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Review

Is the age of genetic surgery finally upon us?

Eric B. Kmiec*

Gene Editing Institute, Center for Translational Cancer Research, Helen F. Graham Cancer Center and Research Institute, 4701 Ogletown-Stanton Road, Suite 4300, Newark, DE, 19713, USA

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ABSTRACT

This review discusses gene editing and its potential in oncology. Gene editing has not evolved faster towards clinical application because of its difficulty in implementation. There have been many limitations of the tools thought to be useful in therapeutic gene editing. However, recently the combinatorial use of multiple biological tools appears to have broken the barrier impending clinical development.

This review gives a short primer on gene editing followed by some of the foundational work in gene editing and subsequently a discussion of programmable nucleases leading to a description of Zinc Finger Nuclease, TALENs and CRISPRs.

Gene editing tools are now being used routinely to re-engineer the human genome. Theoretically, any gene or chromosomal sequence for which a targeting site can be identified could be rendered nonfunctional by the chromosomal breakage activity of Zinc Finger Nucleases, TALENs or a CRISPR/Cas9 system. Since the initial work started on the mechanism and regulation of gene editing, investigators have been searching for a way to develop these technologies as a treatment for cancer. The issue is finding a practical application of gene editing in oncology. However, progressive ideas are working their way through the research arena which may have an impact on cancer treatment.

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Introduction: a short primer on gene editing

Human diseases are often the result of inherent genetic errors in the human genome, theoretically reversible because of the dynamic and malleable nature of DNA. Genetic mutations can manifest as deletions, insertions, inversions, substitutions and, of course, most applicable to cancer genetics, chromosomal translocations. While noble efforts have been made to develop reagents aimed at treating the symptoms, sometimes with the aim of cell destruction, addressing the underlying cause of these diseases has remained elusive. Over the course of the last 30 years, molecular medicine has evolved and gene therapies have now come into play as legitimate treatment options for genetic diseases. In most cases, the goal of gene therapy is to augment the genetic deficiency by

* Tel.: +1 304 840 3375.
E-mail address: eric@GeneEditingInstitute.com.

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adding an exogenous copy of the normal gene to replace the malfunctioning or disabled gene. Broadly speaking, this approach is known as gene addition and is facilitated by viral vectors that infect cells efficiently and deliver the therapeutic payload. By and large, the field remains controversial with clear demonstrations of success, sometimes at enormous cost and deadly consequences [1,2].

The alternative to the gene addition approach is to utilize biological and genetic tools to correct the underlying mutation(s) responsible for the ailment. It is generally recognized that if one could replace a disabled gene with a good copy at the precise site within the context of the chromosome, the genetic disorder would likely be alleviated at least to some degree. While simple in concept, such a strategy would only solve half the problem since one would have to devise efficient methods for delivering these new biological tools into the appropriate target cells. Assuming that the latter problem can be solved, inserting a new gene or editing a mutant gene *may have* enormous advantages to the patient. Most importantly, a functional gene would be placed under the control of the native, normal promoter and so the natural, genetic position in the chromosome is maintained. In the case of pure gene editing wherein a single mutant base pair is exchanged for the normal base pair, a sort of genetic spell check, and the gene is restored to natural function, again maintaining its normal chromosomal position. In addition, chromosomal gene editing maintains the spatial and temporal relationship for inherent gene expression.

Why hasn't gene editing evolved faster toward clinical application?

The answer is simple–if it had been easy to do, it would already been done. My laboratory has worked on this problem for over 25 years and we have created some of the tools that are projected to be useful in therapeutic gene editing [3–6]. Frankly, more importantly, however, we defined some of the limitations of these tools. Until recently, the majority of these molecules showed great promise primarily in the laboratory and in some preclinical settings. Translation into more appropriate targets was quite difficult because the frequency with which these events occurred required laboratory manipulations, not often available in a clinical lab, for detection of a positive outcome. But, now things have changed. The combinatorial use of multiple biological tools now appears to have broken the logjam impeding clinical development.

Some foundational work

As with many genetic technologies, some of the original work that led to the concept of gene editing was actually performed in yeast. Fred Sherman and colleagues in the late 1970s and early 1980s published a series of fascinating papers in which they used a single-stranded oligonucleotide, a synthetic single-stranded fragment of DNA, to mutate a gene in yeast cells that caused it to change its growth requirements in culture [7,8]. Admittedly, and as mentioned above, the frequency with which this event occurred was extremely low, and could be seen only because one can screen billions of yeast cells to find a single converted cell. Yet, this work was pioneering and advanced a novel approach to manipulating a gene existing within the context of the chromosomal. The whole objective was to change a single base within the context of the natural chromosome so that a new protein would be encoded from the transcript of the corrected gene. Our laboratory and others followed up on this work in the early 2000s and identified a number of enzymatic activities that were required for the gene editing reaction to take place [9–13]. If one were then to extrapolate this concept to human disorders caused by single base mutations, the basis for gene editing strategies in human cells can be now put in perspective. Thus, there is foundational data that support the scientific basis for gene editing. Translating all of this from yeast cells to mammalian cells and then into human patients, however, has been *and remains* a monumental task.

Unlike lower eukaryotes, mammalian cells have a paucity of inherent selectable markers, so even if the genetic change occurred, finding the cell in which it occurred has always been problematic. To address this issue, mammalian gene reporter systems that are based on the correction of an integrated copy of a mutated enhanced green fluorescent protein gene were established. Upon correction, regenerated functional protein and green fluorescence were restored in the cells [9]. Using this type of system, workers from many laboratories helped elucidate the mechanism and regulation of gene editing in mammalian cells [14–17]. These studies provided a basic appreciation for the complexity and challenges of gene editing in mammalian cells, and they defined the parameters and limitations of chromosomal manipulation. The concept of using an oligonucleotide to direct single base repair is based on the inherent capacity of the cells own DNA repair system to identify a structural aberration and catalyze a nucleotide exchange [4,5]. In other words, gene editing takes advantage of normal activities of the cells by activating pathways that the cell employs naturally to repair chromosomal aberrations and mutations.

Perhaps, the most vexing problem of these early studies centered on the frequency with which a single base mutation can be corrected to wild type *(normal)* status. It seemed that most laboratories placed this number at between 0.5% and 1%, far below the range that most workers believed to be clinically relevant. A series of adjuvant treatments were developed, some of which improved the frequency 3 to 4 to 5 times higher, but these treatments involved the manipulation of cell cycle or the slowing of the DNA replication fork [5,15,18]. Neither of these perturbations can be used practically *in vivo*, or even in an *ex vivo* strategy, although, by coincidence, the slowing of replication forks actually occurs when some currently approved drugs are used in cancer therapy.

One particularly enticing discovery that emerged from the mechanistic analyses of gene editing pathways centered on the concept of a double-strand break induced by anticancer drugs such as Camptothecin (CPT) [11] increase the frequency of gene editing dramatically. While this manipulation was akin to the cell manipulations described above, it was different in one important way. Most of the reagents used to induce these double strand breaks had actually been approved for use in humans as anticancer treatments. Thus, this discovery provided some insight that a supportive treatment that would improve the frequency of gene editing driven by single-stranded oligonucleotide might elevate the frequency. The argument against such an approach, however, was that by design, these drugs induce DNA breakage at random sites within the human genome with the obvious ultimate goal of killing the cancer cell. Thus, the combination of anticancer drugs aimed at cell destruction with single-stranded oligonucleotides designed to correct a mutation in the chromosomal has been viewed with some skepticism and is somewhat paradoxical. To take advantage of the basic science observation that double strand breaks increase the frequency of gene editing, a specific reagent that could induce a specific double strand break at the exact location of the genetic mutation in the chromosome was needed.

Programmable nucleases

In the last several years, a series of major discoveries have advanced the field of gene editing dramatically, but none more important than the discovery and utilization of programmable nucleases [19–23]. These enzymes can be designed with great