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Gene Swaps May Make Cancers Vulnerable

Howard Hughes Medical Institute (HHMI) researchers at the University of Michigan have identified a set of hybrid genes – created when chromosomes inappropriately swap pieces of genetic material – that may accelerate the growth of prostate cancer, gastric cancer, and melanoma.

The hybrid proteins produced by fused genes are associated with particularly aggressive forms of prostate cancer, gastric cancer and melanoma. Although these hybrid proteins are rare, they may represent attractive targets for new cancer therapies, said Arul Chinnaiyan, an HHMI investigator at the University of Michigan.

Prior to these studies, it was well known that some forms of leukemia, lymphoma, and other blood cancers are caused when chromosomes inappropriately swap pieces of genetic material in a process called translocation. In blood cancers, the reshuffling of broken chromosome segments can force together a promoter (a gene control element) and a cell growth gene, creating a new kind of genetic switch that spurs rapid and uncontrolled cell division. The most famous example of gene fusion is the union of the *Bcr* and *Abl* genes, which triggers the runaway growth of white blood cells characteristic of chronic myelogenous leukemia (CML).

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- Arul M. Chinnaiyan

In contrast to these leukemias and other blood cancers, most solid tumors, such as prostate and gastric cancer, were thought to be caused by mutations that affect one or more growth-regulating genes in the cell. But in 2005, Chinnaiyan and his colleagues at Michigan showed that fusion genes can also spur the development of prostate cancer.

In those earlier experiments, Chinnaiyan's lab showed that gene fusions were present in cells in prostate tumors. In fact, more than half of the prostate cancer samples his team examined contained a fusion between the *TMPRSS2* gene -- which is controlled by male hormones -- and one of several ETS genes that switch on other growth-promoting genes. One of these ETS family members, *ERG*, joined up with the *TMPRSS2* gene in half of prostate cancers the group sampled. When the two genes fuse together, the *ETS* genes come under control of the male hormone androgen. The result is disastrous, as the newly formed hybrid gene behaves like an overactive "on" switch for growth-stimulating genes that can spur tumor growth.

In a study published in *Cancer Cell* on May 18, 2010, Chinnaiyan and his team wanted to unmask the identities of the full spectrum of genes whose behavior is affected by the *TMPRSS2-ETS* fusion. They first focused on the *ERG* gene, which is the ETS family member most frequently fused to *TMPRSS2* in prostate cancer cells. As with other members of the ETS family, *ERG* is a transcription factor, and as such switches on specific genes by first binding to a regulatory sequence nearby on the DNA. To find out which genes *ERG* normally controls, Chinnaiyan's team isolated all of the *ERG* protein from prostate cancer cells. They reasoned that when they pulled out the *ERG*, any DNA that was attached to *ERG* in the cell would also be dragged along. They could then sequence the segments of attached DNA, and come up with a list of genes that are likely controlled by *ERG*. In this way, they uncovered thousands of genes likely to fall under *ERG*'s influence.

They then repeated the experiment to find genes that are controlled by the androgen receptor, a protein that binds to male hormones and activates genes in response. They discovered that *ERG* is recruited to nearly all of the genes that the androgen receptor does.

Additional experiments showed that the activities of the two proteins were tightly linked. Androgen receptor and *ERG* clung to each other in cells, suggesting a functional interplay. The androgen receptor ramps up its own production by cranking up the gene that makes androgen receptor. *ERG* also bound to this gene, and Chinnaiyan's group showed that its presence shut down androgen receptor production. They also found that squelching *ERG* expression restored production of the receptor. The team showed that *ERG* could block other genes activated by androgen receptor, as well.

Doctors commonly treat prostate cancer with drugs that block androgen signaling. These compounds are initially effective, but cancer often returns. The new work suggests that androgen's role in cancer is more complicated than originally believed..

In his view, the gene fusion fosters cancerous growth by activating *ERG* and shutting down genes normally activated by the androgen receptor. He thinks that out of control androgen signaling isn't to blame for prostate cancer. "Androgen receptor signaling is part of normal prostate biology," he says. "Androgen is a good thing."

Chinnaiyan says new drugs that cripple ERG and its related ETS proteins might be an effective strategy for shrinking tumors. However, drug designers have found it difficult to block transcription factors. Because ERG and other transcription factors don't perform a chemical reaction, it's hard to know where to direct a chemical dagger.

Enzymes have historically proven to be much more "druggable," and Chinnaiyan wondered whether the growth of some solid tumors might be driven by gene fusions more vulnerable to intervention.

Chinnaiyan found inspiration in an earlier study in which other researchers had found that a small percentage of patients with lung cancer harbored a gene fusion that included a gene for a kinase protein. Kinases control the function of other proteins by stitching on small molecules called phosphates. One of the truly spectacular success stories in modern oncology is the development of Gleevec, a drug that virtually halts the progress of chronic myeloid leukemia by blocking the wayward kinase that is produced by the *Bcr-Abl* gene. To date, however, there is no effective kinase inhibitor for prostate cancer.

Chinnaiyan says his team scoured their prostate cancer samples for similar genetic alterations. They looked in prostate cancer cells that didn't have any ETS fusions, sequenced active genes, and searched for new kinds of gene fusions. In a small number of test samples, the researchers found two such twinings, they report in *Nature Medicine* on June 6, 2010. Two different prostate tumors held fusions that linked kinase proteins to other molecules. These fusions involved kinases that are part of the so-called RAF signaling pathway, which is known to play a role in the development of malignant melanoma.

Intriguingly, one of the two gene fusions brought a kinase together with an androgen-controlled gene. Chinnaiyan's group was eager to learn how the fusion might activate this protein improperly in prostate cells.

When they analyzed another 349 prostate tumors, they found rearrangements involving RAF kinases in 10 of them. They also found that a small percentage of gastric and melanoma cancers had RAF kinases shuffled. Although the RAF rearrangements were uncommon, they were associated with aggressive forms of cancer that have been difficult to treat.

They found that these fusions caused cultured cells to grow and divide unusually rapidly, and that kinase-blocking compounds stunted this growth. Chinnaiyan notes that companies are already exploring drugs that block the RAF signaling pathway for other cancers. If their development is successful, these compounds could be useful against cancers in which RAF signaling has been accelerated by a gene fusion.

“Broadly, we should think about cancer in terms of the driving molecular lesions,” says Chinnaiyan. “Rather than thinking of it as prostate cancer, we should think of it as a RAF cancer or an ETS cancer.” That frame of mind could lead researchers to new and more effective drugs that could be hand-picked based on the genetics of each prostate cancer patient, he says.