The Heterogeneity of Prostate Cancer: A Practical Approach

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Abstract

Prostate cancer is a paradigm tumor model for heterogeneity in almost every sense. Its clinical, spatial, and morphological heterogeneity divided by the high-level molecular genetic diversity outline the complexity of this disease in the clinical and research settings. In this review, we summarize the main aspects of prostate cancer heterogeneity at different levels, with special attention given to the spatial heterogeneity within the prostate, and to the standard morphological heterogeneity, with respect to tumor grading and modern classifications. We also cover the complex issue of molecular genetic heterogeneity, discussing it in the context of the current evidence of the genetic characterization of prostate carcinoma; the interpatient, intertumoral (multifocal disease), and intratumoral heterogeneity; tumor clonality; and metastatic disease. Clinical and research implications are summarized and serve to address the most pertinent problems stemming from the extreme heterogeneity of prostate cancer.

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Introduction

Prostate cancer is a paradigm tumor model for heterogeneity in almost every sense. Its clinical course differs vastly, ranging from indolent tumors that are clinically completely innocuous and may only be found accidentally at autopsy, to aggressive tumors that metastasize rapidly and can significantly shorten life. It is this dichotomy of “pet type” versus “predator type” cancer that constitutes the Janus-headed face of this disease as mirrored in student textbooks. The morphology is highly heterogeneous as well, earning this tumor its classification as a “pluriform” neoplasm with glandular, cribriform, trabecular, solid, and single-cell tumor patterns, not to speak of metaplastic differentiation, which is less common. This incredible heterogeneity has also slowed the development of an appropriate grading system to allow for a biological classification of prognostic value according to the morphology.

The mode of infiltration of prostate carcinoma is also rather unique, as neoplastic glands mingle with benign glands, rendering the invasion front of this tumor lacerated and indistinct, in comparison to other solid tumors that show a more destructive growth with a more circumscribed invasion front. This may prove challenging to the surgical pathologist, who is confronted with minute tissue biopsies with even more minute patches of infiltrating tumor glands.

It is tempting to hope that the heterogeneity observed in the clinical and morphological spheres could be clarified and managed by an analysis of the molecular background of this disease, but the heterogeneity of this tumor is also found on the molecular level. This article aims to give the reader a broad overview about the challenges of the heterogeneity of prostate cancer, with a focus on the molecular aspects.

Spatial and Morphological Heterogeneity of Prostate Cancer

Spatial Heterogeneity

The heterogeneity of prostate cancer refers to its spatial and morphological heterogeneity. The zone model of the prostate gland, described by McNeal [1], involves the dissection of the prostate into 3 different compartments, or zones, that differ in anatomy and function. The majority of neoplasms (75%) originate from the peripheral zone, but the transitional zone, that has a greater propensity for benign hyperplasia, also gives rise to tumors, though these are less common. This limits the spatial heterogeneity to a small extent. The biopsy regimens of urologists reflect this and sample the peripheral zone more extensively [2].

Prostate cancer has an almost unique infiltration pattern, characterized by a relatively nondestructive growth of intervening stroma between benign preexisting glands, creating irregular tongues of tumor tissue that may extend quite far from the main focus of origin. This is in stark contrast to many other solid tumors (e.g., breast cancer, lung cancer, hepatocellular carcinoma, etc.), that often show a locally destructive yet cohesive growth pattern that results in a round tumor nodule, that can be more easily assessed by radiology and/or biopsy. In this sense, prostate cancer is a very ungrateful target indeed, and this has made it the only neoplasm to be diagnosed by random sampling at biopsy.

The sextant biopsy regimen, described by Hodge et al. [3] in 1989, that takes 3 cores (apical, mid, and basal) from both lobes, was the standard of care for a long time and provided cancer detection rates of 20–35%. As it became apparent that the random sampling of 6 cores missed some clinically significant tumors, more extended biopsy regimens evolved, and nowadays, 10–12 cores are the norm in resource-rich countries [4, 5]. The number of biopsies (>20) was inevitably increased to constitute so-called saturation biopsies, which aim to detect and map any carcinoma, thereby generating the clinical problem

of “overdetection” of insignificant tumors that would probably not have harmed the patient if left undetected [6].

The necessity for random sampling has been challenged by new developments in radiology, with multiparametric MRI evolving as a novel technique of prostate imaging that can detect significant tumors radiologically. However, recent studies comparing MRI-guided biopsies and additional random sampling still demonstrate the value of using both approaches to detect this heterogeneously distributed and differentiated tumor [7].

The multifocality of prostate cancer has long been described and also constitutes a problem for diagnosis and therapy planning [8, 9]. Acknowledging this issue has become more relevant as novel approaches of focal therapy arise [10]. In radical prostatectomy specimens, on average, 2.5 tumor foci can be distinguished, if carefully analyzed [11, 12]. The common multifocality and spatial heterogeneity of cancer distribution implies that pathologists cannot reliably ascertain if several positive tumor cores belong to the same tumor focus and may thus be lumped into a single diagnosis. Hence, common practice in most prostate cancer centers is still the individual reporting of each positive biopsy core. This may change in the future, if detailed imaging or molecular information can be reliably integrated in the diagnostic workup.

The spatial heterogeneity of prostate cancer also has implications for research. Tissue microarrays (TMAs), for example, have become an indispensable tool for cost-effective, high-throughput expression analysis of tumors, as they assemble hundreds of tumors on a few glass slides, that can then be analyzed by in situ techniques like immunohistochemistry or fluorescence in situ hybridization (FISH). The obvious downside is the sampling bias, and the inherent question of whether the minute focus taken of a tumor is sufficiently representative for the whole case, especially when dealing with a highly heterogeneous tumor like prostate cancer. This specific question has been addressed by Rubin et al. [13], who compared the results of Ki-67 expression in a small prostate cancer cohort ($n = 88$), and found that analyzing >3 cores (0.6 mm in diameter) did not necessarily convey relevant information. Alternatively, increasing the number of cases and hence the power of a TMA will statistically smoothen the effects of tumor heterogeneity.

Morphological Heterogeneity

The basic histological categories of adenocarcinoma of the prostate as currently defined by the WHO are surprisingly homogenous (briefly reviewed [14]), with the vast

Fig. 1. Morphologic heterogeneity of a prostate cancer case, illustrating infiltrating glands of different shapes and colors, and also intraductal growth.

majority (>95%) of tumors being classified as acinar adenocarcinoma [15]. As acinar adenocarcinoma of the prostate displays a wide spectrum of morphological patterns (Fig. 1), establishing a system to categorize it further into biologically and prognostically meaningful subgroups, i.e., to establish a grading system, has taken a long time to develop. Broders [16] is often cited as the grandfather of urogenital tumor grading, even though he did not write specifically about prostate cancer. However, his approach to generally classify tumors by the extent of undifferentiated cells deserves credit.

Several grading systems were proposed over the years and, for a while, were used competitively and tested against each other, without demonstrating universal superiority of a single system [17–22]. The system described by Mostofi et al. [23], that classified tumors according to their degree of glandular maturity into well-, moderately, or poorly (G1–G3) differentiated tumors, was first adopted by the WHO and then replaced in 2004 by the Gleason grading system, which became, irrespective of the WHO definitions, the de facto standard in the USA, and, later on, also in Europe.

The concept of the Gleason grading system is simple and appealing, as it focuses exclusively on tumor architecture by categorizing a morphological continuum of dedifferentiating tumor glands arbitrarily into 5 broad categories, called Gleason patterns, ranging from 1 (highly differentiated, well-circumscribed glands) to 5 (a complete loss of glandular differentiation). The ingenious

idea of this grading system is to combine a dominant pattern and a subdominant pattern to describe the whole tumor, which is probably a pragmatic and good fit for modeling a highly heterogeneous tumor like prostate cancer (Fig. 1). It completely ignores all other features that may bear prognostic information like nuclear atypia, stromal response, necrosis, or mitoses; this may be considered a strength of the system. The Gleason grading system underwent further specifications, orchestrated by the International Society of Urological Pathology (ISUP), that aimed to standardize current practice which, over the years, had begun to deviate from the original description by Donald Gleason [24–26]. Despite all efforts, it must be said that the interobserver variability of this powerful grading system was, and remains, moderate, and that further work is necessary to harmonize the human perception of morphological patterns [27–29], or support it with computer-assisted analysis [30, 31]. Alternatively, molecular markers may come to our aid to improve our classification of prostate cancer.

Genetic/Molecular Heterogeneity

A Brief Overview of Prostate Cancer Genetics

The huge efforts of some major scientific projects (The Cancer Genome Atlas [TCGA] and International Cancer Genome Consortium) have provided a deep understanding of prostate cancer genetics through sequencing the cancer genome [32]. The underpinnings of prostate cancer genetics are very complex, however. As is the case with many other tumor types, several genetic anomalies are highly recurrent, and these even allow the classification of prostate cancer into distinct molecular subtypes (7 groups, each based on 1 common recurrent genetic alteration, and an 8th group, “others”) [32] which, to date, actually have absolutely no clinical/practical implications, i.e., they are not relevant for prognosis and do not serve as predictors that would point to implementing any particular therapy. These subtypes probably outline the first oncogenic events that led to the formation of the tumor, and the further “functionality”/aggressivity of the tumor would depend on the subsequent genetic alterations.

Prostate Cancer Genetic/Molecular Heterogeneity Types

At the molecular level, 3 main types of heterogeneity are known: interpatient, intertumoral (in the case of multifocal prostate cancer), and intratumoral. Interpatient heterogeneity assumes that tumors from different pa-

tients will have an unique set of genetic alterations and little commonality between each other [32–34], but would fall into 1 of the 8 TCGA molecular subtypes (see above). Intertumoral genetic heterogeneity outlines the completely different genotypes of the tumors in 1 patient in multifocal disease and in separate cancer foci [33–35].

Probably one of the most intriguing types of the molecular heterogeneity is intratumoral heterogeneity, i.e., the presence of different tumor clones in 1 tumor focus as the result of the tumor evolving. Such clones have different sets of genetic alterations but develop in a certain hierarchy from parent to daughter clones, and therefore have some commonality, i.e., a core set of genetic alterations present in all clones [36]. Importantly, all 3 types of molecular heterogeneity have big implications for clinical and research practice.

Interpatient Genetic Heterogeneity

A clear message as to the uniqueness of every patient's tumor from the major genetic characterization studies [32–34, 37–39] raises the following questions: To what extent should we inform ourselves about the genetic alterations of every single tumor? Should we sequence every tumor's genome completely or retrieve information only about certain genes? Hundreds of studies have been performed to investigate the prognostic role of single genes, mainly those that are recurrently altered in prostate cancer, e.g., *ERG*, *PTEN*, *SPINK1*, and *EZH2* [40–43]. Despite their proven prognostic role, these genes did not find their way into the clinical practice, reflecting the fact that there is no “magic bullet” gene which could describe all the complexity of prostate cancer behavior [41, 42].

Another attempt was to use multigene signatures at the mRNA expression level, using reverse methodology and blindly selecting any of the thousands of genes which are associated with clinical end points (e.g., biochemical recurrence and overall/metastasis-free survival) while not taking into account their biological functions [44]. The commercial attractiveness of this methodology led to the development of some end-products, which are currently used in the clinical setting. However, these multigene signatures have a relatively low success rate in robustly and completely characterizing primary tumors and their behavior [45].

Intertumoral Heterogeneity

Multifocality occurs in 56–87% of prostate adenocarcinomas [46]. Multifocal lesions could imply a level of genetic predisposition in the development of prostate carcinoma [47, 48]. In this scenario, one could expect

that, in cases of multifocal cancer, all tumors will follow similar oncogenic path. However, studies on the genetic similarities of different tumor foci in 1 patient are lacking, and intertumoral heterogeneity should probably be placed in the same set as interpatient heterogeneity, meaning that multifocal tumors in 1 patient are unique. This thesis has found some ground in the scientific works on multifocal prostate cancer [33, 34, 49].

Interpatient/Intertumoral Molecular Heterogeneity versus Standard Morphology

One of the common considerations stemming from interpatient and intertumoral (multifocal disease) molecular/genetic heterogeneity is whether such heterogeneity is reflected in the standard morphological presentation. It is well-known that the Gleason score is a very strong, possibly even the strongest, predictor of the behavior of prostate carcinomas, e.g., extraprostatic extension of the tumor, lymph node metastasis, biochemical recurrence, and metastases development [50, 51].

One recent study showed that modern ISUP grading based on the modified Gleason score [52] shows a strong correlation with the extent and quality of any underlying genetic changes [53]. Typical genetic alterations in prostate cancer are copy-number variations, deletions, or amplifications of chromosome regions with tumor-relevant genes [32, 34, 54]. These chromosome regions are highly recurrent in prostate cancer. Rubin et al. [53] showed a clear trend for the growth of the number of such alterations from ISUP grading group 1 to group 5, with remarkable distinctions across the 5 groups. This implies that the Gleason score is an important morphological surrogate for the underlying genetic heterogeneity, even when it is not clear which genes are in fact crucial for shaping the tumor architecture.

Moreover, the study of Boutros et al. [34] showed that, even in the relatively homogeneous group of Gleason-7 tumors, the genetic diversity is overwhelming. Given the very different microscopic appearance of tumors of a similar grade (ISUP groups “2” and “3” and even group “1” tumors could have a very different microscopic presentation that represents an entire spectrum of architectures), it would be interesting to see if we could link the 2-dimensional microscopic appearance of a tumor even more tightly with its genetic background. However, such morphometric/genetic correlation studies have yet to be conducted.

Intratumoral Genetic/Molecular Heterogeneity

The history of intratumoral genetic/molecular heterogeneity studies extends from the relatively simple at-

Fig. 2. Branched development and clonal hierarchy of the different tumor clones in the prostate from the normal epithelium to the stages of first tumor clone and common ancestor clone, with the eventual appearance of the clone capable of metastatic seeding.

tempts to show this at the immunohistochemistry level (e.g., the marked intratumoral heterogeneity of *ERG* expression in approx. 70% of *ERG*-positive cases [55, 56]) to complex studies with high-throughput techniques (e.g., comparative genomic hybridization and next-generation sequencing studies) [3, 18, 26–28]. These provide the main input to date about the extent of intratumoral heterogeneity.

Crucial for the understanding of intratumoral genetic heterogeneity is tumor clonality (Fig. 2). This term means that, within 1 tumor focus, the evolution of the cancer is a step-wise and branched process from the early parent clones that arise from the normal epithelium and constantly progress to a higher-grade tumor via the accumulation of “driver” genetic alterations [54, 57, 58] which, in turn, provide the “fitness” advantage to a new clone (namely, a change in the birth/death balance of tumor cells towards birth). Many other genetic alterations, which have no influence on the birth/death balance are called “passengers” and are being accumulated in the tumor clones as well [59]. The positive “driver” alterations could occur anywhere in the tumor bulk within the pre-existing clone and give the start to a new, more survivable

clone [60]. Importantly, this also implies that all tumor clones in 1 tumor focus have a common ancestor (with the exclusion of specific circumstances, e.g., collision tumors, when 2 independent tumors coalesce during growth and form 1 focus).

All modern studies show that the clonal hierarchy of prostate cancer is extremely ramified (Fig. 2) [34, 61–63], but the extent of this branching/clonal intratumoral heterogeneity is not well understood. A good study by Lindberg et al. [64], based on the analysis of the chromosome breakpoints (the basis for copy-number aberrations), shows that all 25 analyzed areas from 1 tumor (with sometimes very small distances between the samples) contained different clones. Some of the neighbor clones at the minimal morphologic distance were genetically extremely different. Therefore, it could be stated that prostate cancer is a highly clonally heterogeneous tumor at the genetic and also epigenetic levels [35, 62].

Another interesting and understudied topic is the mechanics of tumor clonality development in prostate cancer at the cellular level. The genetic mechanisms are relatively well-understood, but the relationship between the clones and their spatial distribution are not clear. Some

insights could be acquired from the most recent study on the architecture of prostate cancer [31], which showed that prostate cancer comprises a complex integrative structure of intercommunicating tubules. This implies that, regionally at least, there is an epithelial continuity of all the structural elements (glands). Here, one could, therefore, apply the same mechanical principles as in, for example, colon cancer, or head and neck cancers, where clonal interaction occurs on the surface of the epithelium and has already been studied [65–67]. The fitness advantage that comes with the acquisition of the new “driver” mutation/genetic alteration leads to “clonal sweeps,” the spread of more active clones, and the extinction of weaker clones in the battle for dominance on the inner epithelial surface of the cancer glands. Nevertheless, the complex architecture of prostate cancer hampers the precise understanding of these processes.

Molecular Heterogeneity in Metastatic Disease

Several studies have addressed tumor heterogeneity at the stage of metastatic disease [64, 68–72]; this has major clinical applications. All patients with metastatic prostate cancer experience, sooner or later, the transformation to castration-refractory disease where the therapeutic options are limited. Knowledge about “actionable” mutations/genetic alterations in metastatic tumors could dramatically improve the survival of patients in this era of targeted therapeutic agents [73], especially in view of the fact that approximately 90% of metastatic tumors contain clinically actionable molecular alterations [74].

One of the main questions to answer is whether the metastatic disease is mono- or multiclonal: Is there is one seeding clone in the prostate or several of them? This problem could be solved by next-generation DNA sequencing and a precise analysis of all genetic alterations in a primary tumor and the metastases. Some studies provide evidence of the monoclonal origin of metastases, which seems to be logical [37, 64, 70, 71]. However, another study has been controversial by providing evidence of very complex patterns of metastatic seeding, such as the polyclonal nature of metastases, metastasis as a “pool” of clones for new metastasis, and the metastasis-to-metastasis exchange of tumor clones [72]. Two important conclusions stem from these studies. Firstly, all metastases stem from a common trunk of genetic alterations [37]. Secondly, clonality is a constant process, even at the metastatic site. Therefore, metastasis is a site for the cultivation of new, more aggressive (and resistant) clones.

Another cornerstone study [69], that analyzed the circulating tumor DNA in the blood, provided essential ev-

idence of tumor clonal heterogeneity in a metastatic setting during treatment, making it clear that the resistance to treatment was the consequence of the clonal heterogeneity of prostate carcinoma. Drug therapy leads to the emergence of new (and to a certain extent “old”) clones which, under the pressure of therapy, achieve the possibility for further development. Therefore, metastatic disease is virtually always a process which involves multiple clones with different resistance profiles.

Clinical and Research Implications of Prostate Cancer Molecular/Genetic Heterogeneity

The clinical implications of prostate cancer heterogeneity are diverse. Evaluation of the genetic background of a tumor could change our understanding of the index lesion in multifocal disease. In the past, the Gleason score and tumor volume were the main surrogates for index lesion selection [75, 76]. However, these could recede into the background with the upcoming knowledge of tumor genetics which would be especially important for focal therapy [77]. This would also imply the revision of the current active surveillance concepts for patients with minimal, low-grade prostate carcinoma, which are totally clinically and morphologically based. However, greater knowledge of prostate cancer heterogeneity will probably not lead to changes in the currently implemented biopsy techniques (e.g., extending schemas or sampling more tissue). When there is extensive intratumoral heterogeneity at the stage of primary cancer diagnosis and therapy selection (in the case of not-minimal, high-grade tumors), the Gleason score and tumor volume seem to be robust criteria for primary therapeutic decisions (i.e., surgery or radiation). However, at prognosis, with regard to the presence and potential development of metastases and the response to primary therapy carried out on biopsy material, these should be made important for therapy selection, with inter- and intratumoral heterogeneity being accounted for, because undersampling of more aggressive tumors would potentially drive inappropriate decisions.

Analysis of the properties of a tumor and its propensity for progression after radical treatment, (e.g., prostatectomy, should include the genetic analysis of the tumor tissue obtained from prostatectomy specimens. However, such analyses are senseless when they involve only 1 sample from the tumor, even from the highest-grade area, due to the extreme heterogeneity of prostate cancer. However, to date, it is not yet clear how many samples would be adequate.

The major area for the implementation of tumor heterogeneity-based strategies is metastatic disease. One im-

portant application is the analysis of a primary tumor in the entirety of its clonal architecture (e.g., a prostatectomy specimen or biopsy sample) which can provide essential information for therapy sequencing (e.g., the AR-V7 splice variant of the androgen receptor is associated with resistance to enzalutamide and abiraterone [78]). Whether the metastatic seeding is monoclonal or polyclonal, tumor clones stem from a common trunk genetic alterations/mutations (Fig. 2). When some of these aberrations are therapeutically actionable, therapy would probably be effective against all of the clones, meaning better control. Targeting the subclonal genetic alterations could compromise control and lead to the selection of clones without these alterations. The other important application is the control of therapy efficiency using circulating DNA or circulating cancer cells in the blood. This could facilitate the adjustment of the therapy to target the newly emerging tumor clones and their genetic background.

The implications for research are also manifold. One of the main bottlenecks of current prostate cancer research is the failure to understand the extent of intratumoral heterogeneity and clonality. The main question here is: How many samples from the tumor do we need to say that they represent the tumor in its entirety (so-called saturation)? Importantly, this should be a meaningful (from a clinical and a research point of view) saturation, while, even at the level of single cells, one can find the differences in the number of mutations/copy-number variations/ploidy etc., which do not play any role (they do not define tumor properties and are not therapeutically actionable; many such cells are not cancer stem cells and will therefore out-differentiate and disappear). These data could dramatically change the methodology of the research. Many modern prognostic studies are based on the construction of TMAs which contain only 2–3 samples from each patient. Should it be demonstrated in further studies that intra- und inter-tumoral heterogeneity of prostate cancer warrants many more samples, the whole stratum of prognostic studies from several decades would be wiped out. Biobanking techniques would also be significantly affected [50].

Due to the complexities, studies on clonality evolution in prostate cancer, especially in view of resistance that develops during treatment, are currently lacking. In this area, the mathematic modeling seems to be a worthwhile perspective, similar to in other tumor types [57, 59, 60, 79, 80]; here too, however, the complexity of the tumor architecture could be a major limitation. The extreme heterogeneity of prostate cancer at the metastatic disease stage also warrants further studies. The actionable genetic alterations necessary for the development of new med-

ications and the targeted use of known drugs are nowadays being extracted from high-throughput molecular characterization studies. However, the complex game of clonal interactions during selective pressures and the constant emergence of new resistant clones make these efforts somewhat challenging. Another potential problem for such studies is the necessity to obtain the tissue for molecular analysis from metastases, which is normally not indicated from the clinical point of view [81]. However, some modern research technologies (single-cell sequencing, single-nucleus sequencing, circulating tumor DNA analysis) would support these efforts [69, 82–86]. While the very complex clonal heterogeneity in patients with metastatic disease [72] is under investigation, some studies show that analysis of a single metastasis could be enough to assess major oncogenic driver events because the genomic diversity between metastases is limited [37]. This fact has received confirmation from the evidence in studies on other types of tumors [87].

Conclusion

The heterogeneity of prostate cancer is tremendous and has been recognized, but is still understudied. So far, clinical and research specialists have been operating primarily on the clinical and pathomorphological levels, and we have only just begun to appreciate the issue of the molecular heterogeneity of prostate cancer. The genetic studies conducted on prostate cancer so far only allow a vague approximation of how huge further scientific efforts in this area need to be. Currently, many heterogeneity and clonality issues appear unclear. However, we are confident that the powerful research armamentarium available today will allow for progress in our understanding of the molecular genetic diversity of prostate cancer, that can usher in major changes in clinical practice.

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