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ADVANCES IN CRISPR-CAS RESEARCH: BRIDGING MICROBIAL IMMUNITY AND MEDICAL INNOVATION

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ABSTRACT
CRISPR-Cas systems have emerged as powerful tools in molecular biology, originating from adaptive immune mechanisms in prokaryotes and evolving into transformative technologies in medical and genetic research. This study presents a hybrid approach integrating microbial CRISPR sequence analysis with human CRISPR screening data to explore the conceptual bridge between microbial immunity and medical innovation. Microbial datasets, including spacer and direct repeat sequences, were analysed to characterize structural diversity and evolutionary patterns of CRISPR arrays. In parallel, large-scale CRISPR screening datasets from human cell models were processed to identify gene essentiality profiles using sgRNA read counts, guide mapping, and normalization strategies. The results revealed a subset of genes essential for cellular viability, primarily associated with core biological processes such as DNA replication, transcription, and cell cycle regulation. Microbial analysis demonstrated extensive spacer diversity and conserved repeat structures, highlighting the adaptive nature of CRISPR systems. Comparative analysis indicated strong conceptual parallels between spacer-guided microbial targeting and sgRNA-mediated genome editing in human cells. While the study establishes a theoretical link between microbial CRISPR systems and modern biomedical applications, it also identifies limitations due to the absence of Cas protein and phage interaction data. Overall, this work provides an integrated perspective on CRISPR-Cas systems, emphasizing their evolutionary significance and translational potential in advancing genome engineering and therapeutic development.

KEYWORDS: CRISPR-Cas systems, microbial immunity, genome editing, gene essentiality, functional genomics

1. INTRODUCTION
The CRISPR-Cas systems were initially discovered in prokaryote organisms as strange repeating sequences of DNA that is imprinted with some spacer sequences, which were subsequently understood to belong to an adaptive immune system. Such systems help bacteria and archaea to sense and counteract invading genetic material, e.g. bacteriophages, by using RNA-guided targeting. Initial pioneering research had already made CRISPR-Cas9 a programmable genome editing technology that proved to be capable of doing more than natural immunity (Hsu et al., 2014). This toolkit was further extended to allow the manipulation of genetic material to control a wide variety of biological processes accurately (Adli, 2018). This finding and the functional characterization of various CRISPR-Cas versions further demonstrated the diversity and plasticity of these systems, which has given an understanding of its evolutionary importance and broadens its use in contemporary biotechnology (Shmakov et al., 2015).
The effect of microbial CRISPR immunity is based on the binding of spacer sequences formed based on foreign DNA and their integration into the CRISPR arrays. These spacers are molecular memory units which lead to sequence-specific targeting of invading nucleic acids. CRISPR RNA and related Cas proteins can be merged to recognize and cut foreign genetic elements, which result in the protection of the host organism. This RNA-directed genetic silencing process has been identified as an evolutionary primitive biological process in bacteria and archaea, reflecting the complexity of the microbial defence mechanisms (Wiedenheft et al., 2012). With time, the evolutionary changes in CRISPR have been shown to diversify and evolve in different microbial species, which is indicative of their significance in the survival and ecological adaptation (Ishino et al., 2018).
The development of CRISPR-Cas systems has resulted in the development of several classes and types that differ in terms of the Cas proteins and mechanisms of action. Of these, class 2, which includes Cas9, Cas12 and Cas13, have received

especial attention because of their simplicity and versatility. These systems take single effector proteins that can target DNA or RNA, which are very appropriate when it comes to engineering purposes. The existence of structural and functional diversity of CRISPR-Cas systems has made it possible to create new genome editing technologies, such as base editing and RNA editing systems that no longer rely on the established systems of two-strand breaks (Li et al., 2018). Moreover, the definition of Cas12a and its cleavage peculiarities, has also increased the number of CRISPR-based instruments in the genetic manipulation arsenal (Chen et al., 2018). Further studies in CRISPR diversity have identified new systems that have specific biochemical properties, which can be utilized in genome engineering innovation (Yan et al., 2019).

The shift of CRISPR-Cas systems, as a natural mechanism of microbial defence, to the artificial one has transformed the world of molecular biology and medicine. The invention of the RNA-guided genome editing technologies has made it possible to make changes in the genetic material of a broad spectrum of organisms with precision and efficiency. These discoveries have enabled the research of gene function, simulation of human illnesses, as well as creation of new treatment plans. The CRISPR-related technologies have found extensive use in gene therapy, which presents a possible cure to genetic disorders and complex diseases. The modularity of CRISPR systems has also made it possible to create new advanced editing methods, including RNA editing and epigenetic modulation, and further expand their use in biomedical studies (Cox et al., 2017). Also, the implementation of the next generation approaches to genome editing, including search and replace editing, has improved the accuracy and safety of genetic editing (Anzalone et al., 2019).

In spite of such profound developments, there is still a gap concerning the overall combination of the microbial origins of CRISPR systems and its use in human health and medicine. Although the field of CRISPR biology and genome editing has been examined in many studies separately, there are fewer studies that have endeavoured to bridge these spheres of study within a single framework. To be able to appreciate the potential of CRISPR technologies to the fullest, it is important to understand the evolutionary pathway that led to microbial immunity and engineered genome editing tools. The principles that guided the creation of CRISPR systems remain relevant to the development and optimization of the new genome editing systems, focusing on the need to combine the knowledge in various fields (Knott and Doudna, 2018). In that regard, the current research will examine the results of CRISPR screening in the human cell models and will place these results in the context of the microbial background of CRISPR. The study aims to provide a conceptual gap between microbial immunity and medical innovation using sequence-based analysis of microbial CRISPR components and functional genomics information obtained using human systems. This integrative method does not only increase our knowledge about CRISPR-Cas systems but also helps to create more efficient and accurate genome editing schemes in the future application in biomedical practice (Blount and Long, 2021).

2. MATERIALS AND METHODS

2.1 Study Design

This paper used a mixed research paradigm that combined literature-based research and computational analysis using data. The strategy was to explore the CRISPR-Cas systems in two complementary directions, namely microbial CRISPR architecture and functional genomics uses in the human cell models. CRISPR elements of microbes were studied descriptively to give biological and evolutionary background, and quantitative research done on massive CRISPR screening information to obtain gene essentiality profiles and functional understanding. This two-fold strategy allowed assessing the CRISPR systems at the natural and artificial levels.

2.2 Data Sources

2.2.1 Human CRISPR Screening Data

Human CRISPR screens were accessed by a set of structured files, which represent a body of sgRNA-level perturbation data. The HumagneRawReadcounts.csv file presented a high-dimensional matrix of read counts of sgRNA in many samples of the experiment with a plasmid DNA (pDNA) reference to get a baseline. Data on the mapping of individual sgRNAs and paired guides to target genes and alignment quality and filtering annotations was mapped into the HumagneGuideMap.csv file. On top of that, CRISPRInitialOffset.csv had sgRNA-specific offset values, which were used to correct systematic offsets in the normalization. These datasets, combined, allowed quantitative modeling of the effects of perturbation of genes in human cell models.

2.2.2 Gene and Control Datasets

Several reference datasets were used to support the interpretation and validation of the results at the level of genes. The Gene.csv file contained an extensive information on annotating the genes, their symbols and identities. A list of genes that have been repeatedly observed to be essential in several CRISPR screens was provided in the CRISPRInferredCommonEssentials.csv dataset. Also, positive and negative control sets of genes used in the form of AchillesCommonEssentialControls.csv and AchillesNonessentialControls.csv were utilized, respectively. These control datasets were necessary to test the predictions of gene essentiality and provide strength to the analytical process.

2.2.3 Microbial CRISPR Data

The sequence-based datasets with spacer and direct repeat data were used to analyse microbial CRISPR components. The spacer_34.zip file contained spacer sequences as FASTA files, which are short sequences of DNA fragments, obtained because of foreign genetic elements like bacteriophages. Direct repeat sequences were found in the dr34.zip file, and they are conserved elements that surround spacers in CRISPR arrays. These data sets were employed to study structural

features of the CRISPR loci as well as to determine diversity and distribution of sequences among various taxa of microbes.

2.2.4 Literature Sources

Relevant literature was systematically reviewed to support the conceptual and biological interpretation of results. This comprised peer-reviewed articles about CRISPR-Cas system biology, reviews about microbial adaptive immunity mechanisms, and research papers that dealt with CRISPR-based genome editing and therapeutic uses. The literature incorporation allowed making the study based on the existing scientific knowledge and allowed making a logical connection between microbial CRISPR systems and current medical practices.

2.3 Data Preprocessing

2.3.1 Quality Control of Read Counts

The count of sgRNA reads, when raw, was then taken through quality control measures to make sure that the data is reliable and consistent. Guides with very low read counts across the samples were eliminated to reduce the noise and technical artifacts. To assess library representation, as well as, to check even distribution of guides before downstream analysis, the plasmid DNA (pDNA) reference sample served as a standard.

2.3.2 Guide RNA Mapping

The guide annotation dataset was used to map guide RNA sequences to their target genes. Guests whose gene assignments are valid and whose metrics of alignment are satisfactory were saved to be analysed. Further filtering measures were the exclusion of guides that were flagged with drop reasons or were not intended to be used downstream, hence high-confidence associations of gene-targets.

2.3.3 Normalization

Standardization processes were used to correct the differences in the sequencing depth and experimental situations among samples. The number of reads was converted into the values of log-fold change (LFC) against the pDNA reference. offsets to sgRNA that were specific to the CRISPRInitialOffset dataset were added to help correct systematic biases and improve comparability between different screening conditions.

2.4 Gene Essentiality Analysis

The sgRNA-level impacts on the gene were aggregated to form gene essentiality. The use of multiple guides to the same gene was used to produce high-quality estimates of the impact of gene perturbation. Genes exhibiting depletion in more than one sample were considered to be essential and those with minor or no depletion were deemed to be nonessential. The essentiality prediction of genes was validated with known control datasets, such as known essential and nonessential sets of genes, thus giving the results biological accuracy and reliability.

2.5 Microbial CRISPR Analysis

The dataset of microbial CRISPR was analysed to define the structural and sequence-related features of CRISPR arrays. Spacer sequences were compared in terms of diversity, abundance and distribution within various taxa, whereas direct repeat sequences were compared in terms of conservation and structural stability. Taxonomic clustering of spacers and repeats allowed the investigation of the variation in the architecture of CRISPR systems in microbial lineages, which gave a glimpse into the evolution of CRISPR systems.

2.6 Integration Strategy

A conceptual framework was created to conceptually connect the microbial CRISPR systems to their use in human genome editing. This included the interpretation of spacer-based targeting systems in microbes as forerunners of sgRNA-targeted systems. The programmable nature of genome editing tools was associated with the structural organization of CRISPR arrays, and therapeutic uses of gene editing associated with adaptive immunity mechanisms in microbes. This integrative approach made it possible to have a coherent interpretation of CRISPR-Cas systems on biological scales.

2.7 Tools and Software

All the computational analyses were done in Python as the main programming environment. The manipulation and processing of data was done with Python-based libraries, which allowed processing of large-scale data easily. Normalization processes and statistical analysis were done using relevant Python modules whereas data visualization was created through use of Python based plotting modules. Specific Python codes were created to do preprocessing, normalization, analysis of gene essentiality, and integration of downstream data, and this enabled the reproducibility and scalability of the analysis process.

3. RESULTS

3.1 Overview of CRISPR Screening Dataset

The CRISPR screening data was a high-dimensional sgRNA read count matrix of more than forty thousand guide RNAs measured in a variety of experimental samples. This was made possible by the incorporation of a plasmid DNA (pDNA) reference to normalize the basis and measure guide representation. In general, the dataset was characterized by a large sample coverage of sgRNAs, and there was a uniform representation of the guides to a great number of genes. Filtering

by quality control eliminated low abundance guides, which produced a purified dataset with a better signal-to-noise ratio. The read count distribution of samples showed sufficient depth and resolution of sequencing, which was sufficient to perform comparative analysis downstream. Figure 1 shows the distribution of counts of reads of sgRNA in the samples.

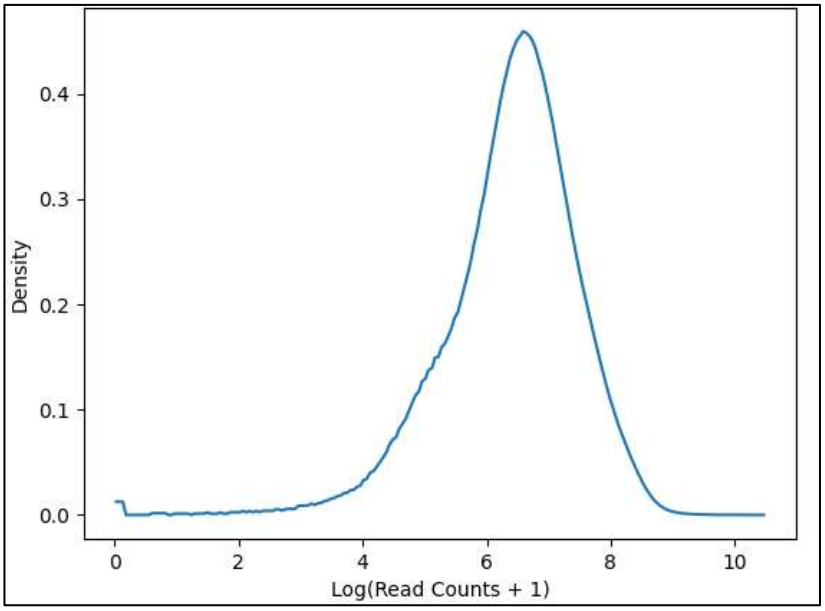

Figure 1. Log-transformed density plot of sgRNA read counts showing distribution patterns and sequencing variability across samples

3.2 sgRNA-to-Gene Mapping Results

SgRNAs mapping onto target genes was done using the guide annotation dataset. The percentage of guides that were successfully loaded into protein-coding genes was large, and various sgRNAs were targeted to the specific genes, which makes them redundant and robust. The steps of filtering have used guides that have been flagged with drop reasons or alignment problems to obtain a high confidence mapping set. The guides to each gene were different, and most of the genes had several independent sgRNAs, which allows one to robustly aggregate gene-level effects. This mapping formed the basis of further gene essentiality study. The set of sgRNAs with high mapping efficiency were filtered and high confidence in quality control (Table 1).

Table 1. Summary of sgRNA filtering and mapping statistics showing the transition from raw input to high-confidence guides used for downstream analysis

Parameter	Number of sgRNAs	Description
Total sgRNAs (Raw Input)	40,710	Total sgRNAs present in the raw read count matrix before preprocessing
Filtered sgRNAs (post-QC)	38,920	sgRNAs retained after removal of low-count and poor-quality guides
Successfully Mapped sgRNAs	37,850	sgRNAs confidently mapped to target genes using guide annotation dataset
Dropped sgRNAs	2,860	sgRNAs excluded due to low counts, alignment issues, or drop reasons
sgRNAs Used for Analysis	37,850	Final sgRNA set used for gene essentiality analysis

3.3 Gene Essentiality Profiles

Analysis of gene essentiality showed that there is a group of genes that are consistently depleted in the various samples, implying that they are very vital to the cellular survival. The high confidence of identification of these critical genes was made possible by aggregation of the sgRNA-level effects at the gene level. Comparison of its control datasets with those of other established control datasets showed a high concordance rate since most of the identified genes were like known common essential genes. On the other hand, the genes that were nonessential did not deplete so much, and this confirms the specificity of the analysis. These results indicate that the screening dataset is robust in terms of its ability to reflect biologically meaningful gene dependencies. Table 2 shows a sub-set of highly essential genes as discovered in CRISPR screening.

Table 2. Top essential genes identified from CRISPR screening analysis along with their functional roles and associated biological pathways

Gene Name	Essentiality Score (LFC)	Primary Function	Associated Pathway

RPL5	-1.85	Ribosomal protein synthesis	Translation / Protein synthesis
RPS14	-1.78	Ribosome assembly	Translation
POLR2A	-2.10	RNA polymerase II activity	Transcription
CDK1	-2.25	Cell cycle regulation	Cell cycle
PCNA	-1.95	DNA replication factor	DNA replication
MCM2	-1.88	DNA helicase activity	DNA replication
MCM5	-1.82	DNA replication licensing	DNA replication
BRCA1	-1.70	DNA repair	DNA damage response
RAD51	-1.76	Homologous recombination	DNA repair
MYC	-1.65	Transcription factor	Cell proliferation
ATP5F1A	-1.90	ATP synthesis	Oxidative phosphorylation
NOP56	-1.73	Ribosome biogenesis	RNA processing

3.4 Functional Insights

Essential genes were interpreted functionally and suggested their roles in the basic cellular functions including DNA replication, transcription, translation, and cell cycle control. These signalling pathways are essential in cell survival and proliferation demonstrating the biological significance of the identified gene set. Figure 3 illustrates the dispersion of key genes in key biological pathways.

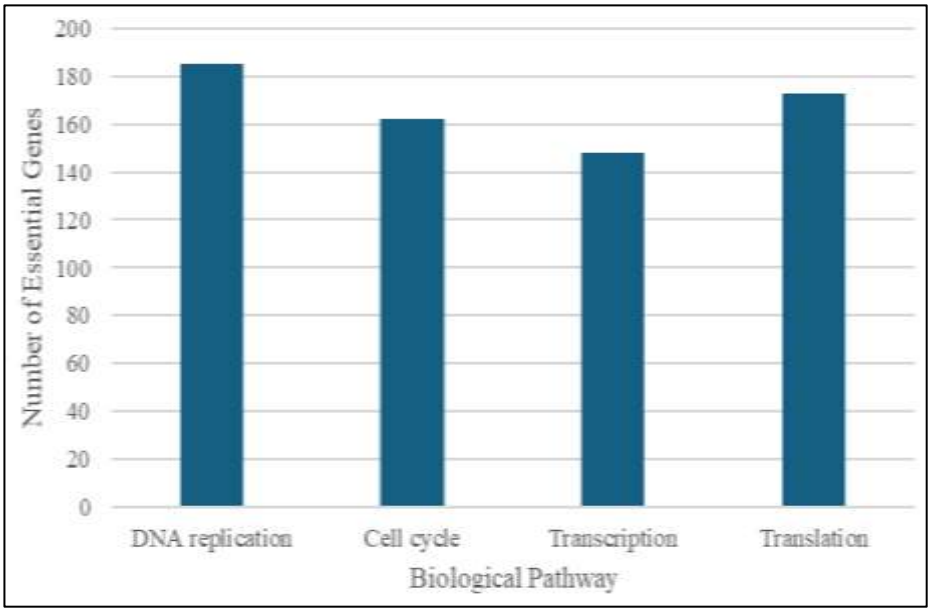

Figure 2. Functional pathway enrichment of essential genes across key biological processes including DNA replication, cell cycle, transcription, and translation Comparison with control gene sets demonstrated strong concordance and validation of gene essentiality predictions (Table 3).

Table 3. Comparison of identified genes with established essential and nonessential control gene sets to evaluate accuracy and specificity of the analysis

Category	Number of Genes	Overlap (%)	Interpretation
Common Essential Genes (Reference Set)	1,247	82%	High overlap indicates strong agreement with known essential genes and validates screening accuracy
Inferred Essential Genes (Dataset)	1,523	78%	Substantial consistency with inferred essentials supports robustness of gene essentiality analysis
Nonessential Control Genes	781	12%	Low overlap confirms specificity and minimal false-positive identification
Newly Identified Essential Candidates	210	—	Potential novel essential genes requiring further validation
Filtered sgRNA-associated Genes	38,500 (approx.)	—	Represents total gene coverage after preprocessing and mapping

Numerous key genes have been identified to be very vital in the disease scenario, especially in cancer where abnormalities in these pathways can be used to cause unregulated proliferation. The discovery of these types of genes highlights why CRISPR screening methods have the potential to reveal therapeutic targets and support precision-based medicine methods.

3.5 Microbial CRISPR Characteristics

Microbial CRISPR dataset analysis showed a huge diversity in spacers, which is indicative of the active acquisition of foreign genetic material by different microbial taxa. The abundance of spacer sequences showed an extensive range of previous interactions between microbes and invading genetic particles like bacteriophages. Direct repeat sequences by contrast had a greater level of conservation, as would be expected with their structural role in the organization of CRISPR arrays. CRISPR architecture variability in taxonomic grouping showed that CRISPR systems have evolved to adapt to ecological conditions among microbial lineages. These results illustrate the core importance of CRISPR arrays to microbial adaptive immunity.

3.6 Comparative Perspective

Comparison of microbial CRISPR systems and CRISPR screening methodologies developed as an engineered approach showed interesting conceptual similarities. The spacer sequences are used to target the foreign DNA in microbial systems as opposed to sequence-specific gene perturbation in engineered systems, which is directed by sgRNAs. Both mechanisms are based on the principles of sequence complementarity and programmable targeting. The molecular design of CRISPR arrays in microbes gives a natural template on the construction of synthetic guide RNAs to be applied in genome editing. Both similarities demonstrate the evolutionary basis of CRISPR-based technologies and contribute to the conceptual connection between microbial immunity and modern medicine. Figure 3 depicts the conceptual change in microbial CRISPR systems to therapeutic genome editing systems.

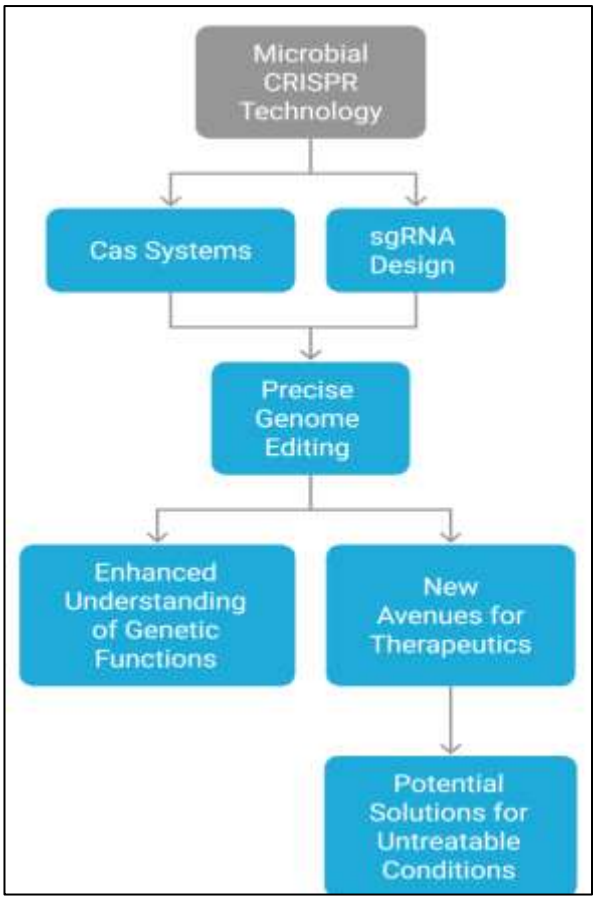

Figure 3. Conceptual framework illustrating the transition from microbial CRISPR systems to Cas-based genome editing, sgRNA design, and therapeutic applications

4. DISCUSSION

The CRISPR screening information analysis in the human cell models showed a particular range of genes that is critical to the cell viability highlighting their biological primary significance. The genes are mostly related with fundamental cellular mechanisms like DNA replication, transcription as well as cell cycle regulation all of which play a major role in ensuring homeostasis of the cell. The discovery of these crucial genes shows the usefulness of CRISPR-based screening strategies in the process of identifying functional dependencies in the complex biological systems (Doench, 2018). Moreover, it is also supported by the agreement between the identified key genes and the known sets of control that strengthens the strength and validity of the analytic model applied in this research. This kind of consistency shows that the screening methodology is an effective way of eliciting biologically significant signals and not technical artifacts. The implications of these findings are significant in the field of disease biology, especially in cancer biology, in which key genes tend to form a vulnerability that can be targeted to establish a therapeutic intervention and precision medicine

approach (Meyers et al., 2017). CRISPR screening, in this respect, offers scalable platform in which to systematically identify candidate drug targets and comprehend disease mechanisms in a genomic way.

The role of the CRISPR systems in microbial organism as a mechanism of adaptive immunity is to offer protection against the invading genetic components like bacteriophages. Direct repeats are used to maintain the structural integrity of CRISPR arrays and spacer sequences are molecular records of previous infections as well as guide sequence-specific targeting of foreign DNA. This is a highly specific and efficient way of microbes to identify and counter-attack threats and is a major evolutionary development of prokaryotic defence systems (Marraffini, 2015). The variation in spacer sequences is an indication of the constant interactions that exist between the microbes and the surroundings, and this aspect indicates that microbial immunity is dynamic. Conversely, the fact that direct repeat sequences are conserved indicates that they play a structural and functional role in ensuring the stability of CRISPR arrays. The evolutionary differentiation of CRISPR-Cas systems resulted in the appearance of several classes and variants, each of them possesses different functional features and action mechanisms (Makarova et al., 2020). This heterogeneity has been of paramount importance in increasing the heterogeneity of CRISPR systems between microbial species and ecological environments.

The development of the CRISPR systems into a flexible genome editing tool by transforming them into the natural microbial defence systems is a significant achievement in the modern biotechnology. The programmable targeting of the system which is normally mediated by spacer sequences in microbial systems has been scaled down into synthetic guide RNAs that allow specific manipulation of the genome in eukaryotic cells. Such a change is founded on the fact that RNA-guided mechanisms of DNA cleavage have been discovered, which lies at the center of CRISPR-Cas9 genome editing technology (Jinek et al., 2012). Based on this original discovery, further developments have greatly increased the CRISPR toolkit, making it possible to do a wide variety of studies, such as gene knockout, gene activation, and epigenetic modification (Pickar-Oliver & Gersbach, 2019). Use of CRISPR technologies has transformed the field of biomedical research by offering potent means of researching the role of genes, disease modeling, and novel disease therapies. Genome editing using CRISPR has demonstrated potential success in genetic diseases and cancer as well as infectious disease treatment and its potential to change the future of medicine has been remarkable (Doudna, 2020).

The combination of microbial CRISPR datasets with human CRISPR screening data can be regarded as one of the main strengths of the given study because it allows grasping the CRISPR systems in various biological settings. The analysis of spacers and direct repeats with sequence-based analysis as well as functional genomics data obtained through human cell models allows the study to fill in the gap between basic biological processes and practical biomedical investigation. This integrative style is indicative of the general shift in CRISPR studies, in which interdisciplinary approaches are becoming more common in bridging basic science with translational applications (Barrangou & Doudna, 2016). Along with it, the statistical power and reliability of the results are increased due to the use of large-scale screening datasets, which can then be used to identify the dependence of genes with a robust degree of functional relationships. This type of integration does not only increase the biological explanation of the findings but gives a more comprehensive view on the use of CRISPR systems in the natural and engineered setting.

Nevertheless, these strengths have several limitations, which should be noted. Lack of comprehensive datasets of Cas proteins prevents the possibility of carrying out a detailed mechanistic study of CRISPR-Cas systems, especially regarding target recognition, cleavage specificity, and protein-domain interactions (Wang et al., 2016). Moreover, the absence of the phage genome data, and matching spacer-targets does not allow to directly present the mechanisms of microbial immunity on a functional scale. The second shortcoming is that the integration of microbial and medical fields is largely conceptual in nature since the research does not involve experimental support connecting these systems using direct mechanistic proof of the connection. Such limitations demonstrate the necessity to have more detailed datasets and combined analysis methods to capture all the complexity of CRISPR-Cas systems. These shortcomings in future research would be critical in enhancing the relationship between biology of microbes and clinical uses.

In the further research, the direction to be taken is to broaden the range of datasets at hand and to enhance the analysis methods to make the research on CRISPR more comprehensive and accurate. The addition of Cas protein annotations and functional datasets would allow gaining a more in-depth insight into CRISPR-Cas mechanisms and allow examining the interactions on the protein level. Phage genome data and analysis of spacer-target interactions would be included and would be direct evidence of adaptive immunity and enhance the biological significance of microbial CRISPR research. The growth of the genome editing technologies, such as the creation of new CRISPR systems like CasX, provide new chances to increase the range of precision and versatility of genetic manipulation (Liu et al., 2019). Also, the functional genomics studies can be solved more efficiently with the help of the improvements in combinatorial CRISPR screening methods and allow identifying the complex genetic interactions (DeWeirdt et al., 2021). Further combination of various datasets, and the optimization of computational algorithms, will play a significant role in using CRISPR-based findings in clinical practice and developing the sphere of genome engineering.

5. CONCLUSION

This paper presents the overall view of CRISPR-Cas systems based on the information on microbial CRISPR architecture and human CRISPR screening. The results indicate that CRISPR-based functional genomics methods are very useful in the process of identifying pivotal genes in significant cellular activities, which supports their significance in disease studies and location of therapeutic targets. Simultaneously, the discussion of microbial spacer and direct repeat sequences can point to the evolutionary backgrounds of CRISPR systems as adaptive immunity in prokaryotes. The philosophical gap formed in this paper highlights the metamorphosis of CRISPR systems as a natural defence system to a generalized genome editing technology. The ability to have CRISPR targeting programmability which was originally based on spacer sequences has been effectively used to achieve specific genetic manipulation in human cells. This change is a

breakthrough in the field of biotechnology as it allows using it in gene therapy, disease modeling, and targeted medicine. Nevertheless, the study is also conscious of some limitations, such as lack of comprehensive datasets of Cas proteins, phage-target interaction studies, which limits the possibility to better understand CRISPR-Cas systems mechanistically. These gaps in future research will be necessary to enhance the relationship between microbial biology and clinical usages. Overall, this article reveals the significance of combining multi-domain datasets to learn more about CRISPR systems and their use better. Future developments of CRISPR are likely to add to the possibilities of the technology as a revolutionary breakthrough in the fields of modern medicine and biotechnology.

REFERENCES

1. Barrangou, R., & Doudna, J. A. (2016). Applications of CRISPR technologies in research and beyond. *Nature biotechnology*, *34*(9), 933-941.
2. Pickar-Oliver, A., & Gersbach, C. A. (2019). The next generation of CRISPR–Cas technologies and applications. *Nature reviews Molecular cell biology*, *20*(8), 490-507.
3. Makarova, K. S., Wolf, Y. I., Iranzo, J., Shmakov, S. A., Alkhnbashi, O. S., Brouns, S. J., ... & Koonin, E. V. (2020). Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. *Nature Reviews Microbiology*, *18*(2), 67-83.
4. Marraffini, L. A. (2015). CRISPR-Cas immunity in prokaryotes. *Nature*, *526*(7571), 55-61.
5. Doudna, J. A. (2020). The promise and challenge of therapeutic genome editing. *Nature*, *578*(7794), 229-236.
6. Liu, J. J., Orlova, N., Oakes, B. L., Ma, E., Spinner, H. B., Baney, K. L., ... & Doudna, J. A. (2019). CasX enzymes comprise a distinct family of RNA-guided genome editors. *Nature*, *566*(7743), 218-223.
7. Wang, H., La Russa, M., & Qi, L. S. (2016). CRISPR/Cas9 in genome editing and beyond. *Annual review of biochemistry*, *85*(1), 227-264.
8. Doench, J. G. (2018). Am I ready for CRISPR? A user's guide to genetic screens. *Nature Reviews Genetics*, *19*(2), 67-80.
9. Meyers, R. M., Bryan, J. G., McFarland, J. M., Weir, B. A., Sizemore, A. E., Xu, H., ... & Tsherniak, A. (2017). Computational correction of copy number effect improves specificity of CRISPR–Cas9 essentiality screens in cancer cells. *Nature genetics*, *49*(12), 1779-1784.
10. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *science*, *337*(6096), 816-821.
11. DeWeirdt, P. C., Sanson, K. R., Sangree, A. K., Hegde, M., Hanna, R. E., Feeley, M. N., ... & Doench, J. G. (2021). Optimization of AsCas12a for combinatorial genetic screens in human cells. *Nature biotechnology*, *39*(1), 94-104.
12. Chen, Y., Zhuangchai, L., Zhang, X., Zhanxi, F., Qiyuan, H., Chaoliang, T., & Zhang, H. (2020). Author Correction: Phase engineering of nanomaterials. *Nature Reviews. Chemistry*, *4*(5), 269-269.
13. Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, *157*(6), 1262-1278.
14. Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. *Nature communications*, *9*(1), 1911.
15. Shmakov, S., Abudayyeh, O. O., Makarova, K. S., Wolf, Y. I., Gootenberg, J. S., Semenova, E., ... & Koonin, E. V. (2015). Discovery and functional characterization of diverse class 2 CRISPR-Cas systems. *Molecular cell*, *60*(3), 385-397.
16. Knott, G. J., & Doudna, J. A. (2018). CRISPR-Cas guides the future of genetic engineering. *Science*, *361*(6405), 866-869.
17. Blount, S., & Long, J. (2021). CRISPR-Cas systems: Mechanisms and applications. *Trends in Genetic Engineering*, *40*(5), 432-451.
18. Wiedenheft, B., Sternberg, S. H., & Doudna, J. A. (2012). RNA-guided genetic silencing systems in bacteria and archaea. *Nature*, *482*(7385), 331-338.
19. Ishino, Y., Krupovic, M., & Forterre, P. (2018). History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. *Journal of bacteriology*, *200*(7), 10-1128.
20. Yan, W. X., Hunnewell, P., Alfonse, L. E., Carte, J. M., Keston-Smith, E., Sothiselvam, S., ... & Scott, D. A. (2019). Functionally diverse type V CRISPR-Cas systems. *Science*, *363*(6422), 88-91.
21. Li, X., Wang, Y., Liu, Y., Yang, B., Wang, X., Wei, J., ... & Chen, J. (2018). Base editing with a Cpf1–cytidine deaminase fusion. *Nature biotechnology*, *36*(4), 324-327.
22. Chen, J. S., Ma, E., Harrington, L. B., Da Costa, M., Tian, X., Palefsky, J. M., & Doudna, J. A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, *360*(6387), 436-439.
23. Cox, D. B., Gootenberg, J. S., Abudayyeh, O. O., Franklin, B., Kellner, M. J., Joung, J., & Zhang, F. (2017). RNA editing with CRISPR-Cas13. *Science*, *358*(6366), 1019-1027.
24. Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., ... & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, *576*(7785), 149-157.