New labeling method expands ability to read DNA modification

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Researchers at Emory University School of Medicine and the University of Chicago have developed a method for labeling and mapping a "sixth nucleotide," whose biological role scientists are only beginning to explore.

The method is described online this week in Nature Biotechnology.

The method allowed the researchers to see for the first time how 5-hydroxymethylcytosine (5-hmC) is distributed throughout the genome. Unlike 5-methylcytosine (5-mC), a chemical modification of DNA that is generally found on genes that are turned off, this extra layer of modification is enriched in active genes. 5-hmC appears to be more abundant in embryonic stem cells and brain cells, compared with other cell types, and its abundance increases substantially as the brain matures.

"The main reason why this DNA modification was not explored previously was because standard approaches didn't detect it. The groups that identified it had to isolate large amounts of DNA and analyze it directly," says co-senior author Peng Jin, PhD, professor of human genetics at Emory University School of Medicine. "I think the beauty of this work lies in how we combined an innovation in DNA chemistry with large-scale genetic analysis to achieve new insight."

In recent years, scientists have been examining the role of methylation, a modification of cytosine, one of the four bases that make up DNA (adenine, thymine, guanine are the others). When stem cells change into

the cells that make up skin, blood, muscle or brain, DNA methylation helps shut inappropriate genes off. Changes in DNA methylation also underpin a healthy cell's transformation into a cancer cell.

In 2009, a second layer of modification on top of 5-mC emerged, with the discovery that 5-hmC was present in mouse brain and especially abundant in Purkinje cells, which are part of the cerebellum. While previous researchers had reported the presence of 5-hmC in human and animal DNA samples, current methods did not allow them to distinguish between 5-mC and 5-hmC.

Seeking to fill this gap, a team at the University of Chicago led by Chuan He, PhD, professor of chemistry, exploited the properties of an enzyme from a bacterial phage, which can attach a chemically modified sugar tag to 5-hmC. They collaborated with Jin, postdoc Keith Szulwach and colleagues to map where 5-hmC appears in the genome and in various cell types.

While the chemical labeling method allows the separation of DNA containing 5-hmC from other DNA, it does not yet provide the ability to see 5-mhC when DNA is read letter-by-letter. He, Jin and their colleagues are working on a higher resolution method for finer analysis.

Mouse embryonic stem cell DNA contains 5-hmC at a level of 500 parts per million, the researchers found. In a mouse's cerebellum, the level rises from 1000 to 4000 parts per million as the mouse becomes an adult and that part of the brain matures. In contrast to 5-mC, which is generally found on genes that are turned off, 5-hmC is enriched on active genes, the researchers found.

"This specific gene enrichment suggests that it is not just an intermediate step when cells need to get rid of DNA methylation, but it may have a unique function in gene regulation," Jin says.

Mutations in the enzymes responsible for converting 5-mC to 5-hmC have also been linked to a form of leukemia, he notes.

The team looked to see which genes tend to acquire 5-hmC as the brain ages, and found an enrichment for genes involved with neurodegeneration, the cell's response to low oxygen and growth of new blood vessels.

"Because the enzymes that convert 5-mC into 5-hmC require oxygen, this may be another way that cells sense and respond to oxygen levels and oxidative stress," Jin says.

Provided by Emory University