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## Cancer Cells Walk the Tightrope

Researchers have shown that the well known tumor-suppressor gene *p53* and its lesser-known cousin, *p73*, are controlled by the same activation switch. Flipping the activation switch begins a process that can instruct a cell to stop growing or to die. As an activator of cell death, the switch may become the target of a new generation of anticancer therapies.

In the October 5, 2000, issue of the journal *Nature*, two Howard Hughes Medical Institute research teams show that a transcription factor called E2F-1 activates *p53* and *p73*. E2F-1 is a cancer-promoting gene, or oncogene. Mutations in the *p53* gene are present in more than half of all human cancers. It is thought that these cancers arise because the crippled p53 protein cannot halt the growth of cells or promote the death of cells that bear activated oncogenes such as *E2F-1*.

Although *p73* is a close relative of *p53*, there were a number of unresolved questions about how it is activated and its role in cell growth. The studies published in *Nature* show that *p73*, like its more famous cousin, is activated by E2F-1 and plays an important role in ensuring the death of cells in which *E2F-1* is overactivated, such as cancer cells or overstimulated immune cells. The research teams were led by HHMI investigators [William G. Kaelin, Jr.](#), at the Dana-Farber Cancer Institute and [Steven F. Dowdy](#) at Washington University School of Medicine. [Tyler Jacks](#), an HHMI investigator at the Massachusetts Institute of Technology, also collaborated on the research.

In one of the *Nature* articles, Kaelin and his colleagues sought to find out whether E2F-1, which was known to activate *p53*, could also activate *p73*. "Early on, we had shown that just as *p73* closely resembled *p53*, it also acted like *p53*, inducing programmed cell death and binding to some of the same DNA sequences and activating the same genes," said Kaelin. Searches by many laboratories for the presence of *p73* mutations in tumors had shown that such mutations are relatively rare in comparison to *p53* mutations. Furthermore, Kaelin's laboratory found that many proteins that inactivated *p53* did not affect *p73*, hinting that *p73* might have a different role. "So, these findings were a conundrum that made us wonder whether *p73* could really be thought of in the same vein as a tumor suppressor like *p53*," said Kaelin.

Employing tumor cell lines that lacked *p53*, Kaelin and his colleagues put *E2F-1* under the control of a genetic switch, called a promoter, that they

could flip on at will. When they switched on *E2F-1*, they found that they were also activating the *p73* gene. Further studies revealed that the *p73* gene contained multiple sites that were turned on specifically by E2F-1.

In other experiments, the scientists manipulated growth conditions in cancer cell lines and measured the effects that those alterations had on levels of E2F-1 and p73. They also studied apoptosis in mouse cells that lacked *p53* and/or *p73* genes, confirming that E2F-1 can regulate p53 and p73 under physiological conditions and showing that p53 and p73 cooperate to induce apoptosis.

By showing that E2F-1 directly activates *p73*, the scientists demonstrated that E2F-1 exerts its effects on p73 in a distinct manner; E2F-1 controls *p53* by activating an intermediate control gene.

"It's becoming very clear that cancer cells walk a tightrope with respect to some of these oncogenes, such as *E2F-1*," said Kaelin. "On the one hand, these oncogenes are driving proliferation, but on the other hand, they can also induce apoptosis. So, this finding is important because it raises the possibility that drugs or other therapies—for example activators of *E2F-1*—might be used to tip the balance in favor of apoptosis." Also, noted Kaelin, it might be possible to kill many cancers by directly activating the *p73* gene.

In the second *Nature* article, Dowdy, Kaelin and their colleagues demonstrated that p73 helps to regulate the number of circulating T cells through a process called T cell receptor activation-induced cell death (TCR-AICD). In TCR-AICD, the immune system induces apoptosis in proliferating T cells that either overreact to foreign proteins or that are no longer needed when an infection has been successfully conquered. Malfunctions in TCR-AICD can lead to autoimmune diseases, lymphomas and leukemias because the process fails to cull unneeded T cells.

In previous work, Dowdy and his colleagues had shown that TCR-AICD operates during the G1 stage of the cell growth cycle. They had also found that TCR-AICD cell death was independent of p53 activity. This led to the question of whether E2F-1 and p73 played a role in TCR-AICD, says Dowdy. In order to investigate that question, the scientists constructed special TAT-fusion proteins that ferried proteins into T cells capable of blocking E2F-1 function, and once inside the cell, blocked apoptosis.

Drawing on Kaelin's findings that E2F-1 activated p73, Dowdy and his colleagues then used TAT-fusion proteins to block p73 function to show that p73 was necessary for TCR-AICD. Moreover, the scientists also showed that mice whose T-cells lacked either *E2F-1* or *p73* genes did not undergo TCR-AICD

"The beauty of these two papers is that taken together they show both the mechanism of E2F-1 and p73, as well as the normal biology of their action in

cells," said Dowdy. "The combination of our data makes us comfortable that the biology is correct."

According to Dowdy, while faulty p73 proteins have not been shown to cause cancer, inactivation of the promoter that drives p73 expression has turned up in human leukemias. Such inactivation would protect blood cells against apoptosis, allowing them to accumulate mutations, proliferate and cause leukemia.

"Whenever you have a situation where a cell that was supposed to be deleted is not deleted, that's a problem because now you have a powder keg just waiting for additional mutations. It's a big problem especially in T-cells that are undergoing rapid division," said Dowdy.