Non-coding DNA reveals a route by which advanced prostate cancer resists treatment

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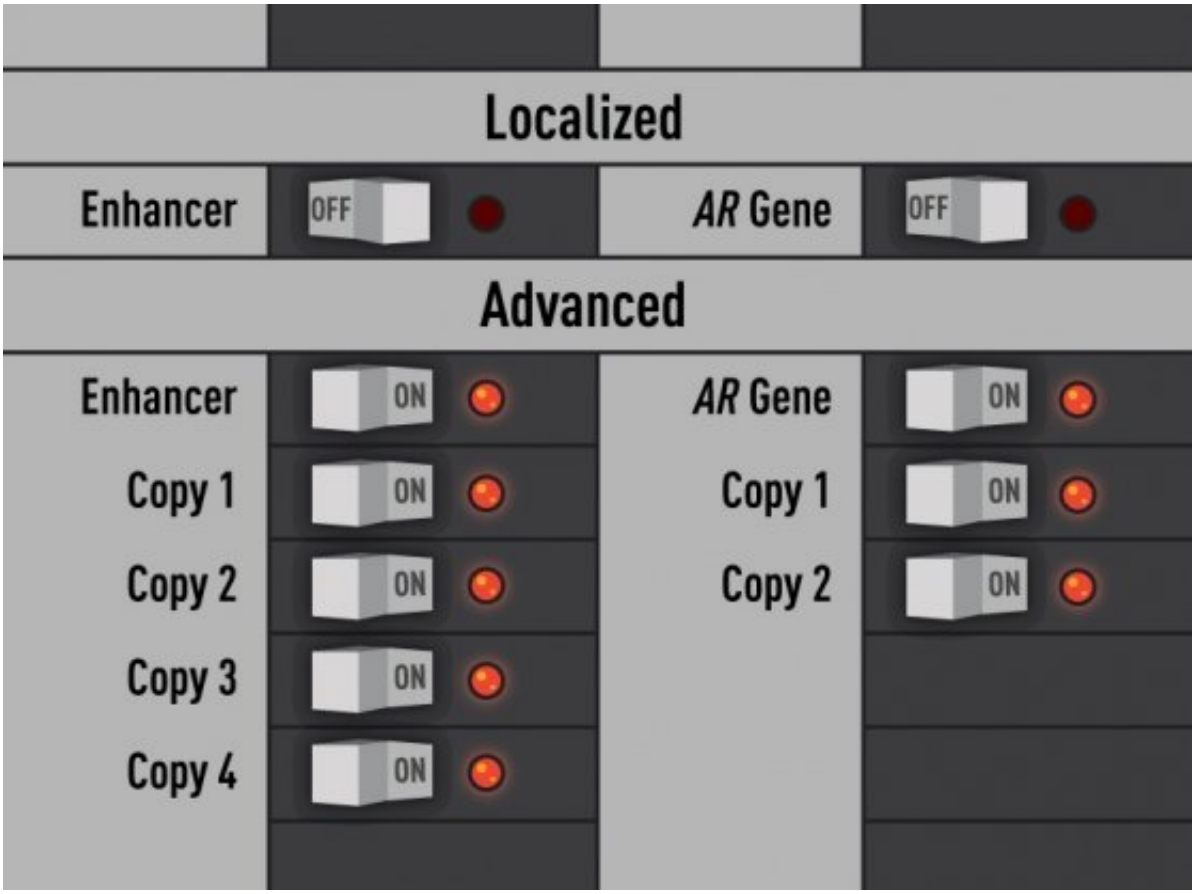

Credit : Susanna M. Hamilton

Two research teams converge on epigenetic switches that feed treatment-resistant metastatic prostate tumors. This research highlights the value of exploring gene regulation and large-scale structural changes in the cancer

genome.

More than three quarters of metastatic prostate cancers that resist hormone-blocking therapies may harbor several duplicates of both the gene for a treatment resistance factor called the androgen receptor (AR) and of a never-before-seen genetic switch, or enhancer, that boosts the gene's expression. The duplications, as well as epigenetic signs that the enhancer is active, appear after tumors are treated with AR-targeting drugs.

These genetic and epigenetic changes in the cancer cells coincide with a unique, widespread pattern of large-scale changes in tumor genome structure, which suggests a breakdown in molecular safeguards that normally keep a cell's genome stable.

These findings were published in concurrent papers in *Cell* by two separate research teams, each led by scientists from at the Broad Institute of MIT and Harvard and the Dana-Farber Cancer Institute.

Both studies reveal important molecular characteristics found in treatment-resistant metastatic prostate cancers, but not early stage tumors. These studies also highlight a way in which these tumors evolve to resist treatment and point out possible clinical opportunities.

Looking beyond genes to the DNA that controls them

Advanced or metastatic prostate cancer is sixth-leading cause of cancer death in the United States, claiming the lives of roughly 30,000 American men every year, according to statistics from the National Cancer Institute. Approximately three million men are living with prostate cancer in the US.

The AR protein is a receptor that provokes growth in advanced prostate

tumors in response to two hormones, testosterone and dihydrotestosterone. Researchers have studied the AR, the gene encoding it (called AR), and its signaling pathways for more than 20 years. Much of the attention to date has focused on the development of therapies that either interfere with its function directly or that reduce the levels of AR-activating hormones in the blood, referred to as androgen-deprivation therapies (ADT).

These two new studies highlight the need to explore regulators of AR, such as enhancers (stretches of non-coding DNA that serve as anchors for proteins that aid genes' expression) and other non-coding genomic or epigenetic elements. They also argue in favor of studying large-scale changes in genome structure that may influence AR activity, and how those changes may relate to disease state.

"We've become very gene-centric in cancer research," said Broad associate member and Dana-Farber cancer geneticist Matthew Freedman, who was the senior author on one of the two Cell papers. "We tend to focus on questions like, 'What are the target genes of copy number gains and deletions?' I believe that as we begin to understand the non-coding genome better, we will see more and more examples of non-coding drivers in cancer."

"The first era of cancer genomics focused primarily on localized prostate cancer, and on coding genetic alterations in localized disease," said Srinivas Viswanathan, a research fellow in the Broad Institute Cancer Program, a prostate oncologist at Dana-Farber, and co-first author—with research fellows Gavin Ha of the Broad and Dana-Farber and Andreas Hoff of the Broad and Oslo University—on the other Cell paper. "But what we see now is that there can be very significant genetic differences between disease states in prostate cancer, and that unique genetic changes—in both the coding and non-coding regions of the genome—can arise with advanced disease and in response to therapy."

Epigenetics brings a new enhancer to light

The team behind Freedman's study, led by co-first authors and postdoctoral researchers David Takeda of the Broad and Dana-Farber and Sandor Spisák of Dana-Farber, identified in ADT-resistant metastatic prostate tumors a stretch of non-coding DNA more than a half-million base pairs away from the AR gene bearing epigenetic marks (chemical tags that flag DNA as being active or inactive) suggesting it was an active enhancer. This enhancer signature was only present in tumor cells from patients with treatment-resistant metastatic, not localized, disease.

Using chromosome conformation analysis (which measures how parts of the genome interact physically), Freedman, Takeda, and Spisák's team found that this non-coding region engages the AR gene promoter, further suggesting that it acts as an enhancer. In addition, they noted that they could curb AR protein production and cell growth in a metastatic prostate cancer cell line by suppressing the enhancer with CRISPR-based genome and epigenome editing reagents. They also found that by adding a single additional copy of the enhancer to the cell line, they could confer increased resistance to enzalutamide, an anti-AR drug currently used for the treatment of ADT-resistant prostate cancer.

"This is a very strong enhancer, and acts like a remote control for AR," Freedman said. "In the future, it may be possible to design small molecules that disrupt proteins that bind to this enhancer, which could suppress AR protein signaling in treatment-resistant metastatic cancers."

Selective duplications

For the other *Cell* study, a team led by Viswanathan, Ha, Hoff, and Broad institute member, Dana-Farber director of cancer genomics, and

study senior author Matthew Meyerson searched 23 biopsy samples from treatment-resistant metastatic prostate cancer patients for structural genomic changes associated with advanced disease. Such so-called structural variations involve long stretches of DNA segments (more than 50-100 base pairs) and can manifest as duplications, deletions, inversions, and more complex alterations.

The Meyerson team's data, generated using long-range linked read sequencing technology developed by 10X Genomics, revealed widespread structural variations, many of which resulted in deactivation of genes known to suppress tumor growth (e.g., PTEN). Their data also uncovered a novel pattern of structural variations known as tandem duplications, with hundreds of such duplications dispersed throughout the genome of localized and metastatic tumor samples. These duplications associated with the functional loss of the gene CDK12, which helps maintain the stability of the genome.

"Tumor cells with this tandem duplicator pattern may be able to coordinately activate many oncogenic pathways at once," Viswanathan explained. "This could also create genomic instability that may render these cells sensitive to certain chemotherapy agents, such as platinum agents, as well as targeted therapies such as PARP inhibitors."

Strikingly, the AR gene and the AR enhancer identified by Freedman's group were the focus of frequent structural change in the study by the Meyerson group. Seventy percent of the samples the Meyerson group studied contained extra copies of both AR and the enhancer; an additional 17 percent (for a total of 87 percent) harbored extra copies of just the enhancer. In none of the samples they studied did they find instances of the AR gene having been duplicated alone without the enhancer as well.

"There is an incredibly complex regulatory network in our non-coding

DNA, much of which is altered in cancer in ways we're just beginning to understand," said Meyerson, who is also director of cancer genomics in the Broad Cancer Program. "Enhancer duplication or modulation occurs in relation to a number of oncogenes, and here we find another example where an oncogene is being activated through this mechanism."

Meyerson and his colleagues validated their findings using blood biopsies (a technique for sampling and isolating tumor cells and DNA circulating in the bloodstream). Applying an innovative approach developed by Ha and the Blood Biopsy Team at the Broad, the team detected alterations in whole genome data from 232 samples of cell-free tumor DNA collected from the blood of patients with metastatic disease. As with the team's solid tumor biopsies, 70 percent of blood biopsies contained extra copies of AR, the AR enhancer, or both.

"The fact that the same genomic rearrangements that were found in surgical tumor biopsies also showed up in blood biopsies suggests that a non-invasive blood test may have value for routinely profiling patients' tumors, and for exploring better and more precise ways to treat them," said Viktor Adalsteinsson, leader of the Broad Blood Biopsy Team at the Broad and a co-author on the Meyerson team study.

In cases where they had pre- and post-treatment samples available, the Meyerson group also saw increases in the number of gene and enhancer copies after therapy with AR-blocking drugs, highlighting how metastatic cells may evolve in response to treatment.

Insights for research and care

The two teams' findings reinforce the observation that treatment-resistant metastatic prostate cancers continue to rely heavily on the AR for their survival and growth, and employ multiple mechanisms to increase its production and activity. They also:

- suggest that amplification of the AR enhancer serve as a biomarker for resistance to treatment with AR-blocking drugs;
- argue for increased use of whole genome sequencing approaches in the care of prostate cancer patients; and
- add to growing evidence that some men with metastatic prostate cancer may benefit from treatments that target genomic instability in cancer cells.

"The majority of advanced prostate cancers remain addicted to androgen receptor signaling as they transform and become treatment resistant," said Mary-Ellen Taplin, a prostate oncologist and director of clinical research at Dana-Farber's Lank Center for Genitourinary Oncology, and a co-author on the Meyerson team's paper. "These elegant studies demonstrate the previously unimagined complexity of alterations in the androgen receptor and provide promising new targets for therapy development."

More information: A somatically acquired enhancer of the androgen receptor is a noncoding driver in advanced prostate cancer. *Cell*. Online June 14, 2018. DOI: 10.1016/j.cell.2018.05.037

Structural alterations driving castration-resistant prostate cancer revealed by linked-read genome sequencing. *Cell*. Online June 14, 2018. DOI: 10.1016/j.cell.2018.05.036.

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