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Reveiw Article

CRISPR-Cas9 as a Potential Cancer Therapy Agent: An Update

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ABSTRACT

Cancer is the second leading cause of death globally and remains a major economic and social burden. Although our understanding of cancer at the molecular level continues to improve, more effort is needed to develop new therapeutic tools and approaches exploiting these advances. Due to its high efficiency and accuracy, the CRISPR-Cas9 genome editing technique has recently emerged as a cancer treatment strategy. Among its many applications, CRISPR-Cas9 has shown an unprecedented clinical potential to discover novel targets for cancer therapy and to dissect chemical-genetic interactions, providing insight into how tumors respond to drug treatment. Moreover, CRISPR-Cas9 can be employed to rapidly engineer immune cells and oncolytic viruses for cancer immunotherapeutic applications. More importantly, the ability of CRISPR-Cas9 to accurately edit genes, not only in cell culture models and model organisms but also in humans, allows its use in therapeutic explorations this review, important considerations for the use of CRISPR/Cas9 in therapeutic properties are discussed, along with major challenges that will need to be addressed before clinical examinations for a complex and polygenic disease such as cancer. This review aimed to explore the potential of the CRISPR-Cas9 genome editing technique as a cancer treatment strategy. Specifically, we will discuss how CRISPR-Cas9 can be used to discover novel targets for cancer therapy and to dissect chemical-genetic interactions.

1. Introduction

In most countries of the world, cancer contributes to early mortality[1]. Cancer incidence worldwide will increase within the next 50 years due to changes in the population, including aging and population growth and differences in cancer rates in various places[2]. Based on the latest incidence trends in the most frequently occurring types of cancer, the number of cancer cases will double by 2070, compared to 2020. Those increases will be greatest in countries with low Human Development Indexes (HDI) in low-resource settings. In contrast, the expected decrease in a national burden can be possible by increasing HDI levels[3]. Although molecular understanding of cancer is improving, new treatments are still needed to exploit discoveries.

In recent years, immunotherapy has significantly increased tumor responses with adverse reactions and reduced toxicity[4,5]. Nevertheless, the development of drug resistance and the high recurrence rate for most cancers call for innovative treatments[6]. Cancer drugs are often given multiple times, but this can lead to increased toxicity and costs, lowering the quality of patients' life. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are a natural defense mechanism against exogenous plasmids and viruses[7]. The CRISPR/Cas system comprises six categories of CRISPR-associated (Cas) proteins, each with its unique functions. Type II systems, with Cas9 protein and single guide RNA (sgRNA), are famous for editing molecules[8]. The type II CRISPR/Cas9 system dominates genome editing today. There are two main classes of Cas protein, as categorizeed by their structural and functional differences. In class 1, multi-Cas proteins are used, while in class 2, a single large Cas protein is used; both types degrade foreign nucleic acids. Of these, two

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main Cas proteins are of interest, namely Cas9 and Cas1-Cas2 complex[9]. The Cas1-Cas2 complex gathers DNA from the invading phage or virus to create a memory. The Cas9 protein is an effector protein guided by CRISPR RNA (crRNA) or RNA (gRNA). It can detect and destroy foreign RNA or DNA that matches the guide sequence, thereby preventing re-invasion by pathogens[10].

On-site and real-time clinical monitoring has advanced significantly by combining biosensors with portable electronic devices. The CRISPR and RNA-guided nucleases (CrRNA-Cas enzymes) provide CRISPR/Cas biosensing science as a revolutionary technique for mobile and on-site healthcare diagnostics and monitoring[11,12]. Among the various CRISPR/Cas systems, CRISPR/Cas13a and CRISPR/Cas12a hybrids are widely used in biosensor design since they can remove both target and non-target DNA[13]. The CRISPR/Cas-based biosensors are ideal for creating diagnostic ultra-sensitive point-of-care systems with enhanced response signals that are cost-effective, portable, reproducible, and highly durable[14].

CRISPR-Cas9 offers the unique clinical ability to identify new molecules for treating cancer and analyze chemical-genetic reactions, revealing how drugs affect tumor growth[15]. Moreover, CRISPR-Cas9 can efficiently construct oncolytic viruses and immune cells for cancer immunotherapy. Furthermore, CRISPR-Cas9's ability to precisely modify genes in humans, cell culture models, and model organisms makes it useful as a therapeutic tool. The Cas9 can be directed to particular sites in a genome by a short RNA search string; DNA sequences within the endogenous genome can now be modified almost anywhere[16]. The Cas9-mediated genetic modification is quick and easy, allowing researchers to understand the structure of the genome in detail and determine causal links between biological phenotypes and genetic variants[17]. This review aimed to explore the potential of the CRISPR-Cas9 genome editing technique as a cancer treatment strategy, discussing its applications in discovering novel targets for cancer therapy, dissecting chemical-genetic interactions, engineering immune cells and oncolytic viruses for cancer immunotherapy, and editing genes in humans.

2. Advancement of gene editing with CRISPR-Cas9 technology

There have been several methods designed to enable effective gene editings, such as zinc finger nucleases (ZFNs), RNA interference (RNAi), or transcription activator-like effector nucleases (TALENs)[18,19]. (RNAi) is activated via double-strand RNA (dsRNA), Originally found in *Caenorhabditis elegans*. After external dsRNA enters cells, it is converted to 21–23 base pairs of small interfering RNAs (siRNAs) via the RNase III family ribonuclease Dicer[20]. Then, the RNA-induced silencing complex (RISC) is activated to the target RNA via siRNAs, ultimately destroying the gene silencing and target RNA. Nevertheless, the study of a particular gene by RNAi is restricted, as RNAi reduces gene expression through transcription[21].

Since its discovery in *Escherichia coli*, the CRISPR mechanism has been a dynamic immunity that protects against invasive foreign DNA[22]. Traditionally, CRISPR-Cas systems are divided into two classes based on six types. Class I (systems I, III, and IV) use different Cas proteins, whereas Class II (systems II, V, and VI) use the same Cas protein through various functions[23, 24]. Thus, Class II CRISPR-Cas is more practical in bioengineering. Among the type II CRISPR Cas system, the most widely used in mammals is CRISPR-Cas9, produced by *Streptococcus pyogenes*[25].

3. The classical CRISPR/Cas9 mechanism

CRISPR/Cas9 is a genetically modified prokaryotic antiviral immune system attacking viral infections and utilizing RNA-guided nucleases to eliminate foreign DNA[26]. This system comprises two parts, namely single-stranded guide RNA (sgRNA) and Cas9 endonuclease. The sgRNA serves as a Cas9 endonuclease, attacking the desired gene's DNA with a specific sequence[27]. Consequently, the DNA cleaves at a series of three base pairs before the "NGG" protospacer adjacent motifs (PAM). CRISPR-Cas9 system makes genetic changes via small inserts or removals (indels) using the high-fidelity homology-directed repair or the relatively error-prone non-homologous end-joining[28, 29].

4. Evolution of the CRISPR-Cas9 and other gene editing systems

In 1987, Japanese researchers found several unusual variants in the *E. coli* genome without examining their significance biologically. In 2002, they were named Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), yet their value was unknown[30]. In 2005, three study groups independently identified CRISPR loci in adaptive immunity, indicating protospacer-adjacent motifs (PAMs) might trigger the class II Cas9 nuclease to cut genomes[31]. CRISPR is responsible for innate immunity, and the sequence of phage genes inserted into bacteria can alter the bacteria's tolerance to phage[32]. The noncoding RNA derived from the CRISPR proto-interregional sequences directed Cas protein to the target region of the genome for defense.

Consequently, transcoding crRNA (tracrRNA) contributes to pre-crRNA maturation and new routes for crRNA maturation [33]. Therefore, *in vitro* studies showed that maturing crRNA created a unique double-stranded RNA bond with tracrRNA via base pair, causing the Cas9 protein to induce double-strand breaks on the DNA mark. The type II Cas method cuts DNA in mammals, leading to CRISPR-Cas9 in gene editing. The Cas9 protein mutant dCas9 (endonuclease-deficient Cas9), without nuclease function, was first discovered[34]. Later, CRISPR interference (CRISPRi) and the CRISPR activation tools were created by combining the dCas9 protein with transcription factors that stimulate or suppress DNA expression[35].

5. Chemical Modifications of CRISPR RNAs to Improve Gene-Editing Activity and Specificity

CRISPR has become a breakthrough method that can revolutionize medicine and biotechnology[36]. Like other nucleic acid methods, CRISPR significantly benefits from chemical innovation to increase specificity and activity for crucial *in vivo* uses[37]. Chemists have recently begun improving different elements of the CRISPR method; the following perspective is focused on chemical modifications of CRISPR RNAs (crRNAs). Early efforts concentrated on existing sugar and structure modifications (2′-deoxy, 2′-F, 2′-OMe, and phosphorothioates) as with other nucleic acid-based approaches[38]. Some important changes in crRNAs have been made with bicyclic (locked) ribose and phosphate backbone replacements (phosphonoacetates and amides). However, chemical developments related to crRNAs are restricted to modifications used in antisense technologies and RNA interference[38]. The positive results of these established modifications underscore the importance of chemists adopting a riskier approach. Further studies are required to investigate most effective chemical methods for advancing the development of CRISPR therapies and other *in vivo* techniques[38]. CRISPR can provide the chance for chemists to combine advances in synthesis and molecular biochemistry for optimizing crRNA-protein interaction rationally.

6. DNA-based knockout-in Oncogenes

Unlike normal genes, oncogenes are controlled in different ways and can lead to cancerous transformations. The CRISPR/Cas9 system can interfere with the expression, deletion, or altering of oncogene activity, resulting in tumor inhibition[39]. The CRISPR-Cas9-mediated deletion of CD-133 reduced vimentin's concentration in cancerous colon cells, especially decreased colony formation, and inhibited cell invasion and migration[40]. The tumorigenic ability of Pancreatic cancer cells is significantly reduced by knocking down miR-3064 with CRISPR/Cas9[41]. Similarly, the growth of lung cancer cell lines H1650, A549, and H1975 was reduced with CRISPR-Cas9 deletions of oncogenic mutant EGFR alleles, with shrinking tumor sizes in mice treated using A549or H1975 cells[42]. FAK gene knockdown with KRAS mutations in NSCLC cells using CRISPR/Cas9 caused noticeable DNA cleavage and improved radiotherapy sensitivity[43]. Additionally, the CRISPR/Cas9-mediated removal of the E3 ubiquitin ligase UBR5 in an animal model of tripled negative breast cancer (TNBC) greatly reduced the size of the tumor and *in vivo* invasion[44]. This research showed that CRISPR/Cas9 gene editing could efficiently identify oncogenes and evaluate the medicinal potency of targeting oncogenes[44].

7. The delivery method of CRISPR Cas9 used in cancer studies

Several delivery methods can efficiently deliver CRISPR parts into cells, including viral delivery methods and nonviral delivery methods.

7.1 Viral delivery system

Adenovirus and lentivirus frequently carry the plasmid CRISPR Cas9 method used to study tumors[45]. The nonpathogenic adeno-associated virus can infect nondividing and dividing cells with low immunity. It was possible to successfully induce liver tumors by changing the Cre-expressing Cas9 mice liver[46]. The researchers used the adenovirus to control the sgRNA library, achieving potential cancer suppressor DNA like Setd2, Trp53, and Pik3r1[47]. With higher infection efficacy and lower immunogenicity, gene expression induced by lentivirus can last longer. After whole-genome CRISPR Cas9 monitoring using a lentiviral vector, a synthetic lethal factor via ATR inhibition *in vivo* and *in vitro* may cause RNASEH2 deficit[48]. Nonetheless, the packaging ability of lentiviruses and adeno-associated viruses was low. Thus, the engineered expressing-Cas9 in mice, modified by adding sgRNA, was designed to defeat the encapsulation problem [49]. A Cre-dependent Cas9 gene knockin mouse was designed by Platt et al. They utilized an AAV vector to add Lkb1, p53, and Kras-mutation to the lung, forming tumors[50]. In contrast to lentiviruses and AAVs, adenoviruses have fewer sequence limitations and larger transgene sizes. The CRISPR part was similarly introduced to mouse tissue via tracheal recombinant adenovirus to cause Eml4-Alk gene interaction[51], leading to lung tumorigenesis with increased penetration. As a result, low tissue orientation and high immunogenicity limit the adenovirus vectors' usage.

7.2. Nonviral delivery systems

Despite their low delivery rates, nonviral delivery techniques pose fewer health risks than virus vectors. Standard nonviral delivery methods include nanoparticles, electroporation, Hydrodynamic injection, and transposon carriers[52]. Chen et al. developed liposome-based hydrogel nanoparticles (LHNPs) using minicircle DNA engineering to carry a CRISPR-Cas9 mechanism against Plk1 (polo-like kinase 1), which effictively inhibited tumor growth[53]. Another carrier to transfer guide RNA (gRNA) libraries for *in vivo* testing is piggyBac (PB) transposons, leading to conversion rates of 86.85-92.43% in mouse livers[54]. Nonetheless, the nonviral delivery system do not often have sufficient level of tissue sensitivity and is overcome by Cas9 protein modification. Asialoglycoprotein receptor (ASGPr) is a c-type lectin that is expressed on the surface of liver cell[55]. In another study, a Cas9 protein was created, carring the ASGPr ligand and forming a combination with sgRNA to transfer the CRISPR-Cas9 part, specifically to liver cells[56].

8. Application of CRISPR -Cas9 in cancer

8.1. breast cancer

Cancer-related deaths among women are

disproportionately caused by breast cancer[57]. CRISPR-Cas9 has been used to prevent various breast cancer types. Annunziato et al. created knockout mice in the cytosine base editor via conditional Cre expression[58]. After direct delivery of the sgRNA encoding vector, efficient point mutations could be induced in one or more endogenous genes (Akt1, Pik3ca) by the CRISPR-Cas9 system. The system successfully influenced the cancer model (triple-negative breast cancer). In addition to the *in situ* development, organoid implantation of mice's breast cancer can imitate the development of breast tumor in humans. Dekkers et al. employed the CRISPR-Cas9 gene editing tool to target four different tumor suppressor genes (RB1, P53, PTEN, and NF1), related to breast cancer. The experiment was performed on breast progenitor cells from six different donors[59,60]. The ER + intracellular tumors were created and long-term cultures were obtained after the 1/6 P53/PTEN/RB1 mutant cell line and 3/6 P53/PTEN/RB1/NF1 mutant cell line were transplanted into mice.

8.2. Lung cancer

Lung cancer is the primary cause of cancer deaths in humans worldwide[61]. The molecular and histopathological characteristics of lung tumors in humans can be induced in mice using chromosome rearrangement and CRISPR Cas9-mediated gene editing. Platt et al. employed the Cas9 system to introduce several sgRNAs into mice using an AAV vector. This resulted in the removal of mutations in lung p53 and Lkb1, as well as the homologous repair-induced Kras G12D mutation[50]. In aother study by Maddalo et al., virus-mediated CRISPR Cas9 was used to induce chromosomal rearrangement, resulting in the expression of the Eml4-Alk fusion gene. This approach was employed to create a mouse model of Eml4-Alk-driven lung cancer, which exhibited a responsive reaction to ALK-inhibitor therapy[62].

9. Conclusion

Cancer is an intense danger to humans and needs a useful method to define its complicated genetic basis as a hereditary disease marked by cancerous cell proliferation. The CRISPR Cas9 gene editing science can be applied widely to cancer research to develop practical therapies and enhance research. In addition to off-target effects and immune reactions, moral considerations are also of concern in this method, which has long been disputed. Although the CRISPR-Cas9 gene editing technique may have certain limitations, it is widely acknowledged that this technology has the potential to significantly improve cancer treatment, despite its limitations.

Declarations

Competing interests

The authors declare that they have no conflict of interest.

Authors' contribution

Conceptualization: [Hassan Borji], …; Methodology: [Soheil Sadr], …; Formal analysis and investigation: [Pouria Ahmadi Simab/ Soheil Sadr], …; Writing - original draft preparation: [All Authors]; Writing - review and editing: [Pouria Ahmadi Simab/Soheil]. All authors checked and approved the final version of the manuscript for publication in the present journal.

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Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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References

1. Lei S, Zheng R, Zhang S, Wang S, Chen R, Sun K, et al. Global patterns of breast cancer incidence and mortality: A population-based cancer registry data analysis from 2000 to 2020. Cancer Commun. 2021; 41(11): 1183-1194. DOI: 10.1002/cac2.12207
2. Soerjomataram I, and Bray F. Planning for tomorrow: Global cancer incidence and the role of prevention 2020–2070. Nat Rev Clin Oncol. 2021; 18(10): 663-672. DOI: 10.1038/s41571-021-00514-z
3. Bray F, Jemal A, Grey N, Ferlay J, and Forman D. Global cancer transitions according to the human development index (2008–2030): A population-based study. Lancet Oncol. 2012; 13(8): 790-801. DOI: 10.1016/s1470-2045(12)70211-5
4. Chang M, Hou Z, Wang M, Li C, and Lin J. Recent advances in hyperthermia therapy-based synergistic immunotherapy. Adv Mater. 2021; 33(4): e2004788. DOI: 10.1002/adma.202004788
5. Sadr S, Yousefsani Z, Ahmadi Simab P, Jafari Rahbar Alizadeh A, Lotfalizadeh N, and Borji H. Trichinella spiralis as a potential antitumor agent: An update. World Vet J. 2023; 13(1): 65-74. DOI: 10.54203/scil.2023.wvj7
6. Sarmento-Ribeiro AB, Scorilas A, Gonçalves AC, Efferth T, and Trougakos IP. The emergence of drug resistance to targeted cancer therapies: Clinical evidence. Drug Resist Updat. 2019; 47: 100646. DOI: 10.1016/j.drup.2019.100646
7. Loureiro A, and da Silva GJ. Crispr-cas: Converting a bacterial defence mechanism into a state-of-the-art genetic manipulation tool. Antibiotics. 2019; 8(1): 18. DOI: 10.3390/antibiotics8010018
8. Karimian A, Azizian K, Parsian H, Rafieian S, Shafiei-Irannejad V, Kheyrollah M, et al. CRISPR/Cas9 technology as a potent molecular tool for gene therapy. J Cell Physiol. 2019; 234(8): 12267-12277. DOI: 10.1002/jcp.27972
9. Pattharaprachayakul N, Lee M, Incharoensakdi A, and Woo HM. Current understanding of the cyanobacterial CRISPR-Cas systems and development of the synthetic CRISPR-Cas systems for cyanobacteria. Enzyme Microb Technol. 2020; 140: 109619. DOI:

10.1016/j.enzmictec.2020.109619
10. Ferreira P, and Choupina AB. CRISPR/Cas9 a simple, inexpensive and effective technique for gene editing. Mol Biol Rep. 2022; 49(7): 7079-7086. DOI: 10.1007/s11033-022-07442-w
11. de Dieu Habimana J, Huang R, Muhoza B, Kalisa YN, Han X, Deng W, et al. Mechanistic insights of CRISPR/Cas nucleases for programmable targeting and early-stage diagnosis: A review. Biosens Bioelectron. 2022; 203: 114033. DOI: 10.1016/j.bios.2022.114033
12. Zhang L, Jiang H, Zhu Z, Liu J, and Li B. Integrating CRISPR/Cas within isothermal amplification for point-of-care assay of nucleic acid. Talanta. 2022; 243: 123388. DOI: 10.1016/j.talanta.2022.123388
13. Wang SY, Du YC, Wang DX, Ma JY, Tang AN, and Kong DM. Signal amplification and output of CRISPR/Cas-based biosensing systems: A review. Anal Chim Acta. 2021; 1185: 338882. DOI: 10.1016/j.aca.2021.338882
14. Wang L, Pan MM, Xu L, Yu X, and Zheng SY. Recent advances of emerging microfluidic chips for exosome-mediated cancer diagnosis. Smart Mat Med. 2021; 2: 158-171. DOI: 10.1016/j.smaim.2021.06.001
15. Zhang H, Qin C, An C, Zheng X, Wen S, Chen W, et al. Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. Mol Cancer. 2021; 20: 1-22. DOI: 10.1186/s12943-021-01431-6
16. Wang JY, and Doudna JA. CRISPR technology: A decade of genome editing is only the beginning. Science. 2023; 379(6629): eadd8643. DOI: 10.1126/science.add8643
17. Jacinto FV, Link W, and Ferreira BI. CRISPR/Cas9-mediated genome editing: From basic research to translational medicine. J Cell Mol Med. 2020; 24(7): 3766-3778. DOI: 10.1111%2Fjcmm.14916
18. Gupta D, Bhattacharjee O, Mandal D, Sen MK, Dey D, Dasgupta A, et al. CRISPR-Cas9 system: A new-fangled dawn in gene editing. Life sci. 2019; 232: 116636. DOI: 10.1016/j.lfs.2019.116636
19. Nargesi S, Kaboli S, Thekkiniath J, Heidari S, Keramati F, Seyedmousavi S, et al. Recent advances in genome editing tools in medical mycology research. J Fungi. 2021; 7(4): 257. DOI: 10.3390%2Fjof7040257
20. Xu W, Jiang X, and Huang L. RNA interference technology. Comprehens Biotechnol. 2019; 2019: 560-575. DOI: 10.1016%2FB978-0-444-64046-8.00282-2
21. Setten RL, Rossi JJ, and Han Sp. The current state and future directions of RNAi-based therapeutics. Nat Rev Drug Discov. 2019; 18(6): 421-446. DOI: 10.1038/s41573-019-0017-4
22. Zheng Y, Li J, Wang B, Han J, Hao Y, Wang S, et al. Endogenous type I CRISPR-Cas: From foreign DNA defense to prokaryotic engineering. Front Bioeng Biotechnol. 2020; 8: 62. DOI: 10.3389%2Ffbioe.2020.00062
23. Liu Z, Dong H, Cui Y, Cong L, and Zhang D. Application of different types of CRISPR/Cas-based systems in bacteria. Microb Cell Fact. 2020; 19(1): 172. DOI: 10.1186/s12934-020-01431-z
24. Katalani C, Boone HA, Hajizade A, Sijercic A, and Ahmadian G. CRISPR-based diagnosis of infectious and noninfectious diseases. Biol Proced Online. 2020; 22(1): 1-14.
25. Janik E, Niemcewicz M, Ceremuga M, Krzowski L, Saluk-Bijak J, and Bijak M. Various aspects of a gene editing system—crispr–cas9. Int J Mol Sci. 2020; 21(24): 9604. DOI: 10.3390/ijms21249604
26. Luthra R, Kaur S, and Bhandari K. Applications of CRISPR as a potential therapeutic. Life Sci. 2021; 284: 119908. DOI: 10.1016/j.lfs.2021.119908
27. Bashir M, Ali Q, Rashid M, and Malika A. Crispr/cas9 in genome editing: A nature gifted molecular tool. Biol Clin Sci Res J. 2020; 2020(1). DOI: 10.54112/bcsrj.v2020i1.18
28. Denes CE, Cole AJ, Aksoy YA, Li G, Neely GG, and Hesselson D. Approaches to enhance precise CRISPR/Cas9-mediated genome editing. Int J Mol Sci. 2021; 22(16): 8571. DOI: 10.3390/ijms22168571
29. Skarnes WC, Pellegrino E, and McDonough JA. Improving homology-directed repair efficiency in human stem cells. Methods. 2019; 164: 18-28. DOI: 10.1016/j.ymeth.2019.06.016
30. Jansen R, Embden JDv, Gaastra W, and Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. Mol Microbiol. 2002; 43(6): 1565-1575. DOI: 10.1046/j.1365-2958.2002.02839.x
31. Ishino Y, Krupovic M, and Forterre P. History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. J Bacteriol. 2018; 200(7): e00580-17. DOI: 10.1128/jb.00580-17
32. Azam AH, and Tanji Y. Bacteriophage-host arm race: An update on the mechanism of phage resistance in bacteria and revenge of the phage with the perspective for phage therapy. Appl Microbiol Biotechnol. 2019; 103(5): 2121-2131. DOI: 10.1007/s00253-019-09629-x
33. Liao C, and Beisel CL. The tracrRNA in CRISPR biology and technologies. Annu Rev Genet. 2021; 55: 161-181. DOI: 10.1146/annurev-genet-071719-022559
34. Xu X, Hulshoff MS, Tan X, Zeisberg M, and Zeisberg EM. CRISPR/Cas derivatives as novel gene modulating tools: Possibilities and *in vivo* applications. Int J Mol Sci. 2020; 21(9): 3038. DOI: 10.3390%2Fijms21093038
35. Schultenkämper K, Brito LF, and Wendisch VF. Impact of CRISPR interference on strain development in biotechnology. Biotechnol Appl Biochem. 2020; 67(1): 7-21. DOI: 10.1002/bab.1901
36. Hu LF, Li YX, Wang JZ, Zhao YT, and Wang Y. Controlling CRISPR-Cas9 by guide RNA engineering. Wiley Interdiscip Rev RNA. 2023; 14(1): e1731. DOI: 10.1002/wrna.1731
37. Akram F, Sahreen S, Aamir F, Haq IU, Malik K, Imtiaz M, et al. An insight into modern targeted genome-editing technologies with a special focus on CRISPR/Cas9 and its applications. Mol Biotechnol. 2023; 65(2): 227-242. DOI: 10.1007/s12033-022-00501-4
38. Rozners E. Chemical modifications of CRISPR RNAs to improve gene-editing activity and specificity. J Am Chem Soc. 2022; 144(28): 12584-12594. DOI: 10.1021/jacs.2c02633
39. Jiang C, Meng L, Yang B, and Luo X. Application of CRISPR/Cas9 gene editing technique in the study of cancer treatment. Clin Genet. 2020; 97(1): 73-88. DOI: 10.1111/cge.13589
40. Kaltschmidt C, Banz-Jansen C, Benhidjeb T, Beshay M, Förster C, Greiner J, et al. A role for NF-κB in organ specific cancer and cancer stem cells. Cancers. 2019; 11(5): 655. DOI: 10.3390%2Fcancers11050655
41. Yan J, Jia Y, Chen H, Chen W, and Zhou X. Long non-coding RNA PXN-AS1 suppresses pancreatic cancer progression by acting as a competing endogenous RNA of miR-3064 to upregulate PIP4K2B expression. J Exp Clin Cancer Res. 2019; 38(1): 1-16. DOI: 10.1186/s13046-019-1379-5
42. Koo T, Yoon AR, Cho HY, Bae S, Yun CO, and Kim JS. Selective disruption of an oncogenic mutant allele by CRISPR/Cas9 induces efficient tumor regression. Nucleic Acids Res. 2017; 45(13): 7897-7908. DOI: 10.1093/nar/gkx490
43. Tang KJ, Constanzo JD, Venkateswaran N, Melegari M, Ilcheva M, Morales JC, et al. Focal adhesion kinase regulates the DNA damage response and its inhibition radiosensitizes mutant KRAS lung cancer. Clin Cancer Res. 2016; 22(23): 5851-563. DOI: 10.1158/1078-0432.ccr-15-2603
44. Liao L, Song M, Li X, Tang L, Zhang T, Zhang L, et al. E3 ubiquitin ligase UBR5 drives the growth and metastasis of triple-negative breast cancerUBR5 is essential for tumor growth and metastasis. Cancer Res. 2017; 77(8): 2090-2101. DOI: 10.1158/0008-5472.can-16-2409
45. Yan J, Kang DD, Turnbull G, and Dong Y. Delivery of CRISPR-Cas9 system for screening and editing RNA binding proteins in cancer. Adv Drug Deliv Rev. 2022; 180: 114042. DOI: 10.1016/j.addr.2021.114042
46. Reiberger T, Chen Y, Ramjiawan RR, Hato T, Fan C, Samuel R, et al. An orthotopic mouse model of hepatocellular carcinoma with underlying liver cirrhosis. Nat Protoc. 2015; 10(8): 1264-1274. DOI: 10.1038/nprot.2015.080
47. Wang G, Chow RD, Ye L, Guzman CD, Dai X, Dong MB, et al. Mapping a functional cancer genome atlas of tumor suppressors in mouse liver using AAV-CRISPR–mediated direct *in vivo* screening. Sci Adv. 2018; 4(2): eaao5508. DOI: 10.1126/sciadv.aao5508
48. Awwad SW, Serrano-Benitez A, Thomas JC, Gupta V, and Jackson SP. Revolutionizing DNA repair research and cancer therapy with CRISPR–Cas screens. Nat Rev Mol Cell Biol. 2023; 13: 1-18. DOI: 10.1038/s41580-022-00571-x
49. Wang SW, Gao C, Zheng YM, Yi L, Lu JC, Huang XY, et al. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Mol Cancer. 2022; 21(1): 57. DOI: 10.1186/s12943-022-01518-8
50. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, et al. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell. 2014; 159(2): 440-455. DOI: 10.1016/j.cell.2014.09.014
51. Rodríguez-Rodríguez DR, Ramírez-Solís R, Garza-Elizondo MA, Garza-Rodríguez MDL, and Barrera-Saldaña HA. Genome editing: A perspective on the application of CRISPR/Cas9 to study human

diseases. Int J Mol Med. 2019; 43(4): 1559-1574. DOI: 10.3892/ijmm.2019.4112
52. Liu W, Li L, Jiang J, Wu M, and Lin P. Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics. Precis Clin Med. 2021; 4(3): 179-191. DOI: 10.1093/pcmedi/pbab014
53. Chen Z, Liu F, Chen Y, Liu J, Wang X, Chen AT, et al. Targeted delivery of CRISPR/Cas9-mediated cancer gene therapy via liposome-templated hydrogel nanoparticles. Adv Funct Mater. 2017; 27(46): 1703036. DOI: 10.1002/adfm.201703036
54. Xu C, Qi X, Du X, Zou H, Gao F, Feng T, et al. piggyBac mediates efficient in vivo CRISPR library screening for tumorigenesis in mice. Proc Natl Acad Sci USA. 2017; 114(4):722-727. DOI: 10.1073/pnas.1615735114
55. Rouet R, Thuma BA, Roy MD, Lintner NG, Rubitski DM, Finley JE, et al. Receptor-mediated delivery of CRISPR-Cas9 endonuclease for cell-type-specific gene editing. J Am Chem Soc. 2018; 140(21): 6596-6603. DOI: 10.1021/jacs.8b01551
56. Rouet R, de Onate L, Li J, Murthy N, and Wilson RC. Engineering CRISPR-Cas9 RNA–protein complexes for improved function and delivery. CRISPR J. 2018; 1(6): 367-378. DOi: 10.1089/crispr.2018.0037
57. Cho B, Han Y, Lian M, Colditz GA, Weber JD, Ma C, et al. Evaluation of racial/ethnic differences in treatment and mortality among women with triple-negative breast cancer. JAMA Oncol. 2021; 7(7): 1016-1023. DOI: 10.1001/jamaoncol.2021.1254
58. Annunziato S, Lutz C, Henneman L, Bhin J, Wong K, Siteur B, et al. In situ CRISPR-Cas9 base editing for the development of genetically engineered mouse models of breast cancer. EMBO J. 2020; 39(5): e102169. DOI: 10.15252/embj.2019102169
59. Sahebi R, Akbari N, Bayat Z, Rashidmayvan M, Mansoori A, Beihaghi M. A Summary of Autophagy Mechanisms in Cancer Cells. Research in Biotechnology and Environmental Science. 2022; 1(1): 28-35.
60. Dekkers JF, Whittle JR, Vaillant F, Chen H-R, Dawson C, Liu K, et al. Modeling breast cancer using CRISPR-Cas9–mediated engineering of human breast organoids. J Natl Cancer Inst. 2020; 112(5): 540-544. DOI: 10.1093/jnci/djz196
61. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394-424. DOI: 10.3322/caac.21492
62. 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(7531): 423-427. DOI: 10.1038/nature13902