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Repairing Without Cutting: A Safer Alternative to Gene Correction?

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doi:10.1038/mt.2014.40

Gene editing is likely to become the next generation of gene therapy, by overcoming the imprecision and the potentially harmful effects of current gene replacement technology. Editing comes in two flavors: gene knockout, which relies on the error-prone repair of a nuclease-directed double-stranded break by nonhomologous end-joining, and gene correction, which uses the homology-directed DNA repair machinery to edit a nucleotide sequence through the use of an artificial donor template. Whereas gene knockout is relatively easy to achieve, gene editing appears to face more serious hurdles, such as the low propensity of somatic cells to utilize homologous recombination (HR) to repair DNA.

A variety of strategies have been developed to increase HR efficiency, such as the use of site-directed nucleases to introduce double-stranded cuts at the target site (e.g., zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats associated with *Cas* genes (CRISPR/*Cas9*))[1] or more recombinogenic donor templates, such as integration-defective lentiviral vectors[2] or adeno-associated viral vectors (AAVs).[3,4] Recent studies have shown that HR-mediated genome editing is relatively efficient in immortalized cell lines but very inefficient in primary cells, particularly in somatic stem cells.[2,5,6] In this issue of *Molecular Therapy*, Melo *et al.* describe the correction of mutations causing junctional epidermolysis bullosa (EB), a severe skin adhesion defect, by using a highly recombinogenic AAV serotype as an HR donor template.[7] They show that correction can be obtained in transplantable keratinocyte progenitors without the need for cutting DNA by a site-directed nuclease, a substantial advantage in terms of biosafety. Although the strategy is probably limited to cells that can be cloned or selected in culture, the article opens interesting perspectives for *ex vivo* gene therapy of inherited skin diseases.

EB is a family of severe and often lethal skin adhesion defects characterized by disfiguring blistering, reduced barrier to infections, and an increased risk of skin cancer.[8] Junctional EB is caused by recessive mutations in any of the three genes (*LAMA2*, *LAMB3*, and *LAMC2*) encoding the laminin 332 heterotrimer, an essential component of the dermal-epidermal junction. Dystrophic EB is caused by mutation in the gene encoding type VII collagen (COL7A1), the ligand of laminin 332. There is no cure for EB, and current therapies are palliative, aimed at treating infections and trauma and maintaining an acceptable quality of life. Gene therapy of EB is based on autologous transplantation of skin derived in culture from genetically corrected epithelial stem cells (EpSCs). After a decade of preclinical investigations, the proof of concept for this form of treatment was established in a pilot clinical trial carried out in 2005 on a patient affected by *LAMB3*-deficient junctional EB.[9,10] Although the study provided clinical validation for the therapeutic potential of gene therapy, safety concerns were raised owing to the use of a Moloney murine leukemia virus (MLV)-derived retroviral vector to deliver the therapeutic gene to EpSCs, which essentially halted the trial. Recent preclinical studies addressed the use of enhancer-deleted MLV vectors or lentiviral vectors to correct human EpSCs, with promising results. However, the propensity of any type of retroviral vector to interfere with gene regulation at transcriptional or post-transcriptional level is still raising safety concerns and therefore mandates careful risk–benefit evaluations.[11] This is true regardless of the strategy used to correct the genetic defect—whether conventional gene replacement, exon skipping, trans-splicing, or RNA interference—because in any case the use of self-renewing stem cells to maintain stable skin grafts requires permanent vector integration.

Genome editing is obviously an attractive alternative. Targeted genetic modification of transplantable EpSCs has been obtained, albeit at very low efficiency, through the combination of zinc-finger nucleases and HR donor cassettes delivered by integration-defective lentiviral vectors or adenoviral vectors,[6] or by the simple use of AAV vectors as efficient HR templates.[12] Melo *at al.* selected a highly recombinogenic AAV serotype, AAV-DJ, to increase HR efficiency without using site-directed nucleases. They reached an efficiency on the order of a few percentage points, a remarkable result although still far from a potentially "therapeutic" threshold. However, EpSCs can be cloned, characterized, and used to produce mono- or oligoclonal skin grafts, so that even a suboptimal technology in terms of gene correction efficiency may be potentially exploitable. The limitation of the cloning approach is the risk that the extensive *in vitro* manipulation required to obtain large surfaces of transplantable skin from single stem cells exhausts their potential and eventually limits their long-term graft-maintaining capacity in patients. Melo *et al.* propose to replace traditional cloning with a rapid selection based on the reconstitution of the adherence capacity of cells corrected in their laminin 332 defect. They show that selection on collagen-coated plastics produces polyclonal populations of primary keratinocytes in which 20% of the cells are corrected by HR. Because

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laminin 332 is deposited at high efficiency at the basal membrane level, 20% of gene correction may be sufficient to rescue the adhesion defect, as they showed in both organotypic cultures and xenografts in nude mice. In addition, keratinocytes with reconstituted adhesion properties undergo further selection after transplantation, as shown by both Melo *et al.* and in a previous study based on retroviral transduction,[13] suggesting that highly recombinogenic AAV vectors could be used to produce stable, genetically corrected skin grafts of therapeutic value for the treatment of junctional EB. These results are very promising, although the transplanted mice were followed for only five weeks, which is insufficient to formally demonstrate gene correction in true EpSCs.

An interesting aspect of the study is the notion that AAV vectors can promote HR more or less efficiently depending on their serotype. Interestingly, the screening carried out by Melo *et al.* showed that transduction efficiency and recombinogenic properties are not correlated—the most recombinogenic serotype, AAV-DJ, transduced primary keratinocytes several times less efficiently than other serotypes, although it favored HR several times more. The molecular basis of "recombinogenicity" is still poorly understood. Melo *et al.* used the same genome, derived from AAV2, in all the vectors, pointing at the capsid as the major determinant of the recombinogenic properties. The simplest explanation for this apparent paradox is that entry and intracellular trafficking may somehow influence second-strand synthesis, and therefore the availability in the nucleus of hairpin-structured single-stranded genomes, an ideal HR template. The study, however, seems to rule out this hypothesis and does not provide alternative explanations for the correlation between serotype and recombinogenic properties.

The work by Melo *et al.* shows that a recombinogenic AAV template alone can favor HR-mediated gene editing at a low but already exploitable efficiency. Provided that gene-corrected stem cells can be enriched for their acquired properties and not lose their tissue-maintaining capacity in the process, the approach could be readily translated into a clinical application, at least in the case of gene therapy for junctional EB. One might be tempted to speculate that combining highly recombinogenic AAV serotypes with HR-stimulating site-directed nucleases would further increase the overall efficiency of the process, making it more widely usable. However, as the authors point out in their discussion, the primary advantage of correcting without cutting is in avoiding all the risks potentially associated with off-target DNA cleavage and the induction of unpredicted patterns of genome repair, such as chromosomal aberrations. Although preliminary and limited to a small subset of potential applications, the work by Melo *et al.* offers a different perspective and puts another brick in the pathway to safe gene correction and safer gene therapy.

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