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COMMENTARY EDITORIAL

Editing the human genome: where ART and science intersect

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Abstract

The rapid development of gene-editing technologies has led to an exponential rise in both basic and translational research initiatives studying molecular processes and investigating possible clinical applications. Early experiments using genome editing to study human embryo development have contradicted findings in studies on model organisms. Additionally, a series of four experiments over the past 2 years set out to investigate the possibilities of introducing genetic modifications to human embryos, each with varying levels of success. Here, we discuss the key findings of these studies, including the efficiency, the safety, the potential untoward effects, major flaws of the studies, and emerging alternative genome editing methods that may allow overcoming the hurdles encountered so far. Given these results, we also raise several questions about the clinical utilization of germline gene editing: For which indications is gene editing appropriate? How do gene-editing technologies compare with genetic testing methods currently used for screening embryos? What are the ethical considerations we should be concerned about? While further research is underway, and our understanding of how to implement this technology continues to evolve, it is critical to contemplate if and how it should be translated from the bench to clinical practice.

Keywords CRISPR · Genetic screening · PGD · Gene editing · ART

The rapid developments of gene editing techniques have led to an explosion of basic and translational research. Among several gene editing techniques, CRISPR (clustered regularly interspersed short palindromic repeats), when coupled with the nuclease Cas9, has forged ahead as the easiest, most cost-effective, and reproducible technique. In the CRISPR/Cas9 system, guide RNA (gRNA) directs the Cas9 nuclease to the desired genomic region where the genetic change is to be introduced and Cas9 creates a double-stranded break. A donor segment of DNA acts as the template for the genetic edit when the double-stranded break is repaired via homology-directed repair. This technology has reduced the barriers to introducing genetic changes directly in the tissue or organism of choice, speeding up our ability to study molecular processes in a number of model systems, including human cell lines. Additionally, the efficiency of CRISPR has inspired scientists to initiate clinical trials aimed at combating genetic diseases, cancer, and infectious disease [1]. It makes sense that the next logical leap, though more ethically fraught, would be *preconception* editing in the embryo, or even in gametes.

Gene editing techniques play an increasingly important role is deciphering the genetic mechanisms underlying normal and abnormal embryo development. These studies seek to explain developmental arrest of human embryos as well as failed fertilization of mature oocytes. The majority of our knowledge of embryo development is based on gene-knockout studies in model organisms, especially mice. However, while this information is invaluable, findings in other species do not guarantee that the same is true for humans [2–4]. Previous methods of generating knockouts in model organisms have not been feasible for study in human embryos. With CRISPR, however, it is possible to introduce genetic changes without having to alter the genome in the ancestors to introduce viruses; the genetic change can be introduced in gametes or in embryos.

In 2016, the HFEA in the UK allowed a group at the Francis Crick Institute to perform gene editing in human embryos with the purpose of identifying genes for embryo

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development [5]. This research was based on information from model systems, as well as gene expression studies [3, 6, 7]. The first findings from this group were published recently [8]. Using the CRIPR technique, the researchers not only demonstrated that *OCT4* was critical for human embryo development, but also that it was obligatory for the very early stage of embryo development, earlier that in the mouse. This breakthrough study is first in what is expected to follow from this and other groups, highlighting the importance of gene editing in searching for genes controlling embryogenesis. In the foreseen future, we might be getting important explanation why seemingly healthy couples experience repeat failure of embryo development.

Outside of these novel studies aimed to understand human embryo development, several recent experiments pioneered CRISPR editing of human embryos with the goal of determining if this technology could be utilized in clinical applications to eliminate pathogenic mutations in affected embryos (Table 1) [9–12]. Two critical challenges of applying the CRISPR technique to embryos are "Repairing" every single cell (preventing "mosaicism") and avoiding gene editing at other, homologous sites in the genome ("off target mutations").

These studies aimed to address four questions:

First, how reliable is CRISPR? All studies succeeded in removing the abnormal mutation and replacing it with a normal one. Since previous experiments on human genome editing (e.g., stem cells) have been successful, this was expected, yet reassuring.

Second, what is the risk of mosaicism? Mosaicism was evident in most experiments when zygotes (either normal bi-pronuclear - 2PN - or abnormal tri-pronuclear - 3PN - embryos) were "CRISPRed." Mosaicism was only absent when CRISPR was injected into a human oocyte along with the sperm that fertilized it.

Third, does CRISPR result in off target mutations? CRISPR led to unintended, "off target" mutations in most, but not all pronuclear experiments, with no such cases when CRISPR was injected into a mature oocyte at the time of fertilization.

Fourth, at what embryonic stage is CRISPR most successful? Experiments utilizing embryos—2PN or 3PN—resulted in either mosaicism or off-target effects, or both. The only stage where CRISPR did not result in either mosaicism or off-target mutations was when CRISPR was injected into a mature oocyte at the time of fertilization.

Taken together, these CRISPR studies illuminate the difficulties in safely editing the human genome in embryos.

In fact, the only experiment, thus far, not found to have deleterious effects was not in pre-embryos but in an oocyte where CRISPR is inserted at the same time as the sperm in a case where the mutation was inherited from the sperm. Clearly, this assertion awaits further scientific confirmation, in addition to investigating other cases in which the oocyte or both oocyte and sperm harbor a mutation. Very recently, the findings from Ma et al. (2017) have come under close scrutiny, with skepticism to the claim that injection of gene-editing machinery into the oocyte that the same time of the sperm eliminates mosaicism and off-target affects. A group of scientists have submitted a preprint of a rebuttal to the findings, which has been submitted to *Nature* for review and publication [13, 14]. The main argument of the rebuttal presented in the preprint surrounds the results from the study reporting that at least a portion of the blastomeres of the CRISPR'ed embryo were corrected using the wild-type maternal allele. The scientists contend that this is not possible given the time and space constraints of normal fertilization and development processes: the maternal and paternal nuclei remain at opposite poles of the egg for a period of time before fusing. As a result, the interpretation that the maternal allele could be used as a template for repair instead of the guide RNA is difficult to fathom. A more logical reasoning, they argue, is that CRISPR introduced a double-stranded break on the

Table 1 Summary of gene editing experiments on human embryos

Study	Embryo type/stage	Gene	Mosaicism detected?	Off-target effects detected?
Liang et al., [9]	Zygote 3PN	*HBB*	Yes	Yes
Kang et al., [10]	Zygote 3PN	*CCR5*	Yes	No (at studied sites)
Tang et al., [11]	Zygote 3PN	*HBB* and *G6PD*	Yes	Not determined
	Zygote 2PN	*HBB* and *G6PD*	Yes	No
Ma et al., [12]	Zygote 2PN	*MYBPC3*	Yes	Not determined
	Gamete 2PN	*MYBPC3*	No	No

paternal allele, causing a deletion, rather than homology-directed repair, resulting in blastomeres that appear wild-type for both alleles, but are actually haploid with the maternal allele at that location. Further studies are warranted to determine whether or not this critique is a more accurate interpretation of the findings.

The hypothesis of the above studies has been that CRISPR can safely correct abnormal gene mutations in the human embryo. So far this null hypothesis has been disproven. The only stage where CRISPR did not falter, based on the interpretation of the authors, was at the time of fertilization. It follows that current research does not support the clinical application of repairing affected embryos. At best, CRISPR may prevent the creation of an affected embryo by altering the genome at the same time the oocyte is fertilized. If we were to use it clinically, it would be in cases where there would be certainty that without CRISPR, the resulting embryo would be affected by the mutated gene. For embryos already affected, current CRISPR technology is too late!

Possible indications for CRISPR of oocytes are rare. One such case would be the extremely rare cases of homozygosity for dominant disorders (which in most cases is lethal), or when both partners are homozygous to a recessive mutation. Either scenario would result in 100% of embryos affected and CRISPR may be the only option to have an unaffected genetic offspring.

It may be argued that, if proven safe, CRISPR could be used on an oocyte at the time of fertilization even in cases where the oocyte or sperm have only a 50% chance to carry a mutated gene.

From a clinical perspective, we should seek to first diagnose the mutation in an embryo prior to treating it with CRISPR. Current IVF technology enables such a diagnosis only at a more advanced stage (ideally, a Day 5 embryo—blastocyst). However, no studies have attempted gene editing beyond the first day of fertilization.

At present, it is difficult to see the clinical advantage of CRISPR over Preimplantation Genetic Testing (PGT). PGD can recognize any known sequence of a pathogenic mutation in the embryo. PGD has allowed us to prevent the transfer of embryos affected with recessive, dominant, and sex-linked disorders, as well as chromosomal aberrations.

Will CRISPR ever become an *alternative* to PGT? Theoretically, it may seem more logical, or ethical, to "fix" the abnormal gene rather than allowing for an affected embryo to be generated only to be discarded. But will this "preemptive" CRISPR ever be as practical and safe as PGT? Replacement of PGT by CRISPR does not seem a viable option at present.

Table 1 demonstrates the progression of CRISPR studies in human embryos. This rapid succession of experiments has been associated with curtailment of untoward effects and increased safety. Even if future experiments find a way to safely CRISPR human embryos, in all likelihood CRISPR will still play, in the foreseeable future, only a minor role in improving the genome of the pre-embryo.

One of the issues with current CRISPR technologies is the inherent inefficiency at repairing double-stranded breaks using homology-directed repair in human cells. This has resulted in mosaicism and failure to insert the desired genetic change. Recently, CRISPR/Cas9 machinery has been modified to induce a genetic change *without* introducing a double-stranded break, called "base editing." This method utilizes cytidine deaminases and guide RNA to convert cytidine to uridine, resulting in a change to thymidine [15]. This technology has been used to efficiently introduce genetic changes into a variety of species, including plant, yeast, sea urchin, mouse zygotes, and human cells and tripronuclear zygotes [15–26]. While this technology still needs to be refined as mosaicism is still observed, it provides a new method that may introduce genetic changes more efficiently without having to damage DNA.

Ethical issues have been raised regarding the abuse of CRISPR technology. Once again, the familiar "slippery slope" and "designer baby" concerns have resurfaced.

Once the technique is improved to a level where the risks are reduced to an acceptable minimum, embryos may be readily subjected to genetic engineering. Issues regarding this emerging technology including—who will set up the guidelines and how strictly will they be enforced—will all have to be resolved. However, these dilemmas are not that different from those already encountered with PGT. For example, should mutations for treatable conditions (e.g., Gaucher's Disease) be edited? Should embryos be engineered if they carry mutations of late onset disease (e.g., Huntington's disease)? And what about mutations that increase cancer risk (e.g., BRCA1), but do not cause cancer in all cases? The concern that CRISPR may be abused to create designer babies ("positive eugenics") seems farfetched at this point. "Desired traits" such as intelligence, physical appearance, and success in life are complex and typically multifactorial—genetic as well as environmental.

As is not uncommon, the ethics of embryo editing lags behind scientific progress. The technology is bound to move forward and hopefully find solutions for the roadblocks encountered so far. The medical field will be responsible to take the appropriate steps to establish strict guidelines and, as much as possible, make sure that the editorial scissors of the human genome is placed in morally responsible hands.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Cornu TI, Mussolino C, Cathomen T. Refining strategies to translate genome editing to the clinic. Nat Med. 2017;23:415–23.
2. Chen AE, Egli D, Niakan K, Deng J, Akutsu H, Yamaki M, et al. Optimal timing of inner cell mass isolation increases the efficiency of human embryonic stem cell derivation and allows generation of sibling cell lines. Cell Stem Cell. 2009;4:103–6.
3. Niakan KK, Eggan K. Analysis of human embryos from zygote to blastocyst reveals distinct gene expression patterns relative to the mouse. Dev Biol. 2013;375:54–64.
4. Blakeley P, Fogarty NME, del Valle I, Wamaitha SE, Hu TX, Elder K, et al. Defining the three cell lineages of the human blastocyst by single-cell RNA-seq. Development. 2015;142:3613–3.
5. Callaway E. UK scientists gain licence to edit genes in human embryos. Nature. 2016;530:18–8.
6. Frum T, Halbisen MA, Wang C, Amiri H, Robson P, Ralston A. Oct4 cell-autonomously promotes primitive endoderm development in the mouse blastocyst. Dev Cell. 2013;25:610–22.
7. Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell. 1998;95:379–91.
8. Fogarty NME, McCarthy A, Snijders KE, Powell BE, Kubikova N, Blakeley P, et al. Genome editing reveals a role for OCT4 in human embryogenesis. Nature. 2017;550:67–73.
9. Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 2015;6:363–72.
10. Kang X, He W, Huang Y, Yu Q, Chen Y, Gao X, et al. Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. J Assist Reprod Genet Springer US. 2016;33:581–8.
11. Tang L, Zeng Y, Du H, Gong M, Peng J, Zhang B, et al. CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein. Mol Gen Genomics. 2017;292:525–33.
12. Ma H, Marti-Gutierrez N, Park S-W, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. Nature. 2017;548:413–9.
13. Callaway E. Doubts raised about CRISPR gene-editing study in human embryos. Nature. 2017;
14. Egli D, Zuccaro M, Kosicki M, Church G, Bradley A, Jasin M. Inter-homologue repair in fertilized human eggs? Cold Spring Harbor Laboratory; 2017;181255.
15. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533:420–4.
16. Chen Y, Wang Z, Ni H, Xu Y, Chen Q, Jiang L. CRISPR/Cas9-mediated base-editing system efficiently generates gain-of-function mutations in Arabidopsis. Sci China Life Sci. 2017;60:520–3.
17. Kim K, Ryu S-M, Kim S-T, Baek G, Kim D, Lim K, et al. Highly efficient RNA-guided base editing in mouse embryos. Nat Biotechnol. 2017;35:435–7.
18. Li J, Sun Y, Du J, Zhao Y, Xia L. Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. Mol Plant. 2017;10:526–9.
19. Li G, Liu Y, Zeng Y, Li J, Wang L, Yang G, et al. Highly efficient and precise base editing in discarded human tripronuclear embryos. Protein cell. Higher Education Press; 2017;1–4.
20. Liang P, Sun H, Sun Y, Zhang X, Xie X, Zhang J, et al. Effective gene editing by high-fidelity base editor 2 in mouse zygotes. Protein Cell. 2017;8:601–11.
21. Lu Y, Zhu J-K. Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. Mol Plant. 2017;10:523–5.
22. Ren B, Yan F, Kuang Y, Li N, Zhang D, Lin H, et al. A CRISPR/Cas9 toolkit for efficient targeted base editing to induce genetic variations in rice. Sci China Life Sci. 2017;60:516–9.
23. Zhou C, Zhang M, Wei Y, Sun Y, Sun Y, Pan H, et al. Highly efficient base editing in human tripronuclear zygotes. Protein cell. Higher Education Press; 2017;1–4.
24. Zong Y, Wang Y, Li C, Zhang R, Chen K, Ran Y, et al. Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nat Biotechnol. 2017;35:438–40.
25. Liang P, Ding C, Sun H, Xie X, Xu Y, Zhang X, et al. Correction of β-thalassemia mutant by base editor in human embryos. Protein cell. Higher Education Press; 2017;1–12.
26. Shevidi S, Uchida A, Schudrowitz N, Wessel GM, Yajima M. Single nucleotide editing without DNA cleavage using CRISPR/Cas9-deaminase in the sea urchin embryo. Dev Dyn. 2017;246:1036–46.