

OCTOBER 25, 2007

Learning How a Cancer Gene Thwarts Cell's Suicide Machinery

Howard Hughes Medical Institute researchers have discovered a surprisingly elaborate network of genes involved in converting normal cells to tumor cells. The new studies found that the cancer-promoting gene *ras*, which is abnormally activated in about 30 percent of human cancers, relies on 28 other genes to switch off the cell's programmed cell death pathway, which helps destroy damaged cells.

Howard Hughes Medical Institute investigator Michael R. Green and his colleagues at the University of Massachusetts Medical School published their findings in the October 25, 2007, issue of the journal *Nature*.

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— Michael R. Green

The conversion of a normal cell to a cancer cell involves a stepwise process that typically requires the activation of oncogenes and inactivation of tumor-suppressor genes and other genes that trigger programmed cell death. When cells are damaged or turn cancerous, the *Fas* gene - which is a tumor suppressor gene — helps ensure that damaged cells die off by activating the cell's programmed cell death machinery.

In their experiments, the researchers sought to understand how the *ras* gene, which promotes cancer, silences *Fas*. During growth and development, certain genes that are to be silenced, or prevented from being expressed, are physically marked by the addition of molecules called methyl groups. This kind of epigenetic control mechanism is separate from other mechanisms that control gene expression, such as regulatory DNA elements that are embedded in the sequences of the genes themselves.

For *Fas* to be activated, segments of *Fas* gene must first be unmasked by the removal of methyl groups. This unmasking allows the gene to be switched on. Researchers knew that *ras* silences *Fas* by shrouding those key gene

segments in methyl groups—called hypermethylation.

There were two broad theories about how this silencing process takes place, said Green. One is that hypermethylation takes place randomly, and that it confers a growth advantage on the cancer cell that allows it to proliferate. And the other is that there is an 'instructive' mechanism by which the *ras* gene epigenetically silences the tumor suppressor gene. Our experiments aimed at trying to decide between these two theories.

In their experiments, the researchers used cultured mouse cells that had the same kinds of genetic changes as cancer cells in the human body. Employing a mass-screening technique, they selectively suppressed each of the roughly 28,000 genes in the cells using RNA interference (RNAi) to uncover critical genes necessary for *ras* to silence *Fas*. In RNAi, double-stranded RNA that matches the messenger RNA produced by a given gene degrades that messenger RNA—in effect wiping out the function of that gene in a cell. After suppressing the genes with RNAi, the researchers then examined the cells to detect which genes' suppression disrupted *ras*'s ability to silence *Fas*. The screening revealed that there were 28 genes that made proteins that were critical for *ras* to silence *Fas*. The researchers called these genes *Ras* epigenetic silencing effectors.

One of the surprises to me was how elaborate this silencing pathway was, said Green. It wasn't just one or two components, it was 28 components and they were all necessary. If you inactivate any one of them *Fas* becomes derepressed in *ras*-transformed cells.

The known functions of the genes indicated that the proteins they made formed a regulatory chain all the way from the surface of the cell, where *ras* works, into the nucleus, where *Fas* is silenced, said Green. Several of the 28 genes in the pathway that Green's group uncovered were not known to be part of the *ras* signaling pathway. In additional experiments, the researchers showed that the silencing pathway does, indeed, directly trigger hypermethylation of *Fas*.

The researchers next tested whether the same pathway might be involved in silencing other genes known to be repressed in *ras*-activated cancer cells. Their experiments revealed that most of the same 28 *Ras* epigenetic silencing effectors were required for silencing these other genes. So we think this is a rather general pathway that acts on many tumor suppressor genes in *ras*-transformed cells, concluded Green.

Overall, our study strongly supports the instructive model for silencing of a tumor suppressor gene in *ras*, said Green. We have clearly defined the components of a pathway for this silencing. And the good news is that this silencing pathway is more complicated and fragile than might have been thought, and that some of its components are potential targets for anti-cancer drugs.

Green noted that anti-cancer drugs that inhibit epigenetic silencing are already in human trials, and even though they are non-specific, they could

point the way to more specific drugs to target the *ras* silencing pathway.

I think now that we have a defined pathway that leads from *ras* to the epigenetic repression of tumor suppressor genes, we can explore the possibility that inhibiting that pathway could produce more selective effects and have better therapeutic efficacy, he said.