Immediate Release

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Researchers at the Gene Editing Institute at Christiana Care Health System develop new system to perform precise 'surgery' on the human genome

Modified CRISPR/Cas 9 technique performs more EXACT gene editing

Wilmington, Del, Jan. 4, 2017 – Molecular biologists at Christiana Care Health System's Gene Editing Institute have developed a new system that allows them to not only repair damaged DNA within human cells, but also to determine when the DNA repair machinery has introduced unwanted genetic changes alongside, or instead of, the desired repair.

A team of researchers led by Eric Kmiec, Ph.D., director of the Gene Editing Institute at the Helen F. Graham Cancer & Research Institute at Christiana Care, published its findings using a modified version of the cutting-edge CRISPR/Cas9 gene editing technique in the Jan.3 issue of the scientific journal PLOS ONE.

The modified CRISPR/Cas9 technique, called Excision and Corrective Therapy, or EXACT, uses a short single-stranded piece of DNA called an oligonucleotide to serve as both a bandage and a template during the repair of a genetic mutation.

"The advancement here is a new concept of using donor DNA as an oligonucleotide to act as a Band-Aid across a gap created by the CRISPR [ribonucleoprotein complex], and then allowing replication to fill in the gap, and then the oligonucleotide dissociates and on you go," said Dr. Kmiec.

The published paper, titled "Insertional mutagenesis by CRISPR/Cas9 Ribonuclear gene editing in cells targeted for point mutation repair directed by short single-stranded DNA oligonucleotides," describes how the EXACT CRISPR/Cas9 technique can be used to repair what are called point mutations—single changes in the DNA code that can render genes non-functional and produce hereditary diseases in humans, such as sickle cell anemia or Gaucher's disease.

The present study follows an earlier report published in the September 9, 2016 issue of the journal Scientific Reports, in which Dr. Kmiec and his colleagues established that their EXACT CRISPR/Cas9 gene editing technique functions using the "Band-Aid template" repair mechanism that they had predicted.

In the PLOS ONE paper, Dr. Kmiec and his colleagues report using a single-stranded DNA template with a pre-assembled CRISPR/Cas9 ribonucleoprotein complex to fix a point mutation in human cells that have been engineered to express a fluorescent protein only if a single change in the DNA that encodes the fluorescent protein can be repaired.

The researchers report that their EXACT gene editing approach does in fact result in a significant amount of point mutation repair, thereby producing cells that make functional fluorescent protein. More importantly, Dr. Kmiec and his colleagues also characterize undesirable mutations that sometimes occur alongside of or instead of the desirable point mutation repair when using the EXACT CRISPR/Cas9 gene editing system. Dr. Kmiec and his co-authors refer to these undesirable side mutations, in which DNA is inappropriately inserted or deleted, as "collateral damage" or "on-site mutagenesis."

"If you lose DNA, even one or two bases, even if you fix the point-mutation the gene is disabled, because the gene can no longer code for the proper protein," said Dr. Kmiec. "So even though you have successfully corrected the gene, the problem is that you've also introduced some sort of secondary mutation at the site, and that causes the gene to be completely non-functional."

As reported by Dr. Kmiec and colleagues, on-site mutagenesis can occur even when repair of the point mutation has not taken place, meaning that CRISPR/Cas9 ribonucleoprotein complexes can produce additional genetic lesions called indels (short for insertions and deletions) at a target site without carrying out the function they were placed there to perform.

In their PLOS ONE paper, the researchers map out exactly where and how indels occur during on-site mutagenesis in greater detail than has been reported previously, examining exactly what happens to both copies of the DNA strand after the CRISPR/Cas9 ribonucleoprotein complex has done its work.

Overcoming the problem of on-site mutagenesis and the genetic scar tissue it leaves behind will be necessary if CRISPR/Cas9-mediated gene therapy is to become useful in the clinical setting. As Dr. Kmiec and his colleagues suggest, solving this problem will not be easy, as the DNA-repair machinery that cells use to perform point mutation repairs is inherently error prone.

Based on the greater mechanistic understanding provided by his recent studies, Dr. Kmiec says he remains optimistic that on-site mutagenesis is a problem that can be overcome.

"We are more optimistic now, seeing this data, that we will be able to fix point mutations efficiently, using this mechanism as opposed to other things that are now being reported in the literature," Dr. Kmiec said. "It's an advance that I think will give people hope that these kind of point mutations can be fixed if we use the proper tools to fix them."

In order to take his CRISPR/Cas9 system into the clinical setting, Dr. Kmiec says it will be necessary to further stabilize the repair complex at the site of the mutation, which should cut down on the occurrence of on-site mutagenesis.

Dr. Kmiec likens the repair process to the way in which a bandage can facilitate wound healing, noting that wounds "heal a lot faster if the bandage stays in place a lot longer. So the more times you wrap it with tape, or in this case, the more stable the binding, the more efficient the point mutation repair is going to be."

To be effective in the clinical setting, Dr. Kmiec and his colleagues also must figure out how to get the CRISPR/Cas9 machinery into the progenitor cells that give rise to mature, therapeutically relevant cells in the body. Dr. Kmiec says this is an active area of research for his laboratory.

Despite all of these challenges, Dr. Kmiec hopes that CRISPR/Cas9 gene therapy with EXACT could be in human clinical trials at Christiana Care within 18 to 24 months.

Dr. Kmiec says he feels confident that clinical trials will be forthcoming in large part because of the ease with which he can collaborate with his clinically oriented colleagues within the Christiana Care Health System.

"Christiana Care is such a great, fully-integrated hospital complex," he said. "I can walk down the hall and talk to the head of hematology here."

Notably, Christiana Care's Gene Editing Institute also recently entered into a partnership with The Wistar Institute in Philadelphia, with a goal of further accelerating research into repairing damage to the human genome.

Dr. Kmiec's colleagues who contributed to the PLOS ONE paper as authors included Pawel Bialk at the Gene Editing Institute, Natalia Rivera-Torres and Kelly Banas at the University of Delaware Department of Medical Laboratory Science, and Kevin Bloh at the Nemours Center for Childhood Cancer Research.

The Gene Editing Institute at the Graham Cancer Center is a worldwide leader in personalized genetic medicine. Founded and led by Dr. Kmiec, the Gene Editing Institute is unlocking the genetic mechanisms that drive cancer and that can lead to new therapies and pharmaceuticals to revolutionize cancer treatment. The Gene Editing Institute also provides instruction in the design and implementation of these precise new genetic tools.

About the Helen F. Graham Cancer Center & Research Institute at Christiana Care
The Helen F. Graham Cancer Center & Research Institute, a National Cancer Institute Community Oncology Research Program, is part of Christiana Care Health System. With more than 220,000 patient visits last year, the Graham Cancer Center is recognized as a national model for multidisciplinary cancer care and a top enroller in U.S. clinical research trials. Its Gene Editing Institute, Center for Translational Cancer Research, Tissue Procurement Center, statewide High-Risk Family Cancer Registry and collaborations with world-renowned scientists at facilities such as the University of Delaware and The Wistar Institute in Philadelphia are opening new avenues to more quickly translate cancer science into cancer medicine.

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