Bladder cancer: Multi-omic profiling refines the molecular view

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Standfirst | Muscle-invasive bladder cancer (MIBC) is a heterogeneous disease for which treatment has, historically, lagged behind that of many other solid tumour types. A more detailed understanding of the biology of individual tumours and the identification of molecular features that provide prognostic and predictive information is key to the application of personalized care for patients with this disease. The recent publication of the complete TCGA study of 412 MIBC samples[1] now provides such data.

Refers to Robertson *et al.* Comprehensive molecular characterization of muscle-invasive bladder cancer. *Cell*. https://doi.org/10.1016/j.cell.2017.09.007 (2017)

Estimates suggest that >400,000 new cases of bladder cancer and 165,000 bladder-cancer-related deaths occur worldwide each year[2]. Approximately 25% of cases are muscle-invasive bladder cancers (MIBC). These comprise a heterogeneous group of tumours, and for patients with metastatic disease, the prognosis is generally poor. Until the recent approval of immune-checkpoint inhibitors for use in some patients with advanced MIBC, no new drugs had been approved for the treatment of bladder cancer in more than two decades and no significant changes in survival outcomes had been recorded[3].

This dismal situation has driven efforts to unveil the molecular features of MIBC, both to fully understand the biology and molecular heterogeneity of the disease, and in association with clinical data, to provide guidance on disease management through the identification of prognostic and predictive biomarkers. In 2014, The Cancer Genome Atlas (TCGA) consortium presented an analysis of molecular data from 131 MIBC that included whole-exome, RNA and microRNA (miRNA) sequencing, in addition to DNA-methylation and protein-expression data[4]. Findings from this and other studies have confirmed that MIBC is not only clinically heterogeneous, but also has a high level of molecular diversity. Notable findings from the initial TCGA study included a high somatic mutation rate, similar to that of non-small-cell lung cancer and melanoma, statistically significant mutations in 32 genes, and sub-classification of MIBC into four distinct subtypes based on mRNA expression, with distinct phenotypes and implications for survival. The presence of considerable mutational diversity was an important finding: only *TP53* was mutated in >40% of samples and the next most frequently mutated gene (*KMT2D)* was mutated in only 27% of samples, with a long 'tail' of genes with infrequent mutations, many of which were plausible oncogenes or tumour suppressors but were not reliably confirmed as statistically significantly mutated genes (SMG). Analysis of many more samples would be needed to develop a robust and fully comprehensive mutational profile for such a diverse malignancy.

Data from an expanded TCGA cohort of 412 tumours have now been published[1]. These data move the field considerably closer to a comprehensive catalogue of molecular features and have enabled the influence of molecular subtype on patient outcomes to be examined at a much greater level of detail. The rich dataset includes whole-exome, mRNA, long non-coding RNA (lncRNA), and microRNA sequencing data, DNA copy number variation and methylation data, and reverse-phase protein array data[1]. The tumours analysed were chemotherapy naive and the majority (86%) were pure urothelial carcinomas. Some samples of urothelial carcinoma with a variant histology were also included, which is representative of the range of phenotypes commonly found in this disease group, thus providing balanced view of the disease at diagnosis.

The large body of whole-exome sequencing (WES) data presented in this study enabled a more detailed analysis of mutational signatures than was previously possible. A high mutation rate was confirmed (with a mean of 8.2 and a median of 5.8 nonsynonymous mutations per megabase) and five mutational signatures were identified. Two of these signatures, both of which are variants of the APOBEC mutagenesis signature[5], accounted for 67% of all single nucleotide variants. Unsupervised clustering analyses revealed four mutational signature clusters. A striking finding is that one of these clusters, consisting of tumours with a high mutational burden, a high APOBEC-associated mutational load and a high predicted neoantigen load was associated with an extremely high 5-year survival probability (75%) compared with the cluster with the lowest mutational burden (22%). This observation is hypothesized to reflect a greater host antitumour immune response. The clinical implications of these features must now be examined further in clinical trials and will be of particular interest in relation to response to immune-checkpoint inhibition.

WES of these 412 tumours revealed 58 SMGs, 18 of which were mutated in >10% of cases. A very high frequency (89%) of alterations in *TP53, RB1* and genes involved in cell-cycle regulation, frequent alterations in chromatin modifiers and regulators, and a broad range of alterations affecting canonical signalling pathways, including mutations or copy-number gains in *EGFR, FGFR3, HER2* or *HER3* were reported. However, given the heterogeneity of MIBC, analysis of an even larger panel will be required to generate a complete catalogue of low-frequency mutations. For example, estimates suggest that, in a tumour type with such a high background mutation frequency, analysis of >3,000 samples might be needed to reliably detect genes that are mutated in 2% of samples[6]. With reduced sequencing costs, this can undoubtedly be achieved within the next few years, ideally using clinically annotated samples obtained from patients participating in trials.

mRNA sequencing data revealed five disease subtypes (luminal-papillary, luminal-infiltrated, luminal, basal-squamous and neuronal) within the dataset. These align closely with the four expression subtypes identified in the 2014 study[4] (FIG. 1). Overall, basal and luminal features divide the tumours into two major groups, as described in the previous TCGA dataset[4] and other studies. Luminal tumours were found to retain aspects of urothelial differentiation, including expression of uroplakin genes and transcription factors implicated in the urothelial differentiation process, while basal tumours were found to express markers characteristic of the nonmalignant basal cells of the urothelium. A key subtype identified in the latest analysis is the luminal-infiltrated subtype, which was characterised by markers suggesting the presence of smooth muscle, fibroblasts and immune cells. This group aligns with the TCGA 2014 subtype II, that has been reported to be sensitive to immune-checkpoint inhibition[7] and the 'p53-like' subtype that has been reported to to be resistant to cisplatin-based chemotherapy[8]. Two additional subdivisions (luminal and neuronal) were described. The neuronal subtype, which has also been described by others[9], comprised approximately 5% of MIBC analysed in this study, and was associated with the worst clinical outcomes of all the expression subtypes. The majority of these neuronal MIBC did not have histological features suggestive of a neuroendocrine origin. Therefore, the expression signature defined will enable the future identification of this aggressive variant.

In addition to mRNA-based subtypes, hypermethylation and hypomethylation, lncRNA, and miRNA clusters were derived. All show overlap with the mRNA-based subtypes, and the lncRNA and miRNA subtypes in particular provide further subdivision of mRNA subtypes. For example, subclassification based on lncRNA clustering revealed a subset within the luminal-papillary tumours associated with more favourable outcomes. The features of these smaller subsets now merit more-detailed evaluation in order to assess their diagnostic and prognostic value.

Around 10–15% of the large group of patients with non-muscle-invasive bladder cancer (NMIBC) are likely to progress to muscle-invasive disease, although what specific molecular features drive such

progression remains unclear. Notably, MIBC with the highest levels of hypomethylation frequently had *FGFR3* alterations and no *TP53* or *RB1* mutations, features that are highly prevalent among NMIBC. Other features of these MIBC, which belonged to the luminal-papillary mRNA subtype, included a low mutation burden, low levels of hypermethylation, a high frequency of *CDKN2A* deletion and better overall survival than other hypomethylation subtypes. Further mining of this extensive dataset might enable robust prognostic signatures to be defined that enable the identification of noninvasive tumours, particularly stage T1 tumours that confer a high risk of disease progression and merit radical surgery early in the course of disease.

The findings of this study have many therapeutic implications. For example, patients with luminal-papillary tumours might benefit from treatment with FGFR inhibitors, and those with luminal-infiltrated tumours from immune-checkpoint inhibition but not cisplatin-based chemotherapy. These observations provide predictive hypotheses that can now be tested by retrospective analysis of samples from previous and ongoing clinical trials, and in novel biomarker-driven trials. Given the biological heterogeneity of MIBC demonstrated by this analysis, the challenge of application of current knowledge in the clinic is considerable. High-quality evidence demonstrating the clinical benefits of the therapeutic hypotheses generated by this and other molecular analyses is now needed. Already, evidence has been presented indicating superior responses of tumours of the basal subtype to neoadjuvant chemotherapy[10], and of the relative resistance of other subtypes to this approach[8]. A finer-grained understanding of sensitivity to the range of chemotherapy, targeted therapy and immune-based therapeutics is now required. To achieve this, the development of subtype classification signatures that are measurable using panels of a limited number of mRNA and/or immunohistochemical markers, robust funding for the routine clinical use of molecular profiling, and close collaboration between scientists and clinicians will be essential to deliver truly personalized care to patients with MIBC.

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Competing interests

The authors declare no competing interests.

Figure 1| mRNA expression-based subtypes of muscle-invasive bladder cancer (MIBC) according to the The Cancer Genome Atlas (**TCGA). a|** mRNA subtypes identified in 131 chemotherapy-naive MIBCs in the 2014 TCGA study[4] and in the extended TCGA panel of 412 MIBC samples published in 2017[1]. **b|** Key features of subtypes defined in the 2017 TCGA mRNA subtype analysis (luminal-papillary, luminal-infiltrated, luminal, basal-squamous and neuronal). Relative mRNA levels of highly expressed genes, carcinoma *in situ* (CIS) signature genes, epithelial-to-mesenchymal transition (EMT)-related genes and claudins, and immune markers are shown. Key genomic alterations and likely relative survival outcomes are also indicated for each subtype.

Author biographies

Carolyn Hurst received her PhD in 1993 from Swansea University where she worked with Professor David Skibinski on the population genetics of marine invertebrates. After finishing her PhD she undertook postdoctoral positions working as a molecular biologist at the Memorial University of Newfoundland, Canada and the University of Greenwich, London. Since 2001 she has worked in Margaret Knowles's group at the University of Leeds, with particular focus on genomics and genome-wide profiling of bladder tumours.

Margaret Knowles received her PhD from the University of London where she worked with Dr Sammy Franks at the Imperial Cancer Research Fund Laboratories (now Cancer Research UK London Research Institute) on carcinogen-induced transformation of epithelial cells. After Postdoctoral work with Professors Marian Hicks and Roger Berry at The Middlesex Hospital Medical School, London she established a group working on the molecular biology of bladder cancer at the Marie Curie Research Institute, Oxted, Surrey. She moved to the University of Leeds as Professor of Experimental Cancer Research in 1997 where her group continues to focus on the molecular features of bladder cancer.