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Seminars Article

Molecular correlates of intermediate- and high-risk localized prostate cancer

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Abstract

Clinicopathologic parameters, including Gleason score, remain the most validated prognostic factors for patients diagnosed with localized prostate cancer (PCa). However, patients of the same risk groups have exhibited heterogeneity of disease outcomes. To improve risk classification, multiple molecular risk classifiers have been developed, which were designed to inform beyond existing clinicopathologic classifiers. Alterations affecting tumor suppressors and oncogenes, such as *PTEN*, *MYC*, *BRCA2*, and *TP53*, which have been long associated with aggressive PCa, demonstrated grade-dependent frequency of alterations in localized PCas. In addition to these genetic hallmarks, several RNA-based commercial tests have been recently developed to help identify men who would benefit from earlier interventions. Large genomic studies also correlate germline genetic alterations and epigenetic features with adverse outcomes, further strengthening the link between the risk of metastasis and a stepwise accumulation of driver molecular lesions. Published by Elsevier Inc.

Keywords: Prostate cancer; Intermediate-risk; Molecular alterations; Gleason grade; Upgrading; Biochemical recurrence

Introduction

Despite being the most common visceral malignancy in American men, with approximately 161,000 new diagnoses in 2017, the fact that less than 20% of men diagnosed with prostate cancer (PCa) die from this disease [1] emphasizes the dual contradictions that while PCa screening and effective interventions are saving lives [2], a substantial proportion of the remaining 80% were never at risk of PCa-specific mortality. For both the patient and his physician, determining which factors to take into account when attempting to predict the likelihood of developing aggressive disease can be a daunting prospect. The challenge of classifying the tumor correctly and determining the risk of disease progression requires adequate tissue sampling as well as applying the appropriate risk-stratification tools.

The frequent and extensive intratumoral heterogeneity of PCa represents a major confounding consideration when assessing an initial diagnosis [3,4]. A total of 85% to 90% of PCas are multifocal [5]. Branching morphogenesis that occurs during the development of the embryonic prostate organ remains at play during development of each individual prostate tumor [6]. Extensive divergence from a common cancer cell ancestor can result in multiple distinct yet coexisting tumor subclones, and prognostic features identified in one of these subclones may not be representative of others. Consequently, disease aggressiveness predicted by the gain of 1 or more molecular alterations must be considered in the context of what may have occurred truncally (i.e., shared by all subpopulations) thus being captured by biopsy, or what is subclonal and may be only present in the unsampled portions of the tumor. Therefore, a deeper understanding of the heterogeneous nature of PCa and mechanisms of molecular progression can improve the application of molecular ancillary tests for risk stratification and patient management.

All prostate biopsies, whether templated or magnetic resonance imaging/ultrasound-fusion guided, suffers from the intrinsic challenge of tumor heterogeneity in that a high-grade component might be underrepresented or overrepresented, due to limited sampling [7,8]. Historically, the greatest value of biopsy was in the positive predictive power of the pathology (tumor grade and volume) for

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disease aggressiveness, guiding patient counseling, and decision making. Historic undersampling is expected to decrease by application of advanced imaging modalities such as multiparametric magnetic resonance imaging, which has an increased sensitivity in detecting higher-grade disease [9–11]. Based on the findings that the adjacent Gleason grade 3 and grade 4 components in intermediate to high-risk PCa are clonally related and share some common molecular features, commercial molecular tests and in-house ancillary tests have been developed and increasingly used to predict final pathology on radical prostatectomy (RP). Moreover, alterations of a handful of driver genes may indicate molecular progression, which we define here as the stepwise accumulation of genomic or genetic alterations that may accompany changes in histopathology grades and are closely associated with disease progression. Finally, molecular alterations detected in the RP tumor tissue can offer prognostic value in determining which patients would benefit from adjuvant radiation therapy (RT).

Gleason grade 3 and 4 components in intermediate- to high-risk PCas are clonally related

Intratumoral heterogeneity was the basis for establishing a pathologic grading system (the Gleason score, GS) 50 years ago [12]. Indeed, the Gleason score's consideration of multiple simultaneous cancer differentiation statuses remains the single best, and most validated, parameter for assessing PCa prognosis [4]. Both the original Gleason scoring system and the modified grade grouping system [13] assign an overall score by adding the most predominant and the second predominant architectural growth pattern, each reflecting distinct underlying molecular alterations. Following a 2014 International Society of Urological Pathology (ISUP) consensus, GS of $3 + 3 = 6$ was reclassified into the lowest grade group (GG) of 1, GS $3 + 4 = 7$ to GG2, $4 + 3 = 7$ to GG3, $4 + 4 = 8$ to GG4, and any amount of Gleason pattern (GP) 5 ($4 + 5$, $5 + 4$, or $5 + 5$), into the highest GG of 5 [13,14]. These grade groupings reflect increased recognition that the quantity of GP4 present (and further distinguishing $3 + 4$ from $4 + 3$) is highly significant, stratifying risk of recurrence-free survival 5 years after RP from ~90% to ~60%. For example, what may be a small group of cancer cells buried within a tumor of GS7 (or their unsampled progenitor) could potentially be the precursor for a distant metastasis; while morphologically identical to their sister cells, these cells could harbor molecular alterations that predispose the patient to an adverse outcome.

It was the Gleason grade's prognostic power that prompted several research groups to assess patterns of mutation and gene expression to ask what makes GP4 behave worse than GP3, and if found together, whether they were clonally related. Next-generation sequencing permitted single-base resolution of the *TMPRSS2-ERG* breakpoint to be used as a definitive clonal marker to demonstrate a common somatic ancestry in ERG-positive GP3 and GP4 foci, in a series of 4 GS7 cancers [15]. Moreover, *PTEN* copy number was noted as a potential mechanism of Gleason progression in 2 cases with clonal *ERG* breakpoints. The finding of clonal relationship between adjacent GP3 and GP4 was confirmed by a study by Kovtun et al., [16] examining global genomic breakpoints in 14 cases of GS7 PCa.

Two independent studies subsequently revealed that the GP3 and GP4 components diverge very early in the development of intermediate to high-risk PCa. Vander-Weele et al. [17] examined the lineage relationship of GP3 and GP4 tumors using whole exome sequencing for higher resolution of shared (and distinct) somatic variants and performing phylogenetic reconstruction in 4 cases of GS7 cancer, including lymph node metastases in 2 patients. In addition to tumors showing evidence of common ancestry, fewer mutations were shared between low-grade and high-grade foci vs. high-grade and metastatic foci, indicating early divergence. A similar conclusion was made in a separate study of 12 GS≥7 cases [18], demonstrating GP3 cancer diverged early from a common ancestor that also had given rise to GP4 disease. Furthermore, the authors identified that increased MYC activity mediates progression from GP3 to GP4 in a subset of tumors. With regard to the precursor lesions of adjacent GP3 and GP4, deeper resolution of data from multiregion sequencing studies suggested that the true origin of these tumors is morphologically normal tissue, representing the "field effect" or somatic mosaicism in the earliest stages of PCa evolution [19,20]. In addition, somatic alterations in high-grade prostatic intraepithelial neoplasia have been shown to indicate a distant clonal relationship between prostatic intraepithelial neoplasia and adjacent GP3 and GP4 disease [21,22]. However, whether alterations present in an aggressive precursor can set a fate of the tumor to progress rapidly is yet to be determined.

Approximately 95% of men with GS6 cancer are cured after undergoing RP [14]. From the perspective of molecular progression, the 5% that do recur may harbor some volume of aggressive disease that establishes a micrometastasis before treatment. These tumor cells may have taken the form of either (A) an unsampled GP4 component or (B) morphologically GP3 tumors that had underwent further occult oncogenic alterations as the source of potentially lethal disease. With the latter being exceedingly rare, the converse argument may also be made about those men with GS $\geq$ 7 ($4 + 3$) PCa upon RP who never experience relapse: (A) either their tumor had aggressive potential but was resected early enough to prevent micrometastastic spread, or (B) despite being morphologically high grade, it had not yet undergone oncogenic transformations required for metastasis, and thus resection could have been delayed. These examples, which are not uncommon,