Akt-mTOR* pathways with significant cross talk between these pathways leading to urothelial cell overgrowth.[4] Overactivity of *HRAS* is found in a large proportion of these cancers, driven either by mutations in the *HRAS* gene or alternative splicing.[5] Receptor tyrosine kinases are also constitutively active in such tumours with 60–70% of these tumours harbouring mutations in the *FGFR3* gene.[6,7] High grade non-muscle invasive and invasive cancers are thought to arise from carcinoma *in situ* or *de novo*. Frequent alterations in the p53 and pRb regions, which are critical in cell cycle control, are found in several of these cancers. Mutations in the *TP53* gene allow unchecked progression at the G1-S and G2-M transitions, possibly through downstream regulation of p21, a cyclin dependent kinase inhibitor,[8] and nuclear accumulation of p53 has now been shown to be predictive of poor outcome in patients with invasive bladder cancer.[9,10] pRb inactivation is also seen in several of these tumours either through pRb gene mutation or hyperphosphorylation in the presence of an intact gene.[11]

Table 1 Clinical and pathological predictive markers of recurrence and progression in non-muscle invasive and muscle invasive bladder cancer

Non-muscle invasive bladder cancer (NMIBC)
1. Number of tumours
2. Tumour size
3. Prior recurrence rate
4. T-stage
5. Presence of concurrent carcinoma *in situ*
6. Tumour grade
Muscle invasive bladder cancer (MIBC)
1. T-stage
2. Lymph node status
3. Variant histology

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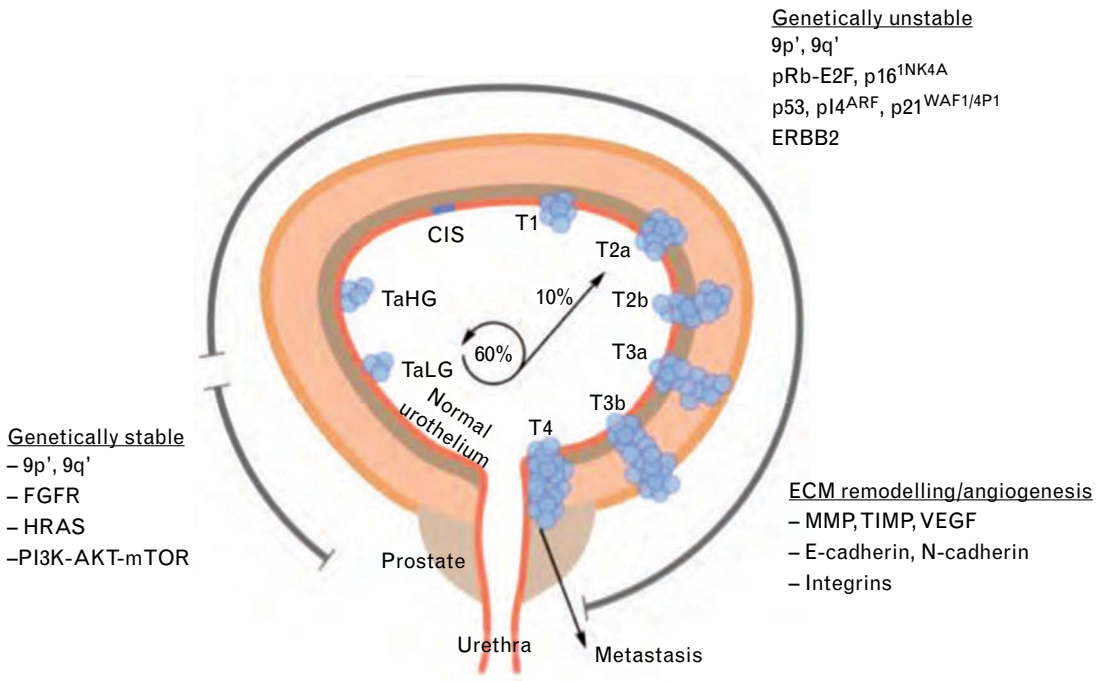

Fig. 1 Dual pathway model of bladder cancer. FGFR, fibroblast growth factor receptor; HRAS, Harvey rat sarcoma viral oncogene homolog; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; TIMP, tissue inhibitor of metalloproteinase; VEGF, vascular endothelial growth factor.

Deletions of both arms of chromosome 9 appear to be early events in both pathways of bladder cancer development.[12] The role of tumour microenvironment and extracellular matrix remodelling is also critical in invasion and metastasis.[13]

More recently, the use of high throughput genomic technologies has demonstrated that the drivers of bladder cancer are likely to be more complex than the traditional dual track model. Epigenetic abnormalities such as DNA methylation, chromatin remodelling and microRNAs also seem to have a large role as drivers of bladder cancer and profiling of such changes hold promise for the development of molecular markers for bladder cancer.

PLATFORMS AND STRATEGIES FOR GENETIC AND EPIGENETIC PROFILING OF BLADDER CANCER

There are two distinct methods used for systematic profiling of genes perturbed in cancer: the global approach and the targeted or pathway-specific approach. The global approach utilises high throughput platforms to profile the genome for significant genetic and epigenetic changes in cancer and includes microarrays and next generation sequencing (NGS). The targeted approach uses low or medium throughput technologies to study an *a priori* panel of genes. The global approach is useful for discovery of novel pathways and the pathway specific approach is ideal for testing specific hypotheses and validation of data from high throughput studies.

Whilst real time quantitative polymerase chain reaction (RT-qPCR) is still considered the gold standard for quantification of gene expression, it is quickly rendered inadequate on a genomic scale. Microarrays remain the most popular choice for genome-wide association studies but are plagued with problems about differing surface chemistry, probe design, labelling techniques and hence normalisation of resulting dataset between runs.[14] There is also the inability to discover novel markers. NGS has paved the way for discovery of novel genes involved in carcinogenesis. These technologies are a critical pillar of this era of cancer biology research and have catalysed our understanding of genome biology and brought us a step closer to applying genomic knowledge in the clinic.[15] However, high throughput platforms such as NGS are associated with their own challenges: they are prone to sequencing errors and a high rate of false discovery alongside problems with complexity and management of data output. This has lead to the development of a systems biology approach to molecular biology where computational methods are increasingly being applied to high throughput data to study the interactions between various components of cellular processes.

GENETIC AND EPIGENETIC BIOMARKERS OF BLADDER CANCER

Chromosomal alterations

Losses on chromosome 9 are the most frequent genetic aberration in bladder cancer. They occur early in neoplasia and may allow for further development of either pathway of cancer development. However, frequent chromosomal alterations have also been found on chromosomes 3, 5, 9, 10, 13, 17 on whole organ genomic mapping studies.[16] The allelic loss of 17p seems to distinguish low grade from high grade urothelial carcinoma 9.[17] Fadl-Elmula and colleagues[18] found that specific losses of material from chromosome arms 11p and 8p, and gains of 8q and 1q seem to be early changes appearing in superficial tumours, whereas losses from 4p and 17p and the formation of an isochromosome for 5p were associated with more aggressive tumour phenotypes.

Cell cycle markers and immunohistochemical panels

Altered cell cycle control is a hallmark of bladder cancer driven by both aberrant signal transduction as well as key alterations in cell cycle molecules such as p53 and pRb. Mutations in TP53,