Hopkins team discovers how DNA changes

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Using human kidney cells and brain tissue from adult mice, Johns Hopkins scientists have uncovered the sequence of steps that makes normally stable DNA undergo the crucial chemical changes implicated in cancers, psychiatric disorders and neurodegenerative diseases. The process may also be involved in learning and memory, the researchers say.

A report on their study appears online April 14 in *Cell*.

While DNA is the stable building block of all of an individual's genetic code, or genome, the presence or absence of a methyl group at specific locations chemically alters DNA and changes the expression of the genes. In a series of experiments, the Johns Hopkins team identified a step-by-step process involving a previously unknown step and two molecules for DNA to change from a methylated to demethylated state. Both methylation and demethylation have long been linked to genetic alterations and a wide range of diseases.

"Anything we can learn from these studies about how to manipulate the process of changing DNA methylation status is going to have implications for human development and disease, including cancer and degenerative disorders," says Hongjun Song, Ph.D., professor of neurology and neuroscience and director of the Stem Cell Program in the Institute for Cell Engineering, the Johns Hopkins University School of Medicine.

First, using human kidney cells in a dish, the Hopkins team focused its

investigation on a tiny region of DNA in the cells' nuclei, specifically watching the actions of one particular chemical base known as cytosine (C). The team added different chemicals to force methylation changes and after watching the fate of methylated cytosine (mC) for two days, and noting that nothing had changed, they then added a protein called TET1 to the cell. As a result, some of the mC became hydroxylmethylated (hmC) and some reverted to plain C, indicating loss of the methyl-group from C in the DNA.

"What this told us was TET1 promotes this process of DNA changing status from methylated to demethylated," Song says.

While only about five percent of human cells progress from hmC to C under natural conditions, the researchers found they could enhance the demethylation process by adding another protein called Apobec1.

"That suggested another clear step in DNA demethylation," Song says. "Cells go from mC to hmC by TET1, and then from hmC to C involving Apobec 1."

Next, they followed up on their own previously published work showing that electrical stimulation like that used in electroconvulsive therapy (ECT) resulted in increased brain cell growth in mice, which likely was an effect of changes in DNA methylation status. The researchers used a genetic tool and PCR-based approach to amplify a tiny region of the genome in dozens of mice, some exposed to ECT-like electrical stimulation and some not, to compare the status of cytosine at similar stretches of DNA in brain tissue. By using genetic sequencing technology to analyze the various states of methylation – simple C, methylated C, or hydroxylmethlyated Cs – in the specific reigons of DNA from brain cells of ECT-exposed mice versus other animals, they found evidence that ECT indeed induces DNA demethylation and identified TET1 as a critical factor for this to happen.

"By identifying two molecules and tying together two pathways needed for DNA methylation status to change, we believe we have shown a unified mechanism that regulates DNA as it goes from a methylated state to a demethylated state," Song says. "This new knowledge gives us an entry point to someday manipulating this fundamentally important process for treating patients with diseases associated with epigenetic abnormality."

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