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The present and future of genome editing in cancer research

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Abstract

The widespread use of high-throughput genome sequencing methods is profoundly changing the way we understand, classify, and treat human cancers. To make sense of the deluge of sequencing data generated in the clinic, more effective and rapid assessments of the functional relevance of newly discovered cancer-associated mutations are urgently needed. In this review, we discuss how genome-editing technologies are responding to this major challenge. Largely focusing on CRISPR-based methods, we will highlight their potential to accelerate discovery, discuss their current limitations, and speculate about future applications.

INTRODUCTION

The convergence of two technological revolutions is radically changing the face of cancer research. By revealing the genetic complexity of human cancers, high-throughput sequencing methods have paved the way for the development of personalized treatments based on the genetic makeup of individual cancers, rather than on their histologic appearance (Meyerson et al., 2010; Simon and Roychowdhury, 2013). Meanwhile, powerful genome-editing tools—CRISPR-Cas9, above all—are providing simple and effective strategies to more accurately model cancer *in vitro* and *in vivo*, greatly accelerating the speed with which new oncogenes and tumor suppressors are identified, novel vulnerabilities are discovered, and more effective treatments are developed. Here we discuss the present and future of genome-editing technologies as they apply to the study of human cancer.

A genome-editing toolbox

Since the deciphering of the genetic code, introducing specific genetic changes in mammalian cells and whole animals has been the dream of molecular biologists. The first successful attempts were through gene targeting via homologous recombination, and culminated with the development of genetically engineered organisms (Capecchi, 1989) [Capecchi reference!]. Although gene targeting in embryonic stem cells has proven highly effective at generating genetically engineered mice, it was with the discovery and engineering of meganucleases (Jacquier and Dujon, 1985; Rouet et al., 1994), proteins capable of recognizing and cleaving specific DNA sequences when expressed in mammalian cells, that the era of somatic genome editing really began. Yet, despite the development of

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improved nucleases such as zinc finger nucleases (Kim et al., 1996) and TAL effector nucleases (Christian et al., 2010; Li et al., 2011), gene-editing methods were not widely adopted due to the relative technical complexity of engineering these enzymes to recognize specific DNA sequences. This dramatically changed with the advent of CRISPR-based systems, in which substrate specificity of the nuclease (Cas9) is directed dictated by Watson-Crick base pairing between a short guide RNA and the target DNA (Jinek et al., 2012). The shift from protein- to RNA-guided recognition of the target DNA represented a critical leap in ease and efficiency, making genome editing available to virtually any laboratory and opening up whole fields of investigation to rigorous genetic analysis.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems are found in bacteria and archaea, where they function as adaptive immune systems against invading nucleic acids (Bhaya et al., 2011). Three major classes of CRISPR systems (and a dozen subclasses) have been discovered, differing from each other in the mechanisms through which the guide RNA is processed, the number and identity of the proteins involved, and the nucleic acid substrate (DNA or RNA). The CRISPR-associated endonuclease *Cas9*, from the type II CRISPR system of *Streptococcus pyogenes*, was the first to be adapted as a genome-editing tool, in part because it consists of a single subunit and has an associated guide RNA with a relatively short recognition sequence (~20 nt)(Cong et al., 2013; Jinek et al., 2013; Mali et al., 2013). In *S. pyogenes* Cas9 is actually associated with two short RNAs: the crRNA, which contains the sequence complementary to the target site, and the tracrRNA, which is required for proper assembly and activity of the complex. However, the system can be simplified for use in mammalian cells by providing a single 'chimeric' guide RNA (gRNA) that contains both the crRNA and the tracrRNA sequences (Cong et al., 2013). For cleavage to occur, the target sequence must be flanked at its 3' end by a specific sequence known as the protospacer-adjacent motif (PAM), which is 'NGG' or 'NAG' for *S. pyogenes* Cas9.

In mammalian cells, the double-strand breaks induced by Cas9 at the target site can be repaired by non-homologous end-joining (NHEJ) or by homology-directed repair (HDR) (Hsu et al., 2014). NHEJ-mediated repair is favored in cells in G0–G1 and often results in the introduction of short indels at the repair site. This makes CRISPR-Cas9 highly effective at creating loss-of-function mutations in protein-coding genes. HDR, on the other hand, is preferentially used to repair DSBs that occur in the S-G2 phase of the cell cycle, when homologous DNA sequences from the sister chromatid are available as a template to repair the lesion. By introducing an appropriate 'donor' DNA bearing homology to the sequence around the cleavage site, HR-mediated repair of CRISPR-induced DSBs can be exploited to introduce or replace specific sequences.

As a testament to its efficacy and versatility, during the past three years, CRISPR-based genome editing has been used to generate site-specific loss-of-function mutations (Cong et al., 2013; Wang et al., 2013), to knock-in alleles (Mali et al., 2013; Xue et al., 2014), to insert or delete large DNA fragments (Groschel et al., 2014; Yang et al., 2014; Yang et al., 2013), and even to model complex chromosomal rearrangements (Blasco et al., 2014; Choi and Meyerson, 2014; Maddalo et al., 2014) (Figure 1).

Although *S. pyogenes* Cas9 was the first programmable endonuclease nucleases to be adapted for use in mammalian cells, it is certainly not the only one. In fact, a race to discover new effectors, and to improve those already available, is currently underway and promises to further simplify and expand the use of genome editing. For instance, protein engineering has been used with success to change the PAM specificity of Cas9 (Kleinstiver et al., 2015), and smaller—and therefore more easily packaged into recombinant viral vectors—Cas9 orthologs have been identified in *Streptococcus thermophiles* (St1Cas9) and in *Staphylococcus aureus* (SaCas9) (Kleinstiver et al., 2015; Ran et al., 2015). Entirely new CRISPR effectors are also being reported, including FnCpf1, a Type II effector from *Francisella novicida* that, by contrast to Cas9, doesn't need a tracrRNA, uses a different PAM sequence, and generates a staggered cut (Zetsche et al., 2015).

In addition, the recently described *Natronobacterium gregoryi* Argonaute (NgAgo) gene encodes for a DNA-guided endonuclease that provides an alternative approach to CRISPR-based genome editing (Gao et al., 2016). Its specificity is directed by a single-stranded 24-nt DNA oligo, which can be easily and inexpensively synthesized and co-transfected with the NgAgo-expressing vector. NgAgo is also appealing because it does not require a PAM motif to cleave its substrate and appears to have low tolerance for mismatches (and therefore less potential for off-targets).

Changing the genome is not the only application of programmable endonucleases. A nuclease-dead version of Cas9 (dCas9) can be combined with modular add-ons such as transactivator (CRISPRa) or repressor domains (CRISPRi), chromatin-modifier domains, and fluorophores, and used to modulate transcription, generate specific chromatin modifications, or to visualize genomic loci respectively, in living cells (Chen et al., 2013; Fu et al., 2016; Gilbert et al., 2014; Gilbert et al., 2013; Hilton et al., 2015; Kearns et al., 2015; Vojta et al., 2016) (Figure 1).

Application of genome-editing tools to cancer research: the present

The availability of powerful genome-editing tools is having an impact on virtually every aspect of cancer research. Here we will discuss three major areas—in vivo cancer modeling, gene discovery, and the exploration of the non-coding genome—that have already been radically transformed by the CRISPR revolution.

In vivo cancer modeling—For nearly three decades, the gold standard for modeling cancer in mice has been to generate transgenic mice by homologous recombination (HR) (Doetschman et al., 1987) or random transgene integration (Brinster et al., 1984). Combined with strategies to temporally and spatially control expression and activation of the transgene, these models have proven essential to define the role of key oncogenes and tumor suppressors, to dissect the interactions between tumor cells and their microenvironments, and to explore new therapeutic avenues (Frese and Tuveson, 2007). However, conventional transgenic methods are costly, time consuming, and technically challenging, often requiring complex breeding schemes to generate animals with the desired genotype. These methods therefore cannot be easily scaled to cope with the rapid increase in mutation data generated by high-throughput sequencing methods. Furthermore, gene-targeting methods by

homologous recombination are harder or impossible to apply to model organisms other than small rodents.

CRISPR-based genome-editing overcomes many of these limitations. The simplicity of the system and its effectiveness in changing DNA sequences not only in the germline, but also in the soma of adult animals (Sanchez-Rivera et al., 2014; Xue et al., 2014), including non-human primates (Niu et al., 2014), offer many advantages. The most obvious is that it accelerates the generation of genetically engineered mice by simplifying the introduction of specific mutations in mouse embryonic stem cells (mESCs) or directly in early stage embryos (Wang et al., 2013; Yang et al., 2013). Perhaps more important—as this was not possible with conventional gene targeting—it enables researchers to modify the genome directly in somatic cells of adult mice. Recent examples of these applications include the generation of novel models of lung, liver, pancreatic, brain, and hematopoietic tumors (Chiou et al., 2015; Heckl et al., 2014; Platt et al., 2014; Sanchez-Rivera et al., 2014; Xue et al., 2014; Zuckermann et al., 2015), as well as the high-throughput *in vivo* testing of potential tumor suppressors (Weber et al., 2015).

Although combined *in vivo* delivery of the programmable endonuclease and its corresponding gRNA(s) has been demonstrated in several tissues, not every tissue can be easily transduced. Furthermore the use of recombinant viruses expressing Cas9 and the gRNAs raises non-trivial biosafety concerns. The recent generation of transgenic mice constitutively or conditionally expressing Cas9 obviates these limitations and substantially expands the number of cell types and tissues that can be easily and safely targeted *in vivo* (Chiou et al., 2015; Dow et al., 2015; Platt et al., 2014).

In addition to allowing the generation of loss-of-function mutations, *in vivo* somatic genome editing can also be used to engineer chromosomal rearrangements, a class of cancer-associated mutations that was previously particularly challenging to faithfully model using conventional transgenic methods. The method consists in co-expressing Cas9 and two gRNAs, so that two simultaneous DSBs are generated at the desired breakpoints. Provided that the two breakpoints are close enough in the nucleus of the cell, the NHEJ machinery will occasionally join them, thus generating the desired rearrangement (Figure 1). Initially tested in cell lines (Choi and Meyerson, 2014), the feasibility of this strategy was first demonstrated *in vivo* by modeling the EML4-ALK inversion, a chromosomal inversion recurrently found in human non-small cell lung cancers (Soda et al., 2007). Two groups used recombinant adenoviral (Maddalo et al., 2014) and lentiviral (Blasco et al., 2014) vectors to deliver the CRISPR machinery to the lung epithelium of adult mice. This resulted in the rapid development of lung adenocarcinomas that harbored the Eml4-Alk rearrangement and responded to targeted therapy with the ALK inhibitor crizotinib (Maddalo et al., 2014).

Finally, it is important to consider that CRISPR-based somatic genome editing and conventional germ line gene targeting methods are not mutually exclusive. On the contrary, their rational combination is one of the most promising new directions in cancer research. It will facilitate studies aimed at modeling cooperating genetic events and investigating the importance of their temporal sequence in tumor evolution (Wang et al., 2013). These techniques can also be extended to *ex vivo* models, as recently demonstrated by elegant

work in which multiple recurrent mutations observed in colorectal cancers were modeled in human intestinal organoids (Drost et al., 2015; Matano et al., 2015).

Gene discovery—In addition to being an invaluable tool to create specific cancer models, CRISPR-based strategies are being increasingly applied to perform targeted and genome-wide functional screens designed to identify new cancer genes and uncover cancer-specific vulnerabilities (Figure 1). Two general strategies have proven particularly useful. The first is to use arrayed libraries to test each candidate gene individually. Although it is more laborious, expensive, and time consuming, the result of the screen is immediately available. Furthermore, virtually any measurable phenotype can be used as the readout of the screen.

The second approach is to use libraries containing pools of lentiviral or retroviral vectors, each expressing a different gRNA. The library is then transduced into the desired cell line. At the beginning and end of the experiment, the relative frequency of each gRNA in the pool is determined by deep sequencing; gRNAs that provide a selective advantage should become enriched in the pool (positive selection), whereas gRNAs that are deleterious for the cell will be depleted (negative selection). Positive selection screens using pooled libraries have been successfully used to identify tumor-suppressor genes involved in tumor growth and metastasis (Chen et al., 2015) and to uncover novel drug resistance mechanisms in melanoma (Shalem et al., 2014; Wang et al., 2014), while negative selection screens have allowed the identification of new therapeutic targets in leukemia (Shi et al., 2015). This strategy is appealing because of its simplicity, and genome-wide pooled CRISPR libraries are already commercially available (Sanjana et al., 2014). Furthermore, focused libraries against a subset of genes can be easily generated starting from on-chip synthetic oligonucleotides.

One limitation of CRISPR-based negative selection screens is that theoretically one in three indels generated by CRISPR-Cas is in frame. This means that in approximately 55% of cells receiving a given gRNA, one or both copies of the targeted gene will not be inactivated. Since for most genes both alleles must be lost for a phenotype to manifest, this greatly complicates the interpretation of depletion screens. This problem can be largely overcome by designing the gRNAs to target essential protein domains, so that even an in-frame indel is likely to have functional consequences. This idea was proposed by the Vakoc group and successfully applied to identify essential chromatin modifiers in leukemias (Shi et al., 2015).

Despite some limitations, high-throughput CRISPR screens have enormous potential (reviewed in (Shalem et al., 2015)), which is further increased by the recent development of CRISPR systems designed to modulate gene activity (Gilbert et al., 2014; Gilbert et al., 2013) and to directly degrade specific RNAs (Figure 1) (Abudayyeh et al., 2016).

Exploring the non-coding genome—Genome editing technologies are also greatly facilitating the functional characterization of the non-coding fraction of the mammalian genome. Long non-coding RNAs (lncRNA), in particular, account for a significant, but poorly characterized, fraction of the human transcriptome, whose role in the pathogenesis of human cancers is becoming increasingly apparent (Sahu et al., 2015; Schmitt and Chang, 2016). lncRNAs are very heterogeneous, differing in subcellular localization of their

transcripts (nuclear, cytosolic, and even mitochondrial), genomic structure, and mechanism of action (Ulitsky and Bartel, 2013). This diversity, and the lack of an open reading frame (ORF), complicates investigation of lncRNAs, as conventional loss-of-function approaches available to the study of coding genes are not always applicable, and great care must be exercised in designing and interpreting functional experiments (Bassett et al., 2014). For example, although RNAi-mediated knockdown has been used with some success to specifically down-regulate lncRNAs, its effectiveness on lncRNAs that have a predominantly nuclear localization is unclear. Furthermore, because the majority of lncRNAs lack clear functional domains, conventional gene-targeting methods aimed at deleting one or two exons, while effective for protein coding genes, are generally not suitable for lncRNAs.

CRISPR-based technologies provide more effective tools to query the biology of lncRNAs. First, using appropriately designed gRNA pairs, entire lncRNA loci can be efficiently deleted, thus guaranteeing the creation of true *null* alleles (Figure 1). Furthermore, methods to facilitate the generation of pooled libraries expressing hundreds or thousand gRNA pairs have been recently developed, enabling the use of this strategy for functional screen of non-coding genetic elements (Vidigal and Ventura, 2015). Second, programmable nucleases can be used to insert floxed transcriptional stop cassettes immediately downstream of a lncRNA promoter, thus allowing complete and reversible gene inactivation without the risk of inadvertently deleting other DNA regulatory elements (Figure 1). This strategy was recently used by the Mendell group to demonstrate the role of the lncRNA NORAD in preserving genomic stability (Lee et al., 2016) and is becoming the gold standard to generate true loss-of-function lncRNA alleles.

Equally useful is the possibility to modulate lncRNA function in cells and animals without directly changing the genome: the use of CRISPRi or CRISPRa to inhibit or activate the transcription of individual lncRNA loci, for example, is particularly appealing and can in principle be scaled to perform cell-based and *in vivo* functional screens (Figure 1).

Analogously, the discovery of C2c2, an RNA-guided RNA endonuclease (Abudayyeh et al., 2016), and the adaptation of Cas9 to target RNAs (O'Connell et al., 2014), will facilitate the functional interrogation of non-coding RNAs and the study of post-transcriptional RNA modifications.

Many of these strategies are also enabling the systematic characterization of *cis*-regulatory regions (promoters and distal elements). Mounting evidence indicates that promoters, enhancers, and insulators are recurrently mutated in cancers (Katainen et al., 2015; Khurana et al., 2016; Melton et al., 2015; Weinhold et al., 2014). By providing an effective way to model these genetic lesions in cells and *in vivo*, genome-editing tools are dramatically accelerating the characterization of this important class of cancer-associated mutations. Recent successes include the functional dissection of an enhancer via CRISPR-mediated saturation mutagenesis (Canver et al., 2015), the demonstration that inversion of a single CTCF site can affect genome topology (Guo et al., 2015), and that a recurrent chromosomal rearrangement promotes leukemia by repositioning a single enhancer (Groschel et al., 2014) (Figure 1).

Applications of genome-editing tools to cancer research: the future

The examples discussed so far highlight the already impressive impact that new gene-editing tools have had on cancer research. Yet, this technology is still young and likely to evolve at a rapid pace. Although predicting the future is notoriously a dangerous exercise, in this section we examine three major areas—modeling specific mutations *in vivo*, manipulating chromatin modifications, and gene therapy applications—that are likely to benefit from the genome editing revolution in the near future.

Modeling specific mutations *in vivo*—A single nucleotide change in codon 12 of the *H-ras* gene was the first oncogenic mutation identified in human cancers (Der et al., 1982; Parada et al., 1982; Santos et al., 1982). Since then, hundreds of activating mutations in oncogenes have been identified, the importance of which cannot be overestimated in the pathogenesis of human cancers. Point mutations are also recognized as an important mechanism for acquired resistance to targeted anticancer therapy and there is a growing need to improve our ability to model this class of mutations *in vitro* and *in vivo*.

Although CRISPR-Cas-mediated mutagenesis is highly effective at generating random indels and loss-of-function alleles, modeling precise gain-of-function mutations is less straightforward as it requires that the CRISPR-induced DSB is repaired by the HDR pathway in the presence of an appropriate repair template (Figure 1) (Mali et al., 2013). Because HDR activity is restricted to cells in the S and G2 phase of the cell cycle, the efficiency of CRISPR-induced HDR in non-dividing cells and *in vivo* is very low. For example, attempts to introduce point mutations in the *β-catenin* gene or to repair a fumarylacetoacetate hydrolase (FAH) mutation in the liver of adult mice by hydrodynamic delivery of CRISPR-Cas9 resulted in ~0.5% of hepatocytes harboring the desired mutation (Xue et al., 2014; Yin et al., 2014).

To improve the efficiency of this technology, several strategies have been proposed. For example, timed delivery of the CRISPR system to cells synchronized in the S phase can substantially increase the rate of HDR (Lin et al., 2014). Alternatively, restriction of Cas9 expression to the S-G2 phase of the cell cycle has been achieved by forcing its degradation in G1 by fusing Cas9 to the N-terminus of Geminin (Gutschner et al., 2016). Other approaches that have shown promising results include pharmacologic or genetic suppression of components in the NHEJ pathway (Chu et al., 2015; Maruyama et al., 2015), as well as optimized design of the single-stranded donor DNA complementary to the non-targeted strand (Richardson et al., 2016). Finally, an elegant alternative strategy that has been recently proposed is to bypass HDR altogether and induce site specific C → T (or G → A) transition by fusing dCas9 with the cytidine deaminase APOBEC1 (Komor et al., 2016).

Despite the progress made, the efficiency of precise gene-editing in most cells and *in vivo* remains too low for many applications. It is likely that further improvements in HDR efficiency will overcome this limitation, for example, by increasing the local concentration of the HDR machinery or of the donor template at the cleavage site.

Manipulating the epigenome—Aberrant gene regulation is a common and important feature of human cancers, and is often reflected by local changes in chromatin modifications

and DNA methylation (Baylin and Jones, 2011). Reproducing these changes *in vivo* is not possible with conventional gene-targeting methods, but could soon be made feasible by clever adaptations of the CRISPR-Cas technology (Figure 1).

Two groups recently showed that dCas9 can be used to modulate transcription and to induce sequence-specific epigenetic modifications (Dominguez et al., 2016; Shalem et al., 2015). Transcriptional activators or repressors can be brought to the target gene by dCas9, to enhance or suppress its expression, respectively, through either direct fusion (Gilbert et al., 2013) or scaffold RNA (scRNA)-mediated recruitment (Konermann et al., 2015) (Figure 1).

Pioneering studies indicate that using a similar strategy to couple chromatin-modifying enzyme and dCas9 can generate local, site-specific, histone or DNA modifications (Figure 1) (Hilton et al., 2015; Kearns et al., 2015; Vojta et al., 2016). In principle, multiplexed epigenetic perturbations could also be obtained by simultaneous or sequential recruitment of multiple epigenetic regulators (Dominguez et al., 2016). Although the repertoire of epigenetic marks that have been modeled is still limited and largely restricted to cell-based systems, it is likely that these strategies will soon be adapted to model more complex transcriptional regulation processes *in vivo*.

Therapeutic applications: CAR T cell therapy—Chimeric antigen receptor (CAR) engineered T-cell therapy, in which patient-derived T cells are modified to recognize and target an antigen expressed by the tumor cells, has been shown to be highly effective in relapsed and refractory B-cell acute lymphoblastic leukemias (ALL) and other B-cell malignancies (Maude et al., 2014). In principle this approach could be adapted to target other cancers (Kershaw et al., 2013) provided that suitable antigens are identified.

Generating autologous CAR-engineered T cells from patients is time consuming and expensive, however, which may limit the widespread application of this promising therapeutic strategy in the clinic. In principle, the use of pre-manufactured universal CAR T cells would address these issues, as they would avoid the need to derive new CAR T-cells from each patient. But to be effective, universal CAR T cells should be also engineered so that they do not attack other host antigens (graft versus host disease, GVHD) and are not destroyed by the host immune system (host versus graft disease, HVGd).

Genome editing could help to achieve this goal. First, CRISPR-Cas9 could be used to genetically ablate the endogenous TCR from the universal CAR T cells (or to directly replace it with the desired TCR), rendering them unable to recognize and attack other antigens in the host. Preventing HVGd seems more challenging, although in principle this could be achieved by deleting the human leukocyte antigen (HLA) genes (Torikai et al., 2013). Clearly, several technical and safety issues must be overcome before this strategy can be applied to the clinic, but this example well illustrates the enormous therapeutic potential of genome editing methods.

Therapeutic applications: repairing cancer-causing mutations—The ability to revert disease-causing mutations in somatic cells has long been the Holy Grail of gene therapy, and genome-editing tools offer a concrete possibility of achieving this goal. For

example, deletion of exon 23 of the mutated dystrophin gene by CRISPR technology can partially rescue the skeletal and cardiac muscle dysfunction in a mouse Duchenne muscular dystrophy (DMD) model (Long et al., 2016; Nelson et al., 2016; Tabebordbar et al., 2016), while correction of a mutation in the FAH gene in the liver has been used to treat a mouse model of human hereditary tyrosinemia (Yin et al., 2016; Yin et al., 2014).

Despite these early successes in pre-clinical models of non-cancer diseases, the idea of using CRISPR-Cas9 to repair or inactivate cancer-causing mutations poses unique challenges. In contrast to DMD and hereditary tyrosinemia, where correction of the defect in a fraction of cells can still have a substantial and lasting therapeutic effect, the nature of cancer requires the gene defect to be corrected in all or nearly all cancer cells, as even a few unedited cells would be enough to cause relapse.

As the current *in vivo* editing efficiency remains well below 10%, this is far from being feasible and several major roadblocks must be overcome. First, the efficiency of CRISPR delivery *in vivo* needs to be greatly increased. The discovery of smaller CRISPR effectors that can be more easily packaged into recombinant viral vectors (Kleinstiver et al., 2015; Ran et al., 2015; Zetsche et al., 2015) is a first encouraging step. An interesting alternative is to combine viral and non-viral delivery methods. For example delivering the Cas9 mRNA packaged into lipid nanoparticles, while the gRNAs and the repair template are packed together in an adeno-associated virus (AAV), was recently shown to significantly improve the efficiency of FAH gene repair in the liver (Yin et al., 2016).

Second, even if the delivery efficiency is increased enough to be effective, safety issues need to be addressed. Other than the dangers associated with random integration of lentiviral and retroviral vectors, undesired off-target effects of CRISPR need to be considered carefully before moving to the clinic (Pattanayak et al., 2013). The spectrum of off-target sites of a specific guide RNA can be experimentally determined by several technologies including “Guide-seq” (Tsai et al., 2015) and “Digenome-seq” (Kim et al., 2015), but improved computational algorithms need to be developed to more effectively predict off targets *in silico*.

Efforts to reduce the off-target rate of CRISPR are also underway. These include the development of more accurate endonucleases generated through structure-guided mutagenesis of Cas9 (Slaymaker et al., 2015) and the use of double CRISPR nickase (Ran et al., 2013) to effectively reduce off-target editing across the genome. Finally, it is important to remember that Cas enzymes are not present in mammalian genomes and when delivered to humans are likely to induce an immune response that could impair treatment effectiveness (Wang et al., 2015).

Concluding Remarks

Few technologies in cancer research have had a more profound and nearly instantaneous impact than the development of CRISPR-based genome editing tools (Dow, 2015). For many groups, ours included, this revolution has meant solution to problems that were previously daunting and has opened up avenues of research once only dreamed about.

As it is inevitable with any technological revolution as it reaches maturity, the current wave of excitement will fade and the race toward the next cool CRISPR application will be replaced by the steady accumulation of new biological insights into how cancers form and evolve. The true benefits of this new and exciting technology are hard to predict at this stage, but it is likely that they will include the identification of novel therapeutic vulnerabilities and the development of more effective treatments.

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Gene editing applications of CRISPR technology				Non-editing applications of CRISPR technology			
	Schematic	Applications	Reference		Schematic	Applications	Reference
Generation of small indels		<ul style="list-style-type: none">• Gene inactivation• Modeling loss-of-function mutation	Cong et al., 2013 Wang et al. 2013	Transcriptional regulation		<ul style="list-style-type: none">• Precise regulation of gene expression• Genome-wide gene activation/repression screens	Gilbert et al., 2013 Gilbert et al., 2014 Konermann et al., 2015 Dominguez et al., 2016
Point mutations		<ul style="list-style-type: none">• Precise mutagenesis• Modeling gain-of-function mutation	Mali et al., 2013 Xue et al., 2014	Chromatin modification		<ul style="list-style-type: none">• Epigenetic regulation on cis-regulatory elements• DNA methylation	Kearns et al., 2015 Hilton et al., 2015 Dominguez et al., 2016 Vojta et al., 2016
Tag/large fragment insertions		<ul style="list-style-type: none">• Protein/lncRNA tag labeling• Fluorescence reporter insertion• Recombination signal (LoxP/Frt) insertion• Insertion of transcriptional STOP signal	Yang et al., 2013 Yang et al., 2014 Lee et al., 2016	Visualizing genomic loci		<ul style="list-style-type: none">• Visualizing repetitive and non-repetitive genetic elements• Revealing chromatin dynamics by live-cell imaging	Chen et al., 2013 Fu et al., 2016
Large fragment deletions		<ul style="list-style-type: none">• Gene Deletion• Investigating higher-order chromatin structure	Yang et al., 2014 Gröschel et al., 2014	Targeting ssRNA		<ul style="list-style-type: none">• Targeting specific RNA• Modulating non-coding RNAs	Abudayyeh et al., 2016
Chromosomal rearrangements		<ul style="list-style-type: none">• Chromosomal rearrangement	Choi et al., 2014 Maddalo et al., 2014 Blasco et al., 2014				
Manipulation of cis-regulatory elements		<ul style="list-style-type: none">• Regulation of gene expression by cis-regulatory element	Guo et al., 2015 Gröschel et al., 2014 Canver et al., 2015				
DNA-guided genome editing		<ul style="list-style-type: none">• DNA-guided DNA editing• Multiplexed gene targeting	Gao et al., 2016				

Figure 1. Genome-editing tools in cancer research
Applications of programmable nucleases of potential relevance to cancer research are shown. The left panel illustrates applications resulting in genetic changes in the recipient cell, while the right panel lists applications that do not modify its genome.