Annals of Integrative Oncology

Short Communication
Published: 04 Jul, 2020

CRISPR/Cas9 and Disease-Specific, Precision-Drugs Development

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Short Communication

CRISPR/Cas9 is a genome-editing tool derived from bacterial adaptive immune system. This natural system was modified for genome editing to introduce site-specific DNA double strand breaks and altering the mammalian genome with high precision [1]. The technology has been reported to be an easy, efficient, and scalable tool for biological research as it allows facile alteration of DNA sequences and modification of gene function [2,3]. It is now being developed further to decrease the size of the Cas9 nuclease and improves its targeting efficiency and accuracy [4].

CRISPR technology has been presented a "simple yet powerful tool for editing genomes". Its many claimed potential applications include correcting genetic defects, and treating and preventing the spread of diseases. To these ends, the technology has been applied to high throughput Gain-of-Function (GOF) or Loss-of-Function (LOF) screening. This is important in the context of identifying pathway elements that may be important in the development and progression of diseases such cancer [5]. For example, The GOF screens can identify positive and negative regulators of cancer; to this end, CRISPR has been applied as a screening tool for this purpose, reducing some of the limitations associated with previously applied approaches, for example off target effects, high-false positive and negative outcomes, etc. [6]. When applied to *in vivo* studies, CRISPR screens can generate information related to so-far elusive subject of complex interactions between the cell and its microenvironment that influence tumor behavior. For example, an *in vivo* CRISPR screen investigated the effect of selected mutagenized genes in tumor growth and metastasis [7]. Similarly, CRISPR-mediated editing has been used for generating disease models, for example for preclinical validation of cancer drug targets [8].

The first application of CRISPR/Cas9 in clinical investigations tested cancer-gene editing in patients with aggressive lung cancer [9]. Immune cells from patients' blood were edited by CRISPR/Cas9 *ex vivo* to disable PD-1 protein [10]. A clinical study that uses a combination of TALENs and CRISPR/Cas9, and targets HPV16 and HPV18 E6/E7 DNA in the treatment of HPV-related cervical neo-plasma is on-going [11].

However, the CRISPR/Cas9 approach to developing high-precision drugs is far from being error-free and reliable; it has been reported to fail some 15% of the time [12,13]. Studies have shown that binding of Cas9 to DSBs limits access of repair proteins to the break site, making the Cas9-DSB-complex formation the rate-limiting step during *in-vivo* genome editing. Further, stereo effects come into play in the translocating efficacy of RNA polymerase [14,15]. An important limitation of the CRISPR/Cas9-mediated editing *in vivo* originates from the humoral and cell-mediated adaptive human immune response to bacterial Cas9 [16]. Also, it has been reported that the repair of DNA breaks introduced by Cas9 can result in extensive deletions and genetic alterations [17]. This may result in unwanted on-target effect of CRISPR/Cas9 such as activation of dormant oncogenes, inactivation of tumor-suppressors genes, affecting other disease-causing factors. Off-target effects of CRISPR/Cas9 editing are also possible and may include incorporation of DNA mismatches in PAM-distal part of the sgRNA sequence [18-22].

The strategies that have been proposed to avoid or minimize off-target effects of CRISPR/Cas9 editing are very similar to what has been stated as the essential requirements for developing disease-targeted precision medicines [23-26], namely the need for selecting unique target sites with no homologies to any other part of the genome. As an example, this might be achieved by fusing dCas9 with sequences that possess high targeting specificity such as FokI nuclease (fCas9) [27]. Further, in terms of drug delivery, the large size of Cas9 makes it difficult to package the protein in low immunogenic AAV vectors used *in vivo* and *in vitro* gene delivery. Also, Cas9 from *S. aureus* and *S. pyogenes* has been shown to cause infectious diseases in humans [8].

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Received Date: *01 Jun 2020*
Accepted Date: *01 Jul 2020*
Published Date: *04 Jul 2020*

Citation:
Petrak K. CRISPR/Cas9 and Disease-Specific, Precision-Drugs Development. Ann Integr Oncol. 2020; 1(1): 1003.

When applied to developing therapies for cancers, CRISPR has not as yet resolved all the issues associated with the lack of understanding of the molecular basis of the disease or helped to bring about cost-effective treatment regimens; the issues of differential mutational load, tumor heterogeneity and therapeutic resistance have not been resolved; neither has it generated the truly disease-targeted cancer therapies. The potential of CRISPR technology to achieve these remains to be demonstrated.

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