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CRISPR technology a silver lining in combating COVID-19 pandemic

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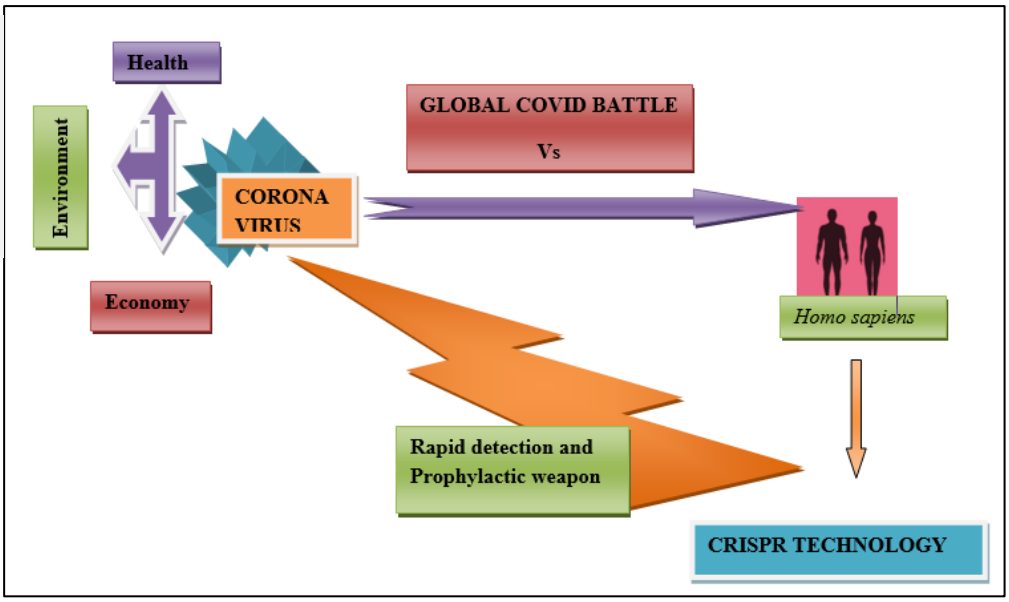
Graphical Abstract
Illustrating the effect of pandemic and significance of CRISPR tool in combating the COVID battle.



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Abstract: Technological advancements have trigged the research arena of life sciences. This has resulted not only in the co-evolution of science and technology but also in building up novel ways to tackle life threatening pandemic like situations. The use of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology in combating the recent COVID-19 pandemic is evidence to this. The technique was originally identified as a microbial adaptive immune system, in which the microbes use RNA-guided nucleases to cleave foreign genetic material. Subsequently, molecular biologists started harvesting this technique in accelerating the genetic engineering/ chimeric DNA methods. During COVID-19 outbreak, the scientists are trying to mould the CRISPR technology in the successful and rapid detection of the virus and also in the treatment of COVID-19 infection. Thus, the application of this technique comes as a ray of hope not only for fighting against COVID but also can be used as weapon for unseen future pandemics.

Keywords: COVID-19; CRISPR technology; prophylactic tool; rapid detection

Introduction

In past four decades the world has been facing the recurrence of large-scale epidemics, as seen in the outbreak of deadly viruses like HIV, SARS, MERS, H1N1, Ebola, Zika and most recently the SARS CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus-2) or COVID-19 [1]. The outbreak of COVID-19 has adversely affected the global health, economy, society and the environment. It has created global chaos since its inception in Wuhan, China in Dec-2019 as perceptible by the disconcerting figure of **2,512,272** global deaths and **113,076,707** confirmed cases (as of 3:09 pm CET World Health Organization (WHO) COVID-19 Dash board data, 27th Feb 2021) [2]. Moreover, the report of new variants of this virus has added more challenges to this current global pandemic.

In the midst of the COVID-19 pandemic, when the world is struggling in all spheres of global health, life, social, economic, financial and environmental crisis, a threat of many more future pandemics in the form of Disease Xs are raising the alarm bells in the medical world, for which one has to be prepared. WHO has introduced the designation of Disease X in the year 2015, for the pandemic caused by an unknown pathogen. Last year's Disease X, has been named as COVID-19 [3]. The scientists including Dr. Dadin Bonkole, who worked for Ebola, have reported a patient

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Zero with this disease is a woman of a remote town in the Democratic Republic of Congo. She showed the early signs of hemorrhagic fever, but her samples were tested negative for Ebola and other diseases with similar symptoms. Researchers are speculating that this first known patient of a new pathogen could be far more contagious than COVID-19 and Ebola [4].

Technologies have always contributed in solving the pandemic like situations. The present article highlights the importance of the emerging role of CRISPR- Cas technique in tackling COVID-19 infections. This technique was identified as a member of microbial adaptive immune system, which uses RNA-guided nucleases to destroy non-self-genetic elements. Here the bacteria store some part of the bacteriophage DNA in them, make gRNA complementary to the bacteriophage DNA and quickly attacks by triggering the Cas9 protein when the phage comes into contact again. The most remarkable feature the scientists have discovered is that this CRISPR technology can be programmed as per one's requirement. This technology has been used in accelerating the genetic engineering researches with fruitful results for the human kind.

Origin and transmission of COVID-19

As far as the emergence and transmission of COVID-19 virus is concerned, the researches in the year 2007, had already warned about the existence of large reservoirs of SARS-CoV like virus in *Rhinolophus*, horseshoe bats as a ticking time bomb. The scientific studies and data, have established the zoonotic origin of this virus, which has evolved either directly or indirectly through the mutation from a beta coronavirus that naturally infects bats and pangolins [5-7]. When a virus encounters a human cell for the first time, it is speculated that it has very recently been transmitted from cells of some different host, say from another animal. This transmission is referred to as host-switching, sometimes delineated as a spillover event [8]. In case of COVID-19 too, this spillover or host switching has occurred due to rapid mutation in small genomes of the zoonotic virus leading to the development of such a coat/spike protein which could bind to human angiotensin-converting enzyme-2 (ACE2) receptors thereby infecting human respiratory epithelial cells and indicating their pandemic potential [9]. The south/southwest China has been mapped as the global hotspot/epicenter for emergence of this pandemic. The major risk factor for rapid transmission has been attributed to the human–animal interactions, bat tourism, live animal markets and wildlife supply chains for human consumption. It has been found that the heavy dependence on the ecosystem harboring abundance and variety of wildlife, which are a reservoir of many pathogens and parasites, always have high potential for switching the zoonotic diseases to the humans. It has also been reported that wildlife species that are under environmental resistance, mainly due to anthropogenic factors, are more likely to excrete large doses of virus; that elevates the risk of spillover and subsequent pandemic [10]. Investigators through their virological and risk mapping studies have indicated a very high threat of further coronavirus outbursts to occur in near future.

Infection cycle of COVID-19

The novel coronavirus causing COVID-19 belongs to a family of positive-sense RNA viruses, which typically attack the upper and lower respiratory tracks and cause infection by direct cytotoxic effects and induce cytokine storms within the host. The infection cycle of SARS-CoV-2 involves: virus attachment to the host cell, entry of its genetic material into the host cytoplasm and synthesis of negative sense genomic and sub-genomic RNAs from which the transcription of viral mRNAs and replication of a new copy of the positive sense viral genomes occurs. Finally budding of the daughter viruses and damaging the host cell [11].

Technological intervention to curb the deadly COVID-19 virus

Tackling any pandemic is a mammoth and herculean task for the front-line workers, medical and paramedical staff, scientists, pharma and biotech researchers, government and the entire society. This has been evident in current pandemic of COVID-19. To prevent mass transmission of the disease it requires rapid detection, isolation, treatment and recovery of the patients. The identification, quantification, isolation, molecular characterization and the biological behavior of the virus within the host cell is extremely vital for drug designing, vaccine development and planning anti-viral intervention strategies. Here comes the role of technologies which elucidate and widen the knowledge about the virus. Various techniques like Real Time PCR (quantitative PCR), TEM, ELISA, Immunoprecipiation, Immunoblotting, Flow Cytometry (FCM), FRET (Fluorescence resonance energy transfer) and Immunohistochemistry (IHC) etc have been used for the qualitative and quantitative study of the virus. All the techniques largely rely upon the availability of the bio-samples. The successful combating of pandemic requires wide-scale biomedical research, which is supported by the creation of biobanks. These biobanks provide access of sufficient biological materials and associated clinical data for the ground level and advanced researches required for the development of intervention strategies to tackle the pandemic situation. The government agencies and international organizations like International Society for Biological and Environmental Repositories (ISBER) and Biobanking and Bio-Molecular Resources Research Infrastructure (BBMRI) play a crucial role as biobanking service provider, which also facilitate disbursing scientific, ethical and legal guidelines for pharmaceutical companies, the R&D and healthcare sectors [12].

qRT-PCR vs CRISPR

The widely used method of the virus detection is real time PCR, which takes normally 4-6 hrs. But the entire turnaround time for screening and diagnosing may reach more than 24 hrs, since it requires access to lot of reagents, elaborate instrumentation and intensive skilled technicians/experts. So much time required only for detection is not viable in a pandemic like situation, where quick detection of results becomes imperative.

Here comes the role of CRISPR technology which serves as a boon and promising tool not only for the rapid diagnosis,

but also for the treatment of COVID-19 cases. This technology does not give false negative or false positive results and has high sensitivity and specificity, in comparison to RT-PCR and immunoassay tests [13].

CRISPR Technology in COVID-19 detection

This technology is like a ray of hope for tackling pandemic situations like COVID 19 and for the first time, this technology is been used in diagnostic and suggested treatment procedure. A large number of global researchers are racing to develop the CRISPR- based COVID-19 detection kits, which offers rapid detection, so that the results can be obtained in less than an hour. This technology is also suggested to be used as a prophylactic strategy to combat novel coronavirus, by another group of researchers [14].

Zhang F et al., have designed a protocol for detection of COVID-19 using CRISPR -based SHERLOCK (Specific High sensitivity Enzymatic Reporter UnLOCKing) technique, using synthetic COVID-19 RNA fragments [15]. They suggest that rapid test can be done to detect the purified RNA from the patient samples without requiring elaborate instrumentation. This test involves isothermal amplification of the nucleic acid extracted from the patient's sample by using recombinase polymerase amplification (RPA) kit. This is followed by detecting the presence of pre-amplified viral RNA sequence using Cas13 (an RNA editing technique) and finally reading out visual result through naked eye using a commercially available paper dipstick. The entire test procedure can be completed in less than one hour and with the aid of most basic biomedical equipments commonly found in every laboratory. The Eli and Edythe L Board Institute jointly operated by MIT and Harvard University, Cambridge had developed this diagnostic kit and which is marketed by Sherlock Biosciences.

Researchers from the University of Connecticut (USA) have designed an All-In-One-Dual CRISPR-Cas12a (AIOD-CRISPR) method. This method utilizes a low-cost hand warmer as an incubator, instead of electrical incubator and the result generating time of as less as 20 minutes. This seems to be a simple, portable and sensitive detection platform which can be used at home, in doctor's chamber and even as mobile testing kit [16]

James P. Broughton et al., have reported CRISPR–Cas12-based DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) technique [17]. In this technique the RNA of the COVID-19 patient is extracted in a universal transport medium (UTM) and subjected to simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT–LAMP), followed by Cas12 detection of predefined coronavirus sequences. Finally, the viral detection is confirmed by the cleavage of a reporter molecule.

Therapeutic application of CRISPR technology

The traditional vaccines approach works by stimulating the human immune system to identify viral coat proteins or attenuated virion and blocking viral entry into cells. But the CRISPR-based strategy is an alternative antiviral approach which recognizes and degrades the genetic material of the intracellular virus and the viral mRNAs. In case of COVID-19, its positive-sense genome and viral mRNAs are targeted together to atrophy the viral genome templates and the viral gene expression finally curbing the multiplication of the virus [18]. Through this alternative approach scientists have shown how a gene editing tool can be used as a molecular weapon to combat against the pandemic battle raised by novel corona virus. This weapon in the scientist's arsenal is like a boon, which will shield human generations from present and future pandemics as well.

Abbott et al., have demonstrated a CRISPR Cas13-based strategy, named PAC-MAN (Prophylactic Antiviral CRISPR in huMAN cells), that can effectively cleave the SARS-CoV-2 sequences and live influenza A virus (IAV) genome inside the lung epithelial cells of the human host [19]. They have designed a group of CRISPR RNAs (crRNAs) as guide RNA, which can target the conserved viral regions and identify functional crRNAs for cleaving SARSCoV-2 with the help of Cas 13 enzyme. Thus PAC-MAN technique is effective in targeting and subsequently destroying the viral genome and preventing its replication. This approach is also effective enough in reducing H1N1 IAV infection. According to the researchers from Standford University, this approach is potentially a rapidly implementable pan-corona-virus inhibition strategy to effectively deal against all emerging pandemic strains of corona viruses. Although this novel technology is a worthy molecular tool, it lacks an effective delivery mechanism. To overcome this challenge, Stanford researchers have collaborated with Molecular Foundry (CA, USA) to use their developed Lipitoids (synthetic peptide), as an effective CRISPR delivery system.

Conclusion

The CRISPR, a gene editing tool can serve dual purposes, not only in rapid viral detection but also as a potential therapeutic tool against COVID-19 pandemic. This tool has added advantages since it is precise, cheap and easy to use. Scientists agree that CRISPR technique has a promising future, where it can be used in correcting genetic disorders and killing the pathogens by targeting its genetic material. CRISPR is a good point-of-care alternative tool in pandemic emergencies where relative shortage of PCR kits is common. Thus, there is no doubt that the scientific community has innovatively used CRISPR Cas technology as a molecular tool for tackling pandemic situations, but if we want to prevent future outbreaks of such pandemics then one has to bring about a major change in the anthropogenic behavior and attitude towards mother earth. This can be done by stopping the following examples, deforestation, devastating the environment, bat cave tourism and banning the wildlife markets for human consumption plus promoting intensive farming. Since there are evidences that conserving natural habitats reduces the risk of disease transmission from wildlife. We must follow the dictum live and let live; otherwise the consequences could be perilous.

Declarations

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