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SPOTLIGHT

Gene editing in human development: ethical concerns and practical applications

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ABSTRACT

The amazing power of CRISPR-Cas9 gene editing tools and other related technologies has impacted all areas of biology today. It has also raised ethical concerns, particularly with regard to the possibility of generating heritable changes in the human genome – so-called germline gene editing. Although technical and safety issues suggest that this approach is far from clinical application, gene editing as a research tool is moving forward in human embryos, non-human primates and in stem cell-derived embryoids. These studies are already providing new information relevant to our understanding of normal human development, infertility, early pregnancy loss and pluripotent stem cell origins.

Introduction

There can hardly be a developmental biologist in the world today who has not had their research impacted by the rapid advances in the technology of CRISPR/Cas9 gene editing. The precision and efficiency of this form of gene editing has revolutionized the generation of defined genetic and epigenetic alterations in cells in culture and in organisms from microbes to plants to animals. Applications underway include improving agricultural crops and livestock, developing new antimicrobials, and attempts to control disease-carrying insects with so-called gene drives (Barrangou and Doudna, 2016). All of these come with their own regulatory and ethical issues that require informed debate. However, worldwide attention has been particularly focused on the issues surrounding clinical application of gene editing to humans.

There are three different ways in which CRISPR/Cas9 gene editing can be applied to human health. First, as a basic research tool for use in human cells or embryos to help understand normal development, model human disease and develop new treatments. Second, for gene editing in somatic cells, either *ex vivo* or *in vivo*, to treat or prevent disease. Third, for gene editing in gametes or embryos with the aim of correcting disease-causing mutations in the next generation – so-called germline gene editing. Ever since the first transgenic mice were made by injection of exogenous DNA into mouse zygotes, there have been discussions around the ethics of germline modification in humans. However, these were rather theoretical and philosophical in nature, given that the science was far from ready to even consider practical application to human embryos. Targeted modification via homologous recombination in embryonic stem cells and generation of germline chimeras made precision editing of the mouse germline possible, but this was not in any way considered a route to human germline editing. All that changed with CRISPR/Cas9. Direct injection of the Cas9 components into mouse zygotes can produce mutations by non-homologous end-joining at close to 100% efficiency, and more precise alterations, such as point mutations and insertions, can be produced at increasingly higher rates, with various new tricks (Plaza Reyes and Lanner, 2017). Suddenly, theoretical discussions on human germline editing are no longer so theoretical.

The first report of gene editing directly in human embryos was published in March 2015. Although the study was carried out in non-viable tripronuclear embryos and demonstrated mosaicism and off-target mutations (Liang et al., 2015), it raised community awareness and sparked a series of responses from organizations and groups worldwide, including calls for a moratorium on all human embryo gene editing. Following an international summit meeting on human gene editing held in Washington in December 2015, the National Academy of Sciences (NAS) and National Academy of Medicine (NAM) established an international working group to consider the scientific, ethical and societal issues raised by human genome editing. The working group reviewed the current and likely future status of the field, the current legal situation internationally and the broad range of ethical debate on the status of the human genome, and received input from multiple sources, including clinicians, ethicists, regulators, patient advocates, industry representatives and the public. The committee's peer-reviewed report was published in February 2017 (National Academies of Sciences, Engineering, and Medicine et al., 2017) and has been widely recognized as a carefully crafted set of guiding principles that can be applied across jurisdictions for the future applications of human gene editing.

The major recommendations of the committee (Fig. 1) have been largely endorsed by various other reports published in the last year (see http://arrige.org/documents.php). Most notably, the report did not support an outright ban on germline gene editing, but proposed a set of stringent criteria and oversight mechanisms that would have to be in place before specific clinical applications could be considered. Extension of human gene editing, either somatic or germline, beyond the treatment or prevention of serious disease, to genetic enhancement strategies was specifically banned. 'Healthy babies not designer babies' was the call. However, the report itself acknowledges that societal norms relating to human gene editing are not uniform worldwide and are not immutable over time. There was a clearly recognized need for further public engagement strategies to help inform the ongoing debate. The year following the report has seen a number of activities in this regard. A new survey of opinions on genome editing carried out by Scheufele and colleagues in the USA suggested that there was a general positive shift in public perception and acceptance of some forms of human gene editing but that all participants supported ongoing public engagement in policy development (Scheufele et al., 2017). A recent call for a 'global observatory' for gene editing (Jasanoff and Hurlbut, 2018)

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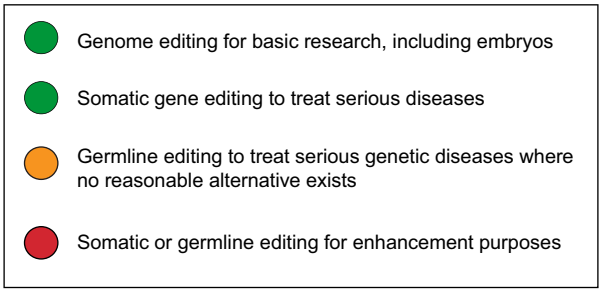

Fig. 1. Major recommendations of the National Academy of Sciences report on human genome editing. The committee did not recommend an outright ban on human germline editing but set out guidelines around when to (1) proceed under existing regulatory processes (green), (2) proceed with caution under stringent oversight and public input (orange) or (3) not proceed at this time (red).

and the establishment of ARRIGE, the Association for Responsible Research and Innovation in Genome Editing (http://arrige.org), both point the way forward for ongoing, broad-ranging international strategies to monitor progress and evaluate ethical and regulatory norms.

Gene editing and human development

While the debate about human germline editing continues, the science of gene editing has not stood still. In terms of human germline-competent gene editing, there have been several additional studies demonstrating feasibility of gene correction in human diploid zygotes (Ma et al., 2017; Tang et al., 2017), as well as the demonstration that new base editing tools can be applied to human embryos to generate specific nucleotide alterations (Liang et al., 2017). In all cases, efficiency is not 100% and mosaicism and unwanted allelic variants remain an issue, making the clinical application of these approaches still a distant prospect.

Application of CRISPR to the study of normal human development *in vitro* has also moved ahead. Despite the widespread prevalence of human *in vitro* fertilization as a means of aiding infertile couples, we still know very little about the molecular events of preimplantation development, implantation and early placental formation in humans. And yet these are the stages that are highly susceptible to disruption leading to early pregnancy loss and later placental insufficiencies in both normal and IVF pregnancies. Most of our understanding comes from the very well-studied mouse system and, indeed, many of the major pathways and events are likely to be conserved. However, it is increasingly clear that there are molecular, morphological and timing differences between mouse and human that may significantly affect not only our understanding of early pregnancy but also the production of pluripotent stem cells suitable for use in modeling development, disease and developing stem cell-based therapies (Rossant and Tam, 2017).

Single-cell RNA-seq analysis can now provide a means of exploring the progression of cell fate specification in the early human embryo and identifying potential functionally significant gene differences from the mouse. CRISPR-Cas9 genome editing represents a useful tool to then test the function of these gene pathways. Several groups have begun to explore this approach, with the first publication last year from the Niakan group targeting the *POU5F1* locus in the early human embryo (Fogarty et al., 2017). Careful analysis of the mutation efficiencies, the morphological defects and transcriptional profile of resulting embryos suggested that embryos fully deficient in POU5F1 showed widespread defects before the blastocyst stage, unlike the mouse in which inner cell mass failure occurred after blastocyst formation in *Pou5f1* mutants (Nichols et al., 1998). This is a surprising result as the general trend in human development is for segregation of expression of early lineage markers to occur later than in the mouse (Wamaitha and Niakan, 2018). Further analysis of gene function into the peri-implantation period can now be considered, with the development of improved culture systems for human blastocyst outgrowths (Deglincerti et al., 2016; Shahbazi et al., 2016). However, further improvements of *in vitro* human embryo cultures would risk violating the long-held 14-day rule, which restricts the *in vitro* culture of intact human embryos to 14 days or the onset of gastrulation. Clearly, there would be great benefit for understanding normal development and for directing pluripotent stem cell differentiation if the genetic pathways that establish and pattern the early germ layers were better understood. Revisiting the 14-day rule is under discussion and debate in several arenas (Pera, 2017).

Although we can expect to see more applications of CRISPR and related approaches to studying human embryo development, there remain many practical hurdles to the widespread uptake of this approach. In many jurisdictions, creation of human embryos specifically for research purposes is banned, restricting gene-editing approaches to potentially less viable, discarded early embryos from IVF programs. Also, in many jurisdictions, gene editing to generate potentially heritable genetic changes is forbidden; this is an area that would need to be revisited before any clinical germline editing could be considered. In Canada, editing to generate heritable changes is subject to criminal prosecution. Health Canada's current interpretation is that it would apply to any attempt at gene editing in the early human embryo, even if the embryos were only studied in culture.

Alternatives to human embryo research

Given accessibility issues and the ethical and legal concerns, can we use surrogate systems to study early human development? Non-human primate embryos certainly provide a useful comparator. Recent studies of pre- and early postimplantation development in macaques have provided interesting clues as to potential differences in germ cell origins between rodents and primates (Nakamura et al., 2016; Sasaki et al., 2016), and gene editing has been applied successfully to non-human primate embryos (Sato et al., 2016), opening up possibilities for exploring gene function in more detail. However, work with these species is slow and expensive and restricted to centers that have major primate colony resources. It cannot replace human embryo research but can be an important complement.

The other complementary approach is the development of embryoids, gastruloids and amniotic sac structures from human pluripotent stem cells. Micropatterned cultures of human pluripotent stem cells can generate organized structures reminiscent of the ordered pattern of cell types in the gastrulating embryo (Tewary et al., 2017; Warmflash et al., 2014). Further development in 3D can produce structures with morphological resemblance to the postimplantation amniotic sac (Shahbazi et al., 2017; Shao et al., 2017). These studies are still at the formative stage, but there are many ways one can envisage these embryoids being developed as models in the future. Because these structures are derived from permanent stem cell lines, gene editing can be readily applied to the starting cell lines to interrogate gene function during early development.

Close comparison between these stem cell-derived constructs and normal human and non-human primate embryo development should aid their development into increasingly powerful and experimentally tractable models in which to study the events of

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early human development. This has raised the question of whether emergent properties in these stem cell-derived entities should bring them under the same ethical limitations as human embryo cultures (Aach et al., 2017; Munsie et al., 2017). Given that they do not have the full capacity to generate the extra-embryonic cell types needed to develop *in utero*, stem cell-derived embryoids can be considered to lack full human potential. However, this remains an area of debate and requires active consideration as the research proceeds.

Human germline gene editing – what does the future hold?

The ongoing development of advanced gene editing tools will certainly be used to enhance our understanding of human developmental programs, whether in human embryos directly or in related mammalian embryos or stem cell-derived embryoids. Whether these developments will lead to safe and acceptable clinical human germline editing remains uncertain, especially if the route to germline editing is restricted to editing oocytes and embryos. The technical, ethical and regulatory challenges remain daunting. Future prospects for germline editing would change dramatically if gene editing could be carried out in stable progenitor cell lines that could produce eggs and sperm *in vitro*. In the mouse, spermatogonial stem cell lines can be derived directly from testis biopsies and give rise to spermatid-like cells that can fertilize eggs and produce viable offspring. A proof of principle study used CRISPR-Cas9 to correct a genetic mutation causing cataracts in mouse spermatogonial stem cells and demonstrated transmission of the corrected allele to the next generation after intracytoplasmic sperm injection into mouse eggs (Wu et al., 2015). Functional oocytes and spermatids have also been produced from mouse embryonic stem cells (Hikabe et al., 2016; Zhou et al., 2016), raising the future possibility of generating oocyte or sperm progenitors from human pluripotent stem cells. To date, however, attempts to generate germline progenitors from human embryonic stem cells have not yet been successful, although early germ cell progenitors have been produced (Irie et al., 2015; Sasaki et al., 2015). As noted earlier, a better understanding of the origins of germ cells in mouse versus human embryos should help promote improved gametogenesis from human pluripotent stem cells. A long-term prospect of patient-specific stem cells being edited *in vitro* and then differentiated into functional eggs or sperm, with validated correction of a heritable defect, is not out of the question. Whether society is ready for this remains an open question.

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Competing interests

The author declares no competing or financial interests.

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