Brief Commentary DOI: 10.18203/issn.2456-3994.IntJMolImmunoOncol20172641

The emerging role of CRISPR-Cas9 in molecular oncology

Radhika A. Vaishnav

Department of Biomedical Research, Harmony Clinic and Vadodara Stroke Center, Vadodara, Gujarat, India

Correspondence to: Radhika A. Vaishnav,
E-mail: radhikavaishnav@gmail.com

It is not uncommon to be curious about the recent hype surrounding the new gene editing player, Cas9, which recognizes and holds into place DNA segments known as clustered regularly interspaced short palindromic repeats (CRISPR). Together, they are known as CRISPR-Cas9 or simply "CRISPR" for brevity. The binding of Cas9 causes the CRISPR sequences to become available for editing.

Discovery of the CRISPR System

In 1987, Japanese researchers studying *Escherichia coli* genes noted a set of 29-nucleotide (nt) repeats, downstream of the "iap" gene they were cloning.[1] These repeats were separated by unrelated, nonrepetitive short sequences (spacers). This was the first report of a CRISPR locus, after which many more reports followed in bacteria[2] and studied primarily in the context of innate and programable bacterial immunity against invading viruses. When infected by viruses, the bacteria that were able to survive would store cleaved pieces of the viral DNA in between the repeats (as spacers). Continuous surveillance of bacterial cell would occur via CRISPR-associated systems (Cas), which would allow matching of all RNA with the viral stored "memory" sequences. Thus, any RNA that matched would be degraded by the bacteria. Subsequently, in 2015, scientists demonstrated the use of CRISPR-Cas9 in the editing of human DNA.[3] Thus, this same principle could be employed across species using small guide RNAs to target, Cas9 to cleave and remove the gene, along with insertion of the desired replacement gene.

CRISPR over the Years

A literature search for "CRISPR" on PubMed turns up a total of 5437 papers as of March 2017, of which 2122 papers were published in the year 2016 alone. Put in the terms "CRISPR oncology" and a total of 348 papers were published and indexed on PubMed. However, very few (65 with the term "CRISPR" and 2 with the term "CRISPR oncology") were published from India and indexed on PubMed. Out of these two, one was a review and only one was an original article.[4,5]

Clearly, the potential for the use of this technique is enormous, and research avenues many.

Shortcomings and Caveats of CRISPR

Although the advantages of CRISPR are many, with potential applications in research and therapy, there are a few caveats to be aware of:

- Possible off-target effects
- Unintended consequences
- Adaptation
- Compensation.

Alternative approaches for gene editing

Although gene editing was being done by laboratories many years before the entry of CRISPR-Cas9, these previous methods were time-consuming, required higher training and were often expensive. Advantages of CRISPR-Cas9 over others like transcription activator-like effector nucleases and zinc finger nucleases are many. It is easier, faster, precise, and more cost-effective. Furthermore, it can be used across species and cross-kingdoms. Clearly, these very attributes have opened up numerous ethical concerns with regard to potentially heritable alterations to the genome. Editing of germline and embryonic DNA could have longstanding effects that are carried forth from generation to generation. Furthermore, the serendipitous discovery of this exciting mechanism is credited to many bright scientists. Not surprisingly, the intellectual property associated with CRISPR is under contention.[6]

CRISPR in Cancer - Bench to Bedside

- **Test new therapeutic targets.** For instance, novel targets discovered using the cancer genome atlas can be assessed.
- **Completely knockout or enhance a gene with high specificity for research.** This can make it possible to study the impact of genes on growth, division and death of cells. One can also distinguish between driver and passenger mutations. Another area is the study of tumor-microenvironment interactions *in vitro* and *in vivo*.
- **Can be used to enhance a gene.** This is a property which sets CRISPR apart from its counterparts, as it can be employed to enhance genes as well.
- **Recreate the steps leading to oncogenic transformation.** Thus, one can recapitulate tumorigenesis – both with regard to the number of mutations and the particular order of the mutations. For example, the use of CRISPR in mouse lung was reported by two groups back-to-back in 2014, for engineering oncogenic chromosomal rearrangements in mice to create a tissue-specific model of Eml4–Alk-driven lung cancer.[7,8]

- **Discover targets for combination therapies.** For example, one approach would be to generate a gene knockout library for a cancer cell line that normally responds well to a drug. Cells from this library that grows in the presence of low dose of the drug are sensitized to the drug therapy (in the absence of only the single gene that was knocked out). These genes can give us mechanistic insights and new targets for designing combination treatments to enhance therapy.
- **Understand underlying mechanisms of drug resistance.** Another approach would be to grow cells in the presence of high dose of a drug. The cells that survive are resistance to the drug. Now CRISPR can be used to knock out one gene at a time, once again to generate a library. The principle is that a gene critical to drug resistance, when knocked out, will obliterate this resistance.

References

1. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the IAP gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli,* and identification of the gene product. J Bacteriol 1987;169:5429-33.
2. Marraffini LA, Sontheimer EJ. CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. Nat Rev Genet 2010;11:181-90.
3. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012;337:816-21.
4. Sachdeva M, Sachdeva N, Pal M, Gupta N, Khan IA, Majumdar M, *et al.* CRISPR/Cas9: Molecular tool for gene therapy to target genome and epigenome in the treatment of lung cancer. Cancer Gene Ther 2015;22:509-17.
5. Mavuluri J, Beesetti S, Surabhi R, Kremerskothen J, Venkatraman G, Rayala SK. Phosphorylation-dependent regulation of the dna damage response of adaptor protein KIBRA in cancer Cells. Mol Cell Biol 2016;36:1354-65.
6. Ledford H. CRISPR, the disruptor. Nature 2015;522:20-4.
7. Maddalo D, Manchado E, Concepcion CP, Bonetti C, Vidigal JA, Han YC, *et al. In vivo* engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature 2014;516:423-7.
8. Blasco RB, Karaca E, Ambrogio C, Cheong TC, Karayol E, Minero VG, *et al.* Simple and rapid *in vivo* generation of chromosomal rearrangements using CRISPR/Cas9 technology. Cell Rep 2014;9:1219-27.

How to cite this article: Vaishnav RA. The emerging role of CRISPR-Cas9 in molecular oncology. Int J Mol Immuno Oncol 2017;2:45-46.

Source of Support: Nil. **Conflict of Interest:** None declared.