PROSTATE CANCER

Clinical hallmarks in whole cancer genomes

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Fraser and colleagues describe the whole-genome sequencing (WGS) profiles of over 200 localized intermediate-risk prostate cancers. WGS has been widely used in research but not, thus far, in clinical settings. Herein, we consider the possible use of WGS in the field of precision oncology.

Refers to Fraser, M. *et al.* Genomic hallmarks of localized, non-indolent prostate cancer. *Nature* **541**, 359–364 (2017)

The emergence of massively parallel sequencing in routine clinical cancer testing raises important considerations as to how, and when whole-genome sequencing (WGS) will enter into the field of precision oncology. Unlike in clinical oncology, both whole-exome sequencing (WES) and WGS have been rapidly adopted by medical geneticists in the management of inherited disorders[1,2]. This lack of adoption by the oncology community reflects several factors: high quantities of good quality DNA are often easier to obtain from buccal smears or blood than they are from cancer tissue samples, which are often limited in quantity and degraded by formalin fixation. In constitutional genetics, a read depth of 20–30× is sufficient to identify clinically relevant single-nucleotide polymorphisms (SNPs), indels, duplications, deletions, and inversions. By contrast, the analysis of DNA from a stromal and subclonal cellular admixture makes variants more difficult to detect, thus raising the coverage requirement to much greater depths (>100×), which are currently difficult to achieve using a cost-effective WGS test[3]. As a result, targeted gene panels remain the current state-of-the-art in clinical cancer sequencing.

Sensitivity is a key challenge to the successful use of clinical testing in precision oncology. The absence of an 'actionable' variant might render a patient ineligible for an approved targeted therapy or entry into a current clinical trial. False-negative results could also occur owing to inadequate on-target coverage (lack of analytical sensitivity), or owing to the limited scope of the particular testing panel (lack of clinical sensitivity). The introduction of WES and/or WGS might increase clinical sensitivity relative to that provided by use of targeted gene panels by identifying non-hotspot mutations in known cancer genes, alterations in non-canonical pathway members, and driver variants that do not change the protein-coding sequence. WGS might also enable detection of additional types of variation, such as complex indels or inversions, which could affect known cancer-related genes. Such findings might indicate the use of a currently available intervention or make a patient eligible for future clinical trials.

> "Imminent declines in the costs of sequencing could enable routine clinical detection of … complex and noncoding variants…"

In a recent publication in *Nature*, Fraser and colleagues describe a bold sequencing effort yielding WGS profiles of >200 patients with localized, intermediate-risk (Gleason score 7) prostate cancer[4]. Data from the study provide several interesting arguments supporting the future use of WGS in the management of patients with certain cancers, especially those with few known recurrent or actionable protein-coding mutations, such as localized, intermediate-risk prostate cancer. No clinical applications for WGS exist in oncology today, although this technology could be adopted for routine clinical testing in the near future, for several reasons.

Less than 10 years ago, most molecular diagnostic laboratories performed each genomic test separately using labour-intensive techniques, such as Sanger sequencing, which provide small amounts of data relative to current methods. More recently, gene panels employing massively parallel sequencing have enabled the routine and simultaneous collection of data on multiple molecular features, which is necessary for the routine genetic evaluation of tumours from patients with breast, colon, or lung cancer. As sequencing costs plummet 5–10-fold in the next few years, the financial argument for applying target enrichment might no longer outweigh the narrow scope and logistical challenges associated with use of gene panels, even compared with WES. Such challenges include the additional costs and labour associated with target enrichment, as well as the requirements for assay redesign because new loci or variants are identified through discovery efforts. Notwithstanding these improvements, the informatics challenges associated with storing and processing WGS data, which can approach 0.5–1 TB of data per patient, could equally be rate limiting.

Fraser and colleagues[4] describe specific single-nucleotide variants (SNVs) and rearrangement signatures among the 200 prostate cancer samples they analysed, which included DNA footprints associated with ageing and a clustered-mutation phenomenon called kataegis (FIG. 1a). The variants that contribute to these signatures are not likely to be cancer drivers but, instead, reflect exposure to a particular mutagen, the presence of specific DNA-repair defects, and/or endogenous mutation processes[5,6]. Such mutation signatures are not currently clinically actionable, although they might, in future, provide biomarkers of a response to cytotoxic chemotherapy, radiotherapy, and/or immunotherapy. One example is provided by the detection of 'BRCAness' signatures in patients that do not have obvious inactivating mutations in homologous DNA-repair pathways[7].

The WGS analyses performed by Fraser and colleagues[4] demonstrate a high burden of complex rearrangements among patients

with localized intermediate-risk prostate cancer, including samples with evidence of chromothripsis (chromosome shattering). The presence of chromothripsis (FIG. 1b), along with other complex rearrangement patterns such as chromoplexy (chains of balanced rearrangements, FIG. 1c) and breakage-fusion bridge cycles, suggests that cancer genomes radically alter their 3D structure[8–10]. Such variants are not detectable using gene panels or even WES, but are highly prevalent among patients with prostate cancer and among those with many other types of cancer. Fraser and colleagues[4] do not identify any candidate drivers among these events, although such alterations might perturb the epigenetics and gene-regulation processes of tumour cells in complex ways that lead to the activation of oncogenic pathways. Alternatively, the presence of such alterations might indicate a particular type of chromosomal instability, which could be linked in future with a response to chemotherapy and/or radiotherapy.

Genomic dark matter — the 98% of the genome that does not encode protein — is a rich ecosystem of regulatory DNA elements that control differentiation and cell-fate in healthy cells, and can be mutated to drive the development or progression of cancer. However, only few noncoding variants of clinical relevance have been identified to date, including telomerase reverse transcriptase (*TERT*) promoter mutations in patients with low-grade gliomas and immunoglobulin rearrangements in those with haematopoietic malignancies[11]. Somatic variations in this part of the genome have only recently begun to be rigorously explored in large, sufficiently powered cancer sequencing datasets, such as the one introduced by Fraser and colleagues[4], or the dataset of nearly 3,000 human cancers that is currently being analysed as part of the international Pan Cancer Analysis of Whole Genomes (PCAWG) effort. Furthermore, noncoding regions are still only beginning to be functionally annotated, an effort that will be dramatically accelerated with the advent of genome-wide genome-editing experiments using technologies such as CRISPR-Cas9.

As Fraser and colleagues[4] show, WGS enables the detection of potentially actionable and recurrent alterations that are invisible to WES, such as inversions in phosphatase and tensin homologue (*PTEN*). Almost all clinical sequencing platforms, including WES, are unable to detect this sort of variant. In addition, Fraser and colleagues[4] demonstrate a loss of *PTEN* expression in samples with these inversions. Fraser and colleagues[4] additionally combine WGS variant data with RNA sequencing and analyses of DNA methylation to orthogonally assess the clinical relevance of noncoding variants. These features were then combined to create a multivariate predictor of disease-free survival that outperforms a previously developed prognostic algorithm.

In summary, Fraser and colleagues[4] have used an ambitious, large-scale WGS analytical approach to study intermediate-risk, localized prostate cancer. Patients with this clinically heterogeneous malignancy are usually treated using surgery or radiation therapy, and would benefit from the availability of better diagnostics that help determine the level of treatment aggressiveness that is required. Through comprehensive analysis of the alterations in DNA from cancers of this type, Fraser and colleagues[4] provide a powerful proof of principle of the application of WGS to a clinically important disease entity. Imminent declines in the costs of sequencing could enable routine clinical detection of the complex and noncoding variants that Fraser and colleagues[4] have identified. The next challenge on the horizon will be how to integrate the analysis and interpretation of these complex data into clinical decision-making.

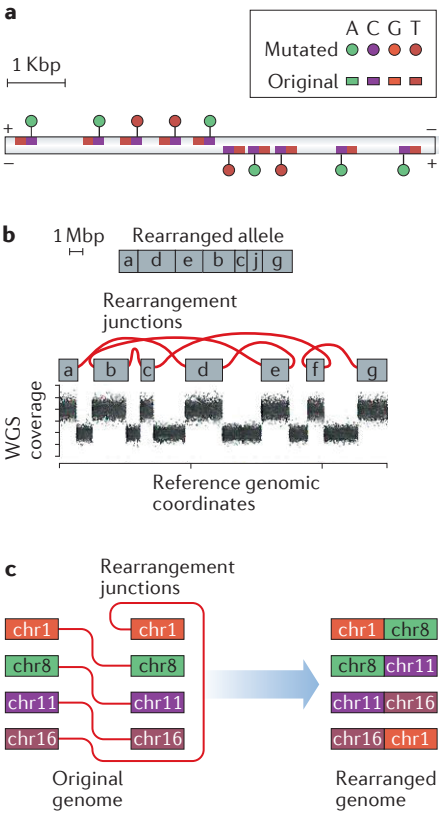

Figure 1 | **Illustration of three complex somatic mutational phenomena frequently seen in the whole genomes of both prostate cancers and those of other tumour types.** **a** | Kataegis refers to clusters of single-nucleotide variants that often occur within 10 kbp of a rearrangement junction consisting of multiple TpC mutations on the same DNA strand. These 'C-strand or G-strand coordinated mutation clusters' are thought to represent footprints of an endogenous mutagenic enzyme called APOBEC[5]. The figure shows one of several possible strand configurations that kataegis mutations (and their associated junctions) can demonstrate. **b** | Chromothripsis refers to 'chromosome shattering', where all, or part of a chromosome undergoes massive DNA breakage, partial deletion, and rejoining of retained fragments in a random order and orientation[8]. **c** | Chromoplexy, which was originally identified in prostate tumours[9] refers to a balanced rearrangement involving three or more loci that are able to trade partners in a genomic 'square dance'.

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