Editorial

The CRISPR road: from bench to bedside on an RNA-guided path

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The development of next-generation sequencing has greatly expanded our understanding of genomic alterations in cancer. This has furthered a need for new research strategies, particularly the generation of animal models for novel genetic alterations such as insertions, deletions or chromosomal rearrangements. As sequencing technology has increased its efficiency and resolution in recent years, the classic generation of genetically engineered mouse models cannot keep up with the pace of new discoveries (1). Gene editing in embryonic stem cells using homologous recombination has provided excellent mouse models to study alterations in tumor suppressor genes and oncogenes, however this approach is time-consuming and costly. Another significant limitation to these models is that germline mutations are constitutively present throughout all tissues in the mouse, limiting their relevance to the organ-specific somatic mutations that produce most human cancers (2). cDNA-based over-expression and RNA interference-mediated knockdown have also allowed scientists to study particular genes or changes in expression of key drivers, but these models are far from physiological (3). Ideally, a successful model system would overcome these key limitations, and would also be able to adapt quickly to accommodate the plethora of new targets and growing diversity of genomic changes that have been implicated in cancer development.

In the last 2 years, a new technology has revolutionized the field of genome editing. In addition to the costly and cumbersome strategies already available, namely Zinc Finger Nucleases (ZFNs) (4) and Transcription Activator-Like Effector Nucleases (TALENs) (5), a novel method was introduced and quickly captured the interest of the community (6). Derived from the immune system of prokaryotes, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9-associated system combines a protein (Cas9) and a single RNA guide (sgRNA) that recognizes a complementary 20-nucleotide genomic sequence downstream a protospacer-adjacent motif (PAM) sequence (NGG) (7). Cas9 is a nuclease that generates a double-stranded DNA break, which the cellular DNA repair system repairs either through non-homologous end joining (NHEJ) or homology-directed repair (HDR), resulting in indels or precise editing respectively. The scientific community quickly embraced the potential of this technique and numerous *in vitro* genetic modifications were generated with ease (8).

One significant advantage of the CRISPR system is that it can be used to model oncogenic chromosomal rearrangements that were difficult to create with previous technologies. The production of mouse models to study such rearrangements is laborious and limited in application, creating non-physiological levels of the oncogenic gene fusions by ectopic over-expression (9). CRISPR overcomes these limitations, as previous work has demonstrated *in vitro* (10). However, the use of this editing technique was not tested *in vivo* on somatic cells until Maddalo *et al.* (11) attempted to replicate the most common *EML4-ALK* variant in non-small cell lung cancers (NSCLCs). An inversion on the short arm of chromosome 2 [inv(2)p21p23] generates the *EML4-ALK* oncogene that can be detected in a subset of NSCLC, most commonly adenocarcinoma, and which causes a constitutive activation of ALK. Although this alteration is only seen in a small percentage of NSCLC cases, it is of special interest as it confers sensitivity to ALK inhibitors, allowing for targeted therapy (12). Secondary mutations in the fusion gene can also promote resistance to these drugs, meaning it is crucial

to model this variant and its influence on drug sensitivity and resistance.

Maddalo *et al.* used a recombinant adenovirus to deliver Cas9 coupled with sgRNAs targeting *EML4* and *ALK* sites, producing concomitant double-strand DNA breaks in specific introns of each gene. Breaks in these sites provoked an inversion that models the human *EML4-ALK* variant. This was first tested *in vitro*, and then the adenovirus was delivered to adult mice by intratracheal instillation, where it also successfully produced the desired inversion. The generation of tumors was fast (large tumors developed 12-14 weeks post-infection) and specific, with histopathological characteristics corresponding to the phenotype observed in human patients with adenocarcinoma. This work was the first to demonstrate that the CRISPR-Cas9 system can be used to engineer large deletions, inversions and chromosomal translocations *in vivo* (13). Other groups have since achieved similar success using CRISPR-Cas9 system to edit the genome *in vivo* (10,13,14).

Maddalo *et al.* also show that the CRISPR-Cas9 system can serve as an appropriate and useful model of cancer drug resistance. After the *EML4-ALK* inversion had been generated *in vivo* and tumors had appeared, the group treated the mice with crizotinib, a dual ALK/MET inhibitor currently in use for ALK^+ NSCLC in human patients (12). They showed that the tumors were sensitive to the drug, achieving complete regression in 6 out of 7 cases, and partial regression in 1 out of 7. This method opens the door to the study of sensitivity and mechanisms of resistance to chemotherapy agents in different subsets of cancer, to determine optimal therapies for each one.

This work demonstrates the CRISPR-Cas9 system can be used to create highly customizable genetic alterations such as chromosomal rearrangements. It is an accessible and expeditious model for tumor initiation and progression, as well as drug susceptibility and resistance in cancer. This method of producing chromosomal rearrangements holds several advantages. Since the rearrangement was induced locally in a group of somatic cells, it generates lesions more similar to those seen in humans. Also, by creating a modification of the endogenous loci, the changes in expression of the fusion oncogene is within physiologic levels, contrary to ectopic over-expression that distorts the phenotype of the cancer. Finally, by using a hit and run system, without modifying the germline, the model can easily be adapted to other species like pigs or non-human primates. Since the publication of this study, numerous groups have optimized the process, achieving multiplex modifications, screening of different mutations, and improved efficiency or specificity of the modifications (15,16).

Precise genome editing enables faster and easier modeling of diseases, promising to fast-track translational research. One possibility offered by this technology is the creation of personalized platforms, building specific models to mimic particular presentations of cancer and test drug sensitivity. Another avenue of research is the *ex vivo* reprogramming of immune cells in immunotherapy. Perhaps even more exciting is the potential of this technology to be used directly as a therapeutic tool to correct mutations or rearrangements in the patient. There are groups that have managed to apply CRISPR-based editing to successfully correct genomic alterations *in vivo*, reverting the affected phenotype (17).

Genome editing using CRISPR-based technology is not without limitations. Currently, the two major challenges that this approach faces are off-target effects and difficulties in delivery. An increasing number of studies show a high frequency of off-target effects using sgRNAs, at an even higher level than is seen with TALENs (18). However, many groups have assembled web-based software programs to assist with designing sgRNA target sites *in silico* to improve specificity (http://tools.genome-engineering.org; http://zifit.partners.org; www.e-crispr.org; http://crispr.mit.edu/; http://crispr.dbcls.jp/; http://crispr.cos.uni-heidelberg.de/help.html). There is some evidence truncated guides may be more specific. A double nickase strategy can also be employed to improve specificity, in which one of the Cas9 cutting sites is mutated so the protein cleaves only one strand of DNA (19). Targeting two Cas9 nickase mutants to adjacent regions of the DNA in order to induce gene editing means reduced likelihood of double strand breaks occurring at off-target sites. Concerning the delivery of plasmids or oligonucleotides, CRISPR faces the same challenges that ZFNs, TALENs, or even shRNA face today. So far, most gene-based therapy research is performed using viral vectors to deliver the system to mammalian cells, which presents some concerns for translational potential (20). However, significant advances in non-viral delivery systems could overcome most of these limitations (21). Developments in nanotechnology and materials have increased alternatives available for gene delivery, but these systems are not yet developed enough to use in patients.

Overall, the work presented by Maddalo *et al.* shows a promising adaptation of a novel technology, the CRISPR-Cas9 system, and its potential for modeling human disease. The past 2 years have changed the landscape of genome editing, and despite a number of limitations, the possibilities

presented by this technology are exciting, extending from basic research to clinical applications. Sánchez-Rivera and Jacks (22) comment, "we envision a new era in cancer biology in which CRISPR-based genome engineering will serve as an important conduit between the bench and the bedside". Each week sees the publication of new solutions similar to the unprecedented modelling of complex chromosomal alterations explored by Maddalo *et al*. As this technology develops, so too will our ability to precisely edit the genome to diagnose, study and treat cancer and other diseases.

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Footnote

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