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Conference report

Enhanced High Mutation Rate and Natural Selection to Produce Attenuated Viral Vaccine with CRISPR Toolkit in RNA Viruses especially SARS-CoV-2

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ABSTRACT:

The best and most effective way to combat pandemics is to use effective vaccines and live attenuated vaccines are among the most effective vaccines. However, one of the major problems is the length of time it takes to get the attenuated vaccines. Today, the CRISPR toolkit (Clustered Regularly Inerspaced Short Palindromic Repeats) has made it possible to make changes with high efficiency and speed. Using this toolkit to make point mutations on the RNA virus's genome in a coculture of permissive and nonpermissive cells and under controlled conditions can accelerate changes in the genome and accelerate natural selection to obtain live attenuated vaccines.

List of abbreviations

SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
sgRNA	single guide RNA
Cas9	CRISPR-associated protein 9
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
dCas9	Deactive Cas9
ADAR	adenosine deamination of RNA
EsCas13d	*Eubacterium siraeum* Cas13d protein
APOBEC	catalytic polypeptide-like
RESCUE	RNA Editing for Specific C-to-U Exchange
PAM	Protospacer Adjacent Motif
REPAIRv1	RNA Editing for Programmable A-to-I Replacement
GeCp	Genome-scale CRISPR/Cas 13 deletion and point mutation

1. Main Text

With the spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and becoming a pandemic crisis, the need for an effective vaccine for viruses has been considerably increased considered and several research studies have been focusing on making a vaccine to prevent the disease (Alturki et al., 2020). Attenuated vaccines are the most effective vaccines for immune system education, stimulation, and immunity (*e.g.* mumps, measles, vaccinia, rubella, rotavirus, and yellow fever) (). Natural selection is one of the best ways to make an effective attenuated vaccine (Fields et al., 2013). For this purpose, the viruses are consecutively cultured *in vitro* and *in vivo* for years to get attenuated by various random genome mutations (Sabin and Boulger, 1973). During long passages in a variety of experimental conditions (*e.g.* low temperature or different cellular and animal hosts), the driving force of natural selection tries to shift the virus populations toward the highest compatibility by fixing adaptive mutations (Dolan et al., 2018; Duffy et al., 2008). This is indeed time-consuming *in vitro* and ineffective oin some viruses especially DNA viruses because it needs to be passaged and purified many times to get an effective live attenuated virus vaccine and difficult to control while manipulated using *in vivo* conditions (Dolan et al., 2018). Therefore, a faster and more efficient system is needed more than ever.

Researchers are using a new toolkit called CRISPR to make targeted changes in the genome. An RNA-designed (single guide RNA [sgRNA]) for the target point is responsible for endonuclease protein (Cas9 [CRISPR-associated protein 9]), Cas12, and Cas13 guidance. By use of the CRISPR toolkit and modified CRISPR toolkit systems, we can knock-in and knock-out genes, transcription activation, and suppression, epigenetic changes, edit the base, and image-specific nucleic acids (Adli, 2018; Jamehdor et al., 2020). Research studies have shown that this system is effective in RNA and DNA editing (Kushawah et al., 2020; Ran et al., 2013). Although this toolkit has worked well in viruses' genome alterations as well, it has two major drawbacks including off-target (attach to other locations and make cuts in them) and delivery of the system to the target tissue (Xu et al., 2019; Zhang et al., 2015).

Type VI (Class 2 Cas proteins) systems are the only prokaryotic CRISPR-Cas immune systems known to target RNA (and not DNA) molecules exclusively, therefore, they show specific potential for RNA detection and manipulation (Konermann et al., 2018). Cas13 is recognized in several classes in which some have been studied (Abudayyeh et al., 2016; Kushawah et al., 2020; Smargon et al., 2017). Scientists by useing of targeted point mutations in Cas13, inactivate RNA cleavage site so, it can bind to RNA but has lost the ability to cut the RNA (called deactive Cas9 [dCas9]). Various effector protein domains have been fused to dCas13 to expand the functionality of Cas13 beyond the RNA cleavage. Fusion of ADAR (adenosine deamination of RNA) enzyme domain with Cas13 resulted in RNA editing (Cox et al., 2017). Recently to edit transcripts bases in mammalian cells, a newly established RNA editing technology called REPAIR is introduced (Cox et al., 2017). Other Cas13 enzymes, the *Eubacterium siraeum* Cas13d protein (EsCas13d) orthologs, the enzymes also do not require a protospacer flanking sequence, so one can target virtually any RNA sequence. Measuring the efficacy of Cas13d demonstrated 92% mCherry protein knockdown (Konermann et al., 2018). dCas13d as a form of deactive Cas13d and fusing to an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) change cytosine to uracil. For virus editing,

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one can use a viral genome-wide library of dCas13b combination in the RNA Editing for Programmable A-to-I Replacement (REPAIRv1) system and dCas13b RESCUE (RNA Editing for Specific C-to-U Exchange) system that derived from dCas13b (derived from Prevotella sep. P5-–125 (PspCas13b)), which does not require Protospacer Adjacent Motif (PAM) fragment, by creating targeted C to U and A to I off-target editing, is a suitable system for generating extensive changes on the RNA viruses genome (Fig. 1) (Cox et al., 2017) In this perspective we think that rate of mutation by CRISPR toolkit library is relatively high but many of these mutations are deleterious and small remaining part of mutations can be effective and functional. However, in some viruses such as influenza and SARS-CoV-2 induced mutation can be tolerated. In addition, the mutation on RNA made by CRISPR can only lead to part of amino acid substitution.

Although having an off-target effect is one of the drawbacks of this technology, it is considered as an advantage since this feature is used to create more mutations in the virus genome. In RNA viruses, a wider range of mutations can be induced with the CRISPR base editing toolkit. (Anzalone et al., 2019). This method of mutant libraries are used for the construction of populations of mutant viruses. These induced mutants include synonymous, non-synonymous, codon-deoptimization, and stop codon. The studies have been shown that these processes or manipulations result in the development of new attenuated variants of SARS-CoV-2 such as potential live attenuated vaccine (Le Nouën et al., 2021). In editing methods, amino acids are also broadly changed and the changes can affect virus protein function and configuration (Cox et al., 2017; Di Giorgio et al., 2020). These methods of mutant libraries are used for the construction of populations of mutant viruses.

After the production of a pool of mutant viruses using the CRISPR system, it should be subjected to the natural selection for new adaptation in the co-culture of “non-permissive” cells such as human fibroblast or MRC5 instead of Vero, Clau-3 (Harcourt et al., 2020). The mutated viruses in non-permissive cells drive to a new situation that is evolutionary different from their origins and are potentially live attenuated.

The mutated viruses in non-permissive cells drive to a new situation that is evolutionary different from their origins and are potentially live attenuated (Fig. 2). In this model, based on the form of selection, can also be selected the other features including viral drug-resistant mutants, low or high virulence mutants, and targeting of tumor cells. iIts results can be rational, especially in oncolytic virus therapy.

2. Conclusion:

Live attenuated vaccines are the best vaccines with a high level of creating immunity. The main problem with making these vaccines is time and the high cost of producing these viruses. By creating a random mutation in the genome of viruses by the CRISPR toolkit and selecting a live attenuated virus adapted to non-permissive cells, we can get these viruses at a low cost and high production speed. In addition, this system can be used to study the evolution of viruses and predict the possibility of changes in the viruses over the coming years. This system has a high potential for application in the laboratory and it is a flexible system that can be useful for manipulating a variety of viruses.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and material

Not applicable.

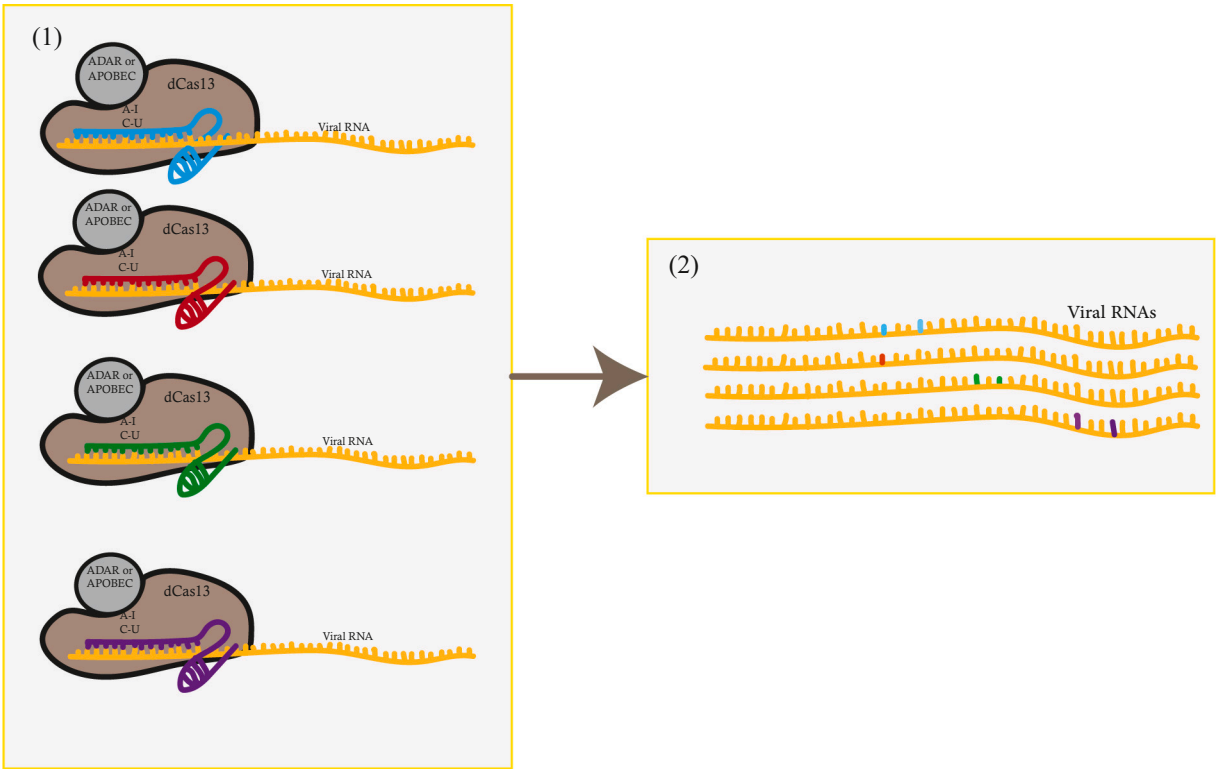

Fig. 1. Generation of random mutations in the genome of RNA viruses with CRISPR toolkit to create live attenuated viruses and natural selection studies. (1) Specific identification of RNA by the dCas13 protein with the guidance of the sgRNA that has been fused to ADAR or APOBEC. (2) Random mutation in the genome of RNA viruses by CRISPR toolkit.

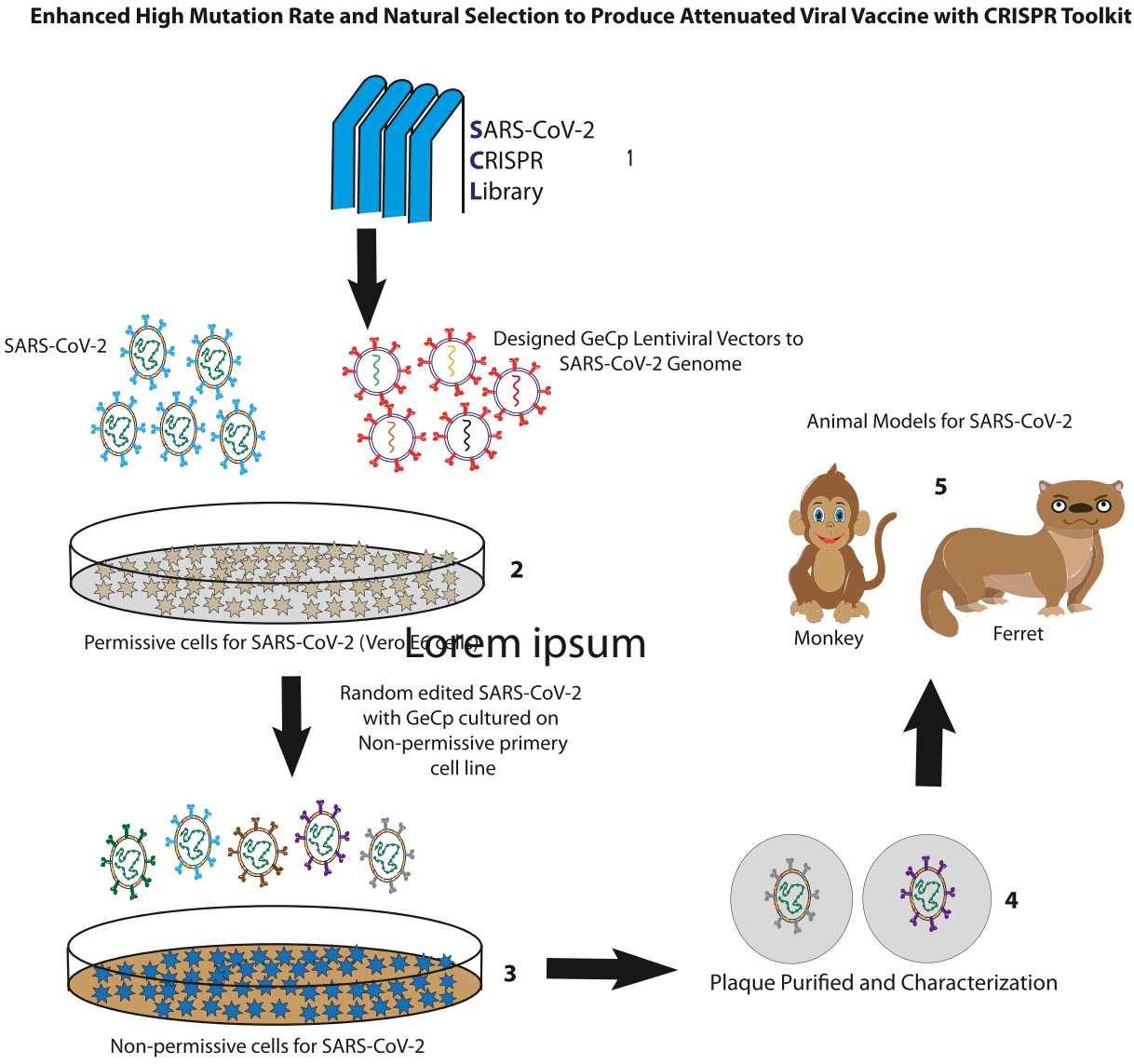

Fig. 2. Enhanced High Mutation Rate and Natural Selection to Produce Attenuated Viral Vaccine with CRISPR Toolkit. 1- CRISPR screening sgRNAs design to create random point mutations in the virus's genome with Genome-scale CRISPR/Cas 13 deletion and point mutation (GeCp). 2- Infect the permissive cells with viruses and GeCp lentiviral vectors. 3- Remove the viruses from permissive cells and infect nonpermissive cells with these viruses. 4- Harvest and purify the replicated viruses in nonpermissive cells. 5- Animal models tests.

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Authors' contributions

All authors conceived, wrote, and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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