An 'evolutionary relic' of the genome causes cancer

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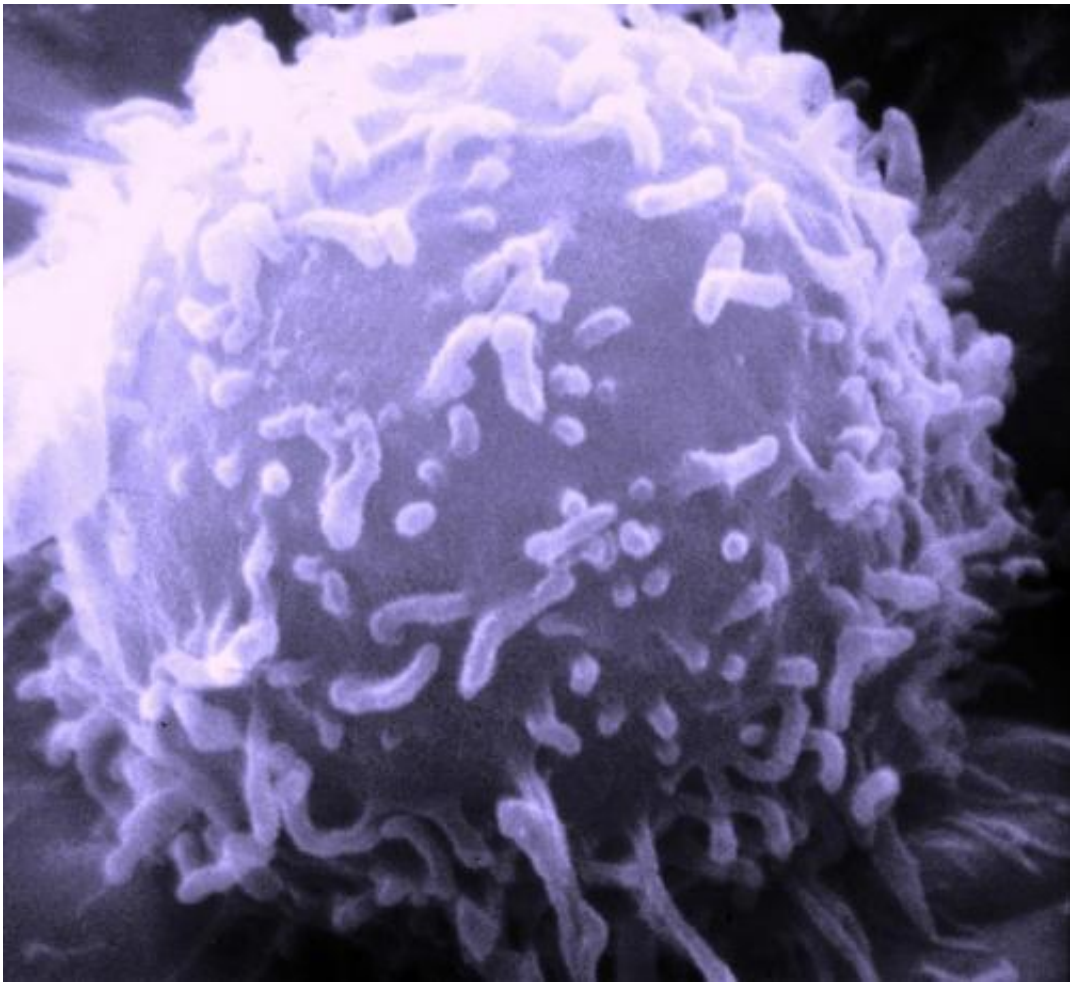

Electron microscopic image of a single human lymphocyte. Credit: Dr. Triche National Cancer Institute

Pseudogenes, a sub-class of long non-coding RNA (lncRNA) that developed from the genome's 20,000 protein-coding genes but lost the ability to produce proteins, have long been considered nothing more than genomic "junk." Yet the retention of these 20,000 mysterious remnants during evolution has suggested that they may in fact possess biological functions and contribute to the development of disease.

Now, a team led by investigators in the Cancer Research Institute at Beth Israel Deaconess Medical Center (BIDMC) has provided some of the first evidence that one of these non-coding "evolutionary relics" actually has a role in causing cancer.

In a new study in the journal *Cell*, publishing online today, the scientists report that independent of any other mutations, abnormal amounts of the BRAF pseudogene led to the development of an aggressive lymphoma-like disease in a mouse model, a discovery that suggests that pseudogenes may play a primary role in a variety of diseases. Importantly, the new discovery also suggests that with the addition of this vast "dark matter" the functional genome could be tremendously larger than previously thought - triple or quadruple its current known size.

"Our mouse model of the BRAF pseudogene developed cancer as rapidly and aggressively as it would if you were to express the protein-coding BRAF oncogene," explains senior author Pier Paolo Pandolfi, MD, PhD, Director of the Cancer Center and co-founder of the Institute for RNA Medicine (iRM) at BIDMC and George C. Reisman Professor of Medicine at Harvard Medical School. "It's remarkable that this very aggressive phenotype, resembling human diffuse large B-cell lymphoma, was driven by a piece of so-called 'junk RNA.' As attention turns to precision medicine and the tremendous promise of targeted cancer therapies, all of this vast non-coding material needs to be taken into account. In the past, we have found non-coding RNA to be

overexpressed, or misexpressed, but because no one knew what to do with this information it was swept under the carpet. Now we can see that it plays a vital role. We have to study this material, we have to sequence it and we have to take advantage of the tremendous opportunity that it offers for cancer therapy."

The new discovery hinges on the concept of competing endogenous RNAs (ceRNA), a functional capability for pseudogenes first described by Pandolfi almost five years ago when his laboratory discovered that pseudogenes and other noncoding RNAs could act as "decoys" to divert and sequester tiny pieces of RNA known as microRNAs away from their protein-coding counterparts to regulate gene expression.

"Our discovery of these 'decoys' revealed a novel new role for messenger RNA, demonstrating that beyond serving as a genetic intermediary in the protein-making process, messenger RNAs could actually regulate expression of one another through this sophisticated new ceRNA 'language,'" says Pandolfi. The team demonstrated in cell culture experiments that when microRNAs were hindered in fulfilling their regulatory function by these microRNA decoys there could be severe consequences, including making cancer cells more aggressive.

In this new paper, the authors wanted to determine if this same ceRNA "cross talk" took place in a living organism—and if it would result in similar consequences.

"We conducted a proof-of-principle experiment using the BRAF pseudogene," explains first author Florian Karreth, PhD, who conducted this work as a postdoctoral fellow in the Pandolfi laboratory. "We investigated whether this pseudogene exerts critical functions in the context of a whole organism and whether its disruption contributes to the development of disease." The investigators focused on the BRAF pseudogene because of its potential ability to regulate the levels of the

BRAF protein, a well-known proto-oncogene linked to numerous types of cancer. In addition, says Karreth, the BRAF pseudogene is known to exist in both humans and mice.

The investigators began by testing the BRAF pseudogene in tissue culture. Their findings demonstrated that when overexpressed, the pseudogene did indeed operate as a microRNA decoy that increased the amounts of the BRAF protein. This, in turn, stimulated the MAP-kinase signaling cascade, a pathway through which the BRAF protein controls cell proliferation, differentiation and survival and which is commonly found to be hyperactive in cancer.

When the team went on to create a mouse model in which the BRAF pseudogene was overexpressed they found that the mice developed an aggressive lymphoma-like cancer. "This cancer of B-lymphocytes manifested primarily in the spleens of the animals but also infiltrated other organs including the kidneys and liver," explains Karreth. "We were particularly surprised by the development of such a dramatic phenotype in response to BRAF pseudogene overexpression alone since the development of full-blown cancer usually requires two or more mutational events."

Similar to their findings in their cell culture experiments, the investigators found that the mice overexpressing the BRAF pseudogene displayed higher levels of the BRAF protein and hyperactivation of the MAP kinase pathway, which suggests that this axis is indeed critical to cancer development. They confirmed this by inhibiting the MAP kinase pathway with a drug that dramatically reduced the ability of cancer cells to infiltrate the liver in transplantation experiments.

The Pandolfi team further validated the microRNA decoy function of the BRAF pseudogene by creating two additional transgenic mice, one overexpressing the front half of the BRAF pseudogene, the other

overexpressing the back half. Both of these mouse models developed the same lymphoma phenotype as the mice overexpressing the full-length pseudogene, a result which the authors describe as "absolutely astonishing."

"We never expected that portions of the BRAF pseudogene could elicit a phenotype and when both front and back halves induced lymphomas, we were certain the BRAF pseudogene was functioning as a microRNA decoy," says Karreth.

The investigators also found that the BRAF pseudogene is overexpressed in human B-cell lymphomas and that the genomic region containing the BRAF pseudogene is commonly amplified in a variety of human cancers, indicating that the findings in the mouse are of relevance to human cancer development. Moreover, say the authors, silencing of the BRAF pseudogene in human cancer cell lines that expressed higher levels led to reduced cell proliferation, a finding that highlights the importance of the pseudogene in these cancers and suggests that a therapy that reduces BRAF pseudogene levels may be beneficial to cancer patients.

"While we have been busy focusing on the genome's 20,000 coding genes, we have neglected perhaps as many as 100,000 noncoding genetic units," says Pandolfi. "Our new findings not only tell us that we need to characterize the role of all of these non-coding pseudogenes in cancer, but, more urgently, suggest that we need to increase our understanding of the non-coding 'junk' of the genome and incorporate this information into our personalized medicine assays. The game has to start now—we have to sequence and analyze the genome and the RNA transcripts from the non-coding space."

Provided by Beth Israel Deaconess Medical Center