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REVIEW

Harnessing the potential of CRISPR-based platforms to advance the field of hospital medicine

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ABSTRACT

Introduction: Clustered regularly interspaced short palindromic repeats (CRISPR) are segments of nucleic acid that play a role in prokaryotic defense and form the basis of a genome editing technology that allows permanent alteration of genetic material. This methodology, known as CRISPR-Cas9, is poised to revolutionize molecular biology, but no literature yet exists on how these advances will affect hospitalists.

Areas covered: These specialists in inpatient medicine care for a wide variety of hospitalized patients, including those with infectious disease, cancer, cardiovascular disease, autoimmune disease, hematologic disease, and a variety of other conditions that may soon be impacted by advances in gene-modifying technology provided by CRISPR-Cas9. A Literature search was performed using PubMed [1 December 2019–17 April 2020].

Expert opinion: This paper reviews the remarkable diagnostic and therapeutic potential of the CRISPR-Cas9 platform and concludes with a look at ethical issues and technical hurdles pertaining to the implementation of permanent gene modification in the practice of Hospital Medicine.

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1. Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) are segments of nucleic acid containing short, repetitive base sequences that play an important role in prokaryotic defense and form the basis of a genome editing technology that allows permanent modification of genes known as CRISPR-Cas9, as well as CRISPR-Cas12a and CRISPR-13 [1–3]. While much has been written about how this novel methodology will contribute to scientific inquiry, no literature yet exists on how these advances will affect hospitalists – specialists in inpatient medicine – who care exclusively for hospitalized patients suffering from a variety of maladies [4–6]. This paper reviews the potential of the CRISPR platform and explores how this technology may soon impact the field of Hospital Medicine, which has emerged as the fastest growing subspecialty in the history of medicine [7]. It concludes with a look at ethical issues and technical hurdles pertaining to the implementation of permanent gene modification in clinical practice.

2. Background

The CRISPR-Cas system evolved in microbes as an immune system in prokaryotes that confers resistance to foreign genetic elements such as those present within plasmids and phages [8,9]. The Cas9 protein performs gene interrogation by unwinding foreign deoxyribonucleic acid (DNA) and determining whether the sequence is complementary to the 20-base pair spacer region of the guide ribonucleic acid (RNA) [10,11]. The CRISPR-Cas9 system can be used to manipulate and excise stretches of DNA that have profound relevance for human health, ranging from aggressive malignancies to mitochondrial storage diseases, as well as potential applications in the prevention and treatment of infectious diseases such as human immunodeficiency virus, influenza virus, and malaria, among many others [12–16]. Other genetic modification systems exist, such as CRISPR-12a and CRISPR-13, but they are outside of the scope of this manuscript, which will focus on CRISPR-Cas9.

Initial applications of gene modification have dealt primarily with conditions that are treated by subspecialty providers (oncologists, rheumatologists, infectious disease specialists), but the CRISPR-Cas9 platform also has profound relevance for primary providers, including pediatricians, obstetricians/gynecologists, primary care physicians, and hospitalists, which will be the focus of this manuscript.

The term 'hospitalist' was coined in 1996 by Watcher and Goldman and since that time, Hospital Medicine has emerged as the fastest growing subspecialty in the history of medicine [17]. There are currently more than 60,000 hospitalists practicing in the United States, and current professional trends suggest that field will continue its extraordinary growth in the decades ahead [7,18]. Hospitalists care for a broad array of medical maladies, ranging from blood clots to pneumonia, and emerging research suggests that many of the medical conditions under the hospitalist's purview could be influenced by advances in the CRISPR-Cas9 [19,20]. Given the rapid evolution of this technology and the widespread appreciation of its potential to alter the treatment of disease, it is necessary for

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Article highlights

- Clustered regularly interspaced short palindromic repeats (CRISPR) are segments of nucleic acid that play an important role in bacterial defense and form the basis of a genome editing technology known as CRISPR-Cas9.
- CRISPR-Cas9 allows for the permanent modification of genetic material and may be harnessed to treat a variety of diseases in humans.
- Many of the applications of CRISPR-Cas9 may ultimately affect hospitalists—specialists in inpatient medicine—who serve as the primary caregivers for patients with a wide variety of maladies, ranging from heart disease to cancer.
- The CRISPR-Cas9 system has been employed to serve as a diagnostic tool for viral infections.
- CRISPR-based human genome editing comes in two forms: heritable germline editing, and non-heritable somatic modifications.
- Germline edits with CRISPR-Cas9 are passed on to future generations.
- In 2019, the World Health Organization recommended against any clinical research on human germline editing until all technical and ethical considerations have been properly vetted.

hospitalists to be familiar with the relevance of CRISPR-Cas9. An overview of the most common conditions cared for by hospitalists is provided below followed by an examination of how management of these diseases may change with advances in somatic and germline gene modification [21].

3. Infectious diseases

The emergence of the hospitalist has altered the care of infectious diseases at many hospitals [22–24]. Patients requiring intravenous antimicrobial therapy are often cared for by hospitalists, with infectious disease specialists serving as consultant (when possible). This means that hospitalists are the primary providers for patients with a wide variety of infectious diseases, which may be broadly divided into the following subgroups: viruses, bacteria, fungi, and parasites [25,26]. Although these groupings have inherent limitations, they do serve to delineate how CRISPR might be utilized to improve the approach to both diagnosis and treatment.

3.1. Viruses

The CRISPR-Cas9 systems evolved in microbes as a defense mechanism against viruses by cleaving nucleic acids from the invading bacteriophage [27,28]. For purposes of pathogen detection, cleavage of a labeled sing-strand RNA or DNA probe generates a signal that can provide a fluorescent readout in a number of portable formats, including the use of disposable paper strips, enabling rapid detection of pathogenic viruses [29]. CRISPR-based tests carry several advantages over traditional microbiological diagnostics such as polymerase chain reaction (PCR), which is generally considered to be the most sensitive and specific test available for viral detection. However, PCR require multiple steps to run the assay, including an upfront nucleic acid extraction and amplification steps, and require dedicated instrumentation [30–32]. By contrast, CRISPR-Cas-based assays can be run directly on primary clinical samples as a single reaction and performed using minimal equipment [33–36]. This is especially relevant for detection of SARS-CoV-2, where testing has been limited in many places due to a lack of testing materials and personal protective equipment [37–39].

As front-line healthcare providers, hospitalists are often the clinicians who must coordinate the work-up of a patient with a suspected infection. Determining the appropriate tests is essential to patient care but also to medical education [40,41]. Hospitalists have emerged as the primary medical educators on the general medicine service at many academic medical centers and they must be familiar with emerging assays to ensure that patients receive proper treatment and that the next generation of clinicians is properly informed [42,43]. This includes diagnostic tests for common and emerging viral pathogens.

The SHERLOCK assay has been successfully used to detect both Zika virus and dengue virus directly from bodily fluids, including urine and respiratory samples, in less than two hours and enabled discrimination among viral serotypes [29,44]. This approach may soon extend to serum samples, and has potential for the rapid diagnosis of human immunodeficiency virus (HIV) as well as hepatitis c virus (HCV) genotype in order to guide the choice of antiviral therapy, which may depend on the presence of single nucleotide polymorphisms [45–48].

Others have used the CRISPR-based DETECTR assay to detect human papillomavirus (HPV) types 16 and 18, which are associated with invasive genital tumors, and highlights the potential use of CRISPR-Cas technologies from infectious diseases to other fields, such as cancer (which will be discussed later in this manuscript) [49,50]. Although more work is necessary to standardize and validate this approach to diagnosis, CRISPR-Cas-based assays show tremendous potential for point-of-care diagnostic because of its ease of implementation, short turnaround time, direct detection from human samples, and colorimetric fluorescent readout [51–53].

3.2. Bacteria

CRISPR-based platforms also have the potential to detect and alter bacterial pathogens, including those that exist as external threats as well those that live within us and comprise the microbiome [54]. This platform also has tremendous potential to identify and eradicate bacterial resistance genes, which enable pathogens to evade or neutralize antibiotics [2]. One such approach, employed by Quan and colleagues, is FLASH (Finding Low Abundance Sequences by Hybridization), a next-generation CRISPR-based diagnostic method that leverages the flexibility and specificity of genetic modification [55]. This approach has been shown to antibiotic resistance genes in saliva and serum and may soon replace multiplex PCR [56]. However, significant hurdles remain. It is unclear how a CRISPR-based platform could remove all clinically-relevant resistance genes in a single bacterium or how this could be scaled up to remove these sequences from an entire population of pathogens.

CRISPR-based methods have also been used to treat drug-resistant bacterial infections. In one example, a fifteen-year-old girl with cystic fibrosis with a disseminated *Mycobacterium abscessus* infection (which included her skin) was treated

with a three-bacteriophage cocktail that had been manipulated using CRISPR [57]. Bacteriophage treatment was associated with marked clinical improvement in this patient who had undergone bilateral lung transplantation and had previously been unable to clear her infection, suggesting that phage therapy may prove to be a useful treatment for drug-resistant bacterial infections for other patients, many of whom are now managed by hospitalists at medical centers around the world [12,58].

3.3. Fungi

Mycotic infections are broadly divided into those caused by yeasts, molds, and thermally-dimorphic fungi [59]. Many of these organisms are ubiquitous in our environment, and preferentially infect patients with immune impairment [60]. These opportunistic pathogens are on the rise, due to advances in the treatment of cancer and autoimmune conditions, which have produced a variety of new drugs that alter the human immune system [61]. The arsenal of anti-fungal drugs is small – there are only three major classes of antifungal drugs – and novel treatment approaches are desperately needed. One such approach involves CRISPR.

Kwon and colleagues provided the first comprehensive analysis and evaluation of different CRISPR approaches for the modification of molds [62]. Guide RNAs were created and CRISPR nucleases were delivered to the filamentous fungus *Thermothelomyces thermophilus* on plasmids. The team was able to generated high numbers of positive transformants that could be useful for high-throughput assays to identify novel antifungal agents. This approach will likely extend to other fungi, and may yield insights into drug resistance associated with mycotic infection, which have emerged as an important cause of morbidity and mortality in immunocompromised patients.

3.4. Parasites

Parasites encompass a variety of human pathogens [63]. Parasitic worms, such as roundworms and flatworms, are amongst the most complex representing the sixth leading cause of morbidity worldwide and treatment options are limited [64]. Several investigators have employed CRISPR to interrupt essential genes in parasitic worms. For example, in the flatworm *Opisthorchis viverrini*, CRISPR-induced mutations were introduced into the *Ov-grn-1* transcript and protein levels were reduced within 48 h of transfection [65,66]. The livers of animals infected with modified worms were less swollen, and their biliary ducts had less scarring compared to the those of animals harboring unmodified worms, suggesting that this CRISPR-based method may serve as an approach to attenuate the pathogenicity of a devastating pathogen that affects humans around the world [67,68].

4. Cancer

The use of CRISPR holds tremendous promise for the treatment of infectious diseases, but many of those assays and therapies are years away. For cancer treatment, CRISPR-based therapies have already arrived [47]. In fact, preliminary results of the first phase-one study using CRISPR to treat cancer were recently revealed. In that study, T-cells from the blood of three patients (two with multiple myeloma and one with sarcoma) and used CRISPR-Cas9 to remove three genes from the cells, including two T-cell receptors as well as programmed cell death-1 (PD-1) [69]. The patients received modified cells after chemotherapy and the CRISPR-edited cells proliferated in all of the patients while no serious treatment-related adverse events were noted. This proof-of-principle study shows that CRISPR-based treatments for cancer are both safe and feasible and will serve as the basis for phase two studies, which will likely begin in the near future [69–72]. It is not yet known how these malignancies evade immune surveillance or how removal of these genes affected the efficacy of T-cells against cancer.

Another CRISPR-based approach to the treatment of cancer is based on the production of next-generation chimeric antigen receptor (CAR) T-cells, which are created to express cancer-targeting receptors [73,74]. CAR T-cell therapy targeting the CD19 antigen has been the most studied and successful due to its specific expression in various forms of leukemia. Recently, investigators treated a patient with aggressive lung cancer with T-cells edited by CRISPR [75]. Clinical trials the safety and efficacy of CAR T-cell immunotherapy for leukemia and lymphoma are ongoing and may soon expand to solid organ malignancies [74,76,77].

5. Heart disease

Heart disease comes in many forms [78,79]. So too, do the treatment options. Recently, CRISPR has been presented as a potential method to remove monogenic cardiovascular disorders from the offspring and subsequent generations of affected families [80]. Heritable cardiomyopathies, such as dilated cardiomyopathy, hypertrophic cardiomyopathy, and Duchenne muscular dystrophy, vasculopathies such as Marfan's syndrome, and infiltrative diseases such as amyloidosis, serve potential candidates for clinical applications of CRISPR-based germline genome editing techniques [81,82]. However, technical and ethical hurdles remain. Among these concerns remains the plight of future generations. Permanently editing the germline will affect unborn children who are unable to consent to such a procedure and its use is currently prohibited [83–85]. But that may soon change.

As the technology advances, CRISPR-based treatments for heart disease may expand beyond heritable cardiomyopathies. It is easy to foresee how gene-editing may be useful for polygenetic syndromes, including hypercholesterolemia, hypertension, and diabetes, among others [80,86,87]. For example, one group used CRISPR-Cas9 genome editing to target genes linked to insulin production, SPRY2, and the functional consequences of SPRY2 knockout and overexpression subsequently assessed using glucose uptake and lipid assays [88]. This work implicates a novel mechanism in the development of glucose intolerance and has profound implications for the treatment of diabetes, which is known to be an important risk factor for heart disease. Research in this area is ongoing [82].

6. Conclusions

CRISPR-based platforms have been described as the greatest scientific discovery of the twentieth century. However, access to these methodologies has been largely consigned to research laboratories. It is incumbent upon clinicians to familiarize themselves with CRISPR to both understand how it may benefit patients and to help identify future areas of inquiry [89]. As technical and ethical questions regarding the use of CRISPR in humans continue to mount, hospitalists must take a more active role in the discussions to ensure that relevant stakeholders have a say in determining how this technology is implemented. CRISPR provides tremendous potential but also comes with serious pitfalls, and the next decade will be an important period to determine how best to harness this platform to advance the field of hospital medicine.

7. Expert opinion

CRISPR-based platforms have profoundly altered molecular biology and are poised to redefine the practice of medicine [48,90–92]. This paper reviews some of the most promising developments in diagnostics and therapeutics that have pertinence to hospitalists – specialists in inpatient medicine – who care for patients with a wide variety of maladies, ranging from infectious diseases to cancer to heart disease [93,94]. It is important for hospitalists to understand this emerging new technology both to ensure that it is used properly and to identify areas where it might be further deployed.

This has become especially relevant with the emergence of the novel coronavirus, SARS-CoV-2. In many medical centers, hospitalists have been the primary physicians responsible for the care of patients with this infection. Diagnostic and therapeutic options have been limited, and hospitalists on the front lines have been forced to make treatment decisions with little or no data. This experience has reinforced the crucial role of the hospitalist in the care of patients with novel infections and it further strengthens the argument that hospitalists must be a part of the future research involving emerging pathogens. Frontline clinicians have a better understanding of the questions pertinent to patient care and can help formulate research projects that will address knowledge gaps. One area that has not yet been full explored is the role of CRISPR-Cas9 in the battle against SARS-CoV-2. Permanent nucleic acid modification may provide unique insights into a pandemic that has already infected more than two million people.

But this area of research comes with certain risks and important caveats [95]. CRISPR-based human genome editing comes in two forms: heritable germline editing, and non-heritable somatic modifications [36,89]. The former results in genetic changes that are passed on to future generations and is currently prohibited to clinical researchers [21,96]. In 2019, the World Health Organization recommended against any clinical research on human germline editing until all technical and ethical considerations have been properly vetted and this policy is unlikely to change in the foreseeable future [97–99].

In fact, some expert now believe that the most relevant hospital applications of CRISPR-Cas9 involve no editing of human DNA. Rather, they see the editing of the microbiota as a far more promising approach, as it would be non-heritable and non-somatic. DNA manipulation may extend to a variety of human pathogens, and may include deletion of viral DNA and cancer DNA to hamper proliferation of malignant cells. As mentioned above, CRISPR-based diagnosis may hasten detection of emerging viral pathogens, such as SARS-CoV-2.

For now, CRISPR-based clinical research will focus on the identification and alteration of infectious pathogens as well as novel treatment options for a variety of diseases ranging from infections to heart disease and cancer [45,100–103].

Recent research indicates that CRISPR has tremendous potential to improve the care of hematologic diseases, such as sickle cell disease and other heritable hemoglobinopathies, as well as autoimmune conditions, such as lupus [35,47,103–105]. But these opportunities must be weighed against potential downstream effects. CRISRP-based platforms have the potential to alter microbiota, which can be associated with adverse health events [106,107]. It will be crucial for hospitalists, who often care for these patients, to take a more involved role in this aspect of clinical research as CRISPR-based approaches will undoubtedly affect many of their patients in the years to come.

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