Review Article

Genomic instability and circulating tumor cells in prostate cancer

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Abstract: Current clinical challenges in the management of prostate cancer patients make the use of circulating tumor cells (CTCs) an attractive option. Tissue biopsies rarely represent the whole tumor, and there is significant intra- and inter-patient tumor heterogeneity even for patients who receive the identical pathological tumor grading. CTCs have been shown to harbor genetic changes found in both the primary and metastatic tumors. They offer the opportunity of multiple and less invasive sampling from the patient's blood, and allow for the monitoring of the dynamic process of tumor evolution. The genomic instability (GI) present in CTCs is poised to allow for more accurate patient management.

Keywords: Genomic instability (GI); circulating tumor cells (CTCs); prostate cancer

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Genomic instability (GI)

GI is a dynamic process that re-organizes the genetic content of affected cells with each cell division. The term "GI" summarizes a complex set of genetic alterations including point mutations, deletions, duplications, amplifications, insertions, translocations, rearrangements and inversions. GI is never static, and it creates and propagates clonal diversity.

The above-listed aberrations may be localized and restricted to certain chromosomal regions. Alternatively, they may occur throughout the genome without an apparent involvement of specific sites. The latter cases make the identification of non-random aberrations that include driver mutations very difficult. The former alterations, when found in each cell or in most of the tumor cells, allow for the identification of driver mutations. Both types of aberrations are found in genomically unstable cells.

Work by Gerlinger *et al.* (1) demonstrated the extent of tumor cell heterogeneity found in a patient's tumor. Intratumor heterogeneity was established following exome sequencing, chromosome aberration analysis, and ploidy profiling (1). The reported intratumor heterogeneity is at the origin of tumor cell evolution that may occur along different paths at each multifocal site, and it therefore may adversely impact on providing personalized medicine options, especially when such decisions depend on tumor cell biopsies. Although Gerlinger *et al.*'s study (1) was carried out with primary renal carcinomas and associated metastatic sites, their findings are widely applicable to other tumors including prostate. Intratumor heterogeneity enables insights into the complexity of the genomic profiles of tumor cells and represents a major challenge to personalized medicine and biomarker development as it is linked to the Darwinian evolution of the tumor cells (1). In this context, Lipinski *et al.* (2) emphasize that cancer is an evolutionary process: mutations, drifts and selection processes are underlying processes involved in tumor development and progression. This concept is not new; it was first experimentally addressed by Boveri (3,4). One hundred years later, ongoing studies have confirmed his findings and models using modern technologies.

Prostate cancer

Prostate cancer is the second most common cancer in men. Nine hundred thousand men are diagnosed worldwide with prostate cancer every year (5), and of these, 250,000 men die of it each year (6).

Prostate cancer is a heterogeneous disease with indolent and aggressive forms. Patients with the same Gleason score often have different outcomes (5,7-10). As Schoenborn *et al.* (9) state, "*Though pathological grading provides a powerful indicator of disease behavior*, *clinical outcomes of tumors with the same histological patterns can vary substantially*." This is due to the fact that the clinical prognostic grouping for localized prostate cancer is imprecise, with 30–50% of patients recurring after image-guided radiotherapy or radical prostatectomy (RP) (5). Close to 20% of intermediate-risk patients have biochemical failure that occurs within 18 months of primary local therapy (5). The consequence of imprecise clinical prognostic grouping is that some indolent tumors are overtreated, while aggressive ones receive no or delayed treatment. The apparent paradox in clinical prognostic grouping of patients and the inter- and intra-personal differences between patients of the same pathology grouping is linked to the level of GI present in the patient's tumor.

Genomic profiles of prostate cancer

When diagnosed, the tumor is multi-focal, and the analysis of multiple biopsy cores suggests genetic heterogeneity from one core to the next for an individual patient and significant differences exist between patients.

As reviewed by Schoenborn *et al.* (9), somatic copy number aberrations are found in >90% of all prostate cancers. Deletions occur more frequently than amplifications and are mostly focal (≤1–5 MB). Deletions are found on chromosomes 6q, 8p, 10q, and 13q and include genes such as *NKX3-1*, *PTEN*, *BRCA2* and *RB1*. Castration-resistant prostate cancer (CRPC) shows frequent amplification of chromosomes X, 7, 8q, and 9q, which include the androgen receptor (*AR*) and *MYC* oncogenes.

Structural aberrations are seen in about 50% of the tumors and involve *TMPRSS2*:*ERG*, where the ERG oncogene is placed under the control of androgen-responsive *TMPRSS2* regulatory elements (11). Rearrangements can also result in new fusion proteins such as *ESRP1*:*CRAF* (12) as well as rearrangements involving other ETS family members (13), and *RAF* kinase gene fusions (14).

Of the determined point mutations, a mutation of MSH6, a DNA mismatch repair enzyme, was linked to a hypermutator phenotype (15-17). MSH6 mutation led to 25-fold more mutations than present in prostate cancers without the MSH6 mutation. Other common mutations include *TP53*, *PTEN*, *RB1* and *PIK3CA* (17-20) and activating mutations of *KRAS* and *BRAF*. Additional recurrent mutations involve *AR* and AR pathways, chromatin modification and transcription in general (9).

The Cancer Genome Atlas (TCGA) Research Network presented a comprehensive analysis of 333 prostate tumors and identified seven molecular subtypes based on the genomic profiling performed (21). The subtypes were defined by the presence of specific fusions or mutations. The fusions included *ERG*, *ETV1/4* and *FLI1*; the mutations included *SPOP*, *FOXA1* and *IDH1*.

Lalonde *et al.* (5) reported on GI and the tumor microenvironment in image-guided biopsies of 126 low- and intermediate risk pre-radiation patients (Toronto cohort) and validated their findings with RP specimen of 154 patients (Memorial Sloan Kettering cohort) and 117 (Cambridge Cohort), respectively.

The authors observed a high degree of genetic heterogeneity in patients with Gleason scores of 6 or 7. Most common were 8p amplifications and 8q deletions, in addition to deletions of 16q23.2 and 6q15. Seventy-six (60%) of 126 low and intermediate risk patients had copy number alterations. Unbiased hierarchical clustering placed patients of the Toronto cohort into four subgroups: group 1 with gain of chromosome 7, group 2 with deletion of 8p and gain of 8q, group 3 with loss of 8p and 16q, and group 4 that was so-called quiet genomes due to few genomic alterations. Patients in the latter subgroup had a significantly better prognosis than those in subtypes 1–3. Lalond *et al.* (5) identified and validated a 100-loci (276 genes) DNA signature that involved 14 chromosomes. This study also indicated that the four GI-derived subtypes were independent of Gleason score, T category, and prostate specific antigen (PSA) in all cohorts as individual Gleason 6 tumors had a higher percentage of genome alteration than some Gleason 7 (4+3) tumors. The percentage of genome alteration was strongly prognostic, independent of clinical covariates, as previously reported (22).

Taylor *et al.* (15) also described genomic profiling that classified patients beyond the classification the Gleason scoring could achieve. *TMPRSS2-ERG* fusion was associated with a prostate-specific deletion at chromosome 3p14. Moreover, DNA copy-number alterations robustly defined clusters of low- and high-risk disease beyond those achieved by Gleason score.

In their study of Gleason 7 disease, Boutros *et al.* (10) examined prostate cancer samples from 74 treatment-naïve patients. Similar to the other studies reviewed above, the authors find significant heterogeneity in the level of GI and

DNA copy number variations within the same patients. In addition to aberrations observed by others, this group also identified focal amplifications of *MYCL*.

Characterization of GI in circulating tumor cells (CTCs) of prostate cancer patients

CTCs were first discovered by Thomas Ashworth (23). These cells originate from the primary tumor and from metastatic sites. They can be isolated from a patient's blood and are often described as "liquid" biopsies in contrast to tissue biopsies. CTCs are present in the blood stream for a limited time; in breast cancer, a half-life of 1–2.4 hours has been reported (24).

Multiple methods exist to isolate CTCs, and the number of cells found in prostate cancer patients is isolation method-dependent [(25-31); for reviews, see (32-34)]. Thus, the numbers of CTCs reported in different studies vary. For example, a recent study reports 16–139 CTCs for non-metastatic prostate cancer patients using a filtration-based device (29). Using near-infrared neodymium tagging, Liu *et al.* (30) identified up to 168±33 for Gleason 6 and up to 420±50 for CTCs of Gleason 7 patients, respectively, with a range of 134–773 CTCs for Gleason 6 to 9. In their study of low and intermediate risk prostate cancer, Shao *et al.* (26) identified live CTCs using near infrared heptamethine carbocyanine dyes in all but one of the 40 patients examined. Stott and colleagues reported similar results using microfluidics; their study cohort had one Gleason 6 patient without CTCs (25). Awe *et al.* (28) found CTCs in all risk groups of prostate cancer using filtration, but no CTC numbers were published. In contrast, when CellSearch was used to isolate CTCs, CTCs were rarely detected in localized prostate cancer (31).

Recent studies published single CTC sequencing to establish the genomic profiles of CTCs and examined CTCs from all risk groups starting with Gleason 6 (35,36). It is anticipated that the presence of CTCs and their genetic profiles may be predictive of patient outcome. Future studies will provide further evidence to the molecular genetic value of CTCs during a patient's disease course.

The analysis of genetic alterations from sources other than tissue biopsies is of key importance as prostate biopsies have a success rate of 60–70% even with CT guidance (9). CTCs provide such an opportunity: Due to the multifocal nature of prostate cancer, the genetic analysis of CTCs allows for an understanding of the GI profile of the tumor they originate from. Several studies emphasize the clinical utility of this approach (9,33,34,37-40). For example, Thalgott *et al.* (41) found for high-risk patients undergoing neoadjuvant chemotherapy/hormonal therapy and RP that those patients with persistent CTCs post-RP developed biochemical recurrence. A summary of clinical applications for CTCs is provided by Alix-Panabières and Pantel (40).

The molecular genetic characterization of CTCs is key to an understanding of their association with indolent or aggressive disease. Genetic approaches have been successful in characterizing CTCs. Genomic data of pooled CTCs and of single CTCs have been reported and technical challenges of sequencing single or pooled CTCs have been discussed (37-39).

Kanwar and Done (33) summarize data indicating that the heterogeneity of CTCs measured by their genetic profiles is representative of subclones in primary tumors and of genetic signatures present in metastases. Data by others also highlight degrees of concordance between the CTCs and the tumor tissue (37). Lack *et al.* (37) studied CRPC and treatment-naïve tissues as well as CTCs using whole genome amplification and exome sequencing. In their pooled CTC study, CTCs had a higher frequency of mutations than tissues in CRPC. These CTCs allowed for the identification of 71% of mutations shared by treatment-naïve and CRPC tissue samples. Lohr *et al.* (38) showed recurrent and non-recurrent CTC aberrations (38). Greene *et al.* (39) examined genomes of prostate cancer cell lines and patient CTCs using array comparative genomic hybridization (aCGH), fluorescent *in situ* hybridization (FISH), and next generation sequencing (NGS)-based approaches. They identified a high level of heterogeneity among the CTCs and classified them by the level of large-scale state transitions (LSTs) as a measure of GI, and through copy number variants (39).

Molecular imaging approaches have classified the level of GI present in CTCs based on three-dimensional (3D) quantitative nuclear telomere imaging and this approach enables subgrouping of patients based on their level of GI. This classification approach uses the quantitative analysis of nuclear architecture in cancer. As postulated earlier (3,4,42), cancer is a disease of DNA organization and nuclear structure. In this context, Adebayo *et al.* (43) used a 3D imaging approach to genetic profiling of CTCs. In this study, 3D quantitative nuclear telomere imaging of CTCs was used to determine the level of GI present in each CTC. Intra-patient tumor cell heterogeneity as well as interpatient heterogeneity was clearly established. A similar approach has been applied to CTCs of high risk prostate cancer

patients undergoing hormone deprivation and radiation therapy who were stratified into three distinct groups based on their 3D nuclear telomeric profiling (44).

Conclusions

CTCs offer the opportunity to examine a patient's tumor without the need for tissue biopsies. CTCs allow us to gain an understanding of the tumor cell genome: CTCs capture the nature of the whole tumor, and their molecular profiles reflect the dynamics of tumor cell evolution. It is anticipated that the molecular genetic profiling of CTCs will unravel the identification of those CTCs that exhibit a high level of GI linked to disease aggressiveness and progression. This approach will also detect those CTCs that are indolent with a low level of GI. The molecular characterization of CTCs may, in the future, enable truly personalized medicine for each patient.

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Footnote

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