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IGIB FINDS A PROTEIN WITH BETTER PRECISION IN GENE-EDITING

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Improving precision: The newly discovered protein can replace the currently used one which has a propensity to cause unnecessary alterations in the DNA. | Photo Credit: vchal

Researchers at the Delhi-based Institute of Genomics and Integrative Biology (CSIR-IGIB) have discovered a protein variant from a different species of bacteria that can edit the DNA with very high precision. In the tool now commonly used for editing disease-causing mutations in DNA (CRISPR-Cas9), the Cas9 protein behaves like a molecular scissors that cuts the DNA at a specific location and inserts a foreign piece of DNA to correct the mutation that causes the disease.

In addition to binding to the intended target on the DNA, the commonly used Cas9 protein from *Strepotococcus pyogenes* bacteria (SpCas9) and its engineered derivative tend to potentially bind to DNA at multiple unintended sites thereby leading to unnecessary alterations in the DNA.

The researchers found their new Cas9 protein, which binds and cuts the DNA, was able to correct sickle cell anaemia mutation in patient-derived stem cells. The protein (FnCas9) used by the researchers to edit the DNA is derived from a bacterium — *Francisella novicida.*

The Cas9 protein is supposed to bind to the DNA only when there is a perfect match between the DNA and the protein, thus reducing the chances of the protein binding at non-target sites on the DNA. But even when three mismatches exist between the protein and the DNA, the currently used SpCas9 protein binds and cleaves the DNA. In contrast, the team led by Debojyoti Chakraborty from IGIB found the new FnCas9 protein showed negligible binding when there exists more than one mismatch in the target DNA. The results were published in the journal *Proceedings of the National Academy of Sciences* (PNAS).

“The high specificity of the new FnCas9 protein arises due to reduced affinity to bind to DNA when there is even a single mismatch. And when there is more than one mismatch, complete absence of binding of the protein to the DNA is seen in many cases,” says Dr. Chakraborty.

“If the Cas9 protein remains bound to DNA at mismatched locations for a long time, there is a possibility that it might cut the DNA at these locations. Also, if it remains bound to DNA, the protein might block the transcription (which is the first step in gene expression) at that location. And if Cas9 is bound at multiple unintended sites then the transcription machinery gets stalled and the expression of genes at these locations might be altered,” Dr. Chakraborty explains.

In nature, DNA often gets damaged and is routinely repaired through one of the two pathways. In the case of the homology-directed repair (HDR) pathway, which is relatively less error-prone, matching sequences are used to repair the DNA. “The FnCas9 protein was found to increase the HDR repair rate fourfold compared to the widely used SpCas9,” says Deepanjan Paul from CSIR-IGIB and one of the first authors of the paper.

The researchers tested the precision of binding and cleavage at the desired sites on the DNA using mouse cell lines (embryonic stem cells and brain cells), human kidney cell lines and induced pluripotent stem cells (iPSc). In the case of human iPS cells, the FnCas9 protein was found to bind to the DNA at the specific site, cut and repair the sickle cell anaemia mutation.

“The correction process is the same for any disease-causing mutation and so our FnCas9 protein should theoretically correct any mutation in the DNA. The efficiency might vary, so we must test it for each disorder,” says Dr. Chakraborty.

The efficiency of any Cas9 protein delivery as well the ability to correct mutations is generally low in the case of iPS cells. The efficiency of correction is about 1.6%. Though the efficiency to correct mutations is low in iPS cells, the corrected cells can be isolated, multiplied and converted (differentiated) into haematopoietic stem cells. Once differentiated into haematopoietic stem cells, they can be transfused into patients.

“Differentiating iPS cells into haematopoietic stem cells is not trivial. Plenty of experimental work is under way to make it efficient for clinical translation,” says Dr. Chakraborty.

Recalling how he started working on FnCas9 protein for genome editing, Dr. Chakraborty recalls that he was looking for a Cas9 protein which can target RNA instead of DNA. There was one study that reported that FnCas9 could potentially target viral RNA. “We were not able to target RNA using FnCas9 proteins. So we started to investigate whether it can target DNA as well since it was not known if FnCas9 can be used for precise gene correction. We found that not only does it target the DNA but does so with very high specificity,” he says.

“We are now proceeding for preclinical studies to establish the efficacy of FnCas9 protein for genome-wide binding and targeting using patient-derived cells and mouse models,” he says.

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