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New study implicates unusual class of circular RNAs in cancer

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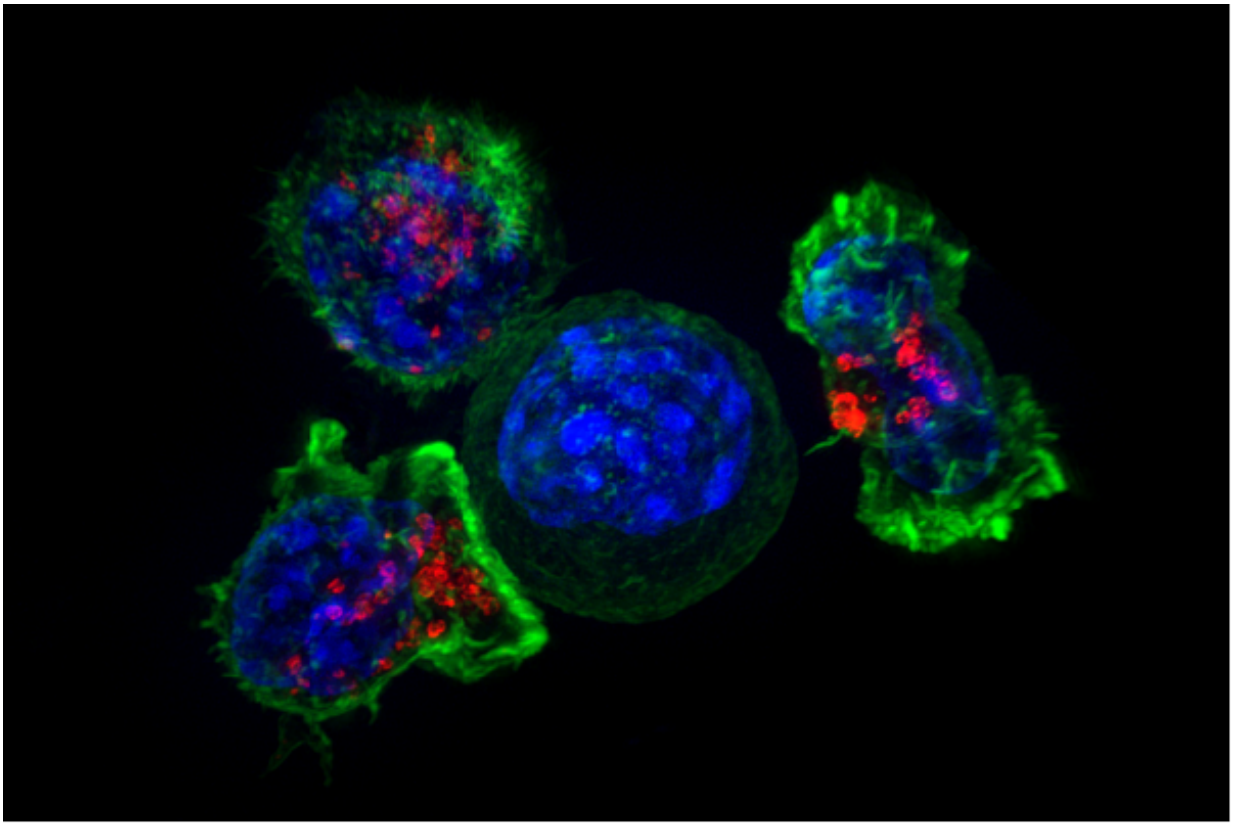

Killer T cells surround a cancer cell. Credit: NIH

Cancer cells are notorious for their genomes gone haywire, often yielding fusion proteins—mash-ups of two disparate genes that, once united, assume new and harmful capabilities. Exactly how such genome

scrambling impacts RNA, particularly the vast and mysterious world of non-coding RNA, has been largely unexplored.

Now, a team led by investigators at Beth Israel Deaconess Medical Center (BIDMC) offers some early answers by studying an intriguing class of non-coding RNAs known as circular RNAs. Published in the March 31 advance online issue of *Cell*, their findings reveal that circular RNAs - like their protein counterparts - are also affected by genomic rearrangements in cancer, resulting in abnormal fusions. Moreover, these fusion-circular RNAs are not mere bystanders; they appear to promote tumor growth and progression, underscoring their role in the disease.

"Cancer is essentially a disease of mutated or broken genes, so that motivated us to examine whether circular RNAs, like proteins, can be affected by these chromosomal breaks," said senior author Pier Paolo Pandolfi, MD, PhD, Director of the Cancer Center at BIDMC and George C. Reisman Professor of Medicine at Harvard Medical School. "Our work paves the way to discovering many more of these unusual RNAs and how they contribute to cancer, which could reveal new mechanisms and druggable pathways involved in tumor progression."

When it comes to RNA, scientists' worldview is in the midst of a significant shift. Long dismissed as a mere messenger, RNA is perhaps best known for its role ferrying instructions from the genome, which is cloistered in the nucleus, to more far-flung parts of the cell, where it is made into protein. Yet only 2 percent of the genome is copied (or "transcribed") from DNA into RNA and then translated into protein. Scientists now recognize that much, if not all, of the remaining 98 percent—which had previously been deemed non-functioning— is in fact transcribed into RNA. The roles this vast swath of so-called "non-coding RNA" might play in human biology and disease now signify an area of intense research.

Curious about the possibility of circular RNAs contributing to cancer, Pandolfi and his colleagues set out to see if they could detect relevant changes in tumors known to harbor distinct fusion proteins, which result when different chromosomes abnormally join together, melding two separate genes into a new centaur-like gene. These chromosomal translocations are common in various types of leukemia, so the researchers examined two types: acute promyelocytic leukemia, which often carries a translocation between the PML and RARα genes; and acute myeloid leukemia, which can harbor a translocation between the MLL and AF9 genes.

The researchers found abnormal fusion-circular RNAs (f-circRNAs), corresponding to different exons associated with the PML-RARα gene fusion as well as the MLL-AF9 gene fusion. (Normally, multiple circular RNAs can be generated from a single gene, so it is not entirely surprising to find different f-circRNAs emerging from the same fusion gene.)

Remarkably, Pandolfi and his colleagues uncovered f-circRNAs in solid tumors, too—in samples from Ewing sarcoma, a form of soft tissue cancer, and lung cancer. Moreover, the team identified them using two distinct methods, PCR-based amplification as well as sequencing-based approaches, underscoring f-circRNAs as bona fide biological entities, rather than experimental artifacts.

"Our ability to readily detect these fusion-circular RNAs—and their normal, non-fused counterparts—will be enhanced by advances in sequencing technology and analytic methods," said first author Jlenia Guarnerio, PhD, also of BIDMC. "Indeed, as we look ahead to cataloguing them comprehensively across all cancers and to deeply understanding their mechanisms of action, we will need to propel these new methodologies even further."

To determine whether f-circRNAs play a functional role in cancer, the

researchers introduced them experimentally into cells, causing the cells to increase their proliferation and tendency to overgrow—features shared by tumor cells. On the other hand, when the researchers blocked f-circRNA activity, the cells' normal behaviors were restored.

The researchers also conducted experiments using a mouse model of leukemia. They focused on a specific f-circRNA associated with the MLL-AF9 fusion gene, called f-circM9. Although insufficient on its own to trigger leukemia, f-circM9 appears to work together with other cancer-promoting signals (such as the MLL-AF9 fusion protein) to cause disease. Additional studies suggest that f-circM9 may also help tumor cells persist in the face of anti-cancer drugs.

"These results are particularly exciting because they suggest that drugs directed at fusion-circular RNAs could be a powerful strategy to pursue for future therapeutic development in cancer," said Pandolfi.

Circular RNAs were first identified more than three decades ago and largely dismissed as a rare cellular oddity. But a study published in 2012 by Patrick Brown's group at Stanford University showed that they are present at high levels in diverse cell types, igniting scientists' efforts to study and understand them. Surprisingly, circular RNAs—are among the most abundant non-coding RNAs in cells, driven in part by the molecules' unusual chemical stability. Unlike linear RNAs, circular RNAs are not susceptible to RNA-degrading enzymes. This ability to persist makes them not only an interesting therapeutic target, but also a potential molecular beacon or biomarker that can facilitate the diagnosis of disease.

"Our knowledge of circular RNAs is really in its infancy," explained Pandolfi. "We know that normally, they can bind proteins as well as DNA and microRNAs, but much more needs to be done to understand how fusion-circular RNAs work. We have only scratched the surface of

these RNAs and their roles in cancer and other diseases."

Provided by Beth Israel Deaconess Medical Center