Experimental Hematology

Experimental Hematology 2017;54:1–3

PERSPECTIVE

Utility of CRISPR/Cas9 systems in hematology research

Daniel Lucas[a], Heather A. O'Leary[b], Benjamin L. Ebert[c,d], Chad A. Cowan[e,f,g], and Cedric S. Tremblay[h]

[a]*Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI;* [b]*Division of Hematology/Oncology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN;* [c]*Department of Medical Oncology, Dana-Farber Cancer Institute and Brigham and Women's Hospital, Boston, MA;* [d]*Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Boston, MA;* [e]*Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Cambridge, MA;* [f]*Broad Institute, Cambridge, MA;* [g]*Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA;* [h]*Australian Centre for Blood Diseases, Monash University, Melbourne, Victoria, Australia*

(Received 20 March 2017; revised 22 June 2017; accepted 23 June 2017)

Since the end of the 20th century, novel approaches have emerged to manipulate experimental models of hematological disorders so that they more accurately mirror what is observed in the clinical setting. Despite these technological advances, the characterization of crucial genes for benign or malignant hematological disorders remains challenging, given the dynamic nature of the hematopoietic system and the genetic heterogeneity of these disorders. To overcome this limitation, genome-editing technologies have been developed to manipulate the genome specifically via deletion, insertion, or modification of targeted loci. These technologies have progressed swiftly, allowing their common use to investigate genetic function in experimental hematology. Among them, homologous-recombination–mediated targeting technologies have facilitated the manipulation of specific loci by generating knock-out and knock-in models. Despite promoting significant advances in our understanding of the molecular mechanisms involved in hematology, these inefficient, time-consuming, and labor-intensive approaches did not permit the development of cellular or animal models, recapitulating the complexity of hematological disorders. On October 26, 2016, Drs. Ben Ebert and Chad Cowan shared their knowledge of and experience with the utilization of CRISPR for models of myeloid malignancy, disease, and novel therapeutics in an International Society for Experimental Hematology webinar titled "Utility of CRISPR/Cas9 Systems in Hematology Research." Here, we provide an overview of the topics they covered, including their insights into the novel applications of the technique and its strengths and limitations.

Over the past 10–15 years, novel genome-editing approaches targeting DNA double-stranded breaks (DSBs) were developed to stimulate DNA repair pathways, namely nonhomologous end-joining (NHEJ) and site-specific homologous recombination (HR). The introduction of DSB stimulates the recruitment of the HR machinery at specific loci. This crucial process involves different targeted nucleases with distinguishing characteristics such as DNA recognition and endonucleic DNA cleavage capacities. The discovery of these targeted nucleases and the subsequent development of engineered nucleases, such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindrome repeats and their associated Cas proteins (CRISPR/Cas9), have enabled the development of novel site-specific gene-editing technologies [1]. However, these technologies generated random insertions or deletions (indels) after the stimulation of error-prone NHEJ in most cases, leading to frameshift mutations. When available, the use of an exogenous donor DNA template surrounding the site-specific DSBs allows this significant hurdle to be bypassed by involving high-fidelity, HR-mediated repair.

Offprint requests to: Heather A. O'Leary, Ph.D., or Cedric S. Tremblay, Ph.D., Division of Hematology/Oncology, Department of Medicine, Indiana University School of Medicine, 980 West Walnut Street, R3-C321D, Indianapolis, IN 46202; Australian Centre for Blood Diseases, Monash University, Melbourne, Victoria, Australia; E-mail: haoleary@iupui.edu; cedric.tremblay@monash.edu

0301-472X/
http://dx.doi.org/10.1016/j.exphem.2017.06.006

Although ZFNs and TALENs have improved our capacity to perform site-specific genome editing significantly, their widespread application in hematology has been restricted by elaborate procedures, high cost, and low specificity. Conversely, the demonstrable efficiency of the CRISPR/Cas9 system offers the greatest flexibility for genome editing by overcoming the limitations of earlier targeted nuclease-mediated methods.

Therefore, the development of the CRISPR/Cas9 system may represent a milestone for medical research, because it allows researchers to develop complex experimental models of benign and malignant hematological disorders. Here, we highlight the implications of this powerful system by summarizing Drs. Benjamin Ebert and Chad Cowan's International Society for Experimental Hematology webinar titled "Utility of CRISPR/Cas9 Systems in Hematology Research," presented October 26, 2016 and moderated by Eirini Papapetrou [2].

Dr. Benjamin Ebert: CRISPR models of myeloid malignancy

Dr. Ebert presented recent work from his group that used CRISPR/Cas9 technology to model myeloid malignancies in vivo. Most myelodyplastic syndrome (MDS)/acute myeloid leukemia (AML) patients have an average of three to five mutations that contribute to disease progression [3]. Further, these mutations are not distributed uniformly throughout the hematopoietic cells of the patient. It is now clear that initiating mutations cause the expansion of preleukemic clones, which acquire additional mutations that lead to MDS and ultimately AML. Leukemic and preleukemic clones with different mutations coexist in vivo, and chemotherapy might eliminate some clones without affecting others. Modeling these complex behaviors using transgenic mouse models is thus not feasible. The Ebert group has recognized the need for models that reflect the genetic complexity and cellular heterogeneity observed in cancer patients. The ideal model would target hematopoietic stem and progenitor cells (HSPCs), work in vivo, and mimic the genetic complexity of human disease. The model should be easily customizable, serially transplantable, and amenable to pharmacological testing. To accomplish this, the Ebert group used CRISPR/Cas9 to model MDS/AML in murine and human HSPCs. This approach uses CRISPR/Cas9 to introduce insertions or deletions in multiple alleles in a single HSPC. They coinfected HSPCs with a lentiviral vector carrying a single RNA guide, the Cas9 cDNA, and an enhanced green fluorescent protein reporter concomitantly with multiple vectors, each expressing a single RNA guide and a red fluorescent protein reporter [4]. Using this strategy, they were able to inactivate, simultaneously and biallelically, *Dnmt3a*, *Ezh2*, *Smc4*, and *Nf1* (eight different alleles) in a single HSPC, leading to leukemia after transplantation in mice [4]. The Ebert group has also used CRISPR/Cas9 to model genetic progression in mice by starting with $Tet2^{-/-}$ HSPCs, followed by the introduction of secondary mutations in six different alleles. They subsequently assessed the effect of these mutations in a primary transplantation, from which mutated HSPCs were harvested and two additional mutations were introduced for testing in secondary transplantations. These experiments confirm the feasibility of using CRISPR/Cas9 to model multiple mutations and leukemic progression in a more expedient fashion than use of conventional gene targeting and breeding strategies in mice will allow. The other significant advantage is that CRISPR/Cas9 can be used in human HSPCs. The Ebert group has used these engineered human HSPCs carrying mutations that were predicted to confer sensitivity to hypomethylating agents (e.g., in the *Tet2* locus) and demonstrated that these mutant HSPCs are more vulnerable to azacitidine in vivo [5]. These experiments indicate that the engineered HSPCs can be used to test new drugs for complex hematological malignancies.

Dr. Chad Cowan: Genome editing from modeling disease to novel therapeutics

Dr. Cowan discussed the use of CRISPR/Cas9 for genome editing to create novel disease/animal models and for potential therapeutic use to bridge biological discovery and clinical therapy. Initial comparison studies of genome editing via CRISPR/Cas9 (an RNA-guided endonuclease) versus transcription activator-like effector nuclease (TALEN, a DNA-binding, motif-based endonuclease) showed that CRISPR/Cas9 had a higher overall efficiency (50%–80%) as well as an increase in homozygous knock-out and knock-in detection and no off-target events. Conversely, TALEN had 0%–30% efficiency, diminished knock-out and knock-in potential, and one on-target translocation detected [6–8]. Therefore, Dr. Cowan's group uses the CRISPR/Cas9 system, which allows them to create clinically relevant, pluripotent, isogenic models (that can be differentiated into hepatocytes or other cells of interest) efficiently with side-by-side mutational analysis without the need for patient recruitment or quality control of multiple induced pluripotent stem cell lines. Dr. Cowan's group has used these models for high-throughput screens to detect genes associated with lipid metabolism (such as *A1CF*) and to investigate the therapeutic potential of CRISPR in the context of HIV. To investigate the therapeutic potential of the CRISPR/Cas9 system, they engineered a novel doubled-guided approach that enhances the efficiency of the CCR5 (the co-receptor for HIV) knock-out in CD34+ HSPCs from mobilized peripheral blood. This strategy resulted in homozygous biallelic editing (40%–50% efficiency), with no change in the in vitro or in vivo multilineage potential of the cells. To confirm the safety of this strategy, target capture sequencing was done to allow for very deep sequencing at specific sites, especially those similar to the guide RNA, because these sites are most likely to have mutations in the CRISPR/Cas9 system. Only one statistically significant mutation was detected, which could

have been eliminated with the use of different software, highlighting that good guide design is absolutely critical when using the CRISPR/Cas9 system. Overall, Dr. Cowan's group has shown that CRISPR/Cas9 system enables the creation of clinically relevant disease models (using cell types that have classically had low rates of efficiency) with very low occurrence of off-target mutations, making it an extremely useful tool for biological discovery with potential for clinical therapeutics.

Summary

These studies describe a few possible applications of the CRISPR/Cas9 system and focus on its use in developing complex experimental models of benign and malignant hematological disorders. This powerful tool may represent a milestone for medical research, because it allows for more accurate modeling of complex multigenic and heterogeneous diseases. The research programs led by Drs. Ebert and Cowan highlight a few of the applications of the CRISPR/Cas9 system, such as development of cell-based, clinically relevant drug screens for complex hematological malignancies. The CRISPR/Cas9 system has triggered a technical revolution in medical research, and we can expect that its exciting possibilities will soon be translated into the clinical setting.

References

1. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol. 2014;32:347–355.
2. Ebert B, Cowan C. Webinar series for: International Society for Experimental Hematology (ISEH). Utility of CRISPR CAS9 Systems in Hematology Research. Available at: http://www.iseh.org/news/350262/Webinar-recording-now-available-Utility-of-CRISPRCAS9-Systems-in-Hematology-Research.htm. Accessed October 26, 2016.
3. Ley TJ, Miller C, Ding L, et al. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368:2059–2074.
4. Heckl D, Kowalczyk MS, Yudovich D, et al. Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. Nat Biotechnol. 2014;32:941–946.
5. Bejar R, Lord A, Stevenson K, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood. 2014;124:2705–2712.
6. Ding Q, Lee YK, Schaefer EA, et al. A TALEN genome-editing system for generating human stem cell-based disease models. Cell Stem Cell. 2013;12:238–251.
7. Ding Q, Regan SN, Xia Y, Oostrom LA, Cowan CA, Musunuru K. Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs. Cell Stem Cell. 2013;12:393–394.
8. Veres A, Gosis BS, Ding Q, et al. Low incidence of off-target mutations in individual CRISPR-Cas9 and TALEN targeted human stem cell clones detected by whole-genome sequencing. Cell Stem Cell. 2014;15:27–30.