Scientists Seek Ban on Method of Editing the Human Genome

By NICHOLAS WADE  MARCH 19, 2015

Scientists Urge Temporary Moratorium On Human Genome Edits

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All Things Considered

FEATURED STORY

Engineering the Perfect Baby

Scientists are developing ways to edit the DNA of tomorrow's children. Should they stop before it's too late?

By Antonio Regalado on March 5, 2015
Outline

➢ Background

➢ Key Issues
  ▪ Ethics
  ▪ Safety
  ▪ Value
CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

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In this report, we used tripronuclear (3PN) zygotes to further investigate CRISPR/Cas9-mediated gene editing in human cells. We found that CRISPR/Cas9 could effectively cleave the endogenous β-globin gene (HBB). However, the efficiency of homologous recombination directed repair (HDR) of HBB was low and the edited embryos were mosaic. Off-target cleavage was also apparent in these 3PN zygotes as revealed by the T7E1 assay and whole-exome sequencing. Furthermore, the endogenous delta-globin gene (HBD), which is homologous to HBB, competed with exogenous donor oligos to act as the repair template, leading to untoward mutations. Our data also

*ostensible application: beta thalassemia

tri-nuclear zygote -- typically arise due to multiple sperm penetration; generally discarded in IVF

[Balakier, Human Reprod (1999)]
CRISPR*-Cas9 genome editing

A BRAVE NEW WORLD OF GENOME EDITING

How the Crispr system derived from bacteria works on human cells to correct genetic defects

~20 bases

1 An RNA “guide” molecule can be programmed to match any unique DNA sequence found in the human genome

2 A special enzyme, called CAS9, can be attached to the RNA guide. Its job is to find the target sequence of DNA

3 The DNA aligns with the target DNA sequence and the CAS9 attaches and cuts both strands of the DNA double helix

4 The DNA cuts can be amended with an extra DNA insertion (above), or a deletion of defective DNA

*‘clustered regularly interspaced short palindromic repeats’
Efficiency currently generally <100\%

CRISPR*-Cas9 genome editing

Various kinds of potential alterations in gene due to “repaired” DNA

NHEJ

HR

gene deleted

knockout

gene inserted

Knockin
McGovern Institute video

https://www.youtube.com/watch?v=2pp17E4E-O8
CRISPR originally a bacterial “immune response” against foreign DNA (e.g., viruses, other microbes)
CRISPR-Cas9 Historical Timeline

1987
First report of CRISPR clustered repeats
Ishino et al.

2002
Coined “CRISPR” name, defined signature Cas genes
Jansen et al.

2007
First experimental evidence for CRISPR adaptive immunity
Barrangou et al.

2009
Type III-B Cmr CRISPR complexes cleave RNA
Hale et al.

2011
tracrRNA forms a duplex structure with crRNA in association with Cas9
Deltcheva et al.
Type II CRISPR systems are modular and can be heterologously expressed in other organisms
Sapranuskas et al.

2013
First demonstration of Cas9 genome engineering in eukaryotic cells
Cong et al.
Mali et al.

2000
Recognition that CRISPR families are present throughout prokaryotes
Mojica et al.

2005
Identified foreign origin of spacers, proposed adaptive immunity function
Mojica et al.
Pourcel et al.
Identified PAM
Bolotin et al.

2008
CRISPR acts upon DNA targets
Marraffini et al.
Spacers are converted into mature crRNAs that act as small guide RNA
Brouns et al.

2010
Cas9 is guided by spacer sequences and cleaves target DNA via DSBs
Garneau et al.

2012
In vitro characterization of DNA targeting by Cas9
Jinek et al.
Gasiunas et al.

2014
Genome-wide functional screening with Cas9
Wang et al.
Shalem et al.
Crystal structure of apo-Cas9
Jinek et al.
Crystal structure of Cas9 in complex with guide RNA and target DNA
Nishimasu et al.

[Hsu, Cell (2014)]
CRISPR-Cas9 genome editing

Among myriad kinds of applications in basic science and in medicine, one is correction of genetic diseases

-- examples:
- Cystic fibrosis
- Muscular dystrophy
- Huntington’s disease
- Beta thalassemia
- Sickle cell anemia
- ...
Overall Context of Gene Therapy

Embryo-Based Gene Therapy

Embryonic stem cell approach

- Blastocysts
- ESCs
- Co-transfection
- Homology Directed Repair
- Genetic Analysis

Zygote approach

- Zygotes
- Microinjection
- Homology Directed Repair
- Preimplantation Genetic Diagnosis
- Genetically repaired embryos

- 1 or 2 blastocysts

Non-Invasive Parental Genetic Testing

- Chorionic Villus Sampling (or Amniocentesis)
- Embryo Transfer

[Araki, Reprod Biol Endocrinol (2014)]
Application to Disease Research – Animal Studies

Example: neurological pathologies, in non-human primates

1 Sperm injection
Lab technicians inject a single sperm into an unfertilized egg.

2 Genome editing
The fertilized egg is injected with “guide” RNAs that target a specific gene, and a template for the DNA-cutting enzyme.

3 Surrogate mother
Researchers transfer healthy-looking embryos, now dividing into many cells, into female monkeys. Typically, three embryos are transferred into a surrogate.

4 Primate babies
The twins Mingming and Lingling are born with multiple genetic changes, the first live primates created in experiments using CRISPR genome editing.

Cell Volume 156, Issue 4, p836–843, 13 February 2014

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu, Bin Shen, Yiqiang Cui, Yongchang Chen, Jianying Wang, Lei Wang, Yu Kang, Xiaoyang Zhao, Wei Si, Wei Li, Andy Peng Xiang, Jiankui Zhou, Xuejiang Guo, Ye Bi, Chenyang Si, Bian Hu, Guoying Dong, Hong Wang, Zuomin Zhou, Tianqing Li, Tao Tan, Xiuqiong Pu, Fang Wang, Shaohui Ji, Qi Zhou, Xingyu Huang, Weizhi Ji, Jiahao Sha
Application to Genetic Disease Correction: Animal Studies

Example: cataracts, in mice
Key Issues

- **Ethics I**
  - germ-line cells vs somatic cells
    - alterations enter human heredity

- **Safety**
  - unintended consequences
    - off-target effects
    - gene co-variation effects
    - general lack of predictive capability

- **Value**
  - actual medical benefit?

- **Ethics II**
  - “desirable” traits?
  - informed consent?
  - socio-economic equity?
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Germ-line vs Somatic Cell Gene Editing

Germ-line =
  • Egg
  • Sperm
  • Zygote
  • Embryo

Gene modification is inherited by off-spring

Somatic =
  • All other tissue/blood cell types

Gene modification is not inherited by off-spring
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Embryonic Blastocysts

What are sources?

Embryonic Stem Cell Sources

Embryodependent

In Vitro Fertilization

Oocyte Sperm

Zygote

Blastocyst

Embryo-independent

Somatic Cell Nuclear Transfer

Enucleated oocyte Somatic cell nucleus

Cloned zygote

Embryo-independent

Nuclear Reprogramming

Ectopic factors Somatic cell

Reprogrammed cell

Pluripotent stem cells

Blood Heart Brain Bone

Tissue-specific differentiation

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Off-Target Effects

- Issue of vigorous research, in quantitative analysis and in enhancement of selectivity
- Currently, significant probability of off-target gene mutations in cells for which the desired gene is affected

http://www.genoway.com/technologies/crispr-cas9-technology.htm
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Gene Co-Variation – Sickle Cell Anemia and Malaria

Sickle Cell hemoglobin gene mutation is found more frequently in areas where malaria is prevalent -- it has favorable selection advantage due to protection against malaria parasite survival within red blood cells

[Piel, Nature Comm (2014)]
General Lack of Predictability
-- dynamic gene network complexity
-- environmental context dependent

Estimated #Protein-Protein Interactions
Fly \(~70,000\)
Worm \(~200,000\)
Plants \(~300,000\)
Human \(~700,000\)

[Max Planck Inst Molec Genetics, Munich]
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Medical Benefit Beyond Current Capabilities?

Preimplantation Genetic Diagnosis (PGD)

While not whole genome sequencing, can examine for particular mutations of concern from parental genetics.

[http://www.californiaivf.com/genetic-diagnosis-PGD-CGH.htm]
DNA Sequencing of IVF Embryos

Researchers are testing whether high-throughput DNA sequencing can help screen out abnormal embryos during in vitro fertilization.

By Susan Young Rojahn on February 14, 2014

IVF embryos: whole genetic code can be scanned for mutations

Last updated: Thursday 12 February 2015 at 12am PST


✧ Can do parental genome sequencing in order to ascertain potential risks for which to examine particular embryo genes
✧ Potential for embryo genome sequencing when parental is not available, or for de novo mutations
Key Issues

- **Ethics I**
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- **Safety**
  - unintended consequences
    - off-target effects
    - gene co-variation effects
    - general lack of predictive capability

- **Value**
  - actual medical benefit?

- **Ethics II**
  - “desirable” traits?
  - informed consent? – individual and progeny
  - socio-economic equity?
Genetic Modifications of Babies

Percentage of U.S. adults saying that changing a baby’s genetic characteristics for each purpose is...

- [ ] Appropriate
- [x] Taking medical advances too far

Survey of U.S. adults August 15-25, 2014. Those saying “don’t know” are not shown.

Pew Research Center
Scientific Community “Pro-Active” Reaction

Biotechnology. A prudent path forward for genomic engineering and germline gene modification.

Baltimore D¹, Berg E², Botchan M³, Carroll D⁴, Charo RA⁵, Church G⁶, Corn JE⁷, Daley GQ⁸, Doudna JA⁹, Fenner M⁷, Greely HT¹⁰, Jinek M¹¹, Martin GS¹², Penhoet E¹³, Puck J¹⁴, Sternberg SH¹⁵, Weissman JS¹⁶, Yamamoto KR¹⁷.

1. Strongly discourage clinical application of this technology at this time.
2. Create forums for education and discussion
3. Encourage open research to evaluate the utility of CRISPR-Cas9 technology for both human and nonhuman model systems.
4. Hold an international meeting to consider these issues and possibly make policy recommendation.

“At present, the potential safety and efficacy issues arising from the use of this technology must be thoroughly investigated and understood before any at-tempts at human engineer-ing are sanctioned, if ever, for clinical testing.”
How did the meeting go? Were there some areas of disagreement?

Doudna: It actually went fairly smoothly. There was definitely very animated discussion. This is a topic that people can feel emotion about. It is pretty profound if you talk about clinical applications that could change human evolution. There were different points of view, but not hugely different. I didn’t hear anybody at either extreme saying things like “We should edit people tomorrow!” or “We have to get rid of this technology.” It was more focused on questions such as “What kind of safety or regulatory matters should be discussed?” It was only a one-day meeting so there wasn’t a lot of time to get into other issues such as gene editing triggering a biological chain reaction where a dominant change could spread through a whole population.
Can you imagine a future point at which you’d support the use of gene editing in humans in a heritable manner? If so, how do we get to that point from where things are today? What do we need to learn first?

Doudna: We need to learn how efficiently it works. What’s the best way to deliver it safely and efficiently? Not only efficiency, but also what are the off-target levels? How do we minimize that? What would be a safe level if any of off-targets? I’d like to see basic research like what happens to the DNA in germ cells or pre-germ cells when a double-stranded break occurs? What is the repair process like in those specific cells? Those answers would be interesting from a basic science perspective as well as informing future potential clinical applications.

I feel uncomfortable imagining widespread gene editing use in humans now, but it is possible that there are going to be certain types of very specific applications that could be envisioned as beneficial in the future. I won’t be able to make a decision of the wisdom of such an approach until we have more data. What are the real risks? There is always a risk-versus-reward kind of consideration. In which cases is the risk worth the payoff?
What do we do if someone goes rogue?

Doudna: That’s one of the purposes of these meetings: to get out in front of that. I can’t guarantee that that might not happen. I can work to form a coalition to say, “here’s our considered view of the technology and here’s what we see as a prudent way to move forward with this”. That’s really the best that we can do. There’s no way to unlearn what is learned. We can’t put this technology to bed. If a person has basic knowledge of molecular biology they can do it. It’s not realistic to think we can block it. Same thing with regulations. To imagine that we could have international regulations, it’s just not realistic, and in any case, how do you enforce them? I wouldn’t feel comfortable hiding away in the lab. The better path is to try to be open and transparent and to educate people who want to understand it. It’s such a wonderful technology in many ways. Like any technology it has the potential to be used for good and not so good. We want to put out there the information that people would need to make an informed decision, to encourage appropriate research and discourage forging ahead with clinical applications that could be dangerous or raise ethical issues.
International Status of Human Germ-line Editing

[Araki, Reprod Biol Endocrinol (2014)]
Status of Human Germ-line Editing in USA

NIH reiterates ban on editing human embryo DNA
Agency issues statement after researchers alter gene in non-viable zygotes.

Sara Reardon
29 April 2015 | Corrected: 29 April 2015

- “NIH will not fund any use of gene-editing technologies in human embryos.”
- “The concept of altering the human germ-line in embryos for clinical purposes has been debated over many years… and has been viewed almost universally as a line that should not be crossed.”
- “…strong arguments against engaging in this activity remain. These include
  ✦ Unquantifiable safety issues
  ✦ Ethical issues presented by altering germ-line in a way that affects the next generation without their consent
  ✦ A current lack of medical applications justifying the use… in embryos.”

[http://www.nih.gov/about/director/04292015_statement_gene_editing_technologies.htm]
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- “Practically, there are multiple existing legislative and regulatory prohibitions against this kind of work.”
- “Dickey-Wicker amendment prohibits use of appropriate funds for creation of human embryos for research purposes or for research in which human embryos are destroyed.”
- “NIH guidelines state that the Recombinant DNA Advisory Committee will not at present entertain proposals for germ-line alteration.”
- FDA has authority to regulate cell and gene therapy products… which would include human germ-line modification.”

[http://www.nih.gov/about/director/04292015_statement_gene_editing_technologies.htm]