'SKIP'-ing splicing forces tumor cells to undergo programmed cell death

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When cells find themselves in a tight spot, the cell cycle regulator p21 halts the cell cycle, buying cells time to repair the damage, or if all else fails, to initiate programmed cell death. In contrast to other stress-induced genes, which dispense with the regular transcriptional entourage, p21Cip1 still requires SKIP, a transcription elongation factor that also helps with the editing of transcripts, to be expressed, found researchers at the Salk Institute for Biological Studies.

In the absence of SKIP, the expression of p21Cip1 is rapidly down-regulated, predisposing cells to undergo programmed cell death, especially when faced with DNA damage-inducing chemotherapeutic agents. Their findings, reported in the April 1, 2011 issue of *Genes & Development*, not only define a new step that controls programmed cell death in cancer cells, but also suggest new approaches to enhance the efficacy of chemotherapeutic drugs.

"Interestingly, SKIP levels decline in cells treated with flavopiridol, which is currently in clinical trials as an anticancer agent for leukemia, and as a combination therapy for solid tumors," says Katherine A. Jones, Ph.D., a professor in the Regulatory Biology Laboratory, who led the study. "Our findings might help explain why flavopiridol works so well in combination with other cytotoxic drugs. Loss of SKIP sensitizes cancer cells to the apoptotic effects of DNA damage-inducing anticancer drugs."

The DNA in our cells is under constant attack from reactive chemicals

generated as by-products of cellular metabolism. In addition, it is assaulted by x-rays, ultraviolet radiation from the sun, and environmental carcinogens such as tobacco smoke. If a cell suffers non-repairable injury it activates a built-in "auto-destruct" mechanism, known as programmed cell death or apoptosis. It eliminates abnormal cells from the body before they can cause disease, including cancer.

As a first-responder, the transcription factor p53 is called to action when cells experience stressful conditions. Depending on the situation, p53 then turns on genes that halt cell division to allow time for repairs, such as p21Cip1, or when all rescue attempts prove futile, order the cell to commit suicide by turning on pro-apoptotic genes such as PUMA.

"It is the timing and duration of expression of the set of p53 target genes that are turned on, which strongly influences cell fate decisions," says Jones, who likes to compare PUMA, which takes its time to come online, and p21Cip1, which quickly snaps into action in response to damage, to Aesop's fable of the tortoise and the hare. "Cell cycle arrest genes are poised for immediate action, whereas pro-apoptotic genes go slow but steady."

Even before cell damage occurs, these genes differ in the number and composition of their pre-loaded transcription complexes. These multi-protein assemblies, called RNA polymerase II (Pol II), slide along the DNA's double helix, reading the genetic code and transcribing it into RNA, which is used as a blueprint to build proteins, or as a switch to regulate other genes. But Pol II is prone to stalling in the middle of transcribing genes and needs to be prodded along by elongation factors such as SKIP. "Under stressful conditions everything changes," explains Jones. "Elongation factors seem to be no longer necessary for transcription."

But SKIP influences gene expression in more than one way. It also plays

an important role during the splicing process, which removes intervening sequences or introns, and joins the remaining pieces or exons to form a mature messenger RNA that can now be used to produce protein.

It is the splicing function that p21Cip1 cannot do without, revealed the experiments by postdoctoral researchers Yupeng Chen, Ph.D., and Lirong Zhang, Ph.D. "SKIP is critical for splicing and expression of p21Cip1, but not for PUMA or any of the other p53-induced genes we looked at in colon cancer and osteosarcoma cells," says Chen. He found that SKIP recruits a critical splice factor to the p21 gene, while the same factor finds its way to the PUMA gene without SKIP's help. As a consequence, the depletion of SKIP induced apoptosis, which was most pronounced in cells subjected to DNA damage.

"Many chemotherapeutic drugs work by inducing apoptosis," says Zhang. "Combining them with small molecules that inhibit splicing of the p21 mRNA may boost their efficiency of forcing tumor cells to undergo programmed cell death."

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