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Researchers Identify Gene's Role in Suppressing Longevity

Researchers have determined that a gene present in mouse cells limits the number of times that a cell can divide. The gene is involved in a process, called senescence, which is thought to ensure that aging cells do not pass on harmful mutations.

The researchers said the gene, known as *SIRT1*, suppresses longevity, and may play a role in regulating the aging process. But they caution against interpreting the results too broadly, because dividing mouse cells in culture are an imperfect model of how aging affects human cells.

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— Frederick W. Alt

There is, however, some indication from the new studies that suppressing *SIRT1* could prove important in techniques that researchers use to generate large numbers of normal cells for research. In this context, the *SIRT1*-deficient cells hold an advantage over other highly proliferative cell types, such as cancer cells, because although they divide indefinitely, they otherwise appear normal.

The research team, led by Frederick W. Alt, a Howard Hughes Medical Institute investigator at Children's Hospital, Boston, and Harvard Medical School, published its findings in the July 2005 issue of the journal *Cell Metabolism*. Katrin Chua and Raul Mostoslavsky in Alt's laboratory were joint first authors of the article, which also included co-authors from the National Institutes of Health and Brigham and Women's Hospital.

The researchers began studying *SIRT1* because they were intrigued by published reports from other research groups that showed that the yeast version of the gene, *Sir2*, extends the ability of cells to replicate. Additional studies had shown that overproducing *Sir2* in the cells of worms and flies

increased the organisms' life span. Such studies had led some to speculate that enhancing the activity of the mammalian version, *SIRT1*, might also promote longevity. For that reason, pharmaceutical companies are exploring drugs that influence *SIRT1* activity, said Alt, who is also scientific director of the CBR Institute for Biomedical Research in Boston.

Alt and his colleagues have been exploring the entire seven-member family of *SIRT* genes because of their potential regulatory roles in the immune system, genomic stability and DNA repair. The enzymes produced by *SIRT* genes, called deacetylases, activate a wide range of target molecules.

In their experiments, Alt and his colleagues studied the effects of suppressing *SIRT1* activity in cell cultures of mouse embryonic fibroblasts. Specifically, they explored the effect of *SIRT1*-deficiency on the ability of the cells to divide in culture.

“We had been studying *SIRT1*-knockout mice, which have a number of defects,” said Alt. “And when we grew embryonic fibroblasts from those mice in culture, we observed that, unlike wild-type cells that only undergo a limited number of divisions before they reach senescence, *SIRT1*-deficient cells continued to grow on and on very well. That was quite surprising, because the findings in yeast and other lower organisms led those in the field to speculate that if you got rid of mammalian *SIRT1*, the cells would senesce sooner. But in fact we got the opposite result; the cells survived and didn't undergo senescence.”

To confirm that it was only suppression of *SIRT1* that affected the cells, and not some secondary effect caused by knocking out the gene early in development, the researchers created mouse cells in which they could switch off the gene at will. Cells from those mice also became “immortalized.” But when the researchers switched *SIRT1* back on, the cells again became subject to senescence.

“So, we showed that, unlike in yeast, mouse *SIRT1* can function to suppress cellular longevity rather than to promote it,” said Alt. “That has been a big surprise to the field since it does not fit with preconceived notions of the role of *SIRT1*.”

In further studies, the researchers found that *SIRT1* might regulate senescence by down-regulating expression of p19^{ARF}, known to be an important mediator of senescence. Indeed, p19-deficiency in cells similarly eliminates senescence in the cell cultures. The researchers also found that *SIRT1* affects a particular response pathway to DNA-damaging oxidation. They found that *SIRT1*-deficient cells, in contrast to normal cells, continued to divide when treated chronically with low-level doses of oxidation-inducing hydrogen peroxide. However, the *SIRT1*-deficient cells had a normal senescence response when exposed to high-level oxidation or the activated cancer gene, *Ras*. Together, these results indicate that *SIRT1* has a specific role in the response to chronic oxidative damage.

According to Alt, the discovery that *SIRT1* deficiency enables cells to proliferate beyond senescence could be valuable to researchers growing cells for study. “Many labs and companies have developed a wide range of modulators of *SIRT1*,” he said. “I think our findings offer one interesting possibility for their use, in that eliminating *SIRT1* could enable the production of large numbers of cells that are relatively normal. “The catch, of course, is that we have only shown this in mouse embryonic fibroblasts so far. It needs to be demonstrated in other differentiated cells and in stem cells,” he said.

“A second major point is that the real utility for this technique would be its use in human cells. And the pathways of senescence are potentially different in human and mouse cells.” Alt also emphasized that senescence is an imperfect model for aging, and findings in cell culture studies are far from directly applicable to effects in whole organisms.

Alt and his colleagues are planning to explore the effects of switching off *SIRT1* in adult mice, since knocking the gene out completely causes abnormal development. Such studies would enable them to determine whether turning off the gene eliminates senescence in wider range of cells. The studies would also permit Alt's team to explore potential roles for *SIRT1* in tumor suppression and other functions.