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Targeted RNA editing with the body's own enzyme activity

Completely new possibilities for research and gene therapy became available following the development of the CRISPR/Cas method for targeted modification of the genome. However, treatment with these molecular scissors is not without risk as potential errors are stored in the genome forever. Scientists from Tübingen have developed an alternative method in which the intervention takes place at the RNA level using the body's own enzymes and is thus reversible. A paper describing the application of CRISPR/Cas to repair a genetic defect in human body cells has just been published.

The scientists from Tübingen who co-authored the recent Nature Biotechnology paper on the results of the RESTORE procedure that used the body's own enzymes for the site-specific editing of RNA for the first ever time (from left to right: Paul Vogel, Thorsten Stafforst, Tobias Merkle)
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For a long time, the ability to "cut and paste" gene segments to specifically alter the genome and cure diseases, for example, was just a pipe dream. Since 2012, when CRISPR/Cas was first shown to be suitable for targeted genome editing, the pipe dream has increasingly entered the realms of possibility. The method can be used to edit the genome and thereby modify and switch genes on and off. However, bacterial gene scissors also entail risks: any errors will be stored permanently in the genome and can thus be passed on to next cell generations. In addition, the method is less suitable for some applications, for example for treating postmitotic cells such as neurons. Moreover, the genome cannot be edited arbitrarily: mutations that would be lethal in the long term or that can be rapidly genetically compensated cannot be effectively introduced into the genome.

That is why Prof. Dr. Thorsten Stafforst and his team of researchers at the University of Tübingen's Interfaculty Centre of Biochemistry (IFIB) have been spending a considerable amount of time working on low-risk alternatives for altering genetic information, i.e. methods that enable the targeted editing of RNA. "Modifications at the RNA level are reversible and therefore safer and can be more finely tuned than modifications at the DNA level," he explains. "Working at the RNA level allows us to insert partial or temporary mutations. This could become interesting, for example, for applications that interfere with cellular signal transduction."

Editing machinery involving guide RNA and correction enzyme

RNA editing involves modifying individual bases of the RNA transcript – i.e. the copy of the DNA. The instructions for building proteins are thus being rewritten while the original message remains in the DNA. In recent years, the IFIB scientists have developed an editing tool in the form of a protein construct which arrives at an RNA target molecule with the help of a complementary guide RNA (gRNA), docks and converts certain bases. The tool is called SNAP-ADAR and is a fusion protein consisting of the SNAP-tag protein and a deaminase called ADAR (adenosine deaminase acting on RNA). "Such enzymes are not found in nature. We use them as tools to try out interventions and use them for basic research tests," says Stafforst. "Our editing tool is a general platform and can be used to efficiently manipulate RNAs in a sequence-specific way."[1]

RNA editing: as a natural (top) and a site-directed process using artificial (bottom left) or endogenous enzymes (bottom right). The RESTORE procedure is particularly attractive for therapeutic applications.
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In addition, the researchers have recently come up with a second approach that has proved very promising for developing therapies and future medical applications. This approach is called RESTORE (recruiting endogenous ADAR to specific transcripts for oligonucleotide-mediated RNA-editing). Initial results of the application of RESTORE to human primary cells have only recently been published.[2] RESTORE works on the principle of recruiting natural ADAR activity in the cells, so that the enzymes in the cells rewrite their own RNA. "We do not need to add an extra enzyme," explains the scientist. "The natural enzymes may not be as good as the artificial ones but this is not a serious limitation. We add chemically stabilized gRNA; the

use of chemically stabilized gRNA is already geared to the possible future pharmaceutical application of gRNA as an antisense therapy. The trick is that we have provided the antisense part of the gRNA with an RNA structure that attracts the cellular ADARs. The sequence of the gRNA must be combined with chemical modifications so that the editing tool works. We have tested this with hepatocytes, but ADAR is expressed ubiquitously, and should therefore work with many cell types."

Recruitment of human cellular enzyme activity

Harnessing the body's own enzymes for the targeted manipulation of RNA has many benefits. For example, a very practical effect is that RESTORE is not a gene therapy, thus eliminating the hurdles of complex, expensive testing procedures. "Our approach has far fewer off-target effects than artificial editing machines; so it is much more precise," adds the professor. "We also have reason to hope that the method could be more efficient than CRISPR/Cas and other methods. There is still a lot of potential for improvement and we are working hard to make the method even more efficient."

A disadvantage of the Tübingen method, however, is that amino acids cannot be exchanged at will. ADARs are only able to convert one single particular base in their target RNA, i.e. adenosine with inosine, which is read as guanosine. "In fact, this means that we are unable to arbitrarily exchange amino acids," Stafforst admits. "This makes the application of the method to monogenic diseases difficult. However, we also see additional application potential in reversible transcriptome interventions that are inaccessible with the gene scissors."

Mutations in somatic cells have already been repaired successfully

In their recent publication in Nature Biotechnology[2], the researchers showed that RESTORE is in principle suitable for treating monogenic diseases. They successfully used natural ADARs to partially repair a mutation called PiZZ, which triggers alpha-1 antitrypsin deficiency, a metabolic disease that causes severe liver and lung damage.

"This is the very first description of the use of the body's own human enzyme system for the manipulation of RNA without overexpression, which was previously not possible but absolutely necessary for artificial editing enzymes." For the therapeutic approach, the gRNA was transfected into primary cells in culture. However, for future therapies it would also be conceivable to apply the gRNA systemically. One of the next steps is to clarify the question of how long the effect can last. "If we manage to keep the stabilized RNA in the cell for a prolonged period of time, the effect will last for a long time, perhaps weeks or months," says Stafforst. "But the question will also be whether this is desirable. Depending on which target you have in mind, you might want to achieve a short-term effect."

The IFIB researchers will continue to work on improving the technology and tools in the coming months and years. Patents have since been filed for the procedures. When asked about commercial exploitation, the group leader replies: "RESTORE is still at a very early stage, but there is clearly potential for commercial exploitation. We are therefore holding talks with potential companies and investors."

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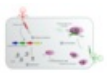
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- Precise RNA editing by recruiting endogenous ADARs with antisense oligonucleotides
- Efficient and precise editing of endogenous transcripts with SNAP-tagged ADARs

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CRISPR/Cas – genome editing is becoming increasingly popular

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