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Turning a Cellular Sentinel into a Cancer Killer

Howard Hughes Medical Institute researchers have developed two strategies to reactivate the *p53* gene in mice, causing blood, bone and liver tumors to self destruct. The p53 protein is called the “guardian of the genome” because it triggers the suicide of cells with damaged DNA.

Inactivation of p53 can set the stage for the development of different types of cancer. The researchers' findings show for the first time that inactivating the *p53* gene is necessary for maintaining tumors. While the researchers caution that cancers can mutate to circumvent *p53* reactivation, they believe their findings offer ideas for new approaches to cancer therapy.

The research was carried out independently by two Howard Hughes Medical Institute (HHMI) research teams led by Tyler Jacks at the Massachusetts Institute of Technology and Scott Lowe at Cold Spring Harbor Laboratory. Both papers were published online January 24, 2007, in advance online publication articles in the journal *Nature*.

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- Tyler Jacks

Although researchers have long known that *p53* inactivation plays a central role in the development of cancer, little was known about whether *p53* inactivation played a role in maintaining cancers. And researchers were not sure whether switching *p53* back on in tumor cells would have any therapeutic effect.

“It had been demonstrated that overexpressing *p53* at very high levels could arrest or kill tumors, said Lowe. “But at such high levels, *p53* might not be working through a physiological mechanism. So, it was an open question

whether restoring the *p53* pathway would have any anti-tumor effect.” For one thing, the high mutation rate in cancers might enable a cancer to switch the *p53* pathway back off, or to circumvent the pathway in some other fashion. For those reasons, researchers were not sure whether the pathway would be a useful therapeutic target.

To reactivate *p53*, Lowe and his colleagues used a genetic technique they had developed to induce an aggressive form of liver cancer in mice. Although they had inactivated *p53* in the mice, they genetically engineered the mice so that they could reverse *p53* inactivation by giving the animals the antibiotic doxycycline. They suppressed *p53* protein levels by using RNA interference (RNAi) that had been modified so that RNAi could be switched off by the antibiotic. The RNA interference technology was developed in collaboration with HHMI investigator Gregory Hannon at Cold Spring Harbor Laboratory.

When the researchers reactivated *p53* in the mice they found that the liver tumors completely disappeared. “This was quite surprising,” said Lowe. “We were working with a very advanced, aggressive tumor, but when we reestablished *p53*, not only did it stop growing, it went away.

“But the second surprise—and perhaps the more scientifically interesting one—was why the tumor went away,” said Lowe. “We expected the tumor cells to undergo programmed cell death, or apoptosis. But instead, we saw evidence for a very different process that *p53* also regulates—senescence, or growth arrest. What really excited us was evidence that this senescence somehow triggered the innate immune system to kill the tumor cells.” Involvement of the innate immune system suggests there may be an unknown mechanism by which cancers can trigger the immune system, he said. Lowe and his colleagues are now exploring how the innate immune system might be enlisted against cancer.

Jacks's team used a different technique to reactivate *p53* in lymphomas and sarcomas. In their experiments, the researchers produced mice whose cells did not have *p53* activity. These mice were genetically engineered so that the drug tamoxifen could be used to switch on *p53* activity.

“When we reactivated *p53* in these mice, we saw two distinct tumor phenotypes,” said Jacks. “In lymphomas the responses were rapid, extensive and were accompanied by the induction of apoptosis. In the sarcomas, the response was less rapid often less extensive and was not accompanied by apoptosis. Instead the cells underwent cell cycle arrest with features of senescence.”

Jacks said that he and his colleagues found no evidence that restoring *p53* affected normal cells. “That gives us a broader therapeutic window in which the cancer cells respond rapidly by one of these two mechanisms, whereas normal cells seem to tolerate reactivation of *p53* quite well,” he said.

In the December 29, 2006, issue of the journal *Cell*, Gerard Evan at the University of California at San Francisco reported that *p53* restoration was effective in killing lymphoma tumor cells.

In a *News & Views* commentary published in *Nature*, Norman Sharpless and Ronald DePinho wrote that “these three papers provide reason for cautious optimism that reactivation of *p53*, and possibly of other tumour-suppressor genes, might be useful in treating certain cancers.” However, they noted that Evan and his colleagues had seen rapid appearance of tumors that progressed despite *p53* expression, indicating that cancers can mutate to deactivate the pathway or circumvent it.

Both Lowe and Jacks said they agreed that therapies aimed at reactivating *p53* would still have to cope with the adaptability of cancers. “For any therapies based on these findings, you would have to imagine that sooner or later, resistance would develop,” said Lowe. “And I think the challenge—which is true for any therapy where you can get resistance—is to come up with combination treatments that take out enough of the cancer cells early on that there aren't enough variants left to get around the *p53* pathway.”

Jacks added that “the main point is that—although there are differences between these mouse models and human cancers—we now can say with some confidence that established tumors are sensitive to *p53* reactivation. There are already a number of treatment strategies being tested to restore *p53* function, and these might well be expected to have therapeutic benefits.”

Lowe emphasized that these new experimental techniques for reactivating *p53* could also be applied to study the effects of other regulatory molecules on cancers. “We think we have a really powerful technology that could be generalized, not only to study tumor suppressors, but also molecules that might be important drivers of tumorigenesis, making them good drug targets,” he said. “For these, we could do the converse experiment, letting the tumor develop and then using our technique to knock out the molecule and see whether the tumor goes away.”

Jacks and his colleagues next plan to study the effects of *p53* reactivation in other cancers. They hope to use the technology to understand why some cancers respond by undergoing apoptosis, while others undergo senescence.