Circumventing Cancer Resistance – Transcript

Introduction

Theme music…

Host:
Traditional and new cancer therapies often become stymied due to tumor resistance, but why resistance arises and how to avoid it remain important questions in the cancer research field. To uncover the ways tumors form, adapt, and ultimately resist treatment, scientists investigate how genetic mutations arise and drive cancer cell evolution.

Welcome to *The Scientist* Speaks, a podcast produced by *The Scientist*'s Creative Services Team. Our podcast is by scientists and for scientists. Once a month, we bring you the stories behind news-worthy molecular biology research.

In this month's episode, Deanna MacNeil from *The Scientist* spoke with Bishoy Faltas, an associate professor of medicine, and cell and developmental biology, and chief research officer of the Englander Institute for Precision Medicine at Weill Cornell, to explore bladder cancer evolution and treatment resistance.

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Bladder Cancer Tumor Evolution and APOBEC Proteins

Narrator (Deanna MacNeil):
When a single cell's DNA in a normal tissue becomes damaged, it may transform into a cancer cell, but the genetic processes that enable a single cancer cell to thrive, form a tumor, and evolve into a disease that evades treatment are highly complex. Much like species evolution, during tumor evolution, cellular lineages diverge under selective pressures to form distinct subpopulations, some of which can resist chemotherapies that are the backbone of cancer care.

Bishoy Faltas studies bladder cancer as a model disease for elucidating the biological mechanisms that drive human cancer cell diversity and evolution and translates foundational insights into precision medicine strategies that overcome treatment resistance. Since his postdoctoral research days in Mark Rubin's laboratory at Cornell University, where he first began studying the clonal evolution of platinum-based chemotherapy resistant bladder cancer, Faltas has pursued the role of a family of proteins called APOBEC, which edit the genomes of both normal and cancer cells.

Bishoy Faltas:
A few years ago, when I was starting my postdoctoral training, I was seeing patients with bladder cancer in the clinic, and at that time, there weren't a lot of effective treatments beyond chemotherapy. I was very interested in dissecting the mechanisms that drive chemotherapy resistance. It became quite clear that there was a very significant presence of APOBEC-induced mutations that are up to about

60%, approximately, of all somatic mutations in a given bladder cancer sample. That triggered my interest in these APOBEC enzymes, and at that time, there were also many others in the field that showed the high prevalence of APOBEC3-induced mutations in several cancers, but bladder cancer stood out.

Narrator:
APOBEC3 cytidine deaminases are mutagenic enzymes that edit cytosines within single-stranded DNA and mRNA to protect cells from viral infection. However, when dysregulated or overexpressed, their DNA editing behavior runs amok, leaving distinct mutation signatures in cancer genomes. Widespread mutations like those left behind by errant APOBEC enzymes can fuel clonal diversity and cancer evolution, making APOBEC3 an enticing candidate for its potential to drive treatment resistance.

Although high APOBEC3 expression and mutational signatures have been observed in different types of cancer, including breast, lung, and bladder cancer, researchers have faced challenges connecting the dots between correlation and causation. This is, in part, related to confounding mutational sources present before, during, and after treatment. APOBEC3 is considered one endogenous source of mutagenesis, yet cancer treatments like platinum-based chemotherapies also cause bursts of mutations throughout the genome that may contribute to clonal diversity and therapeutic resistance. Teasing apart the sequence of mutational events during tumor evolution has historically been a barrier to understanding resistance.

For Faltas, bladder cancer stood out as an opportunity to examine the timing and interplay of these mutagenic processes because of its uniquely high mutational burden, and the mutagenic platinum-based chemotherapy regimen that was the only treatment option available when he began his work.

Faltas:
When I started getting into the field and training in medical oncology, as well as my training in the laboratory, we only had chemotherapy, and almost all patients with metastatic disease eventually developed resistance to chemotherapy. That's partly the efficacy and the mechanisms of the drugs that we use, but also a reflection of the biology of the disease and this very high mutational burden.

Our model at the time, and there were other studies also published by other groups showing that there's these APOBEC3 mutated cancer cell clones under selective pressure from chemotherapy or other types of therapy. And we have more and more evidence that that's the case.

<u>Determining the Timing of Tumor Evolution and Treatment Resistance</u>

Narrator:
In recent work featured in *Nature* in November 2024, the research team investigated this model in tumors that were collected from patients with urothelial cancer, which is the most common type of bladder cancer. They used whole genome sequencing to examine primary and metastatic samples pre-treatment and post-treatment to pick out the effects of chemotherapy and examine the timing of mutational processes related to APOBEC3. In contrast to chemotherapy-induced mutational bursts,

which the researchers observed as hundreds of late subclonal mutations, they found that APOBEC3-induced mutations occurred early in the timeline of clonal evolution.

Faltas:
Bladder cancer is one of the few cancers where APOBEC3-induced mutations are clonal and early. And that's what we showed in our recent paper, that when we look at normal urothelium, and we track these mutations from the tumors back to normal urothelium, we can identify these APOBEC3-induced mutations. And that is consistent also with work by others. Normal urothelium from healthy donors was sequenced, and they were able to also identify APOBEC3-induced mutations in normal urothelium. So, if you put all that together, there's a model that we propose where these mutations actually may contribute to carcinogenesis, and by definition, that means that they are present very early, before the patient receives any treatments, and therefore potentially that APOBEC3-induced heterogeneity contributes to that clonal selection, this clonal diversity, a very diverse landscape that lends itself to the evolution of a treatment-resistant clone.

We think that that's a prominent feature of urothelial cancer, whereas in other cancers that have significant APOBEC activity, for example, lung cancer, there is work that shows that these mutations are late and subclonal. So it's not always clonal in all cancers, and we don't fully understand what triggers APOBEC3 to begin with, to cause these mutations. And my guess is that once these initial triggers are better understood, that may account for these differences in the timing of APOBEC3-induced mutagenesis in different cancers.

Narrator:
According to Faltas, new technologies have been and will continue to be instrumental in studying the timing of tumor evolution and resistance. Mutagenesis events start with rare cellular populations, which require large scale experiments to track as they clonally expand and evolve over time. New genomic technologies have shifted the field away from qualitative studies based on single markers, like APOBEC expression, and towards large scale quantitative datasets. Similar to tracking a family’s lineage back to its earliest ancestors, next-generation sequencing of tumor samples allows scientists to trace post-treatment cell populations back to their progenitors, seeking out the cell that gave rise to resistance and uncovering the mechanisms of treatment evasion.

Faltas:
Now, with the advent of whole genome sequencing and more and better computational pipelines that are able to identify these specific mutational signatures, we are now able to understand that a lot better, to try to understand the sequence of these events, and the interaction between these different mutagenic forces that shape the landscape of the tumor.

We try to really have a comprehensive understanding of not just mutagenic processes and but also structural variants, using genome graph computational tools to understand more complex structural variants, such as extra chromosomal DNA and its role in resistance.

[brief music interlude]

Extrachromosomal DNA in Bladder Cancer

Narrator:
In cancer cells, mutagenesis often leads to large genomic alterations called structural variants, typically consisting of different combinations of DNA gains, losses, or rearrangements. These events can cause entire gene deletions or duplications, known as copy number variations, which may have dire consequences on cellular survival and behavior. One outcome of structural variation that cancer researchers like Faltas are particularly interested in is extrachromosomal DNA or ecDNA.

Most DNA in an individual genome is found in chromosomes, which are large linear strands that are organized in the nucleus and guarded by tightly regulated expression and repair mechanisms that help cells carry out their normal functions and prevent damaged cells from replicating mutations. ecDNA are circular pieces of genetic material that become excised from chromosomes after large DNA damage events. Despite being removed from the chromosome, nuclear ecDNA often contains duplicated or amplified genes that continue to be expressed without the normal chromosomal checks and balances, and these circular fragments can harbor high copy numbers of oncogenes that drive cancer formation and evolution.

Faltas:
Extrachromosomal DNA is not actually new. There were microscopy studies a number of years ago that identified these bodies of what appeared to be extrachromosomal DNA that is within the nucleus, and sometimes these were referred to as double minutes. Over the past few years, thanks to the work of many of my colleagues, we are now recognizing the role of these extrachromosomal DNA events as essentially reservoirs of genetic amplification that plays a role in treatment resistance and in tumor evolution.

There were earlier reports of APOBEC-induced mutations within extrachromosomal DNA. So let me clarify what that means. APOBEC-induced mutations appear as what's called kataegic clusters, essentially a pattern of clustered mutations due to the processivity of these APOBEC3 cytodine deaminases. As they're going through a piece of single stranded DNA, they are processively de-aminating these cytosines, one after another. We and others have looked for this pattern within extrachromosomal DNA. We try to understand whether we can learn anything about the chronology of these two different processes, how they relate to each other.

And indeed, we find that, again, in bladder cancer, APOBEC-induced kataegic clusters within extra chromosomal DNA happens quite early, and that suggests that maybe that APOBEC activity was present at the initial time that extrachromosomal DNA was formed. Or, if you were to go even further, it could suggest that APOBEC3-induced double stranded DNA breaks, which is a phenomenon that's known to happen, could play a role in extrachromosomal DNA biogenesis under certain conditions and in certain cancers. This topological and chronological overlap between APOBEC3-induced mutagenesis and ecDNA biogenesis is quite interesting to us, and is something that we're planning to follow up on.

Narrator:

Faltas also observed that the extrachromosomal DNA became more complex after chemotherapy treatment, incorporating different genomic segments than those present in the early events. Motivated by how the timing of extrachromosomal DNA biogenesis mirrored early clonal APOBEC3-induced mutation signatures, Faltas turned to the copy number variations present in these early structural variants to determine which genes, if any, APOBEC3 might be altering to enable clonal selection and treatment resistance.

Using long-read whole genome sequencing, he and his team found that a gene called cyclin D1, a master regulator of the cell cycle, was frequently amplified in these extrachromosomal DNA-forming structural variants. In order to investigate whether cyclin D1 plays a role in therapeutic resistance for bladder cancer clonal populations, the researchers created cellular models of extrachromosomal DNA with and without cyclin D1 expression and put them through the test of treatment survival in a dish.

Faltas:
What we used was essentially a form of a integrase-deficient, non-integrating lentiviral vector. And we were able to clone cyclin D1 into that to essentially create a model of extrachromosomal DNA. So we generated cells with and without these extrachromosomal DNA events, treated them with chemotherapy, and tracked them over time. What we found was that the cells that were transduced with the cyclin D1 vector that mimicked extrachromosomal DNA had faster G1 to S progression, and they were a lot more resistant to the chemotherapy drug. And then we did the opposite experiment. We found a cell line that has cyclin D1 extrachromosomal DNA, and then we knocked down cyclin D1 in that cell line and here we see that the cells with knockdown of cyclin D1 are a lot more sensitive to chemotherapy. So, when we put all that together, we just really think that this cyclin D1 amplification that's usually part of these very complex structural variants is a driver of resistance, and it occurs in a specific subset of bladder cancers that we're working on studying.

We've known for some time that cyclin D1 is amplified in bladder cancer. The interesting finding here is that in our previous studies that used whole exome sequencing, one couldn't really reconstruct these very complex structural variants because there are a lot of gaps. But using whole genome sequencing, we can get a much more comprehensive picture. And from that, we noticed that essentially, a lot of these cyclin D1 events seem to be involved in these ecDNA-forming structural variants. So that was quite interesting to us.

<u>Precision Medicine Directions for Bladder Cancer and Beyond</u>

Narrator:
The researchers also found that the subclass of bladder cancer tumors with cyclin D1 amplifications in ecDNA often harbored deletions in other key cell cycle regulatory genes from the same pathway, highlighting players upstream and downstream of cyclin D1 as potential targets in cancers that bypass chemotherapy treatment. This includes the kinases CDK4 and CDK6, which are targets of existing inhibitors already used to treat other cancer types. One ongoing next step for Faltas is leading a window-of-opportunity clinical trial, in which patients with localized bladder cancer are receiving treatment with one such CDK 4/6 inhibitor.

There are many future directions in the research laboratory as well, including mechanistic studies that more deeply address outstanding questions, such as how APOBEC3-induced DNA damage drives therapy resistance, what triggers APOBEC3 to go awry in normal urothelial cells, and what role it might play in carcinogenesis. With much work ahead, Faltas is thankful for an interdisciplinary team tackling the challenges of treatment resistance, and looks ever forward to APOBEC-informed precision therapies for bladder cancer and beyond.

Faltas:
This is a massive team effort. This involves a large team of clinicians, laboratory scientists, physician scientists, computational biologists, oncologists and many, many other people to really put this together. We have what is called a rapid autopsy program, where patients and the patients' families have very kindly donated tumors for research, and that is a huge resource for us to try to understand the evolution of these cancers over time and to help other patients who would benefit from this foundational knowledge.

Our model is to use the latest technology to understand cancer evolution, or important biological questions that relate to cancer evolution. I think that the recent advances in whole genome sequencing and the decreasing cost of whole genome sequencing is going to be instrumental in essentially revealing a lot more in the genome. The analysis is not trivial, and the computational power that is required to do all these analyses is quite a lot. I am particularly very grateful for my collaborators, Nico Robine at the New York Genome Center and my collaborator at Weill Cornell Englander Institute for Precision Medicine, Olivier Elemento, who helped us with some of these analyzes.

We have a very active precision medicine program around bladder cancer that we have been building for a number of years, and that includes some of the research efforts that we've mentioned that are sort of the glue that keeps this together, but we also have translational efforts, and we have a research protocol that patients can consent to, and that allows us to study tumors in detail and understand new therapeutic targets. And we actually have the ability to return the information to the patient or to the patient's clinician, and to act on that. We are looking into expanding that into the future, because we think that this is truly important for personalizing medicine for patients with bladder cancer and other cancers as well.

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Outro

Host: Thank you for listening to *The Scientist* Speaks! This episode was produced by the Creative Services Team for *The Scientist* and narrated by Deanna MacNeil. Please join us again in February, as we learn how researchers harness the power of bioelectricity to guide tissue regeneration. To keep up to date with this podcast, follow *The Scientist* on social media and subscribe to *The Scientist* Speaks wherever you get your podcasts.