



The Genomic Architecture of Prostate Cancer

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PROSTATE CANCER AND THE CRITICAL PATH TO EFFECTIVE CANCER TREATMENT

The predominant center of attention in the treatment of cancer must no longer be confined to a solitary focus on the organ in the body in which the cancer arose. Looking at it from a molecular or genetic standpoint, the "critical path" to effective cancer treatment lends more to a focus on tumor dependency that might lead to rational therapeutics (single targeted therapies) and potentially impressive tumor responses. However, these responses are often transient, leading to a need for higher order therapeutic combinations derived from a higher level of knowledge about tumor dependency or resistance, hopefully leading to cure or long-term tumor control. In prostate cancer (PRCA), such a pathway began with a critical tumor dependency (androgen signaling) that has led to development of rational therapeutics (androgen ablation/blockade) resulting in tumor response (lower PSA), and it is known that impressive treatment responses can be achieved with some of these agents. The question remains, however, as to whether we can move to higher order therapeutic combinations by characterizing the genetic characteristics of PRCA that may result in long-term tumor control.

THE LANDSCAPE OF MUTATIONS IN THE EXOME OF AGGRESSIVE PROSTATE CANCER

One study evaluating the genome of PRCA sequenced the exomes of 112 prostate tumor and normal tissue pairs, where the prostate cancers tended to be larger tumors from radical prostatectomy specimens. While there were some previously recognized mutations observed, new recurrent mutations were identified in multiple genes, with *SPOP* being the most frequently mutated gene. Mutations involving the *SPOP* substrate-binding cleft were found in 6% to 14% of tumors across multiple independent cohorts. Recurrent *SPOP* mutations clustered in its substrate binding domain. Prostate cancers with mutant *SPOP* were found to never occur with ETS family gene

rearrangements but did occur with some other genetic alterations. One in particular involved focal deletions in 5nq21.1, which includes a gene called *CHD1*, involved in chromatin regulation; mutations in chromatin regulatory factors have been one of the most striking discoveries in cancer genome characterization. These data suggest that *SPOP* mutations may define a new molecular subtype of prostate cancer [1]. It also has been hypothesized that *SPOP* mutations drive tumorigenesis. Mutant but not wild-type *SPOP* has been found to enable robust prostate epithelial cell proliferation. Of interest is the fact that endometrial cancers also exhibit *SPOP* mutations with an approximate 8% to 10% frequency, but the location of these *SPOP* mutations is completely different, essentially not overlapping with PRCA *SPOP* mutations and not demonstrating a growth phenotype when compared with the PRCA mutations (J.P. Theurillat, M.D., unpublished data). Most important overall is the finding that *SPOP* mutations appear to be driving the proliferation of PRCA epithelial cells, providing a potential new target for therapy in PRCA management.

In another study looking at localized primary tumors versus mCRPC with soft tissue metastases, the primary tumors studied appeared much "quieter" from a genome alteration standpoint compared with the more advanced disease states. However, a notable amount of overlap was noted in the mutated genes in both groups. Mutated genes seen in both primary and advanced disease included *THSD7B*, *SNC11A*, *CDKN1B*, *MLL2*, *MED12* and *SPOP*, potentially providing more targets for future therapies [2]. Protein homeostasis may be another avenue to target for future treatments for PRCA as already seen in other cancers (i.e., proteasome inhibitor therapy with bortezomib for multiple myeloma).

MODELS OF TUMOR EVOLUTION

Genetic exome sequencing not only permits identification of relevant mutations but also provides the ability to understand the evolutionary history of the particular cancer. The cancer genome functions as a molecular fossil record and includes the tumor's mutational

history, clonal history, genomic diversity, and may allow the delineation of treatment-induced bottlenecks in therapy. Questions that arise from such assessments surround the specific evolutionary process that gives rise to individual cancers, and the implications of this process for tumor biology and treatment. The "default" model for tumor evolution is that of "gradualism," similar to the classic Darwinistic model of species evolution. This model is based on the concept that, over time, mutations are accrued incrementally with every cell division, and at some threshold level, the "wrong" set of mutations occurs that gives rise to cancer. Accrued mutations can be affected and elevated by outside factors (e.g., cigarette smoking in lung cancer), so the threshold toward cancer may be crossed earlier in particular organs and malignancies [3]. This model was challenged in a study suggesting that some alterations occurring in cancers were much more radical than those suggested by the gradualism model. This study suggested that rearrangements involving chromosomes may crisscross back and forth across involved regions, creating tens to hundreds of genomic rearrangements, often confined to only 1 or 2 chromosomes. Such changes were considered highly improbable to accumulate over time and instead may have occurred during a single cellular "catastrophe," terming this phenomenon "chromothripsis" [4].

Complex "chains" of genomic rearrangements have also been assessed as a factor in prostate cancer. One study was approached from the focus of several different loci scattered across the genome, on the same or different chromosomes. At some point over the course of cell biology, these loci became physically localized, possibly through transcription. Some of the tumors studied contained complex chains of balanced rearrangements that occurred within or adjacent to known cancer genes, generating "closed chains" in the ETS-positive cancers studied [5]. Compared to the chromothripsis model which reflected tens to hundreds of rearrangements on usually 1 or 2 chromosomes likely caused by mitotic errors, this model delineated chromosome chains with 4 to 12 linked rearrangements, possibly involving multiple chromosomes, likely caused by errors in transcription or chromatin regulation [4, 5].

With the evaluation of all these data surrounding gradual or sudden genetic alteration in PRCA, the question arose as to whether a third tumor evolution model should be invoked, one of "punctuated equilibrium." In this evolutionary model, long periods of stability in the characteristics of an organism are punctuated by short periods of rapid change where new forms may appear [6]. If PRCA actually arises through "closed chain-based" punctuated equilibrium, it may be postulated that these closed chains should be present in a large fraction of PRCAs. Some PRCA genomes should contain multiple independent chains and some should exhibit subclonality, and many chains should contain known cancer genes if this is a true driver model for carcinogenesis.

One study in this area sequenced the genomes of prostate tumors and matched normal tissue in an attempt to characterize somatic alterations and to study how they accumulate both during oncogenesis and cancer progression. The data found substantial translocations and dele-

tions that arose in a highly interdependent manner, with chromosome deletions revealing additional chains. Instead of clean breaks followed by repair, a break might have been followed by a deletion of an intervening sequence followed by misrepair. Deletion bridges may occur, which may be abundant. This prevalent phenomenon of chained rearrangements in PRCA was termed chromoplexy, and this phenomenon was suggested to frequently account for the dysregulation of prostate cancer. The study found that at least 88% of primary PRCA tumors have ≥ 1 chain event, and 65% had ≥ 2 chain events, with some chains exhibiting subclonality. The data support the concept of prostate cancer arising through closed chain-based punctuated equilibrium [7]. The study demonstrated that cancer genes are often interrupted by chromoplexy which has also been hypothesized in other tumor lineages using the study methods, including head and neck squamous cell carcinoma, breast cancer, lung adenocarcinomas, and melanoma. All of these tumor evolution models evaluated suggest a continuum model of tumor evolution, ranging from gradual progression to punctuated progression, to the catastrophe model as involved in tumorigenesis.

WHY TUMOR EVOLUTIONARY HISTORY MAY MATTER

Questions that may be addressed through these evolutionary models include whether mechanisms of tumor evolution might identify new avenues for cancer prevention. Could distinct rearrangement patterns denote novel "synthetic lethal" therapeutic combinations, and might the content and nature of chromosome chains help distinguish more aggressive cancers from those that are more indolent? Are there genes present in chains that are more likely to be "driver" events, allowing for greater precision in therapy and management? Valuable lessons have been learned from PRCA genome characterization, including the genetic definition for a subset of ETS-negative prostate cancer, and the importance of dysregulated protein homeostasis. Research has identified a marked increase in genomic aberrations between primary tumors and mCRPC. Close-chain rearrangements inform a model of punctuated evolution and may be enriched for driver events. None of these observations would have been possible without whole genome sequencing, and these data leave clinicians with an emerging challenge, namely assessing the clinical impact of prostate cancer genomics in the treatment and management of patients with PRCA.

FINANCIAL DISCLOSURES

Dr. Garraway is a consultant/advisor for Novartis, Foundation Medicine, Daiichi Sankyo, Millennium/Takeda, Boehringer Ingelheim; received research funding from Novartis and stockholder with Foundation Medicine.

ACKNOWLEDGEMENTS

This summary was created from the proceedings of Prostate Cancer: Progress and Promise, Part IV which was held after the 2013 Chabner Colloquium, Collaboration in Cancer Drug Trials in Boston, MA. The Society for Translational Oncology received educational grants in support of this activity from Amgen, Lilly, Merrimack Pharmaceuticals, Novartis Oncology, Pfizer and Sanofi Oncology.

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