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CRISPR Screens in Colon Cancer

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Abstract:- CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats – Cas9) is an innovative technology that enables researchers to interrogate the function of genes through precise editing of genes. Researchers have recently utilized this technology to assess the function of all genes in a system using CRISPR-Cas9 screens. These screens are powerful techniques that enable the unbiased interrogation of gene function in various model systems and organisms. These pooled screens employ a predefined set of genetic perturbations called sgRNAs, which are introduced into a pool of cells. This pool of cells, each with its own genetic perturbation, is then grown in competition with each other. After a period of competition, the effects of each perturbation are assessed through the sequence-based counting of each specific mutation, or sgRNA. CRISPR-Cas9 screens have revealed numerous molecular pathways that may confer resistance or sensitivity to various biological challenges. This review will describe how these techniques have advanced our understanding of colon cancer and demonstrate how these screens can be modeled and optimized *in silico* using computational simulation tools. It will further describe how these screens have been applied to living organisms and provide insight into how these may advance our understanding of various biological mechanisms and organ functions.

***Keywords*:-** *Biomedical and Health Science; Genetics and Molecular Biology of Disease; CRISPR; Pooled Genetic Screens; in Silico Simulation.*

I. INTRODUCTION

CRISPR screening has proven to be a promising method for the future of medicine, with still a lot more to come. Essentially the pooling of a multitude of cells to find target genes that respond well to a particular drug or infection, CRISPR screening is conducted with a uniform method in most screens. Traditionally this is done by constructing a pool of oligos, which essentially contains single-stranded DNA complexes that code for the sgRNA of a target gene and contain specific genetic sites so that a lentivirus plasmid can be cloned from the single-stranded DNA [1]. Once these lentivirus plasmids are designed, each with a different genetic makeup, they are injected into a plate of target cells. The RNA is then reverse-transcribed allowing it to amalgamate with the target cell's DNA. Conversely, a new CRISPR screening method is becoming more and more prominent when researchers have already narrowed down the genes they want to test, and there isn't a vast abundance of possible target genes. Here, researchers use a CRISPR gRNA library with one gRNA per cell, and all the cells are separated by wells. The gRNA is introduced into the cell through various methods such as electroporation, lipofection, or viral infection. Upon introduction of these vectors, the gRNA becomes integrated into the cell's DNA. Once the new genes are integrated into target cells, these cells are placed in a competitive cell culture to see which genetic combination has the most selective survival in this specific cell culture. Cell growth and expression can be measured with fluorescent markers and dyes, allowing researchers to see which cells survived and which genes gave the cells a selective advantage over the others. Thus, both processes have various applications, such as those in colon cancer and other life-threatening diseases. For example, researchers can discover genes responsible for a cancer cell's growth and target those genes in future treatments. The results of these CRISPR screens can be portrayed through heat maps and characteristic curves, allowing for more data analysis and plausible future experiments. Overall CRISPR screening is an adequate first step in fabricating a set of experiments that eventually produce a drug that can target a problematic gene.

II. HISTORY OF CRISPR

The history of CRISPR dates back over 20 years ago, in 1987, when the CRISPR-Cas9 function was found in various bacteria and archaea [2]. However, there wasn't much information surrounding DNA sequencing, limiting the possibilities of CRISPR at the time. As the years progressed and more knowledge about the DNA sequence grew, researchers finally understood the purpose of the CRISPR-Cas9 system in bacteria and archaea alike. They realized that the combination of CRISPR and Cas9 proteins could essentially provide immunity to the organism. However, researchers noticed abnormalities in these organisms, such as uncounted repetition of specific sequences [3]. As a product of this, multiple researchers in the early 2000s continued to study the function of the CRISPR-Cas9 system and its possibilities. Makarova concluded that CRISPR could silence genes in organisms coded for the required Cas9 protein [4] [5]. This experiment on a cleave experiment, where target DNA was cleaved by crRNA-tracrRNA-Cas9. After the Cas9 is bound to the target DNA, it could unwind the DNA due to its REC lobe that recognizes the nucleotides. Then the HNH and RuvC domains of Cas9 broke the DNA, essentially allowing the process to silence a target gene. This experiment sheds light on many experiments to come, paving the way for one of the most influential and efficient biomedical research methodologies: CRISPR-Cas9 Screens.

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III. DISCUSSION

➢ *CRISPulator Allows Researchers to Conduct in-Silico Screens to Optimize Future Lab Results*

As technology in this up-and-coming world advances, scientists from all over can harness advanced technology to visualize and assist them in their research. Developed by Tamas Nagy and Martin Kampmann and written by Tamas Nagy, CRISPulator is a new and running innovation that allows researchers to design CRISPR screens in advance digitally. In addition, it will enable researchers to simulate the parameters of CRISPR screens in a digital setting. Despite CRISPR screens' utmost potential, they can become extremely costly and timely, making CRISPulator extremely helpful and viable. For example, researchers can run their desired numbers through the program in their terminal and see which combinations give them the best results. In addition, CRISPulator generates visuals with different colors and presentation methods so that researchers can view where a potential CRISPR screen may take them and where other parameters may benefit and harm them.

➢ *Running Crispulator*

Running CRISPulator requires a series of steps to download. After adding Julia to the computer's PATH, researchers must download Crispulator and run their screens through the Crispulator directory. Then researchers can edit the parameters of a CRISPR screen, and the program will provide them with two images. Edits include changing the number of genes, the number of sgRNAs, and whether the screen is a growth screen or FACS screen [6]. A growth screen measures the cell's ability to grow with or without specific genes. However, this type of screen isn't sufficient for all situations. Hence, researchers also opt for FACS screens, where they screen based on fluorescent activity and use different colors to analyze the activity of a particular gene or gene pathway.

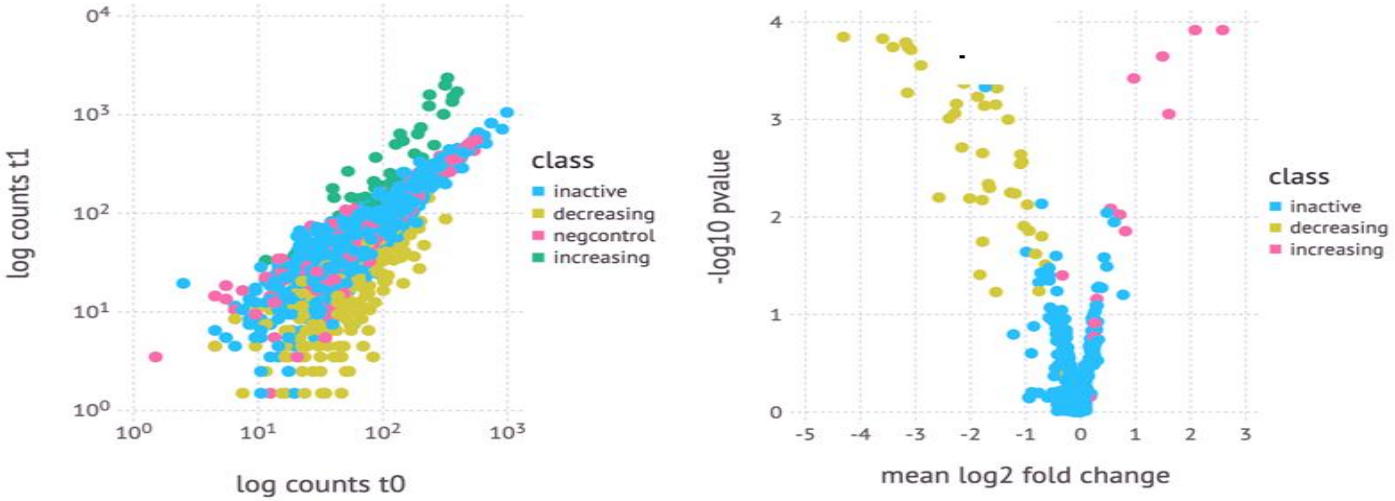

Fig 1 An in Silico Model of a CRISPR Growth Screen. a) A Growth Screen Comparing the Initial Time Point and End Time Points of the Pooled sg RNA (n=500) in the Modeled CRISPR Screen. b) Statistical Significance of the Difference found in a.

With a growth screen of 500 cells, 5 sgRNAs per cell, a transfection selection, and infection value all of 100, CRISPulator generated two images (Figure. 1) [7].

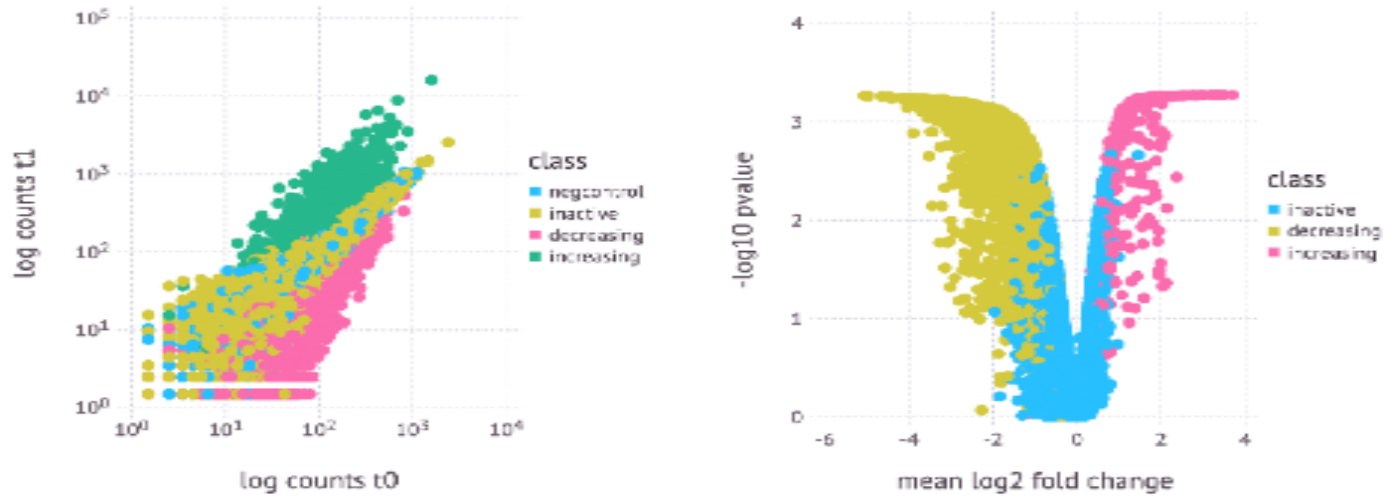

Fig 2 An in Silico Model of a CRISPR Facs Screen. a) A Growth Comparison between the Initial and End Time points of the Pooled sgRNA (n=19114) in the Modeled CRISPR Screen. b) Statistical Significance of the Difference in Terms of Genetic DNA Targets.

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CRISPulator has now given us a breakdown of how each sgRNA will react, as each point represents a different sgRNA. The image on the left is color-coded to express whether they target a positive or negative phenotype. The negative control (pink) points represent the controlled sgRNAs that aren't for a specific target gene. The image on the right shows researchers' calculations for gene phenotypes, and each dot represents a gene. They aim to signify how far each gene is away from the "wild-type" through a volcano plot.

With a FACS screen of 19114 cells, 6 sgRNAs per cell, and transfection, selection, and infection, all values of 100, CRISPulator generates two graphs **(Figure 2)** that look very different from the previous screen.

These images contain a visibly higher amount of spots and a more dense-looking graph. This is because of both the number of cells was increased, as well as the number of sgRNAs. If there are more sgRNAs, there are more dots on the graph on the left. Additionally, if there are more sgRNAs, there are more target genes, which is why the graph on the right is also denser, as that graph indicates genetic phenotypes. Because FACs screens measure fluorescence levels, researchers can use CRISPulator to determine the top and bottom quartile of the cell population, as this is ideal for a FACS screen [8].

Lastly, we conducted a growth screen with 19114 cells, 4 sgRNAs per cell, and transfection, selection, and infection all values of 100 **(Figure. 3).**

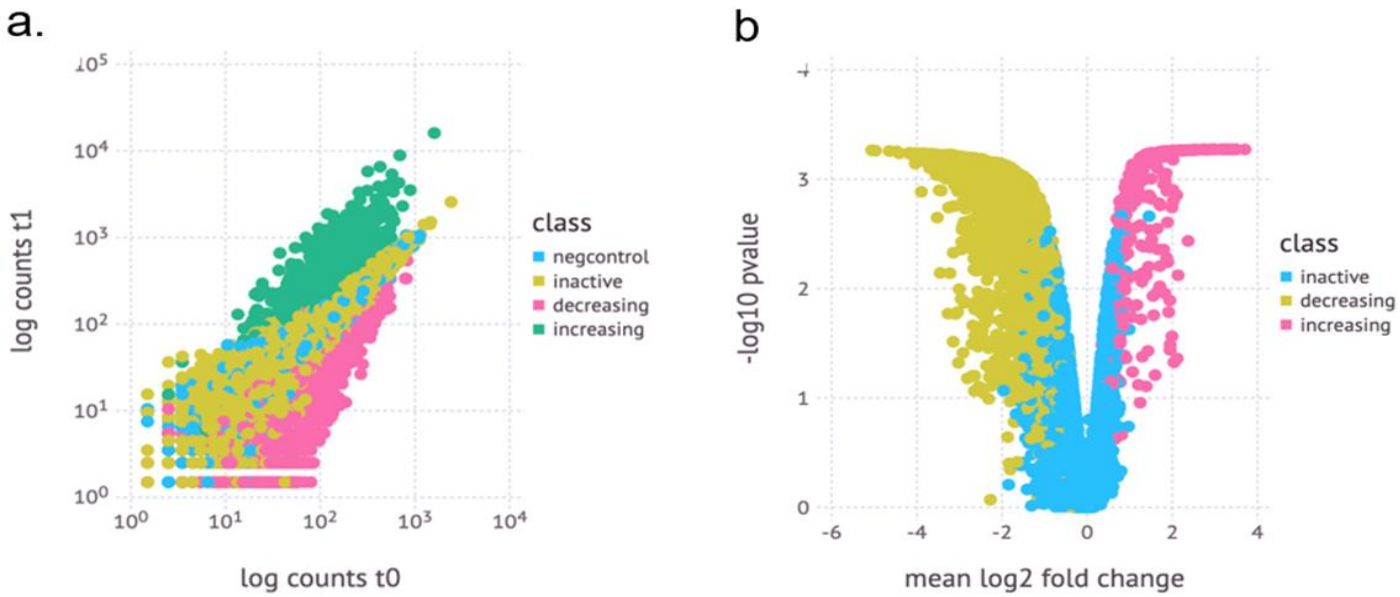

Fig 3 An in Silico Model of a CRISPR Growth Screen. a) A Comparison between the Initial and End Time Points of The Pooled sgRNA (n=19114) in The Modeled CRISPR Screen. b) Statistical Significance of the Difference in Terms of Genetic DNA.

The graphs here look slightly different, as this screen measures the growth of the cells. Growth in-silico screens allow researchers to determine the strength of positive phenotypes, which can be rare in growth *in vitro* screens. Researchers can then edit the numbers to optimize the number of positive phenotypes in growth screens, as they can use these graphs to analyze the results.

➢ *In Vitro Crispr Screen*

The overall methodology of a CRISPR screen follows a complex 4 step process. The initial step is to select an organism from which the samples will be collected, whether human, tissue, or plant tissue [9]. Many cells extracted from the selected organism create the cell culture on which the CRISPR screen will be performed. Once the cells have been chosen, they are usually genetically engineered, through a plasmid, mRNA, or a protein, to transduce the CRISPR-cas9 protein, increasing the efficiency of the CRISPR screen. It is imperative to check the efficiency of the cell's ability to express the CRISPR-cas9 protein at this stage, which can be done by (FLAG-tag) immunoblot which tests the flow of these new proteins [10]. At this stage, if an *in vivo* experiment is being performed, the cells will be transplanted back into the organism, where they will be screened to test the effects of a drug on the organism itself. In an *in vitro* screen, which will be performed on the cell culture outside the living organism, the next step is determining target genes and sequences in the cells ' genomes. Looking at the genome in its entirety, researchers determine which genes they want to target with the sgRNAs, sometimes all in the genome and sometimes a select handful. After the target genes are selected, a pool of sgRNAs must be ready to distribute within the cell culture. This is done by creating a pool of oligos, customized single-stranded DNA strands that correlate with the cells 'targeted genes. Including positive and negative controls in these engineered oligos is crucial, as the screen will not be seen as accurate without them. Once these oligos have been engineered with not only the target gene's sequence but specific genetic sites that allow cloning into a plasmid, they are cloned into plasmids containing lentiviral genes. This is crucial because these lentiviral-gene-containing plasmids are used to produce lentiviruses, which can efficiently carry these genes artificially engineered into the cell culture due to their ability to stay intact when transported through the nuclear membrane [11]. Once these lentiviruses have been

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produced, they can be infected into the aforementioned cell culture. The sgRNAs will detect DNA correlating with their nucleotide, allowing them to attach to the target genes. Once attached, adding tracrRNA will complete the complex by binding the Cas9 protein to the target DNA [12]. The Cas9 protein is a protein that can cut the target gene out, allowing for the screen to begin. A significant factor to consider when infecting the cell culture is the multiplicity of infection (MOI), which is usually set in between 0.3 to 0.5 [13] [14]. This is the number of virions, or the gRNAs, delivered to each cell. If a screen tests 123,411 gRNAs, a screen with a 0.2 MOI would require around 617,055 (123,411 divided by 0.2) cells, ensuring each virion is delivered to a cell [15]. Representation is another factor considered when determining the number of cells to screen. When the screen is running, specific cells can die for uncalculated reasons, which could significantly alter the results. To avoid this, the number of cells injected is increased so that minor blips in the data don't have as heavy of an influence on the actual data. Therefore, instead of 617,055, we multiply the number of cells by 100, giving us an overall 61705500 cells. Once the cell culture has been infected with the lentiviruses, in RNA form, they must be reverse transcribed into DNA for CRISPR to begin. Once CRISPR begins, the goal of the screen is to determine a cell's ability to grow with or without a particular gene.

The cell culture can be examined at different time stamps, examining the progressions of growth or death in cells with different genomes. Examinations can be conducted through multiple methodologies with various complexities, usually following a 5-step process. Beginning with data processing in *in vitro* CRISPR screening pools, where sequencing reads of the cell culture are processed into matrices, a digital and more accessible method of holding the data. These matrices now include data on each gRNA being tested in the CRISPR screen, formed using various digital programs. Following data processing is quality control, which ensures that the collected data is reliable and accurate. This factors in the number of current sgRNAs compared to the initial, consistency of results, expected behavior by positive and negative controls, and high sgRNA representation. After collecting data and ensuring its accuracy, the next step involves ranking each sgRNAs phenotypical effect on the cells, factoring in components such as time-stamps when the screen was examined. After the ranking process is finished, researchers can decipher which genes are most relevant to a cell's proliferation and thus determine possible gene targets for drug treatments. Researchers can then compare these results to other screens ' results to find commonalities and points of comparison. The last step to data analysis for CRISPR screens is presenting the data visually to better overall understanding for a larger audience. Visual interpretations include graphs, heat maps, and volcano plots, all of which assist in finding patterns, consistencies, and outliers which can help determine the viability of a CRISPR screen.

- *Indirect in Vivo Screens Start to become Efficient in Determining the Effects of an Absence of a Gene in a Living Body*

Although *in vitro* screens are used abundantly, they are limited because they are still only done on a plate rather than in an organ. The results found in vitro screens often differ greatly from the same experiment conducted in a living body, such as mice. Due to this inhibition with *in vitro* screens, researchers usually prefer doing at least one *in vivo* screen alongside their *in vitro* screen. Indirect *in vivo* screens are specifically useful for cancer screens. Many cancers inhibit or weaken an organ, a combination of various cells rather than a group of one type of mutated cell. The initial methodology of an *in vivo* screen is similar to an *in vitro* screen, where cells are taken from a specific organ of a specific biological model (such as a mouse). The MOI and representation are usually the same as they would be in an *in vitro screen*: a screen with 123,411 sgRNAs would have the same MOI of 0.2 and a representation multiplier of 100 [16]. After cells are collected, oligos are engineered with desired DNA targets, including the DNA that the researchers want to prevent replication of, as these oligos will then form lentiviral plasmids with gRNAs. However, once these gRNAs are inserted back into the collected cells, the process veers from the *in vitro* process. These cells are now inserted back into the living body the cells were initially collected from, so researchers can study how the engineered cells react in a complex organ system. This screen is named "indirect" because rather than inserting the engineered DNA into the animal, the cells are removed from the animal and inserted back with the engineered DNA. In cancer CRISPR screens, the engineered cells that survived will likely form a tumor, whereas the cells with a nonpreferable DNA makeup due to the sgRNAs will likely die out. This allows researchers to dissect the tumor and examine the cells that have survived in the tested organism. The cell responsible for the tumor's injected sgRNAs will likely become the next target for future tumor suppressors to inhibit such tumors from starting in the first place. Despite its strengths, *indirect in vivo* screens cannot conduct all types of screens, as the cells used in indirect screens must be transplantable, meaning they can be transplanted in and out of the cell. Additionally, many of the organisms that contain these transplantable cells are immunocompromised, thus bringing into the picture ethical considerations.

- *Direct in-Vivo Screens can Further the Progress of in-Vitro CRISPR Screens*

As a result of some of the limitations of *indirect in vitro screens*, researchers have started conducting direct *in vivo* screens. As done with prior screens, the first step is to create a sgRNA library with single strands that correlate with the soon-to-be-tested target genes. The sgRNA is then used to create the lentiviral plasmids. Next, the lentiviral plasmids, such as a liver, are injected into the chosen organ. The sgRNA can assimilate throughout the organ by entering the various cells. After a sufficient period of time, the levels of sgRNA are then examined within the organism. Researchers can now determine which sgRNAs concentration went up, meaning the cells with that specific sgRNA could survive and replicate, and which sgRNAs

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concentration went down, meaning the cells with that specific sgRNA could not survive and had started to die out. One recent discovery using direct *in vivo* screening was the screening of a mouse's liver that aimed to discover information about the mouse's regulation of hepatocyte fitness. Using a targeted and direct *in vivo* screen, researchers induced the mouse liver with sgRNAs from lentiviruses, allowing these single guide RNAs to integrate within every liver cell [17]. Because a mouse's liver cell is covered in hepocotytes, there were many DNA targets that the researchers 'sgRNAs could target [18]. Once the sgRNAs had assimilated throughout the cells 'DNA, researchers could observe which hepocotyte cells were surviving and which were not, leading to the finding of positive and negative regulators of hepatocyte fitness.

- *Science Advances Identifying DLD1 as a Therapeutic Vulnerability in Colon Cancer.*

Colorectal Cancer is one of the world's leading causes of cancer-related deaths, making the disease a vital disease to study. A mutated WNT pathway accounts for most colorectal cancers due to its overactivation. When the WNT pathway is overactivated, there is an excessive amount of **β**-Caratenin in colon stem cells, leading to the development of "colonic polyps" and thus the result of carcinomas. When there is excessive activation of **β**-Caratenin, the expression of various targets is activated through transcription factors. These targets, such as *cMYC*, *AXIN2*, *ASCL2*, *LGR5*, and *CD44*, regulate the proliferation of colon stem cells, making them targets for current research to prevent the overexpression of **β**-Caratenin [19].

Researchers initially used CRISPR Cas-9 Screening to produce colon cancer cell lines on which they could perform the actual screening. Through the screen, researchers hoped to evaluate the activation status of Wnt/**β**-Caratenin in colon cancer cells in the DLD1 line rather than finding a specific gene that regulated it. Using a lentiviral 7× TOP-dGFP mCherry vector where a green fluorescent protein is regulated through 7× TOP-dGFP allows researchers to determine the **β**-Caratenin levels through cell fluorescence. In addition, vector mCherry acted as a fluorescent control, allowing researchers to compare the fluorescent glow of the proteins regulated through 7xTOP-dGFP with the ones of mCherry. Results showed that the lack of **β**-Caratenin led to a lack of GFP Signaling and hence a lower fluorescent glow than that of the mCherry vector. This demonstrated that the DLD1 was indeed a good and effective line with Wnt/**β**-Caratenin activity.

Now that the researchers had the selected cell line of study (DLD1), scientists conducted a whole genome CRISPR Cas-9 screen. They injected the Brunello library of sgRNAs, with 76,411 sgRNAs) to target 19,114 human genes of the DLD1 line [20]. They wanted to determine what **β**-Caratenin regulators could inhibit cell growth in a colorectal cell line. They collected GFP levels, similar to the first step, to determine **β**-Caratenin activity. After the 7-day mark of injecting sgRNAs, they assessed which sgRNAs had increased or decreased GFP levels, with 5% highest GFP being classified as GFP-high and 5% lowest GFP being classified as GFP-low. After the checkpoint at the 7-day mark, the cells continued to grow until the 21-day mark to test cell viability. Whichever cells were still alive were indicators for which regulators resulted in sustained cell growth. The results showed 497 genes vital to DLDI proliferation in colon cancer cells. In addition, the transcription factor TCF7L2 was shown to be a bona fide negative regulator of DLD1. However, researchers also discovered that the lack of TCF7L2 resulted in little to no review, thus making it an unreliable target gene [21]. However, they had now found a viable number of target cells they could further study to produce/release a treatment possibly.

To further test their results, researchers treated two [reporter] DLD1 cell lines with **β**-Caratenin [22]. They also knocked in cassettes with dGFP and red fluorescent protein to visibly compare the levels of dGFP, as the transcription of **β**-Caratenin endogenous was parallel and relative to the transcription of dGFP [23]. This part of the experiment confirmed that adding a **β**-Caratenin sgRNA led to the transcription of a desired target gene, determined with the previous CRISPR screen [24]. Researchers now tested if inhibiting this target gene produced significant results. They did this by knocking in a cassette of dGFP into the mCYC and then using JQ-1 to inhibit the transcription of mCYC, the target gene. When this transcription was inhibited, the cell, like RKO, showed a reduction in GFP levels.

Now, scientists took the 2 DLDI cell lines and the RKO cell line to perform a general CRISPR screen to find genes required for both **β**-Caratenin transcriptional output and growth, as these genes would be targets for the newest treatments and drugs. The results of this CRISPR screen found 476 genes unique to the cells with **β**-Caratenin, compared to the **β**-Caratenin inactive cells.

- The Importance of the MAPK Pathway was determined in Colon Cancer cells Using in vitro screens

Another major mutation found in many colon cancer cases is mutations in the KRAS gene, which can activate the MAPK pathway [25]. Previous studies have tried to repress MEK using MEKi. However, MEKi treatments in clinical trials proved inefficient, as inhibiting MEK resulted in various adverse effects. For example, the inhibition of MEK resulted in the activation of RTKs, which could restart the MEK pathway despite the efforts to inhibit it [26]. Hence, researchers performed a CRISPR knockout to identify oncogenes that improve MEKi resistance in colorectal cancer cells [27]. They also targeted MEK and PLK kinases to determine what type of effect it would have in colorectal cells, both in vitro and in vivo. They used the cell line HCT116, which had medium drug resistance to MEKi treatments [28]. They utilized a representation of 500, thus multiplying the number of sgRNAs by 500 and an MOI of 0.3. Their final number of cells was a grand 2 million, which they then infected with a lentivirus system from the human GeCKO library [29]. These cells were also infected with AZD6244 for seven days so researchers could determine which cells were proliferating, thus producing AZD6244. The active genes in the cells producing

AZD6244 were considered "candidate genes" responsible for MEKi resistance in the RTK Pathway. One of the specific genes they found was GRB7, where the knockout of GRB7 in a cell resulted in a lethal effect in the MEKi of that cell, meaning GRB7 plays a crucial role in MEKi resistance, making it a future drug target [30].

IV. CONCLUSION

Through *in silico* screens, *in vitro* screens, and *in vivo* screens, CRISPR-Cas9 Screens have proven to be a powerful tool in detecting gene targets in cancers, specifically colon cancers. From single-stranded DNA to lentiviral plasmids, researchers can insert sgRNAs attached to Cas9 proteins into cells and cut target genes out, a renowned and developed process. This allows researchers to evaluate how cells react with the addition or removal of different genes: a meaningful discovery for cancer research. In addition, researchers carefully evaluate the number of cells they should use in their culture compared to the number of sgRNAs they are testing, using evaluation methods such as MOI and representation. Recently, researchers have discovered target genes to improve hepacyte fitness and target genes for countering cancer growth in colons. The limits of CRISPR screens are indefinite and possibilities wide, as further advancements in CRISPR screens could ultimately lead to more and more cures for one of the deadliest diseases: cancer.

REFERENCES

[1]. Bock, Datlinger, Chardon, Coelho, Dong, Lawson, Lu, Maroc, Norman, Song, Stanley, Chen, Garnett, Li, Moffat, Qi, Shapiro, Shendure, Weissman, Zhuang, "High-Content CRISPR Screening."
[2]. Ishino, Krupovic, and Forterre, "History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology."
[3]. Jansen, and Embden, and Gaastra, and Schouls, "Identification of Genes That Are Associated with DNA Repeats in Prokaryotes."
[4]. Makarova, Wolf, Iranzo, Shmakov, Alkhnbashi, Brouns, Charpentier, Cheng, Haft, Horvath, Moineau, Mojica, Scott, Shah, Siksnys, Terns, Venclovas, White, Yakunin, Yan, Zhang, Garrett, Backofen, Oost, Barrangou, and Koonin, "Evolutionary Classification of CRISPR–Cas Systems: A Burst of Class 2 and Derived Variants."
[5]. Makarova et al., "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."
[6]. Menasche, Crisman, and Shen, "Fluorescence Activated Cell Sorting (FACS) in Genome-Wide Genetic Screening of Membrane Trafficking."
[7]. Chong, Yeap, and Ho, "Transfection Types, Methods and Strategies: A Technical Review."
[8]. Nagy and Kampmann, "CRISPulator: A Discrete Simulation Tool for Pooled Genetic Screens."
[9]. Miles, Garippa, and Poirier, "Design, Execution, and Analysis of Pooled in Vitro CRISPR/Cas9 Screens."
[10]. Dong and Kantor, "Lentiviral Vectors for Delivery of Gene-Editing Systems Based on CRISPR/Cas: Current State and Perspectives."
[11]. Durand and Cimarelli, "The Inside Out of Lentiviral Vectors."
[12]. Liu, Zhang, Liu, and Cheng, "Delivery Strategies of the CRISPR-Cas9 Gene-Editing System for Therapeutic Applications."
[13]. Gutiérrez Serafín, Pirolles, Yvon, Baecker, Michalakis, and Blanc, "The Multiplicity of Cellular Infection Changes Depending on the Route of Cell Infection in a Plant Virus."
[14]. Shojaei Baghini, Gardanova, Zekiy, Shomali, Tosan, and Jarahian, "Optimizing SgRNA to Improve CRISPR/Cas9 Knockout Efficiency: Special Focus on Human and Animal Cell."
[15]. Wan, Mahara, Sun, Doan, Chua, Xu, Bian, Li, Zhu, Sooraj, Cierpicki, Grembecka, and Firestein, "Genome-Scale CRISPR-Cas9 Screen of Wnt/β-Catenin Signaling Identifies Therapeutic Targets for Colorectal Cancer."
[16]. Kuhn, Santinha, and Platt, "Moving from in Vitro to in Vivo CRISPR Screens."
[17]. Keys and Knouse, "Genome-Scale CRISPR Screening in a Single Mouse Liver."
[18]. Mendoza Banerjee, and Sarkar, "Isolation and Culture of Mouse Hepatocytes and Kupffer Cells (KCs)."
[19]. Wan, Mahara, Sun, Doan, Chua, Xu, Bian, Li, Zhu, Sooraj, Cierpicki, Grembecka, and Firestein, "Genome-Scale CRISPR-Cas9 Screen of Wnt/β-Catenin Signaling Identifies Therapeutic Targets for Colorectal Cancer."
[20]. Sanson, Hanna, Hegde, Donovan, Strand, Sullender, Vaimberg, Goodale, Root, Piccioni, and Doench, "Optimized Libraries for CRISPR-Cas9 Genetic Screens with Multiple Modalities."
[21]. Wenzel, Rose, Haghighi, Lamprecht, Rauen, Freihen, Kesselring, Boerries, and Hecht, "Loss of the Nuclear Wnt Pathway Effector TCF7L2 Promotes Migration and Invasion of Human Colorectal Cancer Cells."
[22]. Sebio, Kahn, and Lenz, "The Potential of Targeting Wnt/β-Catenin in Colon Cancer."
[23]. Campbell, Tour, Palmer, Steinbach, Baird, Zacharias, and Tsien, "A Monomeric Red Fluorescent Protein."
[24]. Hu, Feng, Liang, Du, Li, and Niu, "CRISPR/Cas9-Induced β-Carotene Hydroxylase Mutation in Dunaliella Salina CCAP19/18."
[25]. ZHANG and LIU, "MAPK Signal Pathways in the Regulation of Cell Proliferation in Mammalian Cells."
[26]. Roberts and Der, "Targeting the Raf-MEK-ERK Mitogen-Activated Protein Kinase Cascade for the Treatment of Cancer."
[27]. Zhan, Ambrosi, Wandmacher, Rauscher, Betge, Rindtorff, Häussler, Hinsenkamp, Bamber, Hessling, Müller-Decker, Erdmann, Burgermeister, Ebert, and Boutros, "MEK Inhibitors Activate Wnt Signalling and Induce Stem Cell Plasticity in Colorectal Cancer

[28]. Ma, Xu, Tang, Zhang, Zhao, Wang, and Chen, "Anti-Cancer Potential of Polysaccharide Extracted from Hawthorn (Crataegus.) on Human Colon Cancer Cell Line HCT116 via Cell Cycle Arrest and Apoptosis."
[29]. Sanjana, Shalem, and Zhang, "Improved Vectors and Genome-Wide Libraries for CRISPR Screening."
[30]. Han, Shen, and Guan, "The Grb7 Family Proteins: Structure, Interactions with Other Signaling Molecules and Potential Cellular Functions."