Editorial

Intermediate Risk Prostate Cancer: Disease Heterogeneity Linked to Measurable Biological Features

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Localised prostate cancer (PCa) remains a disease setting where absolute prognostication is difficult, as features that indicate upfront clinical aggression may not be apparent. An individual’s prognosis is currently made based on a multimodal assessment of physical and biochemical disease characteristics and this information is used to predict the likelihood of disease progression [1]. Histopathological assessment of biopsied tissue to identify carcinoma-associated Gleason patterns and TNM tumour classification are standard measures of disease severity [2,3]. Dominant Gleason scores are used to associate patients into D’Amico risk groups, which predict biochemical (prostate-specific antigen) disease recurrence. At diagnosis, 40% of patients present with intermediate risk (Gleason 7 [3+4, 4+3], prostate-specific antigen 10–20 ng/ml, stage T2b–T2c) tumours, a majority of which are indolent and a currently unidentified minority which are not [2,3]. The recently revised ISUP grade group model partially addresses this minority, who are more likely to benefit from active therapy than active surveillance by separating 3+4 (group 2) and 4+3 (group 3) patients. For men who are recommended active therapy (high risk PCa), surgery (radical prostatectomy) and/or adjunct radiotherapy (external beam radiotherapy or brachytherapy) and androgen deprivation therapy (ADT) are potentially curative or allow more than 90% 10 year survival [4,5]. An active area of research is identifying prostate tumours that will ultimately evade therapy or acquire treatment resistance and these may have upfront aggressive features which precede histopathology.

Localised Prostate Cancer Genetics

Genetic marks that are seen at high frequency in advanced PCa, such as Androgen Receptor (AR) mutations and AR splice variant (AR-SV) expression, are not seen in localised, treatment-naive men [6–8]. Instead, early genetic factors to consider that give insight into latent clinical aggression are the presence of TMPRSS2-ERG fusions, which represent 60% of these cases [7]; deletion of PTEN and alteration of TP53 are also significant, occurring in 17% and 8% of cases and become more frequent in advanced disease (>50% of cases). In low–intermediate risk men, it was shown that genetic marks of aggression may involve dysfunction of groups of genes governing genetic stability, such as gene copy number alterations and structural rearrangements, which precede the accumulation of single nucleotide variant changes and pathological aggression associated with high risk to advanced carcinoma [8]. Speaking to this genomic complexity, the use of computationally developed genetic signatures that capture combinations of events to predict for disease relapse, cause-specific survival or metastasis-free survival may have better prognostic value for these men. Relevant to high risk cohorts, the recent ‘Decipher’ test aims to stratify patients based on a 22 gene, RNA-based array associated with risk of disease recurrence [9,10]. The utility of similar multigene signature-based tests, such as the 12-gene ‘Oncotype DX’ and the 31-gene ‘Prolaris’, has shown efficacy in intermediate risk PCa [11–13] and clinical integration of these tests to become standard practice are ongoing.

Genetic Instability as a Driver of Prostate Cancer

The maintenance of genetic stability requires coordinated interaction between sets of genes involved in the DNA damage repair process. Processes include base excision...
Defects and Tumour Hypoxia

Aggressive Collaboration: DNA Repair

Defects in genes that actively sense or repair DNA damage can result in widespread chromosomal alterations, giving rise to genetic instability [14,15]. Importantly, recent evidence supports a role for increasing genetic instability and disease relapse [16,17]. Whole genome sequencing analyses on advanced tumours have shown that copy number changes in key genes that regulate the DNA damage response (DDR) are implicated in metastatic progression [18], the result of which has a profound effect on the oncogenic signalling programme [17]. In localised PCa, somatic mutations in these sets of genes are less common (10% of localised cases), but this becomes more frequent with clinically aggressive disease (27% of metastatic cases) and some of the most frequent of these occur in NBS1, BRCA1, BRCA2, PALB2, CHEK2 and ATM [18], many of which are essential for genetic stability.

In addition, repair fidelity can also be affected by persistent AR signalling. Increased cell killing with hormone therapy is believed to be mediated partially by AR suppression, decreasing the abundance of AR target genes, some of which are DDR proteins [19]. Consequently, increased genome alteration as a marker of genetic instability is observed in metastatic castration-resistant PCa, and patients are said to have acquired a ‘BRCAness’ phenotype, a term that describes a state of dysfunctional DNA repair such that drugs that exploit this acquired vulnerability, such as poly (ADP-ribose) polymerase I inhibitors, have an increased therapeutic ratio [20]. An added complexity in the context of aggressive localised disease is that men with germline BRCA1/2 mutations are also at risk of a specific tumour phenotype associated with a premalignant field defect, which may be characterised by compromised homologous repair [21,22]. These individuals have shown a three- to eight-fold increased lifetime risk of PCa, frequently fail active therapy and have a significantly reduced cancerspecific survival [21–23]. The driver mechanisms in this context are unclear, but suspected changes in tumour biology that implicate a gain of strong oncogenes, such as MYC (Chr8q amplification), and a loss of strong tumour suppressors, such as TP53 (Chr17p loss) and PTEN (Chr10q loss), are strongly suspected to contribute to an aggressive phenotype [23].

Aggressive Collaboration: DNA Repair Defects and Tumour Hypoxia

Tumour microenvironment oxygen availability has been shown to influence tumour physiology and confer susceptibility to DNA damage. Physiological oxygen concentrations in organs are variable and tissues such as the prostate are particularly less oxygenated [16,24]. Chronic and cycling hypoxia (cycles of rapid reoxygenation followed by acute hypoxia) can arise due to fluctuations in perfusion occurring in abnormal and unstable neovascularisation [16]. Fractionated radiotherapy partially circumvents this effect as hypoxic cells become reoxygenated over time and are sensitised during these periods. Prolonged low oxygen exposure can have widely differential effects on tumour cell biology. For example, chronic hypoxia markedly reduces expression of many DDR genes, which can allow mutations to accumulate [25,26]. Hypoxic foci are also known to augment and reduce double strand break repair protein abundance and biophysically resist ionising radiation therapy by decreasing the availability of reactive oxidative species, which induce DNA lesions [16]. Accordingly, localised prostate tumours are focally hypoxic and this is linked to increased genetic instability [27]. Tumour hypoxia may also be a significant risk factor for those individuals with a premalignant field defect (i.e. germline mutations in DDR genes), leading to exacerbation of initiated foci that are already homologous repair compromised. This concept remains to be explored further.

Genetic instability and hypoxia could be independent but synergistic, measurable entities that converge on mutagenesis giving rise to intratumoral clonal diversification (polyclonality), which may strongly contribute to driving upfront clinical aggression in localised disease. Ultimately, the measurement of these entities at clinical presentation may be crucial for precision oncology in this setting. Molecular evidence associates hypoxic foci with high percent genome alteration (PGA) and it has been evidenced as having carcinogenic potential [17,25]. Using this knowledge, Lalonde and colleagues [28,29] derived a 31 locus DNA signature from an assessment of PGA and clinical variables in order to prognosticate these localised tumours. Interestingly, this study also showed that hypoxia was an independent delineator of patients with high PGA and that highly hypoxic regions with high PGA had the most frequent biochemical recurrence (BCR) [28]. This was shown to reliably predict BCR in men who presented with low to intermediate score tumours [28]. Additionally, Yang and colleagues [30] independently showed efficacy of a 28 gene, RNA signature in predicting biochemical and metastasis-free survival, further implicating hypoxia as a delineating factor for prognostication in the localised disease setting. To address mechanisms of aggression, Bhandari and colleagues [31] quantified hypoxia in 8006 tumours across 19 different tumour types and showed that hypoxia exhibited a unique driver mutational signature. In the localised setting, hypoxia was associated with elevated rates of specific genomic alterations, such as chromothripsis, allelic loss of PTEN and shortened telomeres, and that these features are also particularly enriched in polyclonal tumours [31]. As tumour hypoxia is sufficient to facilitate DNA damage [32], it is difficult to assess whether tumour hypoxia is temporally aligned before or after sporadic carcinogenic lesion occurrence.

Selective pressure promoting mutational burden and genetic divergence are thought to give rise to genetically distinct tumour cells that may have differential contextual fitness. Speaking to this concept, Espiritu and colleagues [33] showed genomic evidence of tumour evolution by reconstructing phylogenetic trees from whole genome sequencing of 293 localised tumours with known clinical outcomes and associated increasing intratumoral genetic diversity with poorer overall survival. These data showed
that patients with low risk, monoclonal tumours rarely present with relapse after primary therapy, whereas those who have high risk multiclonal disease frequently have worse disease [33]. Additionally, certain genes are frequently mutated following subclonal diversification, such as \(\text{RB1}, \text{MTOR}\) and \(\text{NKX3-1}\) [33]. It is possible that certain specific genetic losses and gains such as these are sufficient for an aggressive intermediate risk tumour, therefore delineating the mechanisms that give rise to polyclonal disease is of great importance. It is possible that this polyclonal diversification results in the emergence of subpathologies associated with poor outcome, such as \(\text{IDC-P}\). Chua and colleagues [34] also showed that \(\text{IDC-P}\) are frequently seen in men with aggressive disease and in these men, poor outcome is associated with regions of hypoxia and increasing genetic instability, with \(\text{IDC-P}\) bearing increased \(\text{PGA}\). Specifically, these men who also have increased expression of \(\text{SchLAP1}\) have a particularly unfavourable outcome [34]. Future studies will aim to address mechanistic unknowns about the interactions and function of \(\text{SchLAP1}\), \(\text{IDC-P}\) and hypoxia to explain treatment resistance and enable precision medicine for these men (see Table 1 and Fig. 1).

**Final Remarks**

The intermediate PCa setting remains difficult to absolutely prognosticate, as upfront aggression is not easily delineated with any standard method currently available. Observed and validated entities such as genetic instability are measurable, marked by widespread genome alterations and are exacerbated in regions of tumour hypoxia, potentially giving rise to polyclonal disease and subpathologies associated with poor outcome, such as \(\text{IDC-P}\). Men with intermediate risk localised PCa can present with these features without overt pathological phenotypes. Therefore, precision medicine for intermediate risk PCa would benefit from the measurement of these additional features.

**Conflicts of Interest**

The authors declare no conflict of interest.

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**References**


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**Table 1**

| Emerging biological features of aggressive intermediate risk prostate cancer |
|----------------------------------|------------------|------------------|------------------|------------------|
| Adverse biological feature       | Clinical model/assay | Incidence (frequency) 5–10 year BFS | 10 year CSS/MFS |
| None                             | GS + PSA + T-category | 100% (40% of diagnosed [2,3]) | 60% (10 year) [34] | 80%/90% [21,34] |
| Increased hypoxia (<20 mmHg oxygen) | Hypoxia probe (Eppendorf), hypoxia score | 81% [29,35] | 80% (5 year) [34] | 78% (5 year) [35] |
| Increased genetic instability (>7.5% PGA) | SNP array | 25% [8] | 60% (5 year) [29,34] | N/90% [34] |
| Increased polyclonality | SNP array | 59% [33] | 20% (10 year) [33] | N/39% [33] |
| Increased IDC-P | Histopathology, IHC-P | 9% [34] | 40% (10 year) [34] | 50% (5 year) [36] |
| Nimbosis (hypoxia, IDC-P, schLAP1) | Histopathology | 4% [34] | 25% (10 year) [34] | N/60% [34] |
|                          |                  | Germline: 2% [22] |                  | +IDC-P: 20%/N [36] |

BFS, biochemical-free survival; BRCA, breast cancer susceptibility protein; CSS, cause-specific survival; GS, Gleason score; IDC-P, intraductal carcinoma of the prostate; IHC-P, immunohistochemistry; MFS, metastasis-free survival; MLPA, multiplex ligation-dependent probe amplification; N, data not available; PGA, per cent genome alteration; PSA, prostate-specific antigen; SNP, single nucleotide polymorphism; T-category, tumour category (TNM classification).

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**Fig 1.** Biological characteristics as prognostic tools for localised prostate cancer.


