The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(2): S2: 189-193, 2025 www.thebioscan.com

CRISPR-Cas9 Applications Across Different Cancer Types: Targets, Strategies, and Outcomes

Shajin.J[1], Vasanth Albert.R[2], Revanth.R[3*]

[1]Department of Pharmacy Practice, JKKN College of Pharmacy, Kumarapalayam-638183, Tamil Nadu. Email: shajinofficial@gmail.com

ORCID ID: 0009-0009-3481-9122

[2]Department of Pharmacy Practice, JKKN College of Pharmacy, Kumarapalayam-638183, Tamil Nadu. Email: vasanthalbert001@gmail.com

ORCID ID: 0009-0006-9294-8131

[3*]Assistant Professor, Department of Pharmacy Practice,

JKKN College of Pharmacy, Kumarapalayam-638183, Tamil Nadu. Email: revram95@gmail.com

ORCID ID: 0009-0005-3116-4774

DOI: 10.63001/tbs.2025.v20.i02.S2.pp189-193

KEYWORDS
CRISPR-Cas9,
genome editing,
cancer therapy,
gene modification,
gene drive,
off-target effects,
therapeutic applications.

Received on:

12-02-2025

Accepted on:

15-03-2025

Published on:

27-04-2025

ABSTRACT

Targeted gene modification, facilitated by engineered nucleases such as CRISPR-Cas9, has revolutionized therapeutic strategies, offering precise and cost-effective methods for gene editing. The CRISPR-Cas9 system, composed of the Cas9 endonuclease and single guide RNA (sgRNA), allows for targeted DNA cleavage at specific sites, making it a powerful tool for genome editing. This review explores the CRISPR-Cas9 mechanism, which involves three primary stages: recognition, cleavage, and repair, with key repair pathways including non-homologous end joining (NHEJ) and homology-directed repair (HDR). The review further highlights the growing applications of CRISPR technology in cancer therapy, including its use in treating breast cancer, lung cancer, leukemia, liver cancer, colorectal cancer, and prostate cancer. Various CRISPR-based approaches, such as the use of Cas9 nickase (Cas9n) to reduce off-target effects and the manipulation of oncogenes and immune checkpoint genes, show promising therapeutic potential. Despite its transformative impact, the application of CRISPR-Cas9 is not without limitations, including concerns regarding off-target mutations, genetic drift, and ecological implications in gene drive systems. These challenges necessitate further refinement of the technology to ensure its safety and efficacy. Overall, CRISPR-Cas9-based genome editing is a pivotal advancement in cancer therapy, offering new avenues for precision medicine while also raising important ethical and technical considerations.

INTRODUCTION

Targeted gene modification holds promise for therapeutic applications. Recently, engineered nucleases such as zinc finger nucleases, transcription activator-like effector nucleases, and CRISPR-Cas systems have emerged as simpler and more cost-effective methods for precise gene editing.[1] The CRISPR-Cas9 system is made up of the DNA endonuclease Cas9 and single guide RNA (sgRNA). The latter is directed by the former to particular DNA sequences in order to cleave double-stranded DNA at a specified place.[2] A disorder caused by a build-up of genetic and epigenetic abnormalities is cancer. Current cancer therapies are limited due to the intricacy of its mechanism, highlighting the need for alternative therapeutic strategies. Because of technological advancements like CRISPR-Cas9-mediated genome editing, nearly any genomic sequence may be accurately altered, allowing for the correction of cancer-causing mutations and the functional elucidation of genes implicated in carcinogenesis.[3,4,5,6] Cas1 is the most conserved protein that is present in most of the CRISPR-Cas systems and evolves slower than other Cas proteins[7] The CRISPR-Cas system classification has been based on the Cas1 phylogeny. Another significant categorization factor that has been identified is the distinct operon organization of the CRISPR-Cas models in the genomes.[8,9] Ecoli, Ypest, Nmeni,

Dvulg, Tneap, Hmari, Apern, and Mtube were the eight unique subtypes that were first postulated and named after the animals whose genomes encoded a typical system of each subtype. As an illustration, the CRISPR subtype Apern, named after the system found in the Aeropyrum pernix genome, includes several distinct genes: csa1 (CRISPR system Apern gene 1), csa2, csa3, and csa4.[8] Creating a dependable and effective technique for gene editing within living cells has been a persistent objective for researchers in the biomedical field. Once the CRISPR mechanism in prokaryotes was deciphered, scientists recognized its potential utility in humans, plans, and other microorganisms. In 2012, Doudna, J, and Charpentier, E made the groundbreaking discovery that CRISPR/Cas-9 could be applied to modify any targeted DNA simply by supplying the appropriate template.[10] The objective of this review is to explore the mechanisms of genome editing facilitated by CRISPR and its protiens (cas9, cas12, cas13) and underscore its recent applications, considering it as a pivotal scientific discovery of this century. Additionally, the review addresses the existing obstacles hindering the widespread adoption of this technology.

MECHANISM OF THE CRISPR GENONE EDITING:

The process of CRISPR/Cas-9 genome editing majorly used genome editing technique that, can be broadly categorized into three stages: recognition, cleavage, and repair.[11] In the initial step, the designed sgRNA guides Cas-9 to identify the target sequence in the gene of interest by means of its 5' crRNA complementary base pair component. In the absence of sgRNA, the Cas-9 protein remains inactive. The Cas-9 nuclease induces double-stranded breaks (DSBs) at a site located 3 base pairs upstream to the Protospacer Adjacent Motif (PAM). The PAM sequence is a short, conserved DNA sequence (2–5 base pairs in length) positioned downstream of the cut site, with its size varying based on the bacterial species. The widely used Cas-9 nuclease recognizes the 5'-NGG-3' PAM sequence (where N can be any nucleotide base). Upon finding the target site with the appropriate PAM, Cas-9 initiates local DNA melting, leading to the formation of an RNA-DNA hybrid. However, the precise mechanism by which the Cas-9 enzyme facilitates the melting of the target DNA sequence is not yet fully understood. Subsequently, the Cas-9 protein becomes activated for DNA cleavage. The HNH domain cleaves the complementary strand, while the RuvC domain cleaves the non-complementary strand of the target DNA, predominantly generating blunt-ended DSBs. Finally, the host cellular machinery repairs the DSB.[12,13]

In the CRISPR/Cas-9 mechanism, two pathways, namely non-homologous end joining (NHEJ) and homology-directed repair (HDR), serve as mechanisms to repair double-stranded breaks (DSBs) induced by the Cas-9 protein.[14] NHEJ plays a crucial role in repairing double-stranded breaks (DSBs) by connecting DNA fragments through enzymatic processes, even without the presence of external homologous DNA. This repair mechanism operates throughout all phases of the cell cycle and stands out as the primary and efficient cellular repair mechanism. Despite its effectiveness, NHEJ is error-prone, often causing small random insertions or deletions (indels) at the cleavage site. This can result in frameshift mutations or the premature introduction of stop codons[15] HDR stands out as a highly precise mechanism, necessitating the utilization of a homologous DNA template. It is particularly active during the late S and G2 phases of the cell cycle. In the context of CRISPR gene editing, HDR demands a substantial quantity of donor (exogenous) DNA templates that carry the desired sequence. The HDR process achieves accurate gene insertion or replacement by incorporating a donor DNA template with sequence homology at the anticipated double-stranded break (DSB) site.[14,15]

CRISPR FOR CANCER THERAPY:

1) BREAST CANCER:

Cas9n, also known as Cas9 nickase, is a modified version of the Cas9 protein derived from the CRISPR/Cas9 genome editing system through genetic engineering.[16] Cas9 nickase, exhibits a capacity to diminish off-target effects when contrasted with Cas9, demonstrating heightened efficiency and enhanced precision in cellular repair machinery. Its versatility extends to proficient tasks, including the precise introduction of breaks at specific locations in double-stranded DNA. To achieve this, two Cas9n molecules are combined and employed collaboratively.

Mixed-lineage kinase 3 (MLK3) plays a pivotal role as a mitogen-activated protein kinase, acting as a crucial regulator in the metastatic processes of triple-negative breast cancer (TNBC).[18] Research findings have illuminated the pivotal role of MLK3 in triple-negative breast cancer (TNBC). In a study by Rattanasinchai and Gallo, a TNBC model was employed to explore the involvement of MLK3, revealing its contribution to cancer progression through specific signaling pathways. The researchers utilized CRISPR/Cas9n to edit MLK3, leading to a notable reduction in TNBC metastasis.[19]

Cas12 is a gene editing tool it has unique characteristics that significantly contribute to its effectiveness in specific DNA manipulation techniques. This protein demonstrates remarkable versatility, excelling in the precise identification and cleaving of target DNA. As a result, Cas12 emerges as a powerful tool with a broad range of applications, notably in the field of gene editing.[20] To elicit a therapeutic response, the design of a guide RNA (gRNA) is crucial, directing Cas12 to the specified target gene. Upon binding to the target, Cas12 induces double-strand breaks in the DNA, triggering the activation of the DNA repair mechanism. Throughout the repair process, the potential for the incorporation of incorrect nucleotides exists, leading to the occurrence of mutations and a subsequent decrease in the functionality of the gene.[21]

CRISPR/Cas9-based editing of oncogenes in TNBC

Utilizing CRISPR/Cas9 technology, researchers have targeted various genes and proteins in their studies. This includes the muting of ubiquitin-protein ligase E3 component N-recognin 5, the application of CRISPR/Cas9 alongside HMG-box transcription factor 1, the knockdown of CXCR4 and CXCR7 genes, the use of CRISPR/Cas9 in conjunction with the knockdown of integrin α9, and the knockout of Cripto1 through CRISPR/Cas9.[22] These approaches demonstrate the versatility of CRISPR technology in precisely manipulating genetic elements for the accurate therapeutic response.

Table 1: Applications of CRISPR in Different Cancer Types

Cancer Type	Target Gene/Protein	CRISPR Strategy	Outcome/Effect	References
Breast Cancer (TNBC)	MLK3	CRISPR-Cas9n editing	Reduced metastasis	[Rattanasinchai and Gallo]
Lung Cancer	PD-1	CRISPR-Cas9 knockout in T cells	Enhanced anti-tumor activity, minimal off-target effects	[Lu et al.]
Leukemia	PD1, Pbrm1	CRISPR-Cas9 editing	Modest knockout effects; accelerated leukemogenesis with Pbrm1 loss	[Li BE et al.]
Liver Cancer	Fusion oncogenes (e.g., DNAJB1-PRKACA)	CRISPR-mediated deletion	Improved survival, tumor reduction	[Kastenhuber et al.]
Colorectal Cancer	PUM1, DDX5, WHSC1, Caspase-3, Par3L	CRISPR-Cas9 knockout	Reduced proliferation, increased therapy sensitivity, reduced metastasis	[Liu et al., Zhou et al.]
Prostate Cancer	TP53	CRISPR-Cas9 repair (using sgRNA+ssODN)	Wild-type p53 restoration, halted cancer cell proliferation	[Batır et al.]

2) LUNG CANCER

According to Lu et al.'s research, disrupting the PD-1 gene in T cells and subsequently reintroducing the gene-edited cells ex vivo could be a therapeutic approach for non-small cell lung cancer. In a group of patients with advanced lung cancer, a T cell therapy edited using CRISPR–Cas9 and targeting the PD-1 gene showed

minimal off-target effects. This study highlighted the clinical feasibility of CRISPR-based technology in this context. Treatment-related adverse events (AEs) were observed in 11 individuals in a group of 12 patients. Common treatment-related AEs encompassed lymphopenia, fatigue, leukopenia, fever, arthralgia, and skin rash. Additional treatment-related AEs included neutropenia, infusion-related reaction, hyperhidrosis, premature beats, hypertension, increased alanine aminotransferase, increased aspartate aminotransferase, thrombocytopenia, and anemia.

Inhibiting EGFR using CRISPR-Cas9 leads to increased expression of major histocompatibility complex class I, enhancing the recognition and destruction of tumor cells by cytotoxic lymphocytes. Researchers collected the patient's blood, isolated lymphocytes, and treated them with a CRISPR-Cas9 gene-editing system containing a specific sgRNA sequence matching the lymphocytes' programmable death 1 protein (PD-1). Once the system identifies its target sequence, cas9 cuts the DNA, triggering the cell's repair mechanisms. Blocking or disabling the PD-1 gene expression prevents cancer cells from having the receptor necessary for interaction with immune lymphocytes.[24,25]Knocking down the PD-1 protein on immune cells is crucial for initiating caspase activation, a process essential for programmed cell death and increased apoptosis in cancerous cells.[26]

3) LEUKEMIA*:*

Early treatments have involved modifying tumor-infiltrating lymphocytes using ex vivo CRISPR-Cas9 technology to disrupt the programmed cell death protein 1 (PD1) checkpoint molecule. Additionally, a phase 1 study explored T cells expressing recombinant antigen-specific TCRs for anticancer therapy after editing the endogenous TCR and PD1 genes. In both instances, there were modest knockout effects via the repair of CRISPR-induced DNA breaks through non-homologous end joining (NHEJ), and no evident signs of genotoxicity were observed. Similar CRISPR approaches have been employed in hematopoietic stem cells (HSCs) to disrupt B cell lymphoma/leukemia 11A for the treatment of hemoglobinopathies[27,28,29]

Pbrm1 Loss Accelerates Leukemogenesis: By disrupting Pbrm1 (also known as Baf180), the researchers observed an accelerated onset of leukemia. Pbrm1-deficient leukemia cells exhibited reduced immunogenicity, suggesting altered interactions with the immune system. The shortened latency period highlighted Pbrm1's critical role in disease development. Interferon Signaling and MHC II Expression: In the absence of PBRM1, interferon signaling pathways were attenuated. Additionally, major histocompatibility complex class II (MHC II) expression decreased in Pbrm1-deficient leukemia cells. These findings underscore the importance of Pbrm1 in immune responses and tumor surveillance. Transcriptional Control and Immune System Crosstalk: PBRM1 emerged as a key regulator of the interferon pathway. Its impact on transcriptional control sheds light on how leukemia cells interact with the immune system. Understanding these dynamics may pave the way for novel therapeutic strategies.

In summary, CRISPR/Cas9 screening identified Pbrm1 as a novel player in leukemia progression, emphasizing the intricate interplay between transcriptional regulation and the immune response in myeloid leukemia[30]

4) LIVER CANCER:

According to research of Edward R. Kastenhuber in mouse, CRISPR-mediated deletion is a cutting-edge technique used to precisely edit DNA sequences, scientists targeted fusion oncogenes (FOs), which are genes formed by the fusion of two separate genes. The researchers focused on specific introns (non-coding regions) within the genes involved in FO rearrangement. By disrupting these introns using CRISPR/Cas9, they aimed to selectively eliminate FOs in cancer cells. The study demonstrated improved overall survival in mice injected with CRISPR constructs and observed tumor-bearing livers. Sanger sequencing confirmed chimeric transcripts resulting from the fusion of DNAJB1 and PRKACA. Histological analysis revealed tumor characteristics, and the approach holds promise for future cancer therapies.

The significance of this work lies in its proof-of-concept nature. By targeting introns associated with fusion oncogenes, researchers have paved the way for novel strategies to combat cancer. This approach offers a precise means of disrupting FOs selectively within cancer cells, potentially revolutionizing cancer treatment. Additionally, the study sheds light on the biology of fusion oncogenes and their role in tumorigenesis. The findings underscore the potential impact of CRISPR-mediated deletions in advancing our understanding of cancer and developing targeted therapies.[31]

According Wang, D. et al. this study, researchers explored the safety and efficacy of CRISPR/Cas9-mediated genome editing in mouse liver. They used an adenovirus (Ad) vector to deliver Streptococcus pyogenes-derived Cas9 (SpCas9) targeting the Pten gene, which is involved in nonalcoholic steatohepatitis (NASH) and negatively regulates the PI3K-AKT pathway. Despite typical Ad vector-associated immunotoxicity, efficient Pten gene editing was achieved. Mice receiving the Pten gene-editing Ad vector exhibited massive hepatomegaly and NASH-like features. Additionally, humoral immunity against SpCas9 and a potential SpCas9-specific cellular immune response were detected. This study provides a strategy for modeling human liver diseases in mice and emphasizes the importance of considering Cas9-specific immune responses in future CRISPR/Cas9 translational studies.[32]

According to Enache, O. M. et al. investigated the impact of Cas9 on the p53 pathway. Key findings include Cas9 activation leading to p53 pathway activation and the selection of p53-inactivating mutations. Understanding these effects is crucial for precise genome editing and therapeutic applications.[33]

5) COLORECTAL CANCER

Colorectal cancer can be classified into four subtypes. Subtype 1 is linked to microsatellite instability (MSI). Subtype 2 is characterized by a higher likelihood of hypermethylation, a better prognosis, activation of the Wnt signaling pathway, and mutations in TP53. Subtype 3 tumors exhibit mutations in KRAS. Subtype 4 is marked by the activation of the TGF-ß pathway in response to matrix infiltration.[34] Liu et al. investigated the role of PUM1 in the resistance mechanism of colon cancer by knocking out PUM1 and dead-box helicase 5 (DDX5) in trastuzumab-resistant SW480R and Caco-2R cells. They discovered that the proliferation of both cell types was reduced. Additionally, they found that PUM1 can positively regulate DDX5, enhancing cell viability. This suggests that downregulating PUM1 and DDX5 can decrease tumor cell viability, making them potential therapeutic targets to improve the sensitivity of colon cancer to trastuzumab.[35] Liu et al. explored the interaction between HMGA2 and WHSC1 in the regulation of cancer cell growth and found that WHSC1 is a transcriptional target of the oncogene HMGA2, which promotes cancer cell proliferation and metastasis. CRISPR-mediated knockout of WHSC1 in colon cancer cells was shown to inhibit cell proliferation, enhance drug sensitivity, and reduce metastatic potential.[36] Zhou et al. investigated the effects of caspase-3 gene knockout on colon cancer behavior through both in vitro and in vivo experiments. They made several key observations: HCT116 cells with caspase-3 knockout (CASP3KO) showed increased sensitivity to chemotherapy and radiotherapy, suggesting that targeting caspase-3 could enhance cancer treatment efficacy. Additionally, CASP3KO cells exhibited reduced invasiveness compared to control cells and were less likely to develop pulmonary metastases when injected either subcutaneously or intravenously. Overall, these findings highlight caspase-3 as a promising therapeutic target for colon cancer, influencing both treatment response and metastatic potential.[37] The researchers suggest that Par3L could be a promising therapeutic target for colon cancer treatment. They found that colon cancer patients with higher Par3L expression had lower survival rates. Using the CRISPR/Cas9 gene knockout technique, they observed an increase in apoptosis in colon cancer cells when Par3L expression was deleted. Additionally, the loss of Par3L caused abnormal activation of the Lkb1/AMPK signaling pathway, making colon cancer cells more responsive to chemotherapy and radiotherapy. These findings highlight the potential clinical importance of targeting Par3L in colon cancer management.[38]

6) PROSTATE CANCER

The TP53 gene is crucial for synthesizing the p53 protein, which plays a key role in suppressing tumor development by triggering apoptosis, halting the cell cycle, and promoting cellular aging.[39]

The malfunctioning of the TP53 gene is implicated in numerous cancer types, with over 26,000 reported cases of somatic and point mutations in various cancer tissues documented in cancer databases. Consequently, a significant focus of cancer treatment research is on reinstating the function of p53 as a strategy for cancer therapy, employing various methodologies.[40]

According to the Batır et al.[41] research the outcome of the single cell culture analysis, which involved cells transfected with sgRNA2+ssODN2, necessitated a further examination of the wild-type p53 protein in non-dividing cells. This led to the utilization of immunofluorescence analysis on these non-proliferative cells that had been treated with sgRNA2+ssODN2, to confirm the expression of the wild-type p53 protein. The findings revealed the presence of the wild-type p53 protein in these cells. Consequently, the rectification of the TP53 414delC mutation through the CRISPR/Cas9 system was found to halt the proliferation of the PC-3 cancer cells.

LIMITATION:

The utilization of CRISPR–Cas9 technology comes with inherent challenges. It is imperative in studies involving RNA-targeted gene editing with CRISPR-Cas9 to meticulously assess any unintended alterations. The enduring nature of genetic drift within a population may result in the perpetuation of off-target mutations across successive generations. Furthermore, the impact and frequency of these mutations have the potential to escalate over time.[42] Regulating the spread of gene drive characteristics can present significant challenges. Additionally, the eradication of an entire population through gene drive could drastically disrupt ecological equilibrium. There is also the concern of gene transfer to different species within the ecosystem, which could result in the propagation of undesirable traits among related species. In comparison, the incidence of off-target mutations tends to be higher in human cells than in those of zebrafish or mice.[43,44] The employment of CRISPR–Cas9 technology is not without its challenges. It is essential to thoroughly examine any unintended effects in studies that utilize RNA-targeted gene editing via CRISPR-Cas9. Genetic drift's continuous presence within a population may lead to the ongoing occurrence of off-target mutations with each new generation. Additionally, the severity and quantity of these mutations may amplify as time goes on.

FUTURE PERSPECTIVE:

CRISPR-Cas9 holds immense potential in cancer biology due to its adaptability, simplicity, convenience, and efficiency. This technology introduces a groundbreaking approach to cancer treatment by enabling precise modifications to the genome of target cells, a task that was previously challenging. Its versatility, effectiveness, and flexibility position it as a promising future method for cancer care. CRISPR-Cas9 is expected to significantly impact the field of cancer biology, provided researchers develop well-structured strategies and tools for delivering the technology to specific cells or tissues, along with reliable methods to minimize and manage off-target effects.

CONCLUSION

CRISPR-Cas9 technology has become a groundbreaking tool in contemporary oncology, providing accurate, affordable gene-editing methods for the targeting of numerous cancers, such as breast, lung, liver, leukemia, colorectal, and prostate cancers. Main findings indicate that CRISPR-based methods such as Cas9 nickase diminish metastasis in triple-negative breast cancer, disruption of PD-1 gene enhances immune responses in lung cancer, and gene knockouts enhance therapy sensitivity in colorectal cancers. Research also emphasizes new targets such as Pbrm1 in leukemia and disruption of fusion oncogenes in liver cancer, highlighting the diversity of CRISPR. Its potential to revolutionize medicine, however, comes with limitations in off-target effects, genetic drift, and immune activation that must be addressed for efficacy and safety. In total, CRISPR-Cas9 is a potential precision therapy for cancer with implications for future clinical use following strategic optimization.

Acknowledgement

The authors are grateful to JKKN College of Pharmacy, Kumarapalayam, Tamil Nadu, for providing the necessary support and encouragement throughout the preparation of this manuscript.

Conflict of Interest

The authors declare no conflicts of interest regarding this study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Shajin.J and Vasanth Albert.R contributed to the conceptualization, literature review, and drafting of the manuscript.

Revanth.R supervised the work, critically reviewed the content, and provided essential revisions.

All authors read and approved the final version of the manuscript.

REFERENCES

- Kim H & Kim JS (2014) A guide to genomeengineering with programmable nucleases.Nat RevGenet15, 321–334
- F. Jiang, J.A. Doudna, CRISPR-Cas9 structures and mechanisms, Annu. Rev.
- Biophys. 46 (2017) 505–529J.A. Doudna, E. Charpentier, Genome editing. The new frontier of genome en-gineering with CRISPR-Cas9, Science 346 (2014) 1258096.
- P.D. Hsu, E.S. Lander, F. Zhang, Development and applications of CRISPR-Cas9 for genome engineering, Cell 157 (2014) 1262–1278.
- E. Charpentier, L.A. Marraffini, Harnessing CRISPR-Cas9 immunity for genetic engineering, Curr. Opin. Microbiol. 19 (2014) 114–119.
- H. Wang, M. La Russa, L.S. Qi, CRISPR/Cas9 in genome editing and beyond, Annu.Rev. Biochem. 85 (2016) 227–264
- Takeuchi N, Wolf YI, Makarova KS, Koonin EV. Nature and intensity of selection pressure on CRISPR-associated genes. J Bacteriol. 2012;194:1216–1225.
- Haft DH, Selengut J, Mongodin EF, Nelson KE. A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. PLoS Comput Biol. 2005;1:e60
- Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biol Direct. 2006;1:7.
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA – guided. Science. 2012;337(6096):816–822.
- Shao M, Xu T, Chen C. The big bang of genome editing technology: development and application of the CRISPR/CAS9 system in disease animal models. Sci Press Zool Res. 2016;37(2):1–11
- Ceasar SA, Rajan V, Prykhozhij SV, Berman JN, Ignacimuthu S. Insert, remove or replace: a highly advanced genome editing system using CRISPR/Cas9. Biochim Biophys Acta Mol Cell. 2016;1863(9):2333–2344.
- Jiang F, Doudna JA. CRISPR-Cas9 structures and mechanisms. Annu Rev Biophys. 2017;46(1):505–529.
- Liu M, Rehman S, Tang X, et al. Methodologies for improving HDR efficiency. Front Genet. 2019;9:1–9.
- DYang H, Ren S, Yu S, et al. Methods favoring homology-directed repair choice in response to CRISPR/cas9 induced-double strand breaks. Int J Mol Sci. 2020;21(18):1–20.
- Gupta D., Bhattacharjee O., Mandal D., Sen M. K., Dey D., Dasgupta A., et al. (2019). CRISPR-Cas9 system: A new-fangled dawn in gene editing. Life Sci. 232, 116636.
- Yee J.-K. (2016). Off-target effects of engineered nucleases. FEBS J. 283, 3239–3248.
- Cronan M. R., Nakamura K., Johnson N. L., Granger D. A., Cuevas B. D., Wang J.-G., et al. (2012). Defining MAP3 kinases required for MDA-MB-231 cell tumor growth and metastasis. Oncogene 31, 3889–3900.

- Rattanasinchai C., Gallo K. A. (2016). MLK3 signaling in cancer invasion. Cancers 8, 51. 10.3390/cancers8050051
- Pickar-Oliver A., Gersbach C. A. (2019). The next generation of CRISPR–Cas technologies and applications. Nat. Rev. Mol. Cell Biol. 20, 490–507.
- Zhang H., Qin C., An C., Zheng X., Wen S., Chen W., et al. (2021a). Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. Mol. Cancer 20, 126.
- Tiwari PK, Ko TH, Dubey R, et al. CRISPR/Cas9 as a therapeutic tool for triple negative breast cancer: from bench to clinics. Front Mol Biosci. 2023;10:1214489. Published 2023 Jul 4.
- Tiruneh G/Medhin M, Chekol Abebe E, Sisay T, Berhane N, Bekele T, Asmamaw Dejenie T. Current Applications and Future Perspectives of CRISPR-Cas9 for the Treatment of Lung Cancer. Biologics. 2021;15:199-204.
- Castillo A. Gene editing for the treatment of lung cancer (CRISPR-Cas9). Colomb Med. 2016;47(4):178–180.
- Lu Y. PD-1 knockout engineered T cells for metastatic non-small cell lung cancer. ClinicalTrials.gov. 2019.
- Zhao Z, Shi L, Zhang W, et al. CRISPR knock out of programmed cell death protein 1 enhances the anti-tumor activity of cytotoxic T lymphocytes. Oncotarget. 2018;9(4):5208.
- Y. Lu, J. Xue, T. Deng, X. Zhou. Mok,Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. Nat. Med.26,732–740 (2020).
- E. A. Stadtmauer, Y. Zhao, S. F. Lacey. June,CRISPR-engineered T cells in patients with refractory cancer. Science367,eaba7365 (2020).
- A. Sharma, S. Sheth, S. Soni, M. H. Steinberg, D. Wall, A. Yen, S. Corbacioglu,CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N. Engl. J. Med.384,252–260 (2021).
- Li BE, Li GY, Cai W, et al. In vivo CRISPR/Cas9 screening identifies Pbrm1 as a regulator of myeloid leukemia development in mice. Blood Advances. 2023;7(18):5281-5293.
- Kastenhuber ER, Lalazar G, Houlihan SL, et al. DNAJB1-PRKACA fusion kinase interacts with β-catenin and the liver regenerative response to drive fibrolamellar hepatocellular carcinoma. Proc Natl Acad Sci U S A. 2017;114(50):13076-13084.
- Thorsten Kaltenbacher, Löprich J, Maresch R, et al. CRISPR somatic genome engineering and cancer modeling in the mouse pancreas and liver. Nature Protocols. 2022;17(4):1142-1188.
- Enache OM, Rendo V, Abdusamad M, et al. Cas9 activates the p53 pathway and selects for p53-inactivating mutations Nat Genet. 2020;52(7):662-668. doi:10.1038/s41588-020-0623-4
- Wang W, Kandimalla R, Huang H, Zhu L, Li Y, Gao F, et al. Molecular subtyping of colorectal cancer: Recent progress, new challenges and emerging opportunities. Semin Cancer Biol (2019) 55:37–52. doi: 10.1016/j.semcancer.2018.05.002
- Liu Q, Xin C, Chen Y, Yang J, Chen Y, Zhang W, et al. Pum1 is overexpressed in colon cancer cells with acquired resistance to cetuximab. Front Cell Dev Biol (2021) 9:696558. doi: 10.3389/fcell.2021.696558
- Liu HH, Lee CH, Hsieh YC, Hsu DW, Cho EC. Multiple myeloma driving factor Whsc1 is a transcription target of oncogene Hmga2 that facilitates colon cancer proliferation and metastasis. Biochem Biophys Res Commun (2021) 567:183–9.
- Zhou M, Liu X, Li Z, Huang Q, Li F, Li CY. Caspase-3 regulates the migration, invasion and metastasis of colon cancer cells. Int J Cancer (2018) 143(4):921–30.
- Li T, Liu D, Lei X, Jiang Q. Par3l enhances colorectal cancer cell survival by inhibiting Lkb1/Ampk signaling pathway. Biochem Biophys Res Commun (2017) 482 (4):1037–41.
- Chen JD (2016) The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression. Csh Perspect Med.
- Janic A, Valente LJ, Wakefeld MJ, Di Stefano L, Milla L, Wilcox S, Yang HY, Tai L, Vandenberg CJ, Kueh AJ, Mizutani S, Brennan MS, Schenk RL, Lindqvist LM, Papenfuss AT, O'Connor L, Strasser A, Herold MJ (2018) DNA repair processes are critical mediators of p53-dependent tumor suppression. Nat Med 24(7):947
- Batır MB, Şahin E, Çam FS. Evaluation of the CRISPR/Cas9 directed mutant TP53 gene repairing effect in human prostate cancer cell line PC-3. Molecular Biology Reports. 2019;46(6):6471-6484.
- Rodriguez, E. Ethical Issues in Genome Editing using Crispr/Cas9 System. J. Clin. Res. Bioeth. 2016, 7.
- Oye, K.A.; Esvelt, K.; Appleton, E.; Catteruccia, F.; Church, G.; Kuiken, T.; Lightfoot, S.B.-Y.; McNamara, J.; Smidler, A.; Collins, J.P. Regulating gene drives. Science 2014, 345, 626.
- Hwang, W.Y.; Fu, Y.; Reyon, D.; Maeder, M.L.; Tsai, S.Q.; Sander, J.D.; Peterson, R.T.; Yeh, J.R.J.; Joung, J.K. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat. Biotechnol. 2013, 31, 227–229.