Commentary

Molecular heterogeneity of localized prostate cancer: more different than alike

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Introduction

Over the past decade alone, advances in medical and scientific technology have exponentially increased the volume of data available to clinicians. At the forefront of this movement is next-generation sequencing (NGS), which has allowed for molecular analysis of the entire human genome in a matter of hours (1). The application of NGS to prostate cancer (PCa) has undoubtedly revolutionized our understanding of the disease and holds great promise for improving diagnostic and prognostic accuracy (2). Indeed, previous authors have described genetic changes associated with unique molecular subtypes of PCa and have demonstrated that underlying genetic signatures better predicted clinical outcomes compared to traditional factors such as tumor stage, PSA level, and Gleason score (3,4).

It seems only logical that more in-depth characterization of a given cancer would lead to more accurate prediction of its clinical behavior. Nonetheless, the initial surge of data afforded by these technologies likely preceded our understanding of how to use them and interpret their findings, questions that we have more recently begun to explore in greater depth. For example, the multifocal nature of PCa is widely acknowledged (5,6), but questions remain surrounding how to best account for multifocality when standard practice techniques (i.e., biopsy) are associated with gross undersampling of the tumor (7,8). How to best incorporate data from multiple foci presents a limited problem when considering the heterogeneity of Gleason scoring—an extensively validated system unidirectionally associated with prognosis—but is compounded exponentially as we consider various genomic alterations including single amino acid changes, copy number alterations, and gene fusions (9,10)—any of which may differentially impact prognosis and are in most cases poorly established and not yet validated.

Intra- and inter-tumoral genomic heterogeneity in PCa

In their recent article (11), Wei and colleagues have made substantial progress toward better understanding these questions. Using radical prostatectomy specimens from four men who presented with NCCN high-risk (n=3) or intermediate-risk (n=1) localized PCa, the authors performed genomic and transcriptomic analysis specifically aimed at determining the extent of intratumoral (i.e., different regions within a single tumor focus) and intertumoral (i.e., different tumor foci within a single prostate) heterogeneity. In each radical prostatectomy specimen, three independent tissue cores were obtained from the index lesion (determined by size) and an additional core biopsy was obtained from each noncontiguous tumor focus. DNA and RNA were then extracted and analyzed using whole-exome sequencing, single-nucleotide

polymorphism (SNP) array analysis, and RNA sequencing.

Their findings, in summary, demonstrated considerable intratumoral and intertumoral heterogeneity. In one representative case (CAP-003), the mutation profile of one sampled region within the index lesion (Td1b) differed substantially from the other two sampled regions (Td1a and Td1c)—a most straightforward example of intratumoral heterogeneity. Moreover, the four non-index lesions varied considerably in profile—one highly similar to Td1b, another highly similar to Td1a and Td1c, and the final two lesions distinct from the others. Notably, the majority of DNA-level genomic heterogeneity was conserved at the RNA level, along with additional variability detected on RNA sequencing analysis.

The authors next explored the practical implications of their findings in the context of a recently proposed molecular taxonomy for PCa (3), whereby individual tumor foci are classified into one of seven molecular subgroups based on gene fusion status (ERG, ETV1, ETV4 or FLI1 fusions) or somatic mutations (in SPOP, FOXA1 or IDH1). They found that the majority (>60%) of foci could not be classified under any of the proposed subgroups. Analysis of 60 additional patients from four independent studies (12-15) found a similarly low rate of mutually-exclusive concordant classification across tumor foci (28%). As these findings would suggest that the specific tumor focus and region sampled differentially impact risk classification, the authors next quantitated the gene expression signatures of popular tissue-based prognostic tests. While at least two cores from each patient had similar scores for each signature, on the whole there was substantial variability in score range and direction based on the specific tissue sample analyzed. These findings appear to confirm that prognostic information obtained from tissue-based genomic testing varies substantially according to the region and lesion sampled. From this observation, the authors concluded that information from a single biopsy is not sufficient for guiding treatment decisions.

Clinical utility of genomic classifiers in PCa

Although not the first study to demonstrate genetic variability within and between PCa foci (16-18), this study took perhaps the most exhaustive approach to the question—analyzing between five and seven tissue cores from each prostate gland and considering both DNA variability and subsequent RNA expression. Adding these findings to the evolving context of tumor multiclonality and variable biological aggressiveness, it is reasonable to acknowledge that tissue sampling remains a crucial limitation to our ability to accurately predict the clinical behavior of an individual's PCa.

Do these findings suggest that genomic classifiers should not be used clinically? In a word—no. Even considering their limitations, several studies have demonstrated the ability of these tools to provide incremental prognostic data beyond that of existing clinical modalities (19-21). Furthermore, the future of precision oncology is almost certainly based in understanding the genetic and molecular foundations of individual cancers. Nonetheless, these studies are a sobering reminder that integrated genomic approaches remain in their infancy.

Regardless of sampling limitations, the greatest hindrance to widely using genomic data remains our very limited understanding of their specific clinical implications. The reality is that the clinical outcomes associated with even the most well-established genomic alterations are not clearly defined or sufficiently validated (21). As others have proposed (22), research efforts should shift from simply grouping molecular signatures into "high" or "low"-risk to more specifically outlining the functional consequences of specific genetic and molecular features on disease course and responsiveness to therapy (23). Innovative studies linking progressive or treatment-resistant metastases back to a multifocal primary lesion will be crucial in establishing these genotype-to-phenotype relationships (23,24). This is a daunting undertaking, no doubt, but will only grow more feasible as emerging tools such as NGS kits for formalin-fixed paraffin-embedded (FFPE) tissue become commonplace and their costs decline.

Until existing limitations are overcome, the information provided by genomic classifiers should be applied with requisite consideration. The probability that a given tissue sample does not capture the most lethal clone, practically-speaking, simply increases the false-negative rate of genomic testing (in this case, due to sampling error rather than the test itself). As such, findings may be best interpreted like any test of limited sensitivity—a negative result (low-risk classification) does not reliably rule out the presence of high-risk disease (25) and therefore should not be used as the sole basis for deferring aggressive treatment. On the contrary, a positive result (high-risk classification) appears to reliably indicate the presence of high-risk disease (4,19,20) and may be reasonably considered to encourage treatment in those otherwise appropriate for it. Certainly, acknowledging the limitations of these tools will remain an

essential part of physician-patient counseling.

Conclusions

Due to high prevalence and variable clinical behavior of PCa, the ability to accurately predict the clinical course of an individual patient's disease is critical. The sophistication of prognostic assessment has increased remarkably in recent years, yielding a body of data so complex that cancer bioinformaticians are specially trained to synthesize and interpret it. Unfortunately, these technologies remain limited by tissue sampling in the same manner as age-old traditional approaches such as pathologic grading and staging. There is hope that any number of innovations will forestall the limitations of sampling. Nonetheless, the impact of specific genomic findings on clinical outcomes is poorly defined and requires great attention in the coming years.

Ultimately, genomic classifiers represent a clinical tool more powerful (and more complicated) than their predecessors. Like anything of great power—if used properly, this technology has the potential to improve substantially on the *status quo*. If used carelessly, it could have a detrimental effect. The common saying "proceed with caution" may be extreme for these purposes, but the prevailing message stands. It is critical that we utilize these tools with care—mindful of their strengths, shortcomings, and potential influence on the increasingly complex decision-making process.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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