Genome Editing in human embryos

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Gene Therapy Approaches

**Germline Gene Therapy**
- Efficiency: >99%
- Off targets: None

**Somatic Gene Therapy**
- Efficiency: 1% - 100%
- Off targets: No oncogenic mutation
- Immunoresponse
- Deliver

Ma et al. Nature, 2018
Mosaicism in CRISPR-generated mouse, monkey and human embryos

Mouse

Yen, ST. et al. 2014

Monkeys

Liu, HL. et al. 2014

Fogarty, NM. Et al. 2017

Human

OCT4 expression in CRISPR targeted embryos
Onset of zygotic POU5F1 expression

Mosaic blastocysts

Mouse Monkey Human

Yen, ST. et al. 2014

Liu, HL. et al. 2014

Fogarty, NM. Et al. 2017
Generation of complete knockout mice by C-CRISPR

Zuo et al, *Cell research*, 2017

- **Multiple DSBs**
- Chromosome rearrangement
- Chromosome deletion
- Off-target indels

- **Mosaic genotype**
- **Embryonic development**
Unexpected CRISPR on-target effects

Hyunji Lee & Jin-Soo Kim

Cas9 can induce extensive on-target damage, including large deletions, inversions, and insertions.

CRISPR-Cas9 genome editing induces megabase-scale chromosomal truncations
Base editing

DSB -
Target DNA
G
U
Mismatch repair
A
Single base editing

Guide RNA
Cytosine deaminase
CBE
C to T or G to A conversions

Template -
Deaminase

Cytidine deaminase

Cas9 nickase
gRNA

C to T or G to A conversions

Adenine deaminase

T to C or A to G conversions

ABE
Simultaneous inactivation of multiple genes by base editing

Zhang, H. et al., Yang, H# & Zhiyong Liu#, Development, 2018
High mosaicism in base-editing human embryos

Cooperated with Zi-Jiang Chen
Injection of base editors at different embryonic stages

Changyang Zhou

Zhang et al, *Genome Biology*, 2019
Improved base-editing efficiency in human cleaving embryos compared with MII oocytes and zygotes

Zhang et al, *Genome Biology*, 2019
Reveal OCT4 function by base editors

Unpublished data
Correction of a pathogenic heterozygous mutation in human embryos with base editors

Zhang et al., *Genome Biology*, 2019
Low off-target effects by base editing in human embryos

![Diagram showing the process of base editing in human embryos](image)

**Whole genome amplification**

**Random off-targets**

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**Table:**

<table>
<thead>
<tr>
<th>Sample name</th>
<th>SNPs</th>
<th>Indels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variants supported by three algorithms</td>
<td>15345</td>
<td>442</td>
</tr>
<tr>
<td>Filter repeats and microsatellites exons</td>
<td>6966</td>
<td>192</td>
</tr>
<tr>
<td>Protein coding</td>
<td>242</td>
<td>3</td>
</tr>
<tr>
<td>Variants in both samples</td>
<td>168</td>
<td>2</td>
</tr>
<tr>
<td>Predicted offtarget sites (10611)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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Zhang et al, *Genome Biology*, 2019
GOTI (Genome-wide Off-target analysis by Two-cell embryo Injection)

- The same genetic background
- Without genome amplification
- Genome wide and no bias
- Single-cell resolution
- Detection of various mutations

GOTI: 14 SNVs

Traditional methods: 3706 SNVs

A

\begin{itemize}
  \item loxP
  \item CAG
  \item STOP
  \item tdTomato
  \item Ai9
  \item Zygote (Ai9 x C57)
  \item 2-cell
  \item E14.5
  \item Cas9/BE3+Cre
  \item sgRNA
  \item FACS
  \item tdTomato
  \item WGS
  \item Variant calling
  \item SNVs
  \item XX
  \item Indels
\end{itemize}
Cytosine base editor generates substantial off-target single nucleotide variants

Zuo E et al., Science, 2019

CBEs induced SNVs at more than 20-fold higher frequencies

Zuo E et al., Science, 2019
BE3 off-target SNVs were sgRNA-independent

Zuo E et al., Science, 2019
RNA off-targets?

High fidelity base editors?
DNA base editors generate substantial RNA off-targets

- BE3 and ABE induce RNA SNVs
- RNA SNVs are caused by APOBEC1 and TadA

Eliminate RNA off-targets by mutagenesis

Destabilizing the RNA binding capacity of APOBEC1 and TadA

BEs with reduced off-target RNA SNVs while maintaining DNA on-target

Zhou et al., *Nature*, 2019
CBEs with no DNA and RNA off targets

DNA off targets

RNA off targets

Embryonic development

Unpublished data
Off-target analysis in human embryos

- Combined methods
  - sgRNA-dependent: Cycle-Seq, Di-Genome, Chip-Seq ...
  - sgRNA-independent: GOTI, RNA-seq ...

- High fidelity Cas9
- High fidelity fused protein

- New methods
  - Single-cell whole genome sequence
Improve editing efficiency in human embryos

- CRISPR-mediated HDR
  - Recombination factor: RAD51 ...
  - Cell cycle
  - Donor type: ssDNA, dsDNA ...
  - Unwanted alleles

Yao, X. et al. Yang H.#, Dev Cell, 2018
Improve editing efficiency in human embryos

- **Base editing**
  - Different type of conversions: A to C or A to T
  - +1 or -1 editing
  - Efficacy: Different version of deaminases

- **Prime Editing**
  - Different type of conversions
  - No DSB and no template
  - Off targets: reverse transcriptase
  - Efficacy and indels
Non-human primates for human germline gene editing

- Embryonic development
  - Tolerance for gene editing

<table>
<thead>
<tr>
<th>Gene</th>
<th>Injected embryos</th>
<th>Transferred embryos</th>
<th>Recipients</th>
<th>Pregnancy</th>
<th>Fetus</th>
<th>Live-birth</th>
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<tbody>
<tr>
<td>Leptin</td>
<td>163</td>
<td>88</td>
<td>47</td>
<td>6</td>
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<td>2</td>
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</tbody>
</table>

- Off-target evaluation
  - Sequence similarity
Somatic Gene Therapy Now, Germline Gene Therapy in Future?

30,000-50,000 patients in China

New born: 1/5000 – 1/10000
New add per year: 1500 – 2500, (SMA1, 555-925)
Total: 30,000-50,000, (SMA1, 1110-1850)

Treatment for SMA

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type</th>
<th>Targeting</th>
<th>Administration</th>
<th>Phase</th>
<th>Price</th>
<th>Sponsor</th>
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<tbody>
<tr>
<td>Spinraza</td>
<td>ASO</td>
<td>SMN2 mRNA splicing</td>
<td>intrathecal</td>
<td>FDA approved</td>
<td>$125,000-per-vial</td>
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<td>SMN1 replace</td>
<td>intravenous</td>
<td>FDA approved</td>
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<td>Novartis Pharmaceuticals</td>
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<td>oral</td>
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<td>Hoffmann-La Roche</td>
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Acknowledgement

Xuan Yao  Xing Wang  Erwei Zuo  Yidi Sun  Changyang Zhou

ION
Muming Poo
Linyu Shi
Qiang Sun
Zhiqi Xiong
Zhiyong Liu

ShanghaiTech
Pengyu Huang

Shangdong Hospital
Zi-jiang Chen
Meiling Zhang

CAS-MPG
Yixue Li
Yidi Sun

All lab members

Funding: CAS Strategic Priority Research Program, 863, NSFC, China Youth Thousand Talents Program, et al

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