Trends in Biotechnology

Letter
CRISPR-Mediated Gene Editing: Scientific and Ethical Issues

Jarrod Bailey [1,*]

There remains substantial evidence to warrant great concern over the poor efficiency and specificity of clustered regularly interspaced short palindromic repeats (CRISPR)-mediated genetic modification (GM), despite relatively minor improvements compared to other GM methods. These issues cause persistent, adverse, ethical, and scientific consequences for GM animals, which may never be sufficiently resolvable.

An article recently published in *Trends in Biotechnology* discussed how engineering of guide RNAs could improve the efficiency and specificity of CRISPR [1]. While this technique may have great potential for aiding research into the understanding of gene function in the future, and for eventual clinical applications, such as gene therapy, the authors may have been over-optimistic and speculative, and insufficiently critical. While they acknowledge some caveats (only a small set of target genes has been investigated), and the need for further improvements (in increasing efficiency, specificity, and obviating toxicity), it is important to provide more balance, as there exists significant evidence warranting great caution on several levels, which seems underappreciated. This is particularly relevant to the creation of GM animals, which will be an area in which CRISPR is heavily used, and for which there are serious ethical/welfare consequences and considerations.

I recently questioned claims about CRISPR's high degree of efficiency and specificity, and reviewed the evidence supporting a much more prudent approach, which is urged by some stakeholders [2]. To illustrate, recent reports have highlighted slightly improved, though still very poor efficiencies, which when one looks for precision, amount to a few percent at most [3]. Off-target effects are persistent, even with engineered CRISPR components, and can have multiple pathogenic consequences, including cancer. Many off-target effects are missed due to analytical methods that aren't sufficiently comprehensive, and – crucially – some believe they may never be completely removed, however high on-target specificity may become [4,5]. On-target effects may often be more significant than intended, causing large deletions and genomic rearrangements. CRISPR is also more likely to be successful in cells in which the p53 gene is deficient, and so has further cancerous potential – with catastrophic consequences for GM animals and for clinical applications [6,7]. This tumorigenicity does not arise solely from CRISPR, so other contributory factors must be identified. While cells could be selected to avoid this, it is not possible for *in vivo* applications. Perhaps alarmingly, these issues have not led to the cautious approach they justify, and which some scientists urge. This is partly evidenced by clinical trials of the technology, which has been used for various malignancies/cancers.

These concerns are in addition to other long-standing problems associated with the creation of, and experimentation on, GM animals. While these issues could not be expected to be addressed by the authors of the recent *Trends in Biotechnology* paper, they are relevant to the use of CRISPR in science, and substantiate the call for a much more cautious attitude. Poor efficiency and specificity are acknowledged to be a serious welfare issue, with every stage of the creation and breeding of GM animals potentially involving pain and suffering to some degree, and which may

not be fully appreciated and taken into account in harm–benefit analyses [8,9]. There exists significant evidence of failed translation of data from GM animals to human benefit, and of their poor human relevance for many diseases [2]. Of salient concern currently is the increased creation of, and experimentation on, GM monkeys, partly in response to greater appreciation of the inadequacies of GM mice. It has been suggested that they will be more human relevant, but there is little or no evidence to support this. They will be subject to the same inefficiencies and lack of specificity as GM mice, due to the aforementioned (and possibly persistent) inadequacies of CRISPR, and there are myriad confounding differences in gene complement and expression between humans and monkeys, which will always affect – and preclude – their applicability to humans [10].

Finally, it is important to note that many who oppose the application GM technology to animals for ethical and scientific reasons, do not oppose its use in basic science involving cell and tissue cultures, which will become more informative and human relevant with further advances in 3D culture, organoids, body-on-a-chip approaches, stem cells, and so on. These techniques are already being used to investigate gene function, link genetic mutations/polymorphisms with phenotype, attempt gene therapy, etc. It is these human relevant *in vitro* methods that will determine if CRISPR can be sufficiently safe and reliable to be used in human patients (along the lines that Moon and colleagues [1] suggest). There is no scientific necessity to develop CRISPR in animals, or scientific basis to assume that successful and 'safe' gene therapy in, say, mice or monkeys, will translate to the same in human patients.

[1]Cruelty Free International, 16a Crane Grove, London, N7 8NN, UK

*Correspondence:
Jarrod.bailey@crueltyfreeinternational.org (J. Bailey).

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