Commentary

CRISPR made easy in human and murine hematopoietic precursors

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The CRISPR/Cas9 technology has revolutionized gene-editing approaches, by favoring rapid and efficient modifications of gene function, from knock-out to knock-in, and even to silencing or activation of target genes (1,2). In the October 25th issue of Cell Reports, *Gundry et al*. (3) describe a novel and efficient method to engineer hematopoietic stem/progenitor cells (HSPCs), of both human and murine origin. In this study, highly efficient disruption of critical genes for myeloid cell development and function, including *Dnmt3a*, *Eed* and *Suz12* (4), was achieved through virus-free systems. The authors achieve efficient delivery of small guide RNAs (sgRNAs) to murine Cas9-expressing HSPCs by electroporation, with subsequent knock-out of target genes, while maintaining cell viability and colony-forming properties of the edited cells. Targeting of *Eed* and *Suz12* increased proliferation of the edited cells, and their capacity to serially replate, confirming the oncogenic properties of the gene targets. Importantly, the authors report complete virus-free delivery of both Cas9 and sgRNA in human primary T cells and in cord-blood derived HSPCs. By optimizing culture and transfection protocols they could achieve targeting efficiencies of over 80% in these cells. Off-target effects of the sgRNAs were detectable at a very low rate, while multilineage reconstitution capacity of stem cells was maintained.

One of the main advantages of the proposed method is the successful gene-editing of human HSPCs, not only by disruption, but through homology-directed repair (HDR), which allows introduction of point mutations at target *loci*, through co-transfection of a homologous "corrected" template (*Figure 1*). Strategies to increase HDR frequencies are widely studied (5,6) to improve current gene-editing strategies, which generally operate by inducing insertions or deletions (indels) through error-prone non-homologous end joining (NHEJ) of the target allele. To this end, introduction of precise genetic alterations through HDR in mammalian cells has the potential to be exploited for studies of cancer-associated point mutations or correction of disease-associated alterations in the clinical setting, extended, but not limited to, a large number of primary immunodeficiencies (7).

From a clinical standpoint, the virus-free nature of Gundry's strategy represents a major advantage over a number of virus-based, generally retro or lentiviral based strategies (8), and their associated concerns, mainly related to insertional mutagenesis (9), and emergence of replication-competent viruses. Another important advantage of this technique is efficient co-transduction of combinations of Cas9 with multiple sgRNAs and repair templates to combine NHEJ- and HDR-mediated editing strategies. This particularly enables studies of loss-of-function and gain-of-function alterations together, which is highly relevant for cancer research but limited in systems based on constitutive Cas9 expression. It is easy to envision

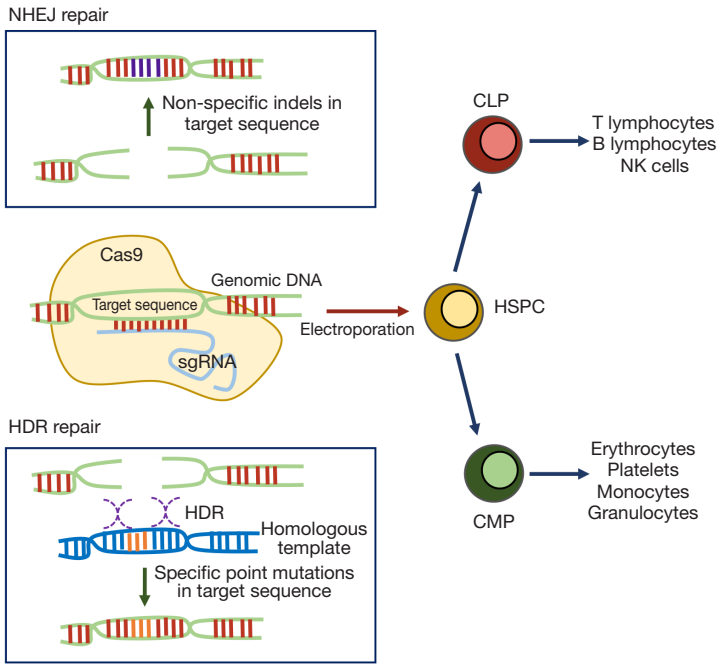

Figure 1 CRISPR/Cas9 gene editing of hematopoietic stem/precursor cells. *Gundry et al.* propose a virus-free strategy through which sgRNA are introduced into HSPC either alone or pre-complexed with Cas9. The sgRNA binds to the corresponding target sequence on the genomic DNA, displacing one strand of the double-stranded DNA target sequence. Such structure is then recognized by the Cas9 enzyme, which operates by cutting the DNA-RNA duplex, causing non-specific insertion/deletions (indels) on the target sequence by NHEJ. Introduction of specific point mutations in the target sequence can also be achieved in this system, through HDR. Such strategy requires co-transduction of a homologous template, containing the desired mutations. Human or murine gene-edited cells maintain engraftment and multilineage differentiation capacity in this system. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; sgRNAs, small guide RNAs; HSPC, hematopoietic stem/precursor cells; NHEJ, non-homologous end joining; HDR, homology-directed repair.

large applicability of this technology to studies of normal and malignant hematopoiesis, and to foresee applications in mouse modeling of myeloid and lymphoid malignancies, in line with previously reported CRISPR-based *in vivo* modeling of myeloid leukemias (10).

Overall, this technology represents a valuable and cost-effective resource, with large-scale applicability and accessibility, and an important tool to model genetic alterations in a wide variety of *in vitro* and *in vivo* settings.

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Footnote

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