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Next-generation Sequencing of Urologic Cancers: Next Is Now

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Next-generation sequencing (NGS) refers to technologies that decipher nucleic acid information at the single-molecule level. This high resolution contrasts with conventional Sanger sequencing, which provides average information on a population of molecules. NGS is revolutionising knowledge of human genome diversity at both the germline level (population) and the somatic level (tumours). Cooperative groups such as the International Cancer Genome Consortium and The Cancer Genome Atlas (TCGA) Research Network provide platforms for effective worldwide collaboration [1]. The genome-wide analysis of tumours (either whole-genome sequencing [WGS] or whole-exome sequencing [WES]) has taken the lead, providing comprehensive genome landscapes, but NGS also allows more targeted analysis of selected genes with high resolution and sensitivity. A major question is whether genome-wide or targeted approaches will be more appropriate to apply in the clinical setting.

Efforts on urologic cancers were preceded by those on colorectal, breast, and pancreatic cancer using Sanger sequencing. Since 2010, WES and WGS of kidney cancer (>500), bladder cancer (approximately 250), and prostate cancer (PCa; approximately 300) have become available. Several common themes have emerged: (1) Tumour site-specific and shared genetic pathways have been identified (Table 1), (2) we are confronted with new tumour taxonomies, and (3) new opportunities for improved management have arisen.

1. Renal cell carcinoma

Until the NGS era, von Hippel-Lindau tumour suppressor, an E3 ubiquitin protein ligase (*VHL*), was the major gene known to be involved in clear cell renal cell carcinoma (ccRCC). Studies from the Wellcome Trust have shown a high frequency of inactivation of polybromo 1 (*PBRM1*; a component of the SWI/SNF chromatin remodelling complex) in 40% of tumours and recurrent mutations in genes involved in histone methylation: SET domain containing 2 (*SETD2*), lysine (K)-specific demethylase 5C (*KDM5C*), and lysine (K)-specific demethylase 6A (*KDM6A*) [2,3]. The BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase) (BAP1) deubiquitinase has been found to be inactivated in 15% of tumours [4]. Intriguingly, *VHL*, *PBRM1*, and *BAP1* are on chromosome 3p, the most common arm-level loss in ccRCC. *PBRM1* and *BAP1* mutations are generally exclusive, and several retrospective studies suggest that BAP1 loss is a promising marker of poor prognosis [5,6]. The TCGA study confirmed these findings, identified *SETD2*-associated DNA methylation subsets, and provided evidence of multiple RNA-based tumour subtypes characterised by mutations in chromatin remodellers/*PBRM1*, *CDKNA* and phosphatase and tensin homolog (*PTEN*), and mechanistic target of rapamycin (serine/threonine kinase) (*MTOR*) and *BAP1* [7]. In addition, signatures have been identified that are independently associated with outcome, including those revealing metabolic remodelling of tumours and having potential as therapeutic targets [7,8].

Ongoing studies of ccRCC from France and China and the TCGA project on papillary tumours will support a more detailed understanding of renal cell carcinoma (RCC) genomic diversity.

2. Bladder cancer

A thorough knowledge of the genomic landscape of urothelial bladder cancer (UBC) is still lacking. More than

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Table 1 – Summary of the main pathways/genes altered in urologic tumours, identified using next-generation sequencing

	Renal cell carcinoma (>500)	Urothelial bladder cancer (approximately 250)	Prostate cancer (approximately 300)
P53	*TP53*	*TP53, MDM2*	*TP53*
Histone modifications/ chromatin remodelling	*PBRM1, SETD2, AID1A, KDM5C, KDM6A*	*ARID1A, KDM6A, CREBBP, EP300, MLL1-3*	*MLL2*
PI3K/mTOR	*MTOR, PIK3CA*	*PIC3CA, PTEN, TSC1*	*PTEN*
Transcription	*VHL/HIF1a*	*RXRA, ER*	*AR, MED12, SPOP*
Cell cycle	*CDKN2A*	*CDKN2A, CDKN1A, cyclin E, E2F3, MYC*	
Hypoxia	*VHL/HIF1A*		
Receptor tyrosine kinases		*FGFR3, ERBB1, ERBB2, ERBB3*	
Cohesin		*STAG2*	
DNA repair		*ATM, ERCC2, FANCA*	
Oxidative stress		*NFE2L2, TXNIP*	
Genomic rearrangements	*SFPQ-TFE3*	*FGFR3-TACC3, ERBB2*-associated	*TMPRSS-ERG* and related
Other genetic features		*APOBEC* signature hypermutation	Punctuated evolution C-class tumours, with few SNVs

SNV = single-nucleotide variant.

50% of UBCs are low-grade, non–muscle-invasive bladder cancers (NMIBCs), yet only few of their genomes (approximately 10) have been sequenced. A Chinese project has focused mainly on high-grade NMIBC and muscle-invasive bladder cancers (MIBCs), whereas the TCGA has exclusively analysed MIBC [9–11]. Therefore, we still await a detailed comparison of NMIBC and MIBC.

WES has revealed mutations in chromatin modifiers/remodellers, including AT rich interactive domain 1A (SWI-like) (*ARID1A*), *KDM6A*, CREB binding protein (*CREBBP*), E1A binding protein p300 (*EP300*), *MLL1–3*, and nuclear receptor corepressor (*NCOR*) [9–12]. Recent studies have highlighted alterations in stromal antigen 2 (*STAG2*) and other genes involved in chromosome segregation acting as tumour suppressors [10–12]. The mechanisms through which these genes participate in UBC are unknown; *STAG2* inactivation is preferentially associated with low-grade NMIBC. Nucleotide excision repair (excision repair cross-complementation group 2 [*ERCC2*], associated with a distinct mutational pattern) and homologous recombination (ataxia telangiectasia mutated [*ATM*], Fanconi anaemia, among others) pathways also are frequently mutated [10–12]. The TCGA has identified mutations in nuclear factor, erythroid 2-like 2 (*NFE2L2*) and thioredoxin interacting protein (*TXNIP*), involved in the response to oxidative stress, in 15% of tumours. An apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B (*APOBEC3B*)–associated signature accounts for >50% of single nucleotide variants (SNVs). MIBC subgroups have been defined based on copy-number changes as well as RNA expression [11]. These studies have suggested putative therapeutic targets including receptor tyrosine kinases (fibroblast growth factor receptor 3 and ERBB1, ERBB2, and ERBB3, both through mutations and gene rearrangements), nuclear receptors, heat shock proteins, and proteins involved in glycolysis and cell cycle. Sequencing of tumours from exceptional-responder patients has uncovered the predictive potential of tuberous sclerosis 1 (*TSC1*) mutations for response to everolimus [13].

The completion of the TCGA project and the expansion of the Chinese initiative will provide a more comprehensive map of aggressive tumours. The UROMOL Consortium plans to complete RNA-sequencing analysis of 1000 NMIBCs. New projects are required to determine the genomic landscape of specific tumour subtypes, such as micropapillary, plasmacytoid, and squamous carcinomas, and of tumours from selected patient populations (eg, nonsmokers, women).

3. Prostate cancer

PCa was the last of the common tumour types to have its genome sequenced [14]. Initial studies focused on a broad-based characterisation of PCa. The identification of complex multichromosomal genomic rearrangement loops [14] and of recurrent speckle-type POZ protein (*SPOP*), forkhead box A1 (*FOXA1*), *MLL2*, and mediator complex subunit 12 (*MED12*) mutations has shed new light on PCa genetics [15,16]. The PCa genome appears to be characterised by rare SNV and frequent copy-number aberrations and genomic rearrangements, termed a *C-class* tumour type. These rearrangements seem to arise in a punctuated manner, driving clonal expansion and evolution [17].

Initial sequencing surveys did not provide deep insight into either PCa treatment or prognosis. Subsequent work has focused on specific disease subgroups. An elegant study of pretreatment metastatic PCa revealed mutational profiles comparable to earlier stage disease and the functional impact of *FOXA1* mutations in androgen responsiveness [18]. Another focused report of 11 early-onset tumours showed distinct, age-related, mutational profiles as well as sustained dysregulation of androgen signalling [19].

The studies discussed have set the stage for statistically well-powered, genomically comprehensive projects interrogating well-defined clinical cohorts (Fig. 1). These large-scale projects will answer fundamental questions about PCa, including its mutation-driving profiles. By integrating these diverse data sets, we will begin to assemble a

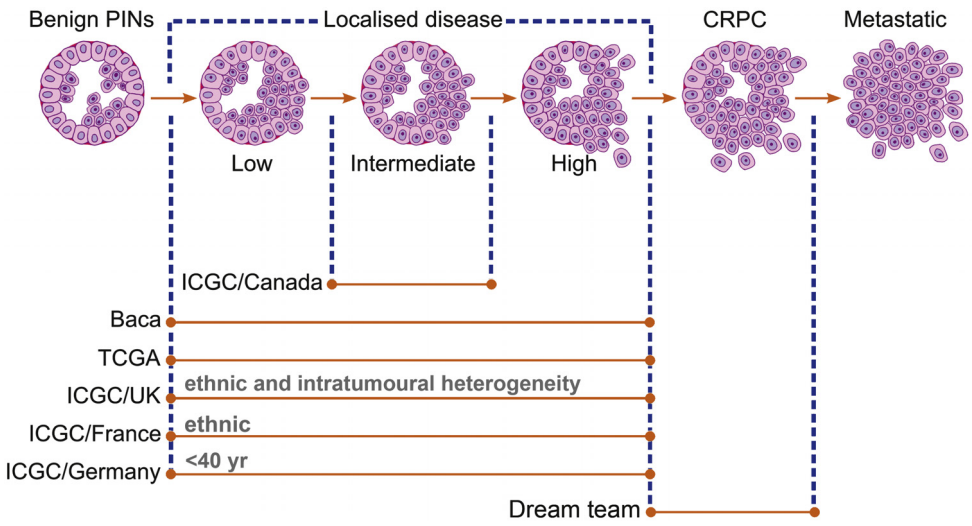

Fig. 1 – Summary of ongoing projects in prostate cancer (PCa) using next-generation sequencing. A French project focuses on PCa within the French Caribbean islands; a British project studies intratumoural and ethnic heterogeneity; a German project analyses early-onset PCa; and a Canadian project focuses on intratumoural heterogeneity and risk stratification of intermediate-risk PCa. The US Cancer Genome Atlas Research Network project provides a large-scale survey across all localised risk groups and will serve as a useful validation cohort. Finally, Stand Up to Cancer has created two PCa "dream teams," one focusing on metastatic disease and the other on linking genomic profiles to treatment selection.
CPRC = castration-resistant prostate cancer; ICGC = International Cancer Genome Consortium; PINs = prostatic intraepithelial neoplasia; TCGA = The Cancer Genome Atlas.

temporal and spatial understanding of the evolution of the PCa genome.

4. NGS: Beyond description and towards patient management

There is a substantial expectation that the power of NGS will be revealed at additional levels, including understanding tumour progression, aetiology, prevention, and early detection.

Studies in ccRCC have shown the complex phylogeny and branching evolution of tumour subpopulations and the occurrence of convergent evolution (ie, distinct subclones of the tumour independently acquiring different mutations, converging in functional inactivation of a given gene) [20]. Most genetic alterations in a tumour, in fact, appear to be subclonal, and intratumoural heterogeneity reveals patterns that are parallel to those observed when comparing tumours from different patients (M. Gerlinger, unpubl. data). Similar diversity has emerged from single-cell exome sequencing in RCC [21].

A case in point is the study of aristolochic acid–associated (AAA) upper urinary tract urothelial tumours (UTUCs) [22]. AAA-UTUCs were first reported in the context of Balkan nephropathy and were found to display an A>T mutational signature in tumour protein p53 (*TP53*). Two recent WES and WGS studies have confirmed this signature at the genome-wide level and have shown a high mutational rate (150 mutations per megabase pair [Mbp] compared with 111 mutations per Mbp in melanoma and lower rates in tobacco-associated cancers) as well as a preference for splice acceptor site mutations. Interestingly, the culprit genes involved in AAA-UTUC overlap with non-AAA urothelial cancers [22,23].

5. Conclusions

NGS technology is already providing new knowledge about the molecular pathogenesis of cancer. The common players involved in the major urologic tumours have likely been identified, but the "long tail" of mutations remains poorly characterised. It may well be that many new and targetable mutations remain to be discovered and that differences according to tumour subtype or population characteristics (sex, age, environmental exposure) will emerge. The recent approval of NGS technology for clinical application by the US Food and Drug Administration sets the stage for a transformation in precision medicine.

Conflicts of interest: The authors have nothing to disclose.

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