Cancer Treatment and Research Communications 26 (2021) 100304

Contents lists available at ScienceDirect

Cancer Treatment and Research Communications

journal homepage: www.sciencedirect.com/journal/cancer-treatment-and-research-communications

Milestones of CRISPR/Cas9 system, promises and roadblocks

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A B S T R A C T

In this issue of Cancer Treatment and Research Communications, Sarkar et al. describe CRISPR/Cas9-based gene-editing from its discovery to current views. Originally a bacterial adaptive immune system, CRISPR/Cas9 was adapted to mammalian cells as a tool to perform a wide range of actions given its capability of accurately targeting specific DNA loci. While the CRISPR/Cas9 system is readily used in laboratories as a research tool for a few years now, the first clinical trials have recently started utilizing this system in translational medicine. Although several obstacles have been resolved related to the usage of CRISPR/Cas9 on mammalian cells, the scientific community is facing new challenges with this technology.

Main text

Development of effective and safe technologies is essential in fulfilling humankinds' dream to treat and prevent diseases. Great minds' deep curiosity and careful observation of nature revolutionized research and therapy in medical sciences. Several research tools and drugs currently in use were the result of such studies. One of the latest gifts from nature was the Nobel winning CRISPR/Cas9 gene-editing method. Originally, CRISPR/Cas9 provides adaptive immunity in bacteria against invading organisms, where it identifies and attacks certain DNA sequences to clear the infection.

Scientists adapted this system to mammalian cells as a tool with promising capabilities due to its ability to accurately target specific DNA sequences. As Sarkar et al. mentioned, CRISPR/Cas9 is an easy, cost effective and faster tool in comparison to previous gene-editing methods [1]. Moreover, just after its first application on mammalian cells less than 10 years ago, several diseases were investigated by CRISPR/Cas9-mediated gene-editing and new tools have been developed for several purposes including but not limited to genetic manipulation, transcriptional modulation, and DNA labelling. It was quite exciting for researchers to incorporate CRISPR/Cas9 system into their research for those reasons. However, just like any other tool humankind explored, CRISPR/Cas9 also brought several challenges.

Theoretically, the protospacer adjacent motif (PAM) sequence, a specific gRNA targeting gene of interest and active Cas9 protein are enough for this gene-editing technology to work. However, several problems have been encountered through the years related to using this technology on mammalian cells. Sarkar et al. describes those problems as on-target damage, off-target editing, low efficiency and problems with introduction of the system into the human body. Even though it has been successfully incorporated into laboratory research, direct gene-editing on human embryos has been found risky due to unwanted chromosome alterations caused by inaccurate gene-editing [2]. With large data obtained from studies using CRISPR/Cas9 for gene-editing, several strategies are being developed to overcome those problems and ameliorate the efficiency of the CRISPR/Cas9 system. Since the obstacles are mostly related to the specificity of the system, strategies for improvement are particularly related to gRNA design and delivery of the required particles to the target cells [3]. In addition to the direct gene-editing related problems mentioned above, some unresolved issues related to CRISPR/Cas9-based gene-editing were presented to scientific community in recent years. For example, presence of wild type p53 has been shown to inhibit CRISPR/Cas9 based gene-editing in human pluripotent stem cells due to its function in DNA repair mechanisms [4]. Moreover, as one study showed that more than half of the donors investigated showed antibodies against *S. aureus* and *S. pyogenes* Cas9, it has been hypothesized that this may not only cause for low editing efficiency but may also lead strong immune responses in patients [5]. According to those results, it seemed like many obstacles should be overcome before the first gene-editing in human embryos can be performed. However, despite the scientific concerns, a controversial experiment has been done using CRISPR/Cas9-mediated gene-editing to promote HIV resistance in a human embryo in 2018 [6]. One year later, the people responsible for this experiment have been sentenced and fined. In addition to safety and health concerns, serious ethical matters have been raised related to gene-editing in humans. Before any next attempts, clear answers should be given to the problems mentioned above and a world-wide consensus with clear guidelines and restrictions should be reached [7].

Although -safe- gene-editing in embryos does not seem plausible in

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https://doi.org/10.1016/j.ctarc.2021.100304

Available online 5 January 2021
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the near future, several clinical studies utilizing CRISPR/Cas9 have been performed in recent years as described by Sarkar et al. The majority of the ongoing clinical trials using CRISPR/Cas9 are focusing on treatment of a variety of cancer types. In these studies, *ex vivo* approaches have been preferred as genetic manipulation is applied on somatic adult cells. As an example, some of the leading clinical studies are using gene-editing on T cells *ex vivo* prior to reinjection in the patient. A recent phase-I trial (NCT03081715) has shown promising results with respect to safety.

Overall, given the current rate of developments in the field of gene-editing, we can expect to hear more about the CRISPR/Cas9 technology in the near future. Still, there is a long way to go before this promising tool can be safely applied in humans. Hopefully, once safety and ethical concerns are resolved, CRISPR/Cas9-mediated gene-editing will serve as a reliable tool to treat and prevent human disease.

Author contribution

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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