Cancer Cell
Previews

Molecular Archeology: Unearthing Androgen-Induced Structural Rearrangements in Prostate Cancer Genomes

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http://dx.doi.org/10.1016/j.ccr.2013.01.019

In this issue of *Cancer Cell*, Weischenfeldt and colleagues report on the whole genome sequencing of 11 early-onset prostate cancers. Compared to elderly onset prostate cancer, these tumors demonstrate enrichment for androgen-driven structural rearrangements involving ETS family genes. This study confirms observations that prostate cancer manifests discrete genomic subclasses.

Prostate cancer remains the most common type of cancer and a frequent cause of cancer-related mortality in men worldwide. Although prostate cancer predominantly affects older men, a subset of prostate cancer arises in men below the age of 50. Early-onset cancers often display hereditary links to germline mutations; well-known examples include *BRCA2* mutations in breast and ovarian cancers and *p53* mutations in Li-Fraumeni syndrome. Among common solid tumors, prostate cancer has been shown to have the largest estimated effect of heritability (Lichtenstein et al., 2000). Despite suggestions that hereditary prostate cancer genes exist, most studies have failed to yield reproducible germline variants that account for early onset prostate cancer. Rare germline variants have been discovered, such as in *HOXB13*, but may only account for a small percentage of early-onset prostate cancer cases. No single germline variant accounts for a substantial proportion of prostate cancer.

In the search for a genetic basis for early-onset prostate cancer, Weischenfeldt et al. (2013) describe, in this issue of *Cancer Cell*, whole genome sequencing of 11 early-onset prostate cancer (median age 47 years) selected from a German cohort. They compared their results to seven previously published whole genomes meeting their definition for elderly-onset prostate cancer (median age of 65 years) (Berger et al., 2011). A side-to-side evaluation revealed a statistically significant increase in somatic structural rearrangements (SRs) in general and balanced rearrangements in particular in the 11 early onset-prostate cancer tumors as compared to the elderly-onset prostate cancer tumors. Interestingly, 91% (10/11) early-onset prostate cancer harbored an ETS gene rearrangement involving either *ERG* (n = 9) or *ETV1* (n = 1), which is significantly higher than the estimated 50% for all prostate cancers (Rubin et al., 2011; Tomlins et al., 2005).

Androgen stimulation and genotoxic stress (e.g., radiation) have previously been shown to induce the *TMPRSS2-ERG* gene fusion, the most common prostate cancer ETS rearrangement (Mani et al., 2009). By exploiting a map of publicly available androgen receptor (AR) binding sites, Weischenfeldt et al. (2013) observed that the early-onset prostate cancer tumors exhibited SR break points situated nearer to AR binding sites than those in the seven elderly-onset prostate cancer tumors from Berger et al. (2011). This finding raises the possibility that androgen stimulation preferentially induces certain types of SRs in early-onset prostate cancer, leading to a cascade of oncogenic molecular events such as recurrent ETS rearrangements. The authors conclude that early-onset prostate cancer may be more likely to harbor androgen-driven SRs, whereas elderly onset prostate cancers display a distinct rearrangement landscape.

The notion that androgen-triggered DNA damage might explain the majority of early-onset prostate cancer is certainly intriguing. However, comprehensive assessment of causal components should also account for how prostate cancer is often diagnosed. Today, most prostate cancer is detected through prostate-specific antigen (PSA) screening. Widespread screening has increased the detection of low risk cancers. In fact, recent U.S. and European screening studies have questioned if the benefits of PSA screening outweigh the attendant morbidities and costs. Relevant to the Weischenfeldt et al. (2013) study, prostate cancer detected by PSA screening may be diagnosed up to 15 years earlier than may have been the case without PSA screening. Thus, a 50-year-old man diagnosed with prostate cancer by PSA

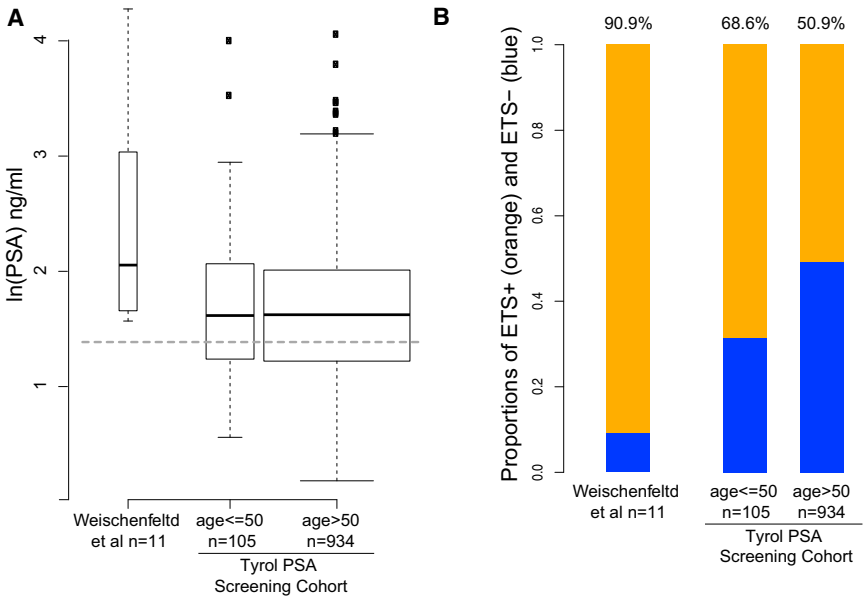

Figure 1. Comparison of PSA Levels and ETS Rearrangement Frequencies of the 11 Cases from the Weischenfeldt and Colleagues Study and Data from the Tyrol PSA Screening Population

(A) Significantly higher PSA levels were found in the Weischenfeldt et al. (2013) cases than observed in the Tyrol PSA screening population of men with prostate cancer. The width of the boxes reflects differences in sample sizes: the dotted horizontal gray line corresponds to 4 ng/ml, and age indicates years.

(B) The percentage of ETS rearrangement positive prostate cancer in the 11 whole genomes from the Weischenfeldt et al. (2013) study is shown compared to men from the Tyrol screening cohort stratified by age. The numbers at the top of each bar indicates the percent of cases that are ETS positive.

screening might not have presented clinically until he was 65 years old. In such a case, the "early onset" versus "elderly onset" comparison might represent a distinction without a difference.

PSA vagaries aside, results of the Weischenfeldt et al. (2013) study also suggest that the constellation of genomic alterations (i.e., increased somatic androgen-related SRs and ETS rearrangements) influence earlier clinical detection. We recently completed a population-based study determining *ERG* overexpression, used as a surrogate for *ERG* rearrangements, in 1,039 radical prostatectomy tumor samples from the Tyrol PSA screening cohort (Schaefer et al., 2013). This study showed that early *ERG* rearranged tumors manifest clinically at lower PSA levels, and their prevalence is age-dependent. Figure 1 shows the comparison of PSA levels and the *ETS/ERG* rearrangement frequencies of the 11 cases from the Weischenfeldt et al. (2013) study with the Tyrol PSA screening population, distinguishing between men of 50 years of age at diagnosis or younger versus patients above 50 years of age and using the cutoff defined by Weischenfeldt et al. (2013). Their ten ETS rearrangement positive cases demonstrate significantly higher PSA levels than those observed in prostate cancer cases in the Tyrol PSA screening population. The higher PSA level, high tumor grade, and stage might therefore reflect a particularly virulent subset of prostate cancer identified from a larger population of men with early-onset disease diagnosed by PSA screening. The question of whether or not a similar virulent, high-PSA subset may also exist among elderly prostate cancer patients—and at what prevalence—will be an important follow-up question.

A corollary of the Weischenfeldt et al. (2013) study findings posits that SRs arising from androgen-driven DNA damage also give rise to the *TMPRSS2-ERG* (and other ETS) rearrangements. This important study extends previous work that ETS-rearranged prostate cancer comprises a distinct molecular subclass. The *TMPRSS2-ERG* rearrangement can be observed in the prostate cancer precursor lesion (Perner et al., 2006) and is associated with distinct somatic copy number aberrations (Demichelis et al., 2009). *TMPRSS2* is highly androgen regulated and drives *ERG* expression in the fusion gene. Berger et al. (2011) noted that *ERG*-rearrangement positive cases contained somatic SR breakpoints located near AR binding sites, whereas ETS-negative prostate cancer harbored SR breakpoints significantly distant from AR binding sites. They also observed that these SRs in *ERG* rearranged tumors could exist as interwoven chains that involved cancer related genes, suggesting a possible selective growth advantage (Berger et al., 2011). More recent work suggests that ERG overexpression may mediate three-dimensional DNA conformational changes through active transcription, putatively facilitating SRs under genotoxic conditions (Rickman et al., 2012). The Weischenfeldt et al. (2013) study now suggests an alternative scenario where androgenic events drive SRs, which leads to ETS rearrangements. This would imply the early-onset "gene" might really represent a susceptibility to androgenic DNA damage.

Disentangling the effect of cancer screening from age may prove challenging for the foreseeable future, given widespread PSA testing. Regardless, Weischenfeldt et al. (2013) confirm observations that prostate cancer manifests discrete genomic subclasses. The telltale molecular fingerprints are emerging through advances in whole genome sequencing that encompass important noncoding regulatory regions not captured by exome sequencing and innovations in large data analysis. Future studies should help elucidate the genomic events predisposing to androgen-driven SR breakpoints, genomic events that may trigger a cascade of prostate cancer alterations including the recurrent *TMPRSS2-ERG* rearrangements, and the development of balanced chained loop rearrangements. One can imagine that germline polymorphisms (i.e., SNPs or copy number variants) could deleteriously hinder DNA repair mechanisms, thus phenocopying BRCA2 deficiency. Epigenetic or environmental effects may play some modifying role in this process. Knowledge of somatic SRs may also have important implications with regards to diagnosis and response to targeted treatment after disease progression. Overall, the

Weischenfeldt et al. (2013) study extends a growing paradigm regarding the links between complex rearrangements and prostate carcinogenesis, while also considering the age dimension as a possible player in the spectrum of clinical features that contribute to disease biology.

ACKNOWLEDGMENTS

Work cited in this article was supported by the Starr Cancer Consortium (to M.A.R., F.D., and L.A.G.), the Prostate Cancer Foundation (to M.A.R.), US Department of Defense Synergy awards (PC101020 to F.D., L.A.G., and M.A.R.), and the Early Detection Research Network (U01CA111275 and NCI EDRN to F.D. and M.A.R.). L.A.G. is an equity holder and consultant in Foundation Medicine, a consultant to Novartis and Millenium/Takeda, and a recipient of a grant from Novartis. F.D. and M.A.R. are listed as co-inventors of the patent on the detection of gene fusions in prostate cancer, filed by The University of Michigan and the Brigham and Women's Hospital. The diagnostic field of use for ETS gene fusions has been licensed to Hologic Gen-Probe. The authors would like to thank Helmut A. Klocker and Scott M. Tomlins for their thoughtful comments.

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Growth Factor Receptors Define Cancer Hierarchies

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http://dx.doi.org/10.1016/j.ccr.2013.01.020

Normal and neoplastic tissues display cellular hierarchies that integrate extracellular cues to maintain tissue function through bidirectional signals mediated via cell surface proteins. Two papers in *Cancer Cell*, one in this issue (Day and colleagues) and one in a recent issue (Binda and colleagues), describe how Eph receptor tyrosine kinases critically define and regulate the growth of cancer stem cells.

Tumors display cellular heterogeneity through the integration of multiple supportive cell types—vasculature, stroma, and immune components—as well as diversity within the neoplastic compartment derived from genetic and epigenetic variability. Cancers co-opt transcriptional programs normally active in development and wound responses, processes in which stem and progenitor cells contribute, so it is not surprising that cancers display characteristics of stem and progenitor cells.

Recent data from human and murine models support the presence of cellular hierarchies in some advanced cancers with cancer stem cells (CSC) at the apex (Chen et al., 2012; Singh et al., 2004). The CSC field currently lacks a coherent set of criteria to define these cells. Many reports mistakenly hold that CSCs simply represent cells that form spheres in culture and tumors upon transplantation (i.e., tumor initiating cells). Rather, CSCs also mimic normal stem cells to create a dysfunctional cellular hierarchy with non-tumorigenic cells derived from the self-renewing CSC. To fulfill this feature, the CSC hypothesis needs to employ strategies to prospectively segregate tumorigenic and non-tumorigenic cells or perform functional lineage tracing studies. A critical aspect of these studies is the requirement to identify and separate discrete populations and perform functional analyses. In response, researchers have defined a number of surface molecules that are preferentially expressed by CSCs and can be interrogated in live cells. At first blush, the increasing number of these markers may engender skepticism as to the validity of the CSC hypothesis, but this viewpoint is derived from our desire to impose simplicity on an inherently complex and dynamic system. First,