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The CRISPR-Cas Technique for Gene Editing and its Impact on Society

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From time to time, the biological sciences undergo a technological breakthrough. Examples of this type of innovation in recent decades are the advent of the recombinant DNA techniques, the cloning of the first mammal from adult cells, the establishment of the first cultures of stem cells derived from human blastocysts and the generation of induced pluripotent stem cells from adult cells.

These advances have given rise to active debates that have transcended the scientific community. The most frequent concerns have been the bioethical implications of the use of the novel technologies with humans and their possible impact on the environment. The media, both written and visual, disseminate these breakthroughs to the general public, sometimes with fruitful creativity and imagination. In turn, scientists normally take the initiative in convening committees and proposing guidelines for the proper application of the new technologies. A paradigmatic case of this type of response was the Asilomar conference in 1975. Noteworthy, this Pontifical Academy has played a leading role organizing workshops on topics such as GMOs and stem cells, publishing statements with conclusions and recommendations. On the other hand, governments dictate public policies to assure safety benefits to society. For example, there are regulations for genetically modified crops issued by the FDA and the European Commission. The protocols for human gene therapy and the use of human embryonic stem cells are also subject to strict oversight. The challenge for the regulatory agencies is to respond in due time with guidelines that are supported on solid scientific evidence.

We are now witnessing another breakthrough that seems to surpass the traditional recombinant DNA techniques in terms of versatility and accuracy, namely, the so-called CRISPR-Cas technology. CRISPR is an acronym for Clustered Regularly Interspaced Short Palindromic Repeats. These correspond to DNA sequences in bacteria and archaea that, in conjunction with Cas (CRISPR-associated) proteins, participate in the recognition and elimination of invading foreign DNA species, thus acting as an adaptive immune system. In particular, the CRIPR-Cas9 system offers a simple procedure for introducing various modifications at targeted loci in all kinds of living cells. This system relies on base complementarity between an engineered guide RNA and a specific DNA sequence in the genome, followed by double strand cleavage by Cas9. The double strand breaks are later repaired by one of several pathways that may lead to gene mutation or replacement.

Recent developments have allowed the expansion of the genome-editing repertoire of the CRISPR-Cas system. These include modification of the guide RNA to improve recognition specificity, amendment of the Cas9 enzyme to create new docking sites in the genome and deactivation of the nuclease activity of the Cas protein. A nuclease deficient Cas enzyme can be fused to another enzyme to correct a mutant base or to introduce nonsense mutations at selected sites, without cleaving the genome.

Both social media and the scientific community reacted very quickly upon the advent of the CRIPR-Cas technology. Their main apprehension is the possibility of editing the human germ line,[1] either for therapeutic or enhancement purposes. The scientific community has taken several initiatives, consisting mainly in deliberations comprising both scientific and bioethical arguments, while at the same time encouraging further scientific research. A milestone in these actions was a meeting held in Napa, California, in January of 2015, which is reminiscent of the Asilomar meeting. Convened by the Innovative Genomics Institute,[2] the group released a statement in which, among other recommendations, it proposed not to conduct germline genome modification, at least for the moment. A similar conclusion was reached at the First International Summit on Human Gene editing, convened in Washington DC in December of 2015 by the U.S. National Academies of Sciences and Medicine, the Chinese Academy of Sciences and the Royal Society. The second version of this meeting will take place in Hong Kong later this month.

A voluntary moratorium as an effective way to discourage human germline modification has been supported by several scientists. For example, Edward Lanphier and four colleagues have argued that heritable human genetic modifications pose serious risks and the therapeutic benefits are tenuous.[3] The NIH, for its part, has announced that, although it will continue to support a wide range of innovations in biomedical research, it will not fund any use of gene editing technologies in human embryos.

However, there are some disagreements among scientists, as illustrated with these headlines taken from *Nature* magazine. Thus, Henry Miller from Stanford University claims that germline gene therapy should be used sparingly and with scrutiny, pushing the frontiers of medicine to rid families of monstrous genetic diseases.[4] In a similar stance, geneticist George Church argues that banning human germline editing could put a damper on the best medical research, driving the practice underground.[5] In a more cautious position, Jennifer Doudna, a prominent leader in the field, calls for urgent ethical discussions and avers that a complete ban is impractical given the ease of use of the technique.[6]

In view of these disputes, it may be worthwhile to find out what science academies and societies have said in this respect. I have selected two of them based on their ample representativeness. According to the US National Research Council,[7] the ethical norms and regulatory regimes developed for somatic cell therapy are appropriate for the management of somatic genome-editing applications. In the case of germline editing for therapeutic purposes, research trials might be permitted, but only following much more research and, even then, only for compelling reasons and under strict oversight. In contrast, genome editing for enhancement should not proceed at this time, and public discussions should precede any decisions about such applications. On the other hand, The European Society of Human Reproduction and Embryology and the European Society of Human Genetics released a joint report asserting that although clinical germline gene editing would be totally premature, it might become a responsible intervention in the future, but only after adequate preclinical research conducted under societal oversight. It adds that the present prohibition in Europe[8] of germline modification needs renewed discussion among relevant stakeholders.

In the meantime, while these deliberations have been taking place, some research centers have dared to take the first steps. One would have thought that since somatic gene therapy offers much less bioethical apprehensions than germline gene editing, the first attempts would be directed to treat or prevent disease in infants and adults. Indeed, Companies such as Editas Medicine, CRISPR Therapeutics and Intellia Therapeutics, as well as research groups at various universities, are engaged in programs aimed at treating patients suffering from different diseases. To my knowledge, there are no publications in the scientific literature reporting actual trials. There is only a very short note that appeared in *Nature* describing the treatment of a cancer patient in Chengdu, China, and a report in the Wall Street Journal earlier this year noting that there are presently in China 86 cancer or HIV patients being treated using the CRISPR-Cas technology.

In contrast, scientific journals disclose that gene editing in human embryos is an active area of research, especially in China. To date, experiments have been intended to test the efficacy of the technique rather than to establish pregnancies by transferring the modified embryos. The first report appeared in 2015,[9] implying that experimentation started as soon as the CRISPR-Cas technique was harnessed for genome editing in eukaryotic cells.[10] Early results obtained in this work and a couple of subsequent attempts[11],[12] revealed several non-targeted gene modification events, as well as mosaicism when the embryos were allowed to further develop *in vitro*. The protocols applied in subsequent instances were aimed primarily at overcoming these flaws.

One of them involved the simultaneous injection into oocytes of sperm plus the editing components.[13] This particular work, conducted by Shoukhrat Mitalipov's team in the USA,[14] resulted in more efficient editing, although the interpretation of the results was openly debated by another group. In turn, a report by Junjiu Huang's group from Guangzhou, China, deals with the amendment of the mutation responsible for thalassemia, a 'recessive' disease that is caused by having two faulty copies of a gene.[15] Because it would be difficult to find dozens of embryos that have this rare double mutation, the team developed embryonic clones from their patient's skin cells. There is also a publication by a British team in which, rather than targeting a disease, they investigate the function of the pluripotency transcription factor OCT4 during human embryogenesis.[16]

The CRISPR-Cas technique exhibits some improvements, but it is still far from reaching a safety stage that would permit the transfer of the modified embryos, or even its application in adult gene therapy. For example, it has recently been shown that cleavage of the DNA by Cas nuclease induces the p53-mediated DNA damage response leading to a selection against cells with a functional p53 pathway.[17],[18] The authors showed that inhibiting DNA damage signaling improves the efficiency of editing in normal cells. However, inhibition of p53 leaves the cell transiently vulnerable to the introduction of chromosomal rearrangements and other tumorigenic mutations. In addition, work conducted with mouse and human cells has shown that CRISPR/Cas editing leads to large deletions and complex rearrangements both at target and distal sites.[19] Still another setback to be solved, at least in protocols aimed at somatic gene therapy, is the identification in humans of pre-existing immunity to Cas9 proteins.[20] It is for reasons such as these that *The New York Times* felt compelled to publish an extensive article cautioning about the present limitations of the CRISPR-Cas technology.[21]

It is likely that several features of CRISPR-Cas are still unknown to us. Hence, it would be advisable for the moment to moderate expectations and proceed with caution, especially when it comes to extrapolating the results obtained in the lab to the treatment of adult patients or human embryos. In particular, human embryo

editing constitutes a clear example of science advancing faster than ethical reflection and legislation, with the further complication that due to different views regarding the moral status of the human embryo, general agreements are difficult to reach. More than ever before, the scientific community should be aware of its great responsibility in guiding society in safeguarding human dignity.

[1] Early-stage embryos, eggs, sperm and the cells that give rise to them.

[2] Academic partnership between UC Berkeley and UCSF whose mission is to develop and deploy genome engineering to cure disease, ensure food security and sustain the environment for current and future generations.

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[4] Miller,H. Germline Gene Therapy: Don't let good intentions spawn bad policy. *Issues*, Volume XXXII (3), Spring 2016.

[5] Church, G. Encourage the innovators. *Nature* 528, S5, 2015.

[6] Doudna, J. Embryo editing needs scrutiny. *Nature* 528, S6, 2015.

[7] The USA National Academies of Sciences, Engineering and Medicine.

[8] Clinical Trials Regulation EU N° 536/2014, article 90.

[9] Liand, P. *et al.*, CRISPR/Cas-mediated gene editing in human tripronuclear zygotes. *Protein Cell* 6, 363-372, 2015.

[10] Cong, L. *et al.*, Multiplex genome engineering using CRISPR/Cas systems. *Science* 339, 819-823, 2013.

[11] Kang, X. *et al.*, Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. *J. Assist. Reprod. Genet.* 33, 581-588, 2016.

[12] Tang, L. *et al*., CRISPR/Cas-mediated gene editing in human zygotes using Cas9 protein. *Mol. Genet. Genomics* 292, 525-533, 2017.

[13] Ma, H. *et al*., Correction of a pathogenic gene mutation in human embryos. *Nature* 548, 413-419, 2017.

[14] Oregon Health & Science University.

[15] Liang, P. *et al*., Correction of b-thalassemia mutant by base editor in human embryos. *Protein Cell* 8, 811-822, 2017.

[16] Fogarty, N.M.E. *et al*., Genome editing reveals a role for OCT4 in human embryogenesis. *Nature* 550, 67-73, 2017.

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[19] Kosicki, M. *et al.*, Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. *Nature Biotechnol*. 36, 765-771, 2018.

[20] In the form of both anti-Cas9 antibodies and T-cells: Porteus *et al.*, bioRxiv preprint first posted online Jan. 5, 2018; doi: http://dx.doi.org/10.1101/243345

[21] Zimmer, C.A. Crispr conundrum: How cells fend off gene editing. *New York Times*, June 12, 2018.