"CRISPR /Cas9-mediated Gene Editing in Human Tripronuclear Zygotes" (2015), by Junjiu Huang et al. [1]

By: Zhu, Meilin

In 2015, Junjiu Huang and his colleagues reported their attempt to enable CRISPR/cas 9-mediated gene editing in nonviable human zygotes for the first time at Sun Yat-Sen University in Guangzhou, China. Their article, "CRISPR /Cas9-mediated Gene Editing in Human Tripronuclear Zygotes," was published in Protein and Cell. Nonviable zygotes are sperm [2]-fertilized eggs that cannot develop into a fetus [3]. Researchers previously developed the CRISPR/cas 9 gene editing tool, which is a system that originated from bacteria as a defense mechanism against viruses. In their article, Huang and his team demonstrate that CRISPR/cas-9 gene editing can be used to correct a mutation in zygotes, or sperm [2]-fertilized egg [4] cells. However, they report that using CRISPR/cas 9 to edit those nonviable human zygotes led to off-target changes and, therefore, to unintended mutations in the human genome [6]. Before Huang and his colleagues' experiment, CRISPR/cas 9 had never been used on human zygotes. In their article, Huang and his colleagues demonstrated the need to improve CRISPR/cas 9 gene editing accuracy before using it for gene therapy to treat and correct genetic diseases in humans [6].

Huang and his colleagues worked at the Sun Yat-sen University in Guangzhou, China. With a background in cellular and molecular biology, Huang was a principle investigator in the university's department of reproductive medicine. Huang and his colleagues' past research involved studies on stem cells [7] and embryonic development.

In the article, the authors discuss the CRISPR technology that was used to edit the DNA of human nonviable zygotes. DNA is a double-stranded nucleic acid, which contains genetic information for cells. CRISPR for gene editing enables scientists to change the code and thereby genetic information in DNA. CRISPR gene editing technology works by using Cas 9 protein and a guide RNA. Cas 9 is a protein derived from bacteria that is able to cut double stranded DNA. RNA is a single-stranded nucleic acid that translates genetic information from DNA into directions for making proteins. The guide RNA is a molecule that scientists engineer to direct Cas 9 protein to cut a specific DNA sequence. The authors explain that they select RNA sequences that match the DNA sequences they want to modify. Then use those guide RNA molecules to direct a Cas 9 protein to change the DNA. The Cas 9 cuts double-stranded DNA, leaving a space for a new sequence of DNA to be inserted. With that, the authors show how CRISPR technology is used to edit DNA.

The authors begin the article with an introduction of the CRISPR/cas 9 gene editing mechanism. Huang and his colleagues also address some ethical concerns of using human zygotes in that section. In the results section, Huang and his colleagues delineate their results for editing nonviable zygotes. They also describe their analysis of off-target editing that led to unwanted mutations or changes in the zygotes' DNA. The authors also include a discussion section, where they summarize the results and evaluate the success of using CRISPR/cas 9 in nonviable human zygotes. Finally, in the last section, they describe their materials and methods for the experiments.

In the first section of their paper, the authors introduce CRISPR/cas 9 gene editing and summarize the development of CRISPR/cas 9 as a gene editing tool. Scientists before Huang and his colleagues engineered the CRISPR/cas 9 system derived from bacteria into a gene editing tool. CRISPR sequences are repeated DNA sequences in bacteria and archaea used to identify viruses for immunity. Cas proteins are CRISPR associated proteins. They state that from past research they knew that cas 9 protein could recognize and cut DNA. Next, they discuss that RNA molecules, single-strands of genetic code, help cas 9 proteins identify a specific portion of DNA, which the cas 9 proteins then cut. Then, Huang and his colleagues note how researchers Jennifer Doudna and Emmanuelle Charpentier had proposed using CRISPR/cas 9 as a tool for editing genomes in 2012. They also mention how researchers had demonstrated the use of guide RNAs with cas 9 to cut and repair double-stranded DNA in human cell lines. Huang and his colleagues refer to that method of using guide RNA and cas 9 to edit DNA as CRISPR/cas 9 gene editing.

Also in the introduction, Huang and his colleagues discuss the ethics of their experiment. They state that they obtained eighty-six tripronuclear human zygotes from an in vitro fertilization [8], or IVF, clinic in Guangzhou, China. Tripronuclear zygotes are egg [9] cells that are fertilized by two sperm [2] cells instead one sperm [2] cell. Because they are fertilized by two sperm [2] cells, tripronuclear zygotes cannot develop into a fetus [3]. Therefore, they are nonviable human zygotes. IVF clinics help women who have trouble getting pregnant by fertilizing eggs with sperm [2] in a test tube before inserting them into the women to help them get...
pregnant. The authors state that tripronuclear zygotes occur in those test tubes two to five percent of the time and are usually discarded by IVF clinics because they are nonviable. Huang and his colleagues reference studies to support those claims. They emphasize that tripronuclear human zygotes fail to develop into fetuses and are often discarded in IVF clinics. They also affirm that use of nonviable human zygotes serve as an ideal model system for studying the effects of CRISPR/cas 9 gene editing on human embryonic development.

Next, Huang and his colleagues assert their aim and methodology to investigate the efficiency and fidelity of CRISPR/cas 9 gene editing in normal human cell lines and nonviable human zygotes. They describe their intent to edit the human beta-globin, or HBB, gene found in all human cells. The HBB gene codes for a part of a protein called hemoglobin, which helps red blood cells carry oxygen. First, the authors explain how they designed three different possible guide RNAs to guide the cas 9 protein and correctly target the HBB gene. Then, they discuss testing two of the guide RNAs to determine which RNA molecule most accurately targeted the HBB gene.

To determine which guide RNA most accurately targeted the HBB gene, Huang and his colleagues explain how they tested the guide RNAs in a normal human cell line. A cell line is a culture of cells that all have the same genetic makeup. The researchers state that they injected two groups of cells with guide RNA molecules used for CRISPR/cas 9 gene editing. The first group of cells received guide RNA 1 and the second group of cells received guide RNA 2. While evaluating which guide RNA worked better, the authors were concerned about accuracy of editing, meaning that they wanted to ensure their guide RNA only directed the CRISPR/cas 9 system to edit the desired gene and not any other genes. The authors explain that they predicted seven possible off-target sequences. Modifying off-target sequences leads to off-target editing. That happens when the guide RNA directs cas 9 to edit the wrong gene. Off-target editing usually leads to unwanted DNA mutations.

In their article, the authors outline how they analyzed the genomes of the cells after the injection of the two different guide RNAs to determine which worked better. They looked for the seven off-target sequences that they predicted. Finding the off-target sequence would indicate inaccurate gene editing by the RNA-guided cas 9. They report that guide RNA 1 targeted the correct DNA sequence more accurately than RNA guide 2. From testing the guide RNAs in a normal human cell line, Huang and his colleagues conclude that using RNA guide 1 is better than using RNA guide 2 in the nonviable human zygotes.

After discussing which guide RNA was chosen, the research team discuss how they tested their selected guide RNA in the non-viable human zygotes. They injected the guide RNA, cas 9 protein RNA, Green Fluorescent Protein, or GFP, RNA, and a single stranded DNA template into the nonviable human zygotes. GFP RNA codes for a protein that will fluoresce, or glow, green, indicating that the cell successfully took up the RNA molecule and is using the RNA to make proteins that fluoresce. Huang and his team used the GFP protein to identify which zygotes successfully contained the guide RNA, cas 9 protein RNA, and GFP RNA. Using the fluorescence indicator from zygotes with GFP, they identified zygotes of which the CRISPR/cas 9 system successfully edited the genome. Fifty-four nonviable zygotes were identified with GFP fluorescence and collected for analysis.

Next, Huang and his team discuss their analysis of those fifty-four zygotes’ genomes to determine if the HBB gene was edited successfully. Huang and his colleagues determined which zygotes were correctly repaired in the HBB gene by cas 9 by examining the target HBB gene as well as any off-target gene editing and other possible mutations. Correct cleavage and repair of the HBB gene would indicate successful gene editing using CRISPR/cas 9. Off-target editing and mutations would indicate negative side effects of using CRISPR/cas 9 gene editing. They report that the CRISPR/cas 9 system successfully edited about fifty-two percent of the zygotes HBB gene. About fourteen percent of zygotes were correctly repaired and edited with the DNA template and about twenty-five percent of zygotes were incorrectly repaired and edited with another gene similar to HBB. Huang and his colleagues conclude that their results show the possibility of using CRISPR/cas 9 gene editing on nonviable human zygotes but also demonstrate the high probability of creating unwanted mutations in the cell.

After explaining their results, Huang and his colleagues conclude that many obstacles, such as off-target editing and mutations, still exist for using CRISPR/cas 9 gene editing in nonviable human zygotes. The authors recommend that to use CRISPR/cas 9 to edit human zygotes, researchers need to reduce the number of mutations that happen in DNA repair. However, the authors note that some of the mutations they noted in their zygotes arose due to the natural processes of DNA repair in cells and should be considered in human genome editing. In their article, Huang and his colleagues explain that CRISPR/cas 9 gene editing in zygotes was inefficient and created unwanted mutations in their genomes.

Huang and his colleagues reported the first ever attempt to utilize CRISPR/cas 9 gene editing on human zygotes. Many ethical concerns surrounded Huang and his colleagues’ experiments because scientists had never used CRISPR/cas 9 to edit human zygotes before. Zygotes are sperm and fertilized egg cells. Huang and his colleagues stated that to conduct ethical experimentation with fertilized egg cells, they chose to experiment with tripronuclear zygotes. However, when attempting to publish their study, Huang and his colleagues’ manuscript was rejected by top journals, such as Nature and Science, because of the ethical concerns surrounding their study. In the same year as Huang’s study, David Baltimore, a leader in ethical discussion
on biotechnology, warned scientists to not implement CRISPR/cas9 editing in human clinical trials before there were more thorough investigations of the biotechnology. Huang and his colleagues’ study underlined the complications in using CRISPR/cas9 for editing \textit{genes} in human zygotes, and the study prompted some researchers to use CRISPR/cas9 in \textit{viable} \textsuperscript{11} human embryos.

**Sources**


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**Subject**
Zhongshan da xue (Guangzhou, China) \textsuperscript{14} Doudna, Jennifer A. \textsuperscript{15} Charpentier, Emmanuelle \textsuperscript{16} Clustered Regularly Interspaced Short Palindromic Repeats \textsuperscript{17} CRISPR-Cas Systems \textsuperscript{18} Zygote \textsuperscript{19} Mutation \textsuperscript{20} Gene Editing \textsuperscript{21} DNA \textsuperscript{22} beta-Globins \textsuperscript{23} scFV green fluorescent protein, recombinant \textsuperscript{24} RNA, Guide \textsuperscript{25}

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