Perspective: Welcoming a New Age for Gene Therapy in Hematology

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Our capacities to understand and manipulate mammalian genomes are accelerating at an astounding pace. In 2007, Capecchi, Evans and Smithies were awarded the Nobel Prize in medicine for their work on gene targeting, which showed that embryonic stem cells could be modified by homologous recombination (HR) with engineered template DNA to alter virtually any gene and create mutant mice. This work revolutionized biology by allowing investigators to study the in vivo consequences of selected gene alteration. However, the efficiency of HR in embryonic stem cells is unpredictable, depending on the target gene and HR template. More importantly, spontaneous HR occurs at very low rates in most somatic cells, restricting the use of standard gene targeting for most laboratory and clinical applications. This limitation is being overcome by genome editing technologies, which markedly enhance the capacity to alter cellular genes with laser-like precision. Four review articles in this edition of BLOOD summarize the field of genome editing, focusing on their potential for treating hematological disorders.

In 1994, Jasin and colleagues reported the use of a rare-cutting yeast endonuclease system to create DNA sequence-specific double stranded DNA breaks within a Neo selection gene in mouse 3T3 cells. They showed that the cleavage occurred at high frequency and that the DNA breaks were repaired by two basic mechanisms: blunt ended non-homologous end joining (NHEJ) during which insertions or deletions (indels) occurred at the site of repair, and end joining at short regions of DNA homology. Moreover, the double-stranded DNA break increased the frequency of HR with an exogenous DNA template by more than 100-fold. The authors speculated that: “The enhanced gene targeting that we detect in this model system may have applications for creating targeted mutations in loci of interest.”

Subsequently, numerous investigators have developed a diverse and rapidly growing set of different nucleases that introduce double-stranded DNA breaks at specified nucleotide sequences, thereby disrupting genes by NHEJ or enhancing their modification by template-guided HR. The use of these nucleases to precisely alter DNA, which defines the concept of genome editing, is effective for most cell types. These technologies are now an integral toolkit for many laboratories where technicians and trainees can learn the basic methods in a few weeks, and use this approach for various applications ranging from the introduction or removal of somatic mutations in cell lines to engineering complex, polygenic germline mutations in mice. The remarkable ease of genome editing is enhancing laboratory research broadly and fundamentally. The same technology has powerful clinical implications. Indeed, the convergence of genome editing with rapidly advancing methods to identify mutations that contribute to human disease and health related traits represents a “perfect storm” to create synergy for developing new disease therapies through targeted genetic manipulation of somatic cells.

We are pleased to introduce this series of reviews that summarize conceptual, clinical and ethical aspects of genome editing relevant to hematological diseases:
Hoban and Bauer provide a primer on the “molecular toolbox” of genome editing, describing the various nucleases and their potential applications for biomedical research and treatment of blood disorders. Of note, they discuss how high throughput mutational screening using the CRISPR-Cas9 nuclease can identify new gene regulatory elements, which themselves represent therapeutic targets for genome editing. In addition, they highlight the potential synergy between genome editing and other emerging treatment modalities in hematology, such as immunotherapy.

Canver and Orkin discuss the potential use of genome editing for treating common β-hemoglobinopathies, specifically sickle cell disease and β thalassemia. They show how our deep understanding of these diseases and globin gene regulation provides numerous therapeutic options. For example, it may be possible to correct βglobin gene mutations directly by HR to restore the synthesis of normal adult hemoglobin (α2β2). Alternatively, it is possible to disrupt one of several genetic control elements that drive the developmental switch from γ to β globin, which typically occurs around birth. Consequently, reactivation of fetal hemoglobin (α2γ2) can reduce or eliminate the pathophysiology and symptoms of β hemoglobinopathies.

Wang and Cannon discuss the use of genome editing for treating HIV/AIDS, noting that this disorder represents the first-in-man application of therapeutic genome editing with 7 clinical trials now either completed or in progress. Current trials focus on rendering T-cells resistant to HIV infection by disrupting the gene encoding its co-receptor, CCR5. However, insights into the biology of HIV, including identification of host cellular restriction factors, are providing new strategies for genome-editing mediated therapies.

Finally, Kohn, Porteus and Scharenberg outline ethical and regulatory considerations for clinical implementation of genome editing. They highlight the fundamental differences between germline and somatic cell gene editing, noting the myriad of practical and ethical issues that currently prohibit intentional modification of the germline. They suggest that preclinical testing requirements for somatic cell genome editing become progressively focused and streamlined as
information emerges for specific strategies, target genes and nucleases. This rational approach should allow patients with a wide range of rare diseases to benefit from genome editing as safely and expeditiously as possible.

All four articles discuss two fundamental problems that limit clinical applications of genome editing: First, new methods are required to optimize the efficiency of genome editing in clinically relevant target cells. For example, many therapies for blood disorders are limited by the relatively low frequency of HR in hematopoietic stem cells. Wang and Cannon describe some promising new technical advances that address this problem. Second, new computational and experimental approaches are required to predict, detect and limit undesirable off-target genetic alterations conferred by gene editing nucleases. In particular, improved cell based assays are required to identify genetic mutations and rearrangements that may occur at very low frequencies but nonetheless have the potential to be deleterious, for example, by conferring a selective clonal growth advantage to cells and thereby promote malignant transformation or autoimmunity.

The exciting new field of genome editing represents a quantum leap for biology and medicine. In this regard, it is important to note that key tools of this technology arose from basic studies on the structure of zinc finger protein-DNA interactions and adaptive immunity in bacteria, fungi and algae. This illustrates once again how general pursuit of knowledge can yield unexpected practical benefits and underscores the importance of academic basic science, not only for its intrinsic virtues, but also for maintaining a strong pipeline to fuel translational and clinical research.
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