Researchers discover hidden genetic influence on cancer

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In findings with major implications for the genetics of cancer and human health, researchers at Beth Israel Deaconess Medical Center (BIDMC) and two other science teams in New York City and Rome have uncovered evidence of powerful new genetic networks and showed how it may work to drive cancer and normal development.

Four papers published online Oct. 14 in the journal *Cell* describe aspects of what may be a fundamentally new dimension of genetic activity that involves a vast posse of RNA molecules interacting and manipulating the molecular endgame behind the scenes. Each paper used a different approach, strengthening the basic discovery of the new RNA network.

In the half-century old central dogma of molecular biology, DNA issues its genetic blueprint to messenger RNA, which relays the orders to the protein-making machinery of the cell. The new studies suggest a significant new role for RNA on top of its traditional middle-management job: The RNA of one gene can control and be controlled by dozens or hundreds of RNAs of other genes.

In the case of a major tumor suppressor gene, PTEN, a shift in the associated RNA network appears to be as malevolent as a mutation in the gene itself in human prostate and colon cancer cells, in glioblastoma cells, and in a mouse model of melanoma, according to three of the papers.

The findings may enlarge the framework for investigating how tumors

form and progress, who is at risk for cancer, and how to find and disable the essential misbehaving molecules that drive the growth and spread of cancer.

"For instance, we now know that the PTEN tumor suppressor gene is talking to a vast unrecognized RNA network," said Pier Paolo Pandolfi MD PhD, director of the Cancer Genetics Program at BIDMC and George C. Reisman Professor of Medicine at Harvard Medical School, and the senior author of two of the papers. "The RNAs talk through a new language. If this language is broken and the RNA network is perturbed, PTEN goes down, and this has devastating consequences. But it's incredibly exciting for therapeutic possibilities. You may be able to rewire the crosstalk between the RNAs for cancer prevention and therapy."

Scientists typically use genetic studies to probe how changes in the DNA code influence the action of the proteins. Targeted therapies have arisen from efforts to counteract the effect of problematic proteins, yet most of the genetic determinants of cancer remain a vexing puzzle. The newly discovered RNA network could explain much of the elusive genetic variation underlying cancer and other diseases, say authors of the papers.

The new RNA regulatory network also appears to extend into the massive non-protein-coding region of the human genome and plays an important role in normal muscle development, suggests another related paper in *Cell*. Because humans share so many protein-coding genes with other organisms, including worms and yeast, this large portion that is transcribed into non-coding RNA makes the human genome distinctive. Much of the function of that non-coding RNA has been a mystery.

"Almost all of the scientific analysis of cancer genes focuses on the protein-coding genes," Pandolfi said, referring to the two percent of the human genome where instructions are passed from DNA to RNA to

proteins. "We know that nearly half of the genome is transcribed into RNA that doesn't code for protein. Through this new 'language' of RNA, we can functionalize this space."

How it works

The newly discovered network of RNA molecules converse through tiny targeted molecules called microRNAs, Pandolfi and his colleagues have found. RNAs share a vocabulary composed of specific sequences along their strands called microRNA response elements (MREs). RNAs compete for certain matching microRNAs. Once attached, microRNAs disable their host RNA molecules. It works through simple math: An increase in RNA can sponge up more microRNA, allowing other RNA to go about their business unhindered.

Scientists have known for a decade that microRNA can block RNA and prevent it from being translated into proteins. Some research has advanced to harnessing specific small microRNA molecules as experimental therapeutic tools to block individual protein-coding genes. What's new in the Cell papers is the idea of reverse logic -- that a large RNA network uses microRNA as a regulatory language.

Tantalizing hints of this newfound regulatory network have shown up in recent studies from several labs. Last year, Pandolfi's group reported that both PTEN and its nemesis, the common cancer-promoting gene KRAS, have doppelgangers known as pseudogenes in the non-coding regions of the genome, which act as decoys for targeted microRNA, greatly influencing the activity of the two cancer genes.

This August, Pandolfi and his co-authors named this RNA language and network activity "competing endogenous RNA" (ceRNA, pronounced SIR-na) in a *Cell* essay. The paper synthesized the emerging experimental evidence in a new theory. They proposed that ceRNA

activity greatly expanded the functional genetic information in the human genome and played important roles in diseases, including cancer.

The ceRNA hypothesis adds a major new layer to the highly regulated basic players defined by the central dogma of molecular biology – DNA, RNA and proteins. Other more established regulatory networks that keep cells healthy -- and break down in disease -- include small molecules added to proteins, such as the recycling label called ubiquitin. Another layer called epigenetics acts on the DNA and its packaging to lock or unlock certain genes.

The findings in the papers

Two of the *Cell* papers use a combination of bioinformatics and experimental evidence to connect the PTEN tumor suppressor gene to a network of several hundred RNA molecules in close communication.

One of the new papers from the Pandolfi lab linked about 150 new genes to the tumor suppressor PTEN in human prostate and colon cancer cell lines. Working with a collaborator at Jefferson Medical College in Philadelphia, postdoctoral fellow Yvonne Tay and her co-authors scanned the RNA transcripts of protein-coding genes based on their MRE sequences and then tested a few of the results. "Surprisingly, PTEN can be regulated by a lot of other genes through the ceRNA network," Tay said.

In an independent paper, a team in the lab of Andrea Califano at Columbia University in New York evaluated glioblastoma RNA and microRNA expression data from The Cancer Genome Atlas, a public database. They found a network in which more than 500 genes regulate PTEN. Of these, 13 are frequently deleted in glioblastoma and seem to work together through the microRNA language to squelch the tumor suppressor activity as if the tumors had mutations or deletions of PTEN

itself.

"All these papers address different aspects of this compelling story and reinforce each other," said Califano, who also found RNA networks that appeared to communicate by other means. "PTEN is just an example. In each cell, different cliques of genes are connected by this microRNA-mediated network, including all the established oncogenes and tumor suppressors. This layer explains a significant amount of genetic variability in cancer. It allows genes that have nothing to do with the typical oncogene or tumor suppressor to gang up and regulate it. The discovery of this network allows us to discover genes never before associated with a tumor type or disease."

In a second paper from the Pandolfi group, mutations in the PTEN RNA network speeded up the growth of cancer in a mouse model of melanoma. Postdoctoral fellow Florian Karreth and his co-authors discovered possible new PTEN ceRNAs in a mutagenesis screen of a mouse model of melanoma. With the help of a bioinformatics team from the University of Turin, they did an in-depth analysis of one ceRNA (ZEB2) that is reduced in human cancer and verified that its reduction accelerated cancer progression in the mice. Interestingly, while the ZEB2 ceRNA opposes melanoma by sponging the microRNAs that would otherwise repress PTEN's tumor suppression activity, the ZEB2 protein is known to promote other cancers. "It is astonishing that RNA and protein molecules encoded by the same gene can take part in opposing biological processes," Karreth said.

The final study extends functional evidence of the new RNA network phenomenon to the normal differentiation of human muscle cells and to the large realm of human non-coding RNAs. Irene Bozzoni's group at the Sapienza University of Rome found that a long non-protein coding RNA works similarly as a decoy for microRNAs in normal muscle differentiation in mice and humans. In Duchenne muscular dystrophy,

the decoy RNA is missing at a crucial time, preventing muscle cells from maturing.

"This explains in part why Duchenne cells have trouble, and it gives us another circuitry to attack in order to cure the disease," said Bozzoni, who heard about Pandolfi's ceRNA hypothesis at a meeting last year. "We have been working on noncoding RNA and microRNA for quite a long time. This cross-talk of RNAs through microRNAs is a revolutionary idea."

More information: In the paper, "Coding-Independent Regulation of the Tumor Suppressor PTEN by Competing Endogenous mRNAs," Tay and Pandolfi's co-authors include BIDMC colleagues Ugo Ala, Lev Kats, Leonardo Salmena, Florian Karreth and Laura Poliseno; University of Turin collaborators Paolo Provero and Ferdinando Di Cunto; and Children's Hospital Boston researchers Shen Mynn Tan and Judy Lieberman. (DOI 10.1016/j.cell.2011.09.029)

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