Genome Medicine

RESEARCH HIGHLIGHT

Genome interrupted: sequencing of prostate cancer reveals the importance of chromosomal rearrangements

Akash Kumar[1], Jay Shendure[1] and Peter S Nelson*[2]

Abstract

A recent study involving whole genome sequencing of seven prostate cancers has provided the first comprehensive assessment of genomic changes that underlie this common malignancy. Point mutations were found to be infrequent but changes in chromosome structure were common. Rearrangements were linked to chromatin organization and associated with regions involved in transcription factor binding. Novel candidate prostate cancer genes were also identified, highlighting the importance of genome sequencing to identify oncogenic changes that are otherwise invisible to detection.

From genetics to genomics in prostate cancer research

Prostate cancer is diagnosed in more than 200,000 men and accounts for more than 30,000 fatalities in the USA every year [1]. The course of this disease is remarkably heterogeneous; some cancers remain asymptomatic for decades, while others rapidly metastasize to bone and other tissues, resulting in substantial morbidity and mortality. Although several genetic abnormalities have previously been identified in prostate cancer, including recurrent rearrangements involving the androgen-regulated serine protease gene *TMPRSS2* and members of the *ETS* family of oncogenic transcription factors [1], a complete view of the prostate cancer genome has been lacking.

Major advances in DNA sequencing technology have recently enabled the exploration of the genetic underpinnings of cancer to an unprecedented level of detail. The genomes of a number of carcinomas, including breast, lung and skin, have been sequenced (Table 1). These studies have provided fascinating insights into tumor biology, and have identified new leads for diagnosis and therapy [2-4]. A recent *Nature* article by Berger and colleagues [1] details the first whole genome study of prostate cancer. The work builds on earlier findings concerning the genetic make-up of cancers and highlights aspects that appear to be unique to prostate tumors.

Relatively few point mutations in prostate cancer

Berger *et al.* [1] sequenced the genomes of seven high-grade aggressive primary prostate cancers and corresponding normal tissues by generating approximately 30-fold genome coverage of paired-end, short-read sequencing on the Illumina platform. After mapping reads to the human reference genome, they found that each cancer genome possessed on average less than one somatic point mutation per megabase. This mutation rate is far lower than that previously seen in genomes of lung cancer and melanoma (Table 1), but similar to that reported for breast cancer and acute myelogenous leukemia [2,3,5]. This pattern supports the notion that the mechanisms underlying the genesis of prostate cancer do not include common environmental carcinogens, such as UV radiation in melanoma or tobacco exposure in lung cancer, which result in DNA point mutations. The tumors had an average of 20 non-synonymous (protein-changing) substitutions per genome. Despite this relatively low mutation frequency, two genes, *SPTA1* and *SPOP*, were recurrently mutated. *SPOP* has been shown to interact with a cell-death-associated protein Daxx, and *SPTA1* encodes a scaffold protein involved in determining cell morphology. The specific mechanism by which these genes influence tumorigenesis remains to be established. Additionally, genes encoding proteins involved in chromatin remodeling, antigen processing and heat shock were enriched for mutations, suggesting that these pathways may be relevant in prostate tumorigenesis.

*Correspondence: pnelson@fhcrc.org
[2]Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N., Seattle, WA 98109, USA
Full list of author information is available at the end of the article

Table 1. Genome alterations reported in published whole genome studies

Tumor type	Number of tumors sequenced	Approximate number of point mutations per tumor	Average number of non-synonymous mutations per tumor	Number of rearrangements per tumor	Reference(s)
Lung (non-small-cell)	1	50,000	302	43	Lee *et al.* (2010) [8]
Melanoma	1	30,000	187	74	Pleasance *et al.* (2010) [2]
Lung (small-cell)	1	20,000	94	58	Pleasance *et al.* (2010) [3]
Breast	2	6,000	~30	40	Ding *et al.* (2010) [4]; Shah *et al.* (2009) [9]
Prostate	7	4,000	20	~100	Berger *et al.* (2011) [1]
AML	1	1,000	8	ND	Mardis *et al.* (2009) [5]

AML, acute myelogenous leukemia; ND, not determined.

A larger role for rearrangements

Perhaps the most striking finding of the study by Berger *et al.* [1] concerned chromosomal alterations. Each cancer genome contained an average of 100 inter- and intra-chromosomal rearrangements. Unsurprisingly, three tumors contained rearrangements involving *TMPRSS2* and *ERG* (*ETS*-related gene) - an event previously reported to occur in approximately 50% of primary prostate carcinomas [6]. Of great interest, the investigators reported that a subset of rearrangements participated in a 'closed chain' pattern in which multiple inter- and intrachromosomal locations exchange breakpoint arms without any loss in total genetic material (Figure 1). This pattern is distinct from one in which all breaks occur as reciprocal pairs, and the authors hypothesized that these events may be due to the simultaneous disruption of many co-localized chromosomes through a mechanism depicted in Figure 1. Genomic insults such as genotoxic damage produced by oxidative stress or ionizing radiation can induce DNA breaks. Subsequent reshuffling of chromosomal material may help to drive the derangement of many genes in parallel, the importance of which is bolstered by the recent findings of 'chromothripsis' or chromosomal 'shattering' in other tumor types [7]. In contrast to the events seen in chromothripsis, the rearrangements seen in these prostate cancers affected multiple chromosomes at once. This pattern of rearrangement suggests a unique mechanism of tumorigenesis within prostate cancer that may be associated with the known influences of androgen receptors and other regulators of gene expression.

A potential mechanism for rearrangements in hormone-driven cancers

It was noted by Berger *et al.* that closed chain rearrangements could occur if chromosomes were spatially co-localized before rearrangement; this phenomenon has been seen to occur through androgen receptor-induced 'transcription hubs' that approximate intra- and intergenic regions under common regulatory control (Figure 1). The investigators looked for a correlation between the locations of breakpoints and specific chromatin marks in a similar *TMPRSS2-ERG* fusion-positive prostate cancer cell line and found a significant association between rearrangements in some *TMPRSS2-ERG* fusion-positive tumors and open chromatin marks within the cell line [1].

In contrast, tumors without *TMPRSS2-ERG* alterations demonstrated an inverse correlation between open chromatin and rearrangements, indicating that these tumors either possess different patterns of chromatin organization from that of the cell line or preferentially rearrange in regions of closed chromatin [1]. Directly exploring chromatin structure in these and additional prostate tumors, and measuring the overlap between rearrangement breakpoints and chromatin state may address this question.

Interestingly, Berger *et al.* [1] found that associations between open chromatin and rearrangements also extended to breast cancer. Of 18 previously sequenced breast cancer samples, 16 exhibited rearrangements that overlapped significantly with open chromatin regions in the prostate cancer cell line as well as with estrogen receptor binding sites. This pattern was not observed in lung cancers or melanoma, suggesting that it may be most relevant to hormone-driven cancers, such as breast and prostate cancers, which involve nuclear hormone receptors.

Candidate genes identified by whole genome sequencing

Whole genome sequencing also identified a set of novel genes recurrently disrupted by rearrangements [1]. *CADM2*, encoding a cellular adhesion molecule, was rearranged in three out of seven tumors studied and rearrangements were seen in an additional six out of ninety prostate cancers using fluorescence *in situ* hybridization; as some rearrangements are too complex to resolve via the fluorescence *in situ* hybridization method, this number is likely to be an underestimate. Similarly, the gene *MAGI2* was recurrently affected by a copy-neutral rearrangement. *MAGI2* is predicted to be

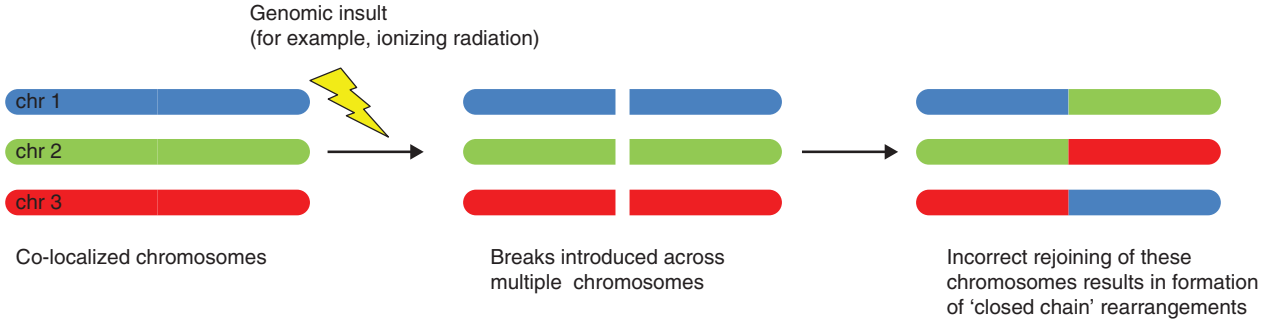

Figure 1. Schematic diagram illustrating the hypothesized mechanism of 'closed chain' rearrangement formation. Chromatin structure or transcription factor binding causes certain chromosomes to be physically near each other. A genomic insult such as ionizing radiation may disrupt these co-localized chromosomes simultaneously. DNA repair machinery can incorrectly join chromosome fragments, resulting in chimeric chromosomes containing disruptions in multiple genes. The figure is adapted from Figure 2A in Berger *et al.* [1].

involved in the phosphatidylinositol 3-kinase pathway based on known interactions with *PTEN*. Additionally, mutations in *PTEN* and *MAGI2* within the seven genomes appear to be mutually exclusive, further suggesting the involvement of the phosphatidylinositol 3-kinase pathway as a driver of prostate carcinogenesis and a potentially important therapeutic target. As pointed out by the investigators, the fact that *MAGI2* was disrupted in a copy-neutral manner would likely make it invisible to detection methods other than whole genome sequencing.

Implications for future prostate cancer research and treatment

The analysis of prostate cancer genomes highlights the utility of whole genome sequencing as a discovery tool in cancer. Berger *et al.* uncovered novel candidate oncogenes using this approach, identified a new pattern of chromosomal rearrangement and provided insights into the mechanisms by which rearrangements may arise. As the search for additional tumor-promoting and tumor-suppressing genes continues, this work exemplifies the power of detailed genomic characterization to identify genes disrupted by rearrangements that remain undetectable by other approaches.

Additional genomic studies of prostate cancers are needed before its landscape and mechanisms of tumorigenesis can be said to be well understood. The finding that *TMPRSS2* fusion status appears to influence a tumor's pattern of chromosomal rearrangement is interesting. It will be important to characterize the chromatin state of fusion-negative cells to help resolve the question of whether rearrangements in prostate cells have a tendency to occur in regions of open chromatin. Sequencing other types of tumors can determine whether the closed-chain patterns of rearrangements are present in other hormonally driven cancers or rather are unique to the prostate. In this regard, a comparison of estrogen-receptor-positive and estrogen-receptor-negative breast cancers may be informative. The Berger *et al.* study was not sufficiently large to rule out the possibility of new recurrently mutated genes in prostate cancers, and examining the genomes of additional tumor samples, especially metastases, may uncover new therapeutic targets. However, the data do suggest that few specific genes will be mutated at high frequency in primary prostate cancers, and support the concept of pathway-based analyses that involve assessing alterations in multiple genes that each may influence the activation state of a given network. Lastly, it will be important to establish the functional relevance of both rearrangements and point mutations identified through cause-effect experiments in preclinical models of prostate cancer.

The future of cancer management will likely be governed by partitioning tumors into categories or classes based on their constellation of mutations, structural alterations and epigenetic states that control oncogenic pathways. Of critical clinical utility will be those genomic features that are prognostic and those that are amenable to pharmacological control. In the simplest case, large subtypes of cancers are driven by single gene alterations that are directly targetable, such as the Bcr-abl fusion protein of chronic myelogenous leukemia, and others where mutations in a particular pathway are common, but do not represent the single accelerator of tumor growth, such as *EGFR* mutations in subsets of lung cancer. More likely, as emphasized by whole genome analyses of epithelial tumors, cancers such as those arising in the prostate are influenced by a collection of relatively rare mutations. Clinical assessment of tumors in this scenario will require methods to comprehensively assess their genomes in order to prescribe the most appropriate therapeutics. The diversity and complexity of tumor genomes coupled with the increasing affordability

and utility of the whole genome sequencing approaches indicate that these technologies will be increasingly used and have an important future in the management of cancer patients.

Abbreviations

AML, acute myelogenous leukemia; Daxx, death-domain associated protein; ***ERG***, ***ETS*** related gene; ER, estrogen receptor; PI3K, phosphatidylinositol 3-kinase pathway.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We would like to thank S Salipante, J Hiatt and N Krumm for helpful discussions. Our research on prostate cancer genomics is supported in part by the US Department of Defense, the Prostate Cancer Foundation, and the NCI Pacific Northwest Prostate Cancer SPORE. AK is supported by an Achievement Rewards for College Scientists fellowship. Our funding agencies have not exerted any influence over this article.

Author details

[1]Department of Genome Sciences, University of Washington, 3720 15th Avenue NE, Seattle, WA 98105, USA. [2]Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N., Seattle, WA 98109, USA.

Published: 19 April 2011

References

1. Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, Sboner A, Esgueva R, Pflueger D, Sougnez C, Onofrio R, Carter SL, Park K, Habegger L, Ambrogio L, Fennell T, Parkin M, Saksena G, Voet D, Ramos AH, Pugh TJ, Wilkinson J, Fisher S, Winckler W, Mahan S, Ardlie K, Baldwin J, Simons JW, Kitabayashi N, MacDonald TY, *et al.*: **The genomic complexity of primary human prostate cancer.** *Nature* 2011, **470:**214-220.
2. Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, Greenman CD, Varela I, Lin M-L, Ordonez GR, Bignell GR, Ye K, Alipaz J, Bauer MJ, Beare D, Butler A, Carter RJ, Chen L, Cox AJ, Edkins S, Kokko-Gonzales PI, Gormley NA, Grocock RJ, Haudenschild CD, Hims MM, James T, Jia M, Kingsbury Z, Leroy C, Marshall J, Menzies A, Mudie LJ, *et al.*: **A comprehensive catalogue of somatic mutations from a human cancer genome.** *Nature* 2010, **463:**191-196.
3. Pleasance ED, Stephens PJ, O'Meara S, McBride DJ, Meynert A, Jones D, Lin M-L, Beare D, Lau KW, Greenman C, Varela I, Nik-Zainal S, Davies HR, Ordonez GR, Mudie LJ, Latimer C, Edkins S, Stebbings L, Chen L, Jia M, Leroy C, Marshall J, Menzies A, Butler A, Teague JW, Mangion J, Sun YA, McLaughlin SF, Peckham HE, Tsung EF, *et al.*: **A small-cell lung cancer genome with complex signatures of tobacco exposure.** *Nature* 2010, **463:**184-190.
4. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, Harris CC, McLellan MD, Fulton RS, Fulton LL, Abbott RM, Hoog J, Dooling DJ, Koboldt DC, Schmidt H, Kalicki J, Zhang Q, Chen L, Lin L, Wendl MC, McMichael JF, Magrini VJ, Cook L, McGrath SD, Vickery TL, Appelbaum E, Deschryver K, Davies S, Guintoli T, Lin L, *et al.*: **Genome remodelling in a basal-like breast cancer metastasis and xenograft.** *Nature* 2010, **464:**999-1005.
5. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehaunty KD, McGrath SD, Fulton LA, Locke DP, Magrini VJ, Abbott RM, Vickery TL, Reed JS, Robinson JS, Wylie T, Smith SM, Carmichael L, Eldred JM, Harris CC, Walker J, Peck JB, Du F, Dukes AF, Sanderson GE, Brummett AM, Clark E, McMichael JF, *et al.*: **Recurring mutations found by sequencing an acute myeloid leukemia genome.** *N Engl J Med* 2009, **361:**1058-1066.
6. King J, Xu J, Wongvipat J, Hieronymus H, Carver B, Leung D, Taylor B, Sander C, Cardiff R, Couto S, Gerald W, Sawyers C: **Cooperativity of *TMPRSS2-ERG* with PI3-kinase pathway activation in prostate oncogenesis.** *Nat Genet* 2009, **41:**524-526.
7. Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, Pleasance ED, Lau KW, Beare D, Stebbings LA, McLaren S, Lin M-L, McBride DJ, Varela I, Nik-Zainal S, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Quail MA, Burton J, Swerdlow H, Carter NP, Morsberger LA, Iacobuzio-Donahue C, Follows GA, Green AR, Flanagan AM, Stratton MR, *et al.*: **Massive genomic rearrangement acquired in a single catastrophic event during cancer development.** *Cell* 2011, **144:**27-40.
8. Lee W, Jiang Z, Liu J, Haverty PM, Guan Y, Stinson J, Yue P, Zhang Y, Pant KP, Bhatt D, Ha C, Johnson S, Kennemer MI, Mohan S, Nazarenko I, Watanabe C, Sparks AB, Shames DS, Gentleman R, de Sauvage FJ, Stern H, Pandita A, Ballinger DG, Drmanac R, Modrusan Z, Seshagiri S, Zhang Z: **The mutation spectrum revealed by paired genome sequences from a lung cancer patient.** *Nature* 2010, **465:**473-477.
9. Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A, Delaney A, Gelmon K, Guliany R, Senz J, Steidl C, Holt RA, Jones S, Sun M, Leung G, Moore R, Severson T, Taylor GA, Teschendorff AE, Tse K, Turashvili G, Varhol R, Warren RL, Watson P, Zhao Y, Caldas C, Huntsman D, Hirst M, Marra MA, Aparicio S: **Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution.** *Nature* 2009, **461:**809-813.

doi:10.1186/gm237
Cite this article as: Kumar A, *et al.*: Genome interrupted: sequencing of prostate cancer reveals the importance of chromosomal rearrangements. *Genome Medicine* 2011, **3:**23.