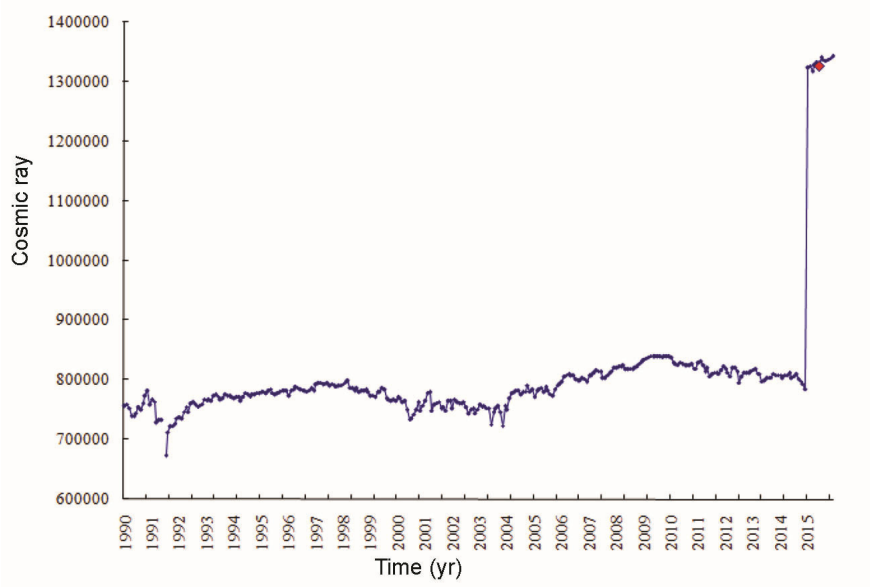

Figure 2. Association between cosmic ray and 2015 Zika virus outbreak. Red square represents onset of the 2015 Zika virus outbreak.

24) that peaked in 2014 showed the lowest sunspot number recorded since 1906, with many consecutive days of very low sunspot numbers in 2014/15 (ref. 5). Cosmic rays, particularly galactic cosmic rays, can reach a maximum intensity when the earth's magnetic field is weakening dramatically and the sun is least active. A new study revealed that solar radiation and cosmic rays are physical mutagens of natural genetic mutation/recombination, and can lead to the emergence of some modified viruses such as those responsible for pandemic influenza[6].

Thus the ZIKV outbreak in 2015 may have been linked to a systematic increase in the flux of cosmic rays and a general decline of sunspot activity with an accompanying weakening of the magnetic field around the earth[7]. Hence we propose that a surveillance of magnetic field, sunspot numbers and cosmic ray activity may serve as a potential warning of future pandemics. Together with other epidemiological data, such information might prove to be a useful factor for strategic disease control planning of ZIKV, as well as other pandemic-causing viruses.

Competing interests: The authors declare that no competing interests exist.

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Jiangwen Qu is in the Department of Infectious Disease Control, Tianjin Centers for Disease Control and Prevention, No 6 of Huayue Road, Hedong District, Tianjin, China; N. Chandra Wickramasinghe is in the Buckingham Centre for Astrobiology, University of Buckingham, Hunter Street, Buckingham, MK18 1EG, United Kingdom.*
**e-mail: ncwick@gmail.com*

CRISPR-Cas: the molecular scissors for genetic disease surgeries?

Naresh L. Selokar

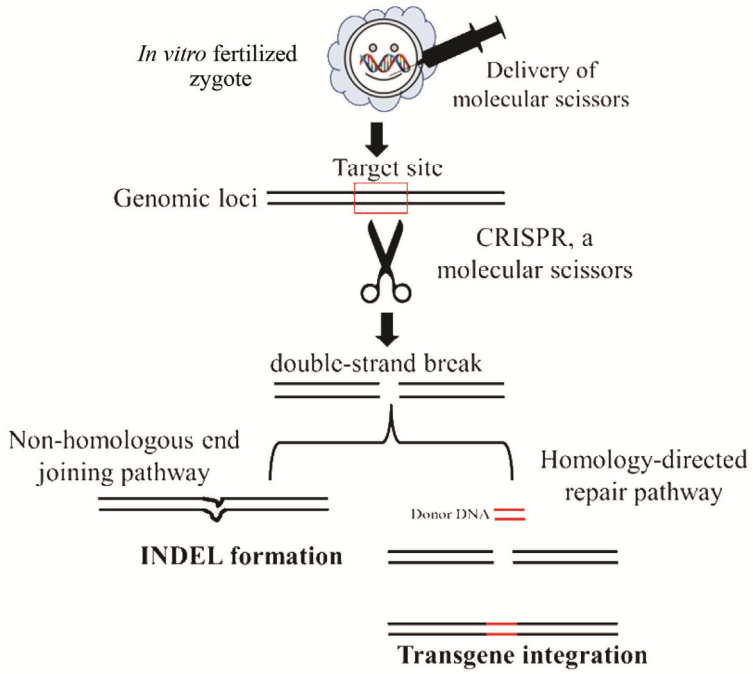
CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is an emerging biomedical tool, which allows researchers to manipulate DNA sequence(s) for desired gene(s) function. Genetic disorders, namely sickle-cell anaemia and β-thalassemia, had corrected in the stem cells using CRISPR. Therefore, researchers are much optimistic that CRISPR can prevent and cure human diseases in the coming future. However, it is a debatable question that whether this tool is safe and effective for use in human.

The news of CRISPR-Cas (clustered regularly interspaced short palindromic repeats and its associated protein nuclease) mediated gene editing in human embryos is a hot and exciting topic of discussion in the scientific fraternity, which prognosticates that many genetic diseases can be cured using CRISPR technology. CRISPR is a molecular tool (see a knowledge box) that can potentially allow the precise changes in the genome by adding, removing or altering the nucleotides in organisms, from a tiny *Drosophila* to a gigantic elephant. In 2013, for the first time, scientists used CRISPR to edit

human cells[1]. Following this, within a short period of time, this novel technique has spread like a forest fire, and has emerged as a precise, cheap, easy to use and powerful genome-editing tool. In humans, the best recent example of CRISPR application is the correction of mutated blood-forming stem cells to treat the world's leading blood genetic diseases such as sickle-cell anaemia[2] and β-thalassaemia[3]. It is imagined that a clinician would inject CRISPR corrected blood-forming stem cells directly into a patient which would eventually make healthy blood cells recover from a disease state. At present, CRISPR is a preferred choice over other genome-editing tools (zinc finger proteins and transcription activator-like effector nucleases) because of its simplicity and low experimental cost involved in making CRISPR cassettes.

Despite its potential applications, many ethicists have shown their public fear about the use of CRISPR in clinical therapies. They expressed concerns that if genome editing is applied in the clinics to treat human genetic diseases, it would also encourage introducing, removing or improving specific human traits for non-medical reasons like intelligence and emotion. Further, the unequal access to technology may incite the phenomenon of genetic classism among the people[4]. Therefore, ethicists have been warming that editing of human embryos would have lifelong and unintended threatening consequences.

A knowledge box:



The working principle of CRISPR: The CRISPR-Cas editing system consists of two components, (1) a guide RNA sequence and (2) a Cas endonuclease enzyme (Cas9 is commonly used). A guide RNA sequence recognizes matching sequences in the genome and forms a complex with the Cas endonuclease enzyme that creates double-stranded breaks in the genome. Double-stranded breaks can be repaired by two pathways in mammalian cells: the non-homologous end joining pathway in which a double-stranded break is repaired by non-specific addition and/or deletion of nucleotides, which results in the disruption of the gene function. Another pathway is homology-directed repair in which corrected DNA sequence is delivered into targeted cells to replace mutated DNA sequence to enable tailor repairs. CRISPR-Cas, clustered regularly interspaced short palindromic repeats and its associated protein nuclease.

The regulations regarding human embryo editing vary from country to country. In the USA, federal funds do not support research on human embryos; however, private funds can be used after taking regulatory approval. Germany, France and Australia have banned CRISPR technology for its clinical uses, but permitted its uses for research purpose; whereas Asian countries like India, China and Japan do not have stringent regulatory controls over the gene editing of human embryos[4]. Notably, in 2016, China launched a first CRISPR gene editing trial in humans to cure cancer[5]. Despite extraordinary hope to cure devastating human diseases by CRISPR, the technique needs to answer many scientific questions (e.g. off-target effects which may cause germline mutations) followed by regulatory approval by authorities and final acceptance by patients.

Indian scientists can contribute significantly to human embryo editing research since India has a large number of infertility and IVF clinics. Additionally, a large number of sperms, oocytes and embryos of livestock (sheep, goats and buffalo) and laboratory animals (mice and rats) are available to conduct proof-of-principle studies. In the future, common genetic diseases such as sickle-cell anaemia, thalasseamia, haemophilia and Down syndrome in India might get top priority for correction using CRISPR gene editing. Further, there is a blooming opportunity for Indian scientists to explore CRISPR to manipulate mosquitoes and insects in such a way that these organisms cannot serve as intermediate hosts to transmit any vector-borne diseases, since India has been endemic to deadly vector-borne diseases such as malaria, dengue, chikungunya and Japanese encephalitis.

In October 2017, the national guidelines for stem cell research have been published by the Indian Council of Medical Research[6]; wherein the genome modification procedures including gene editing (CRISPR) of stem cells, germ-line stem cells, gametes and embryos are restricted only to *in vitro* studies. For such *in vitro* experiments, only spare embryos, germline cells and gametes need to be used and their sources should be clearly defined. Genome modified human embryos should not be cultured beyond 14 days of post-fertilization. In addition, the research related to human germline gene

therapy, reproductive cloning and clinical trials involving xenogeneic cells are strictly prohibited. The genome editing research requires thorough review and approval by the institutional committee for stem cell research (IC-SCR), the institutional ethics committee (IEC) and the institutional biosafety committee (IBSC), and finally by the review committee on genetic manipulation (RCGM). Despite stringent regulations, some infertility and IVF clinics indulging in unregulated ethical practices of IVF and preimplantation genetic diagnosis may commercially exploit CRISPR technology to earn illegal money from potential patients. Therefore, regulatory agencies need to be more alert, active and vigilant to avoid unintended use of CRISPR by intruders.

The results of several embryonic editing studies in laboratory animals and a few studies in human embryos have opened up a ray of hope that any genetic disease can be cured using CRISPR gene editing. However, more translational studies are needed to confirm that CRISPR approach is highly efficient and safe; also issues of off-target, mosaicism and germline mutation should completely be erased. Despite the scientific receipt, this novel technique needs to undergo a long and hard battle of ethical endorsement followed by people's acceptance and their preparedness for its clinical reality.

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Naresh L. Selokar is in the ICAR-Central Institute for Research on Buffaloes, Hisar 125 001, India; Present address: Department of Biotechnology, Friedrich-Loeffler-Institute, Mariensee, 31535 Neustadt, Germany.
e-mail: selokarnareshlalaji@gmail.com